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Influence of estradiol and exercise on foot laxity, ankle laxity, and plantar loading in college athletes

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This thesis is dedicated to my mother Karoline Silke for her unconditional love and support.

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Abstract

As female participation in athletics has increased, it has become apparent that sex differences exist in sports-related injury types and frequencies. The purpose of this study is to examine the effects of exercise and the sex hormone estradiol on sex differences in foot and ankle laxity and plantar pressure.

We measured serum estradiol, pre- and post-fatigue ankle and toe laxity, and pre- and post-fatigue plantar pressure in 47 subjects (34 female, 13 male) once a week for 12 weeks. We compared laxity and plantar pressure in females not using oral contraceptive (NOC) to females using oral contraceptives (OC) and males. Relative estradiol level was regressed against plantar pressure variables and laxity pre- and post-exercise.

The results of this study demonstrated that females had higher ankle and first metatarsophalangeal (MP1) joint laxity than males. There were no differences in ankle and MP1 laxity between OC and NOC groups or across the menstrual cycle. There was no relationship between estradiol levels and ankle/MP1 joint laxity. The exercise protocol caused an increase in MP1 joint laxity but did not alter ankle laxity. Despite no changes in speed, stride length, or step length, post-exercise peak pressure in the toes and metatarsals 1 and 2 regions decreased while peak pressure in the medial midfoot increased. Contact area increased in metatarsals 1 and 2 and toe 1 while maximum force remained the same. There was no relationship between peak plantar pressure and joint laxity in any foot regions or in either joint. Males had higher toe 1 peak pressures than females and metatarsal 1 and 2 peak pressures were higher in the NOC group compared to the OC group. Peak pressure was not different across the menstrual cycle for any foot region.

Joint laxity is increased by repetitive loading from exercise and higher levels of estradiol in females than males. Influences in joint laxity are not evident at the lower levels of sex

hormone involved in monthly fluctuation. Increased joint laxity may increase the risk for ligamentous injury which might explain why females have higher rates of ankle sprains compared to males, especially after exercise. Additionally, joint laxity and muscular fatigue in response to repetitive loading from exercise potentially increase bone strain and raise the risk for stress fractures by providing less support to the bone. The metatarsals 1 and 2 might be a common site for stress fractures because maximum force remains the same after the supporting muscles are fatigued and surrounding soft tissues are stretched out. Repetitive loading also increases medial midfoot pressure which raises the risk for shin splints and patellofemoral overuse injury. Prevention strategies will likely need in incorporate strength and endurance training as well as optimizing joint laxity.

Introduction

Increased involvement of females in athletics and military employment has brought to the forefront sex differences in types and frequencies of sports-related injuries. The National Collegiate Athletic Association (NCAA) reports that more than 100,000 women participate in collegiate level sports each year. The 2007 NCAA Injury Surveillance System reported that female participation in collegiate athletics has increased 80 percent from the late 1980's. The increased female involvement in sports and athletics has been accompanied by noticeable differences in male and female injury rates.

Despite similar sports and exposure frequencies, females tend to experience different injuries than males and, in general, are injured more frequently than males in the lower limb (Almeida; 1999; Deitch, 2007) as summarized in Table 1. The most common lower limb sports-related injuries among all athletes are anterior cruciate ligament (ACL) tears, knee pain, stress fractures, and ankle sprains (Almeida, 1999; Borowski, 2008; Iwamoto, 2008). It is well known that females sustain more ACL injuries than males (Agel, 2005; Arendt, 1999; Arendt and Dick, 1995; Bjordal, 1997; Borowski 2008; Chandy, 1985; Dehaven and Lintner, 1986; Deitch, 2007; Ferretti, 1992; Gray, 1985; Gwinn, 2000; Hootman, 2007; Iwamoto, 2008; Lanese, 1990; Lindenfeld , 1996; Malone, 1993; Messina, 1999; Mihata, 2006; Mountcastle; 2007; Myklebust, 1998; Oliphant and Drawbert, 1996; Prodromos, 2007; Ristolainen, 2009). This is especially true in sports such as soccer and basketball where there is a lot of cutting (Arendt, 1999). Anterior patellofemoral pain is also reported to be more common in female athletes (Almeida, 1999; Fulkerson, 2002; Iwamoto, 2008). Stress fractures in the tibia and metatarsals are shown to be 10 times more likely in female athletes and military personnel than in male counterparts, especially in track/field, basketball, soccer, and basic training (Iwamoto, 2008; Protzmann, 1977). The foot, especially the metatarsals, is more commonly stress fractured than the tibia (Arendt, 2003). Within the foot, females tend to suffer from more metatarsal 2 stress fractures than males (Hame, 2004). The rates of ankle injuries among males and females tend to differ by sport. For example,

Waterman *et al.* (2010) found that athletes participating in rugby, cheerleading, basketball, soccer, and lacrosse have higher incidences of ankle sprains compared to those involved with track sports. In basketball and soccer, females suffer from more ankle sprains than males (Beynnon, 2005; Chandy, 1985; Hosea, 2000; Messina, 1999; Ristolainen, 2009). Furthermore, Hosea *et al.* (2000) reported that female athletes have a 25% greater risk of sustaining grade I ankle sprains compared to their male counterparts.

Understanding the etiology of sex differences in injury rates and ultimately reducing injury frequency in female athletes requires the investigation of normal lower limb mechanics and injury mechanism. Several intrinsic and extrinsic factors influence the development of sports-related injuries. Extrinsic factors are those which can be externally controlled. Common extrinsic factors that influence injuries include training techniques, neuromuscular coordination (Posthuma, 1987; Sarwar, 1996), shoe design (Wiegerinck, 2009) and biomechanics (Chaudhari, 2007; Milner, 2006). Intrinsic factors are those within the body that cannot be directly manipulated. Anatomical and geometric characteristics (ie: q-angle) (Crossley, 1999; Giladi, 1987; Messier, 2008; Tumia and Maffuli, 2002), bone density (Crossely, 1999) and hormonal influences (Beynnon, 2005; Hewett, 2007; Zazulak, 2006) are intrinsic factors that have been associated with the sex bias in sports-related injuries. It is likely that a combination of multiple factors ultimately influence the progression of injuries. This study will specifically focus on examining the roles of hormonal influences and muscular fatigue on potential risks for sports-related injuries.

Table 1. Summary of literature reporting injury frequencies in males and females.

Source	ACL Injuries	Stress Fractures	Ankle Injuries	Muscle Injuries
Agel *et al.*, 2005	F			
Arendt and Dick, 1995	F			
Arendt *et al.*, 1999	F			
Bennell *et al.*, 1996		N		
Beynnon *et al.*, 2005				
Basketball			F	
Soccer, Lacrosse			N	
Bjordal *et al.*, 1997	F			
Borowski *et al.*, 2008	F	M	N	
Cameron *et al.*, 2010			F	
Chandy *et al.*, 1985	F		F	M
Dehaven and Lintner, 1986	F			
Deitch *et al.*, 2007	F			
Ferretti *et al.*, 1992	F			
Gray *et al.*, 1985	F			
Gwinn *et al.*, 2000	F			
Hame *et al.*, 2004		F		
Hootman, 2007	F		N	
Hosea *et al.*, 2000			F	
Iwamoto *et al.*, 2008				
Basketball, Volleyball	F	N	N	N
Tennis	N	N	N	N
Skiing	F	N	N	N
Track/Field	N	F	N	N
Lanese *et al.*, 1990	F		N	
Lindenfeld *et al.*, 1996	F			
Malone *et al.*, 1993	F			
Mihata *et al.*, 2006				
Basketball, Soccer	F			
Lacrosse	N			
Messina *et al.*, 1999	F		F	
Mountcastle *et al.*, 2007	F			
Myklebust *et al.*, 1998	F			
Oliphant and Drawbert, 1996	F			
Porter *et al.*, 2005		M		
Prodromos *et al.*, 2007				
Basketball, Soccer, Handball	F			
Lacrosse, Rugby, Indoor Soccer	N			
Protzmann *et al.*, 1977		F		
Putukian *et al.*, 1996	N			
Ristolainen *et al.*, 2009	F		F	M
Waterman *et al.*, 2010			N	
Zelisko *et al.*, 1982				M

F=females have higher injury rate; M=males have higher injury rate; N=no difference in injury rate. Differences repeated were all statistically significant.

Effects of Sex Hormones on the Skeletal System and Injury

The menstrual cycle is usually divided into follicular, ovulatory, and luteal phases. Just before ovulation, there is a surge of estradiol followed by an acute drop in estradiol levels. During the luteal phase, estradiol, progesterone, and relaxin levels slowly rise and then fall just before menstruation (Figure 1).

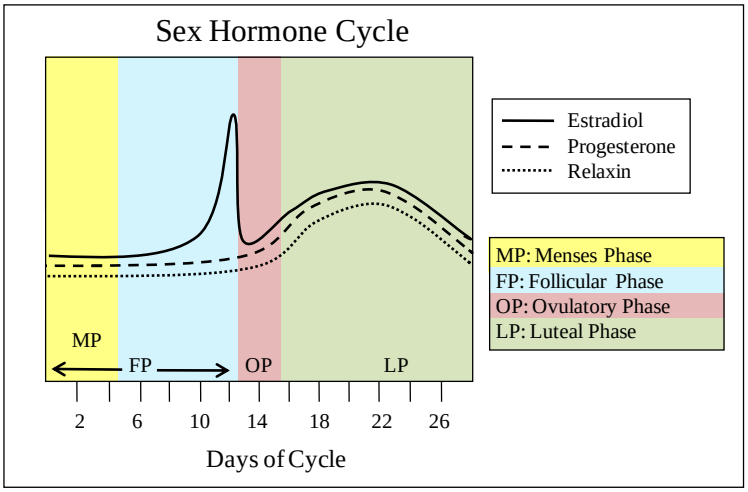

Figure 1. Estradiol, progesterone, and relaxin levels across 28-day menstrual cycle.

These sex hormones influence neuromuscular properties such as strength (Sarwar, 1996), relaxation rate (Jaskolska, 2007; Sarwar, 1996), musculotendonous stiffness (Eiling, 2007, Granata, 2002), and fine motor control (Posthuma, 1987). Sex hormones have also been shown to influence tendon and ligament properties including tendon strain (Bryant, 2008) and knee joint laxity (Beynnon, 2005; Deie, 2002; Park, 2009; Shultz, 2010; Wojtys, 2002; Zazulak, 2006). Due to the high incidence of ACL injuries among female athletes, the relationships among knee joint laxity, the menstrual cycle, and ACL injury rates have been researched extensively (Hewett, 2007; Zazulak, 2006). Several studies have observed a pattern in the rates of ACL injuries across phases of the menstrual cycle, however there is no consensus for which phase has higher injury rates. For example, Adachi *et al.* (2008) and Wojtys *et al.* (2002) found that ACL tears occur significantly more often in the ovulatory phase. Myklebust *et al.* (1998) and Arendt *et al.* (1999) found higher ACL injury rates occurring during the late luteal and follicular phases. Other

studies show that ACL tears occur with increasing frequency only during the follicular phase (Myklebust, 2003; Slauterbeck, 2002). These discrepancies may be explained by the use of inconsistent methods for identifying menstrual cycle and variable phase characteristics (Vescovi, 2011). Therefore, although the relationship between phase and ACL injury rates has yet to be clearly defined, these studies suggest that there may be a relationship.

Numerous *in vitro* studies illustrate a direct effect of sex hormones on tissues properties. One of the primary functions of estradiol and relaxin is to increase the laxity of the pelvic ligaments during childbirth by decreasing collagen in these ligaments. Sex hormones will, to a lesser extent, affect other heavily collagenated tissues in the body during phases of high concentrations. Several studies have demonstrated the presences of sex hormone receptors, particularly for estradiol, in various joint tissues (Liu, 1996; Sciore, 1998; Wiik, 2009). The presence of estradiol and relaxin hormones within the tissue ultimately alters total protein composition by decreasing collagen content (Abubaker, 1996; Fischer, 1973; Liu, 1997). Collagen is a protein that primarily functions to maintain strength and stiffness within the tissue and is found in muscles, tendons, and ligaments. Estradiol decreases collagen content in these tissues by inhibiting collagen synthesis (Fischer, 1973; Kwan, 1996; Liu, 1997). Liu *et al.* (1997) found that collagen synthesis of the rabbit ACL was decreased by more than 40% when estrogen levels were increased from 0.025 to 0.25 ng/ml (within normal physiological levels). Fischer (1973) revealed that, in the presence of extremely large amounts of estradiol (10^5 ng/mL), collagen synthesis was reduced in tendon tissue at 24, 48, and 144 hours and in fascia at 144 hours. Alternatively, Hansen *et al.* (2008) sampled interstitial fluid anterior to the patellar tendon and found that estradiol increased tendon collagen synthesis. However, when estradiol was administered with the combination of exercise, tendon collagen synthesis actually decreased (Hansen, 2008), suggesting that exercise has an important influence on soft tissue properties. Additionally, Abubaker *et al.* (1996) demonstrated that estrogen combined with progesterone significantly decreased collagen content of ovariectomized female and orchiectomized male rats,

which illustrated the importance for understanding the influence of combined sex hormones. Relaxin also downregulates collagen expression (Samuel, 1996; Unemori and Amento, 1990) by inhibiting the expression of procollagenase (Palejwala, 2002), an enzyme involved with the degradation of collagen. It appears that the effects of relaxin (Palejwala, 2001) and estradiol (Fischer, 1973) on collagen content are tissue dependent. For example, relaxin inhibits procollagenase in endothelial cells and stimulates it in cervical fibroblasts (Palejwala, 2001). However few studies have been done to test the effects of relaxin on skeletal tissue in relation to sports injury risk despite the significant role relaxin plays on stretching out the pelvic ligaments during birth.

Due to the effects of sex hormones on collagen content, it is likely that, on a smaller scale, laxity of all ligaments increases during the menstrual phases of high estradiol and relaxin (the pre-ovulatory, and luteal phases) thereby causing cyclic changes in joint laxity. If joint laxity increases, the soft tissues may be at a higher risk for being injured (Myer, 2008; Skinner, 1986). A joint with higher laxity will be less stable and may affect surrounding joints and bones (Hertel, 2000), especially in the foot and ankle where joints are close together. It is somewhat debated whether a more flexible joint has positive or negative effects on the distal structures. On one hand, a joint with more distensible ligaments and tendons has better shock absorbing capabilities helping to attenuate lower limb loading. On the other hand, a more flexible joint is less stable during weight-bearing activities causing muscles to work harder to control for the excessive motion. Most likely there is an optimal joint laxity value for a lowest risk of injury. For example, having an excessively flexible foot arch (low-arched) increases the chances of sustaining a metatarsal stress fracture (Simkin, 1989, Sullivan, 1984), while an arch that is highly rigid (high-arched) raises the risk for tibial stress fractures (Matheson, 1987; Simkin, 1989; Sullivan, 1984). Thus, if sex hormones are causing soft tissue properties of a joint to shift towards laxity values that are too high, there may be negative secondary effects on the surrounding bones, especially if muscles are fatigued and the ability to dampen bone loading is

decreased. Higher peaks and frequencies of bone loading ultimately increase the risk for an overuse injury (Hreljac, 2004).

A number of studies have investigated the relationship between joint laxity and menstrual phase, however the results remain inconclusive (Table 2). If estradiol affects joint laxity by way of collagen breakdown, it would be expected that joint laxity would be highest just before ovulation (highest estradiol peak) and during the luteal phase (estradiol and relaxin peaks). There is some literature that supports this (Table 2). Deie *et al.* (2002) found the highest anterior knee laxity in both the luteal and ovulatory phases compared to the follicular. Park *et al.* (2009) observed that anterior knee laxity increased in the ovulatory phase while Shultz *et al*. (2005) and Heitz *et al*. (1999) reported that knee laxity was highest during the luteal phase. Also, several studies have found no differences in anterior knee laxity across any phase of the menstrual cycle (Belanger, 2004; Eiling, 2007; Hertel, 2006; Karangeanse, 2000; Pollard, 2006). Additionally, no correlation has been found between sex hormone level and joint laxity (Arnold, 2002; Beynnon, 2005). However, most of the studies that have found a pattern of higher laxity across the menstrual cycle have found high laxity in phases with higher estrogen in contrast to the phase(s) being compared (Table 2). This suggests that estrogen is indeed influencing joint laxity. Further research must be done using larger sample sizes, consistent and reliable methods for determining menstrual phase, control for cycle inconsistencies, and accounting for menstrual history and activity level (Vescovi, 2011; Zazulak, 2006).

Table 2. Summary of joint laxity changes across menstrual cycle phases.

Source	Cycle Division (days post-menses)	Days of Data Collection	Relationship Found
Belanger *et al.*, 2004	Follicular (1-9) Ovulatory (10-14) Luteal (15-28)	20 total visits: 2 times per week for 10 weeks	N
Beynnon *et al.*, 2005	Early Fol. (1-3) Late Fol. (11-13) Mid. Luteal (20-22) Late Luteal (27-28)	5 total visits: 1 per phase, 2 in early follicular	N
Deie *et al.*, 2002	Follicular (1-9) Ovulatory (14) Luteal (15-28)	2-3 visits a week over four weeks	O, L>F*
Eiling *et al.*, 2007	Menses (1-5) Follicular (6-13) Ovulatory (14) Luteal (15-28)	4 total visits: day 1, mid-follicular, ovulation, mid-luteal	N
Heitz *et al.*, 1999	Menses (1-3) Follicular (9-13) Luteal (19-24)	8 total visits: days 1, 23-Oct	F,L>M*
Hertel *et al.*, 2006	Mid. Follicular (7-10) Ovulatory (13-15) Mid. Luteal (21-24)	3 total visits: once per phase	N
Karangeanse *et al.*, 2000	Follicular (1-11) Ovulatory (14) Luteal (15-28)	5-8 total visits: 1-7 days apart	N
Park *et al.*, 2009	Menses (1-5) Ovulatory (14) Luteal (15-28)	3 total visits: days 5, 14, 21	O>L, F*
Pollard *et al.*, 2006	Early Follicular (1-7) Mid-Follicular (8-12) Mid. Luteal (21-23)	5 total visits: days 1, 10 12 post menses; 7, 9 post ovulation	N
Shultz *et al.*, 2010	Menses (1-5) Ovulatory (14) Luteal (15-28)	14 total vists: days 1-6 post menses; 1-8 post ovulation	L5<L8
Shultz *et al.*, 2005	Menses (M) (1-5) Ovulatory (O) (14) Early Luteal (EL) (19-23) Late Luteal (LL) (24-28)	20 total visits: first 5 days after onset of each phase	O5, LL1, LL2>M3*

*Higher laxity is found in phases with higher estradiol levels than the compared phase(s).

Designated days in cycle division column were assigned starting with menstruation based on a 28-day cycle to facilitate comparison. Relationship found column refers to which phase(s) had highest laxity (M=menses, F=follicular, O=ovulatory, L=luteal) in relation to which phases. Shultz *et al.* 2005 and Shultz *et al.* 2010 compared individual days which were included in the relationship found column. All joint tested were knees, Beynnon *et al.* (2004) tested the knee and ankle.

Most studies focus on knee laxity, and less attention has been given to the foot and ankle. However these regions are common sites for injury, and sex differences exist in rates of injuries (Table 1). The ankle joint functions to transmit body weight from the vertical to the horizontal

direction. The foot and ankle bear high loads because as force is transferred from the ground to the body, the foot and ankle experience all of the ground reaction forces with little attenuation. Ligaments surrounding the ankle joint are essential for maintaining stability and adaptive weight-bearing (Bonnel, 2010). The knee studies suggest that during the menstrual cycle, hormones affect joint laxity and a small number of studies have examined this relationship on the ankle. Beynnon *et al.* (2005) investigated the relationship between ankle laxity and levels of estradiol and progesterone and found no correlation. Bryant *et al.* (2008) demonstrated that females taking oral contraceptives (OC) (experiencing low and consistent levels of estradiol) have lower Achilles tendon strain (displacement divided by resting tendon length) compared to females not on oral contraceptives (NOC). However, they did not find cyclic changes in Achilles tendon strain across the menstrual cycle among females NOC. Wilkerson *et al.* (2000) found there to be significant differences between males and females in mean ankle ligament laxity. These studies suggest that, while hormones may not have an acute effect on joint laxity, there may be a prolonged consequence of exposure to sex hormones that ultimately influences joint properties (Bryant, 2008; Park, 2009; Shultz, 2005). This may help explain why females have higher joint laxity than males (Arnold, 2002; Beynnon, 2005; Pollard, 2006; Rozzi 1999; Shultz, 2005). Further research on the sex hormones and ankle joint properties is needed in order to associate the menstrual cycle with ankle injuries.

Effects of Muscular Fatigue and Repetitive Loading on Injury Risk

Muscular fatigue and repetitive loading during exercise are also key factors for influencing the risk for sports injuries, particularly overuse injuries such as stress fractures (Orava, 1978). Tibial and metatarsal stress fractures are the most common overuse injuries among athletes (Arendt, 2003; Brukner, 1996) and recovery time is longest from these types of injuries. Intense exercise during practices, games, or training regimes results in repetitive and high impact forces on the lower limb. During running, impact forces on the lower limb can reach

up to 5 times the body weight. Frequent loading results in microdamage occurring to structures of the leg and foot, especially bone, at a faster rate than they can heal (Messier, 2008; Milgrom, 2002; Weist, 2004). With insufficient bone remodeling, such microdamage often coalesces into macrodamage and ultimately develops into stress fractures (Bennell, 1999; Hreljac, 2004; Wen, 2007). Hreljac (2004) suggested that there is an injury threshold shaped by the combination of frequency and magnitude of impact forces (Figure 2). The curve below suggests that with higher frequency (related to run duration) lesser amounts of stress will induce an injury. The region above the curve represents the amounts of stress and frequencies that will cause a stress fracture.

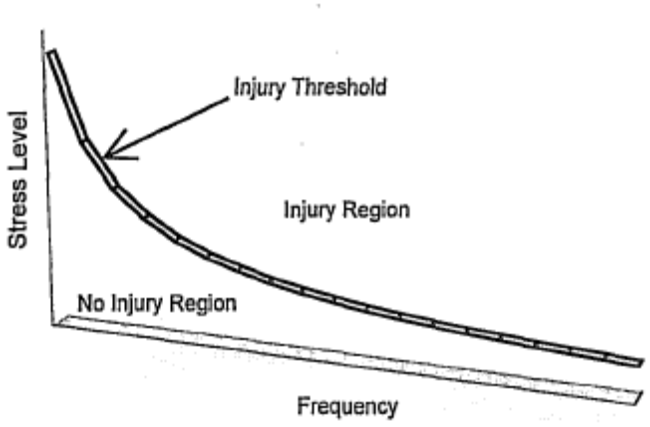

Figure 2. Fatigue curve from Hreljac *et al.* (2004) showing the relationship between stress and frequency in stress fracture risk.

When the high frequency and impact forces during exercise are coupled with muscular fatigue, the susceptibility for stress fracture injuries may be even greater. Muscles function to help dampen the impact forces on the bone of which they cross (Ferris, 1996; Yoshikawa, 1994). Figure 3 illustrates this using the metatarsal 1 and flexor hallucis longus muscle during toe-off. The ground reaction force causes the metatarsal bone to bend, producing a compression force on the dorsal side and a tension force on the plantar side. Bones are weakest under tension forces and are therefore most susceptible to injury (Yamada, 1970). Flexor hallucis longus produces forces in the opposite direction of the impact force dissipating the tension/compression forces (Yoshikawa, 1994). However, as the muscle becomes fatigued, it is less able to dampen the

lower limb impact forces resulting in larger strain being placed on the bone (Donahue and Sharkey, 1999; Ferris, 1996) and increasing the risk for a stress fracture injury. The foot is especially at a higher risk for stress fracture because during only moderate exercise, compression and tension strains are high in the metatarsals than the tibia (Milgrom, 2002). A non-invasive method for estimating strain placed on bones of the foot is by measuring plantar pressure. Strain follows similar patterns as plantar pressure. Increases in strain (Ferris, 1996) and peak plantar pressure (Sharkey, 1999) values following exercise have been shown in the same foot regions, suggesting strain and peak plantar pressure are related. Specifically, peak plantar pressure in the forefoot and medial midfoot regions increases following exercise (Ferris, 1996; Nagel, 2008; Weist, 2004; Wu, 2007).

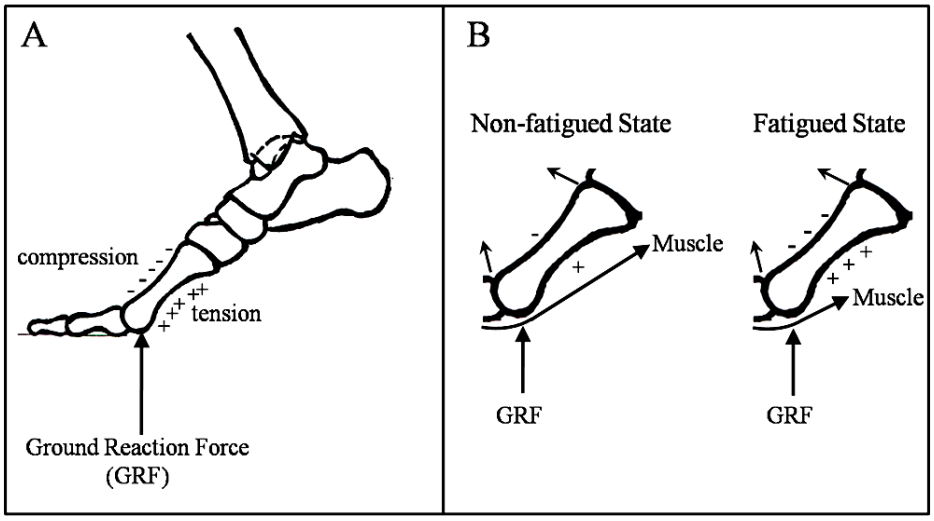

Figure 3. A) Compression and tension forces on metatarsal 1 due to a ground reaction force from landing. B) Representation of compression and tension forces on metatarsal 1 resulting from a non-fatigue and fatigued flexor hallucis longus muscle. Compression strain is represented by the minus sign and tension strain is represented by the plus sign. Less symbols and smaller arrows represent lower strain and forces, respectively.

In addition to muscular fatigue, repetitive loading during exercise also affects the properties of tendons and ligaments. It is well known that joint laxity in the knee increases after exercise (Johannsen, 1989; Nawata, 1999; Pollard, 2006; Rowe, 1999; Sakai, 1992; Skinner, 1986), and the threshold for maximum ligament laxity change is relatively low (Nawata, 1999). Ligaments and tendons experiencing large tension forces during exercise will undergo

microdamage, reducing the integrity of the tissue and increasing laxity (Pollard, 2006). It is likely that if knee laxity increases after repetitive loading, ankle and foot laxity would do the same. Furthermore, potential changes to ankle and foot laxity in response to exercise may be exacerbated by hormone-related effects (Carcia, 2004), however little research has been done on ankle and foot laxity in response to exercise and the menstrual cycle. It is likely that the potential increases in ankle and foot joint laxity contribute to changes in load distribution under the foot. Further research should examine how hormones and exercise affect ankle and foot laxity as well as how these two factors affect plantar loading.

In summary, female athletes are susceptible to different sports injuries compared to men, especially ACL tears and lower limb stress fractures. The menstrual cycle and exercise are two factors that appear to influence joint laxity and injury incidence. A better understanding of how these variables influence the risk for injury will provide insight into the etiology and prevention of sports injury. The purpose of this study is to investigate the effects of exercise and hormones of the menstrual cycle on ankle and toe laxity and plantar pressure distributions.

Predictions

1) Exercise targeting foot and ankle muscle fatigue will increase first metatarsophalangeal (MP1) dorsiflexion range of motion (ROM) and ankle laxity.
2) Females will have higher ankle laxity and MP1 dorsiflexion ROM than males.
3) Estradiol will affect ankle laxity and MP1 dorsiflexion ROM.
    a. Females on oral contraceptives (OC) will have lower ankle laxity and MP1 dorsiflexion ROM than females not on oral contraceptives (NOC).
    b. Ankle laxity and MP1 dorsiflexion ROM will be highest during the ovulatory and luteal phases of the menstrual cycle when estradiol levels are high. This will especially be the case for females NOC.

   c. Estradiol levels will be positively correlated with ankle and MP1 dorsiflexion ROM laxity in females.

4) Males and females will have different plantar pressure distributions that are associated with regions frequently injured.
   a. Females will have higher metatarsal 1 and 2 peak pressure than males because of the higher rate of metatarsal 1 and 2 observed in females compared to males (Hame, 2004)
   b. Females will have higher medial midfoot peak pressure than males because high medial midfoot peak pressure is associated patellofemoral pain and patellofemoral pain is more common in females than males (Almeida, 1999; Fulkerson, 2002; Iwamoto, 2008).
   c. Males will have higher lateral midfoot and metatarsals 3-5 peak plantar pressure than females. Males tend to load the lateral midfoot and metatarsal region more than females (Sims, 2008), have larger relative lateral midfoot regions (Wunderlich and Cavanagh, 2001) and suffer from more 5^{th} metatarsal stress fractures than females (Borowski, 2008).
5) Exercise will increase peak plantar pressure in the metatarsal 1, metatarsal 2, toe 1, toes 2-5 in a similar manner to previous studies (Weist, 2004; Wu, 2007). Medial midfoot region will also increase because pronation increases after exercise (Weist, 2004).
6) Peak plantar pressure will increase with increased joint laxity.
   a. Ankle laxity will positively correlate with medial midfoot, forefoot, and toe peak plantar pressures regions because decreased ankle stability will increase pronation and force transferred from the ankle to the forefoot during push-off.
   b. MP1 dorsiflexion ROM will positively correlate with metatarsal 1 peak plantar pressure because there will be higher dorsiflexion during toe-off which will increase contact time.

7) Peak plantar pressure will increase under conditions of high estradiol.
    a. Females on OC will have lower peak plantar pressures than females NOC.
    b. Peak plantar pressure will be lowest during menstruation and highest during ovulation.
    c. Peak plantar pressure will positively correlate with estradiol levels in females.

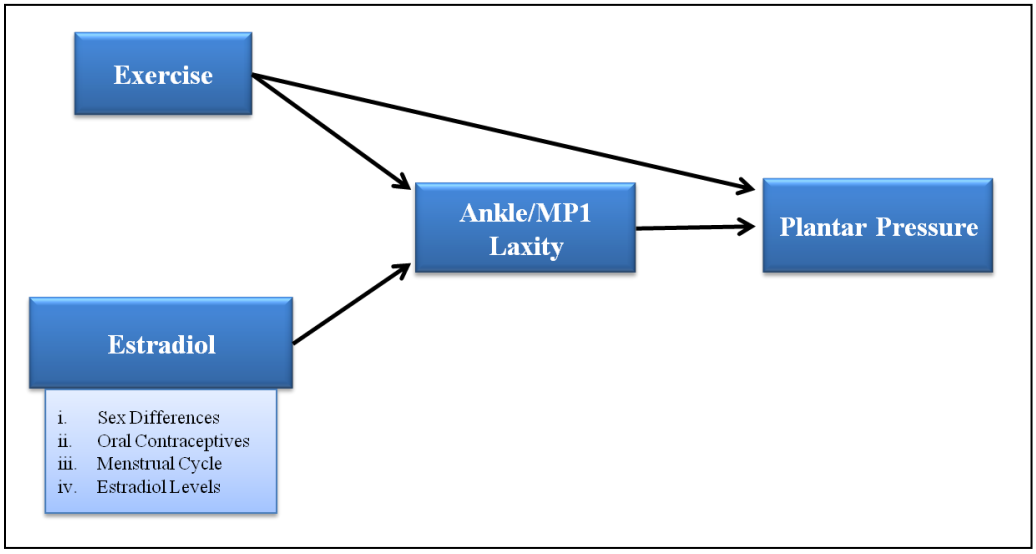

Figure 4. Overview of predictions.

Methods

The Institutional Review Board on the Use of Human Subjects approved all procedures used in this study and each subject provided informed consent.

Design: Over the course of 13 months, we tested 47 subjects up to 12 times. Assuming a 28-day menstrual cycle, each subject came in three times for each menstrual quartile. We made efforts to test the participants at the same time and day each week consecutively, however visits were inconsistent for many subjects.

Visits: Each visit included a questionnaire about activity, menstrual cycle, and oral contraceptive use for the past week; blood sampling for hormone analysis; fatiguing exercises; pre- and post-exercise ligament laxity; and pre- and post-exercise barefoot plantar pressure distribution. We also took measurements of height and weight to account for size differences.

At each visit, subjects self-reported the first day of their last menstruation. Over 12 weeks, at least 2-3 full menstrual periods were recorded for each female subject from which average cycle length (in days) was calculated per subject. We placed each visit into a menstrual quartile by counting the days since the subject's last menstruation and dividing that by her average cycle length and expressing as a percentage. We placed the percentage values into quartiles as illustrated in Table 3.

Table 3. Percent of menstrual cycle conversion to quartile denotation and menstrual cycle phase.

Percent of Menstrual Cycle	Quartile Denotation	Menstrual Phase
1-25%	A	Menses, Early Follicular
26-50%	B	Late Follicular, Ovulation
51-75%	C	Early Luteal
76-100%	D	Late Luteal

Subjects: We recruited fifty three subjects to participate in the study. A total of six subjects dropped out due to injuries (occurring outside of the study) or scheduling conflicts leaving a total of forty seven subjects with at least four visits completed to be included in the analysis (34 female, 13 male). Ages ranged from 18-26. Females were divided into two groups: oral contraceptive (OC) users and non oral contraceptive (NOC). Subjects incorporated in this study were members of club and varsity sports teams including soccer (3 total; 2 male, 1 female), ultimate Frisbee (6 total; 5 female, 1 male), triathlon (9 total; 6 female, 3 male), rugby (4 female), and lacrosse (1 female), cheerleading (7 total, 4 female, 3 male), field hockey (1 female), and football (1 male). Twelve subjects (10 female, 2 male) were classified as recreational athletes who run or participated in a lower limb-stressing activity 4-5 times a week. There were three participants from JMU's ROTC program (2 female, 1 male). Athletes were excluded if they had a current injury or obtained an injury during the course of the study.

Hormone Analysis: At each visit, we took a 10-mL blood sample and immediately centrifuged the sample, removed the plasma, stored the plasma in a -70^{o}C freezer. We assayed plasma samples in triplicates to determine concentrations of estradiol using Enzyme Immunoassay (EIA Cayman Chemical, Ann Arbor, Michigan). Due to an anticipated high variation, we standardized the estradiol levels by calculating the percent of the lowest recorded estradiol value. We used these "relative" levels to compare across subjects.

Joint Laxity. We measured joint laxity at the ankle and first metatarsophalangeal (MP1) joint each week before and after exercise. We used an ankle arthrometer (Blue Bay Research Inc., Milton, FL) to quantify relative displacement of the talotibial joint during anterior drawer loading. Ligament laxity is defined as the estimated displacement of the talus on the tibia and calculated from 0 N to 125 N. We estimated the laxity of the structures surrounding the first metatarsophalangeal joint by measuring the dorsiflexion range of motion (ROM) (degrees)

between the first toe and metatarsal as the subject pulled his/her hallux back as far as possible (Figure 5). We then subtracted the measured angle from 180^{o}, so a smaller value indicated lower laxity.

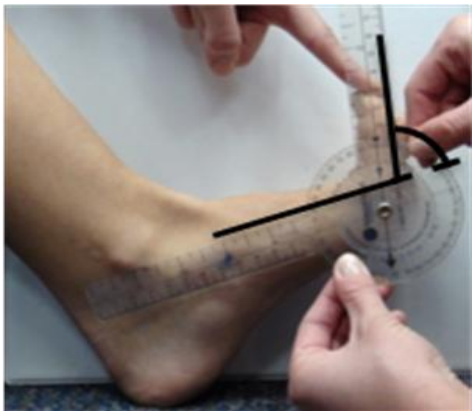

Figure 5. Angle measured for quantifying MP1 dorsiflexion ROM.

Plantar Pressure. We measured plantar pressure distribution during barefoot walking at a self-selected pace before and after exercise using the Novel EMED-ST (Novel Inc., Munich, Germany) plantar pressure system collecting at 50 Hz. We measured both right and left feet and averaged the values. For analysis, we divided plantar pressure data into 8 anatomical regions including hindfoot, medial midfoot, lateral midfoot, metatarsal 1, metatarsal 2, metatarsal 3-5, toe 1, and toes 2-5. We quantified peak plantar pressure, contact area, maximum force, and pressure time integral for each region. To compare differences in contact area after exercise between sexes, we calculated the ratio of post-exercise to pre-exercise contact area.

Stride Characteristics. For each pressure pad step, we recorded videos at (120 Hz) using Sony Handycam HDR-CX550 (Sony Electronics Inc., San Diego, CA). We placed a wooden board of known length in the background of each video in order to calibrate lengths. Stride length and average speed were measured using the program ProAnalyst (Xcitex Inc., Cambridge, MA). The points at initial heel down for steps before the pressure pad, on the pressure pad, and

after the pressure pad were tracked in order to measure speed and step length for one stride. The hip was also tracked at the three steps in order to measure average velocity over the stride.

Exercise Protocol. The subjects ran up and down two flights of stairs a total of ten times. The participants then completed a modified beep test designed by a JMU undergraduate student, Sarah Cebulski. This test involved jumping over four 1-foot hurdles that are set 2 feet apart. The participant jumped the hurdles in between the time of two beeps. The beep test had 16 levels with 12 jumps within the level. The time allotted for jumping decreased with each level, forcing the subject to increase effort. When the subject missed two beeps in a row, he/she was considered fatigued and allowed to stop. The subjects wore shoes during the stairs for safety purposes but were asked to jump the hurdles barefoot in order to target foot and calf muscle fatigue.

Figure 6. Participant jumping over hurdles.

We assessed fatigue in three ways: first we obtained lactic acid levels before and after exercise. We loaded a small sample of blood from a finger stick into a portable lactic analyzer (LactatePro, Arkray Products, Kyoto, Japan) which measured lactate concentrations. We considered lactate values above 3.0 mmol/L to represent fatigue. Second, we asked the athletes to rate their own level of fatigue by using the Rate of Perceived Exertion Scale (6-very, very light up

to 20-very, very hard). Third, we recorded the beep test level achieved to insure similar levels of fatigue were achieved in each visit.

Data Analysis. We performed all analyses using the statistical program JMP. Individuals and visits were repeated measures. We used a two-way repeated measures analysis of variance (ANOVA) to test for differences in estradiol levels between sexes and between OC groups. We used multiple analysis of variance (MANOVA) to compare estradiol levels across menstrual quartiles in females. We used a repeated measures ANOVA to compare differences before and after exercise in velocity, stride length, step length, as well as lactic acid levels. We also used an ANOVA to examine the effects of sex differences and exercise on ankle laxity and first metatarsophalangeal (MP1) joint range of motion (ROM) as well as the effects of oral contraceptives (OC) and exercise on laxity. We also used a repeated measures ANOVA to compare differences in peak pressure, contact area, maximum force, and pressure time integral in all eight regions (toe 1, toes 2-5, 1^{st} metatarsal, 2^{nd} metatarsal, metatarsals 3-5, medial midfoot, lateral midfoot, and heel) between sexes as well as before and after exercise. We used a MANOVA to test for differences in ankle, MP1 laxity and peak pressures in all eight regions across the menstrual quartiles before and after exercise in females. We used a significance level of 0.05. Lastly, we used a least squares regression to examine the effects of estradiol level on ankle and toe laxity as well as to examine the relationship between ankle/ toe laxity and peak plantar pressure.

Results

Estradiol Levels. Overall estradiol levels were compared between males and females as well as between females on OC and females NOC. Females had significantly higher estradiol levels than males ($p<0.01$). Females on OC had significantly lower estradiol levels than females NOC ($p<0.01$). Estradiol levels were compared across menstrual quartiles in OC and NOC groups to determine if the estradiol levels were fluctuating as expected. There were no differences in estradiol levels between any menstrual quartile in the OC group or NOC group (Figure 7).

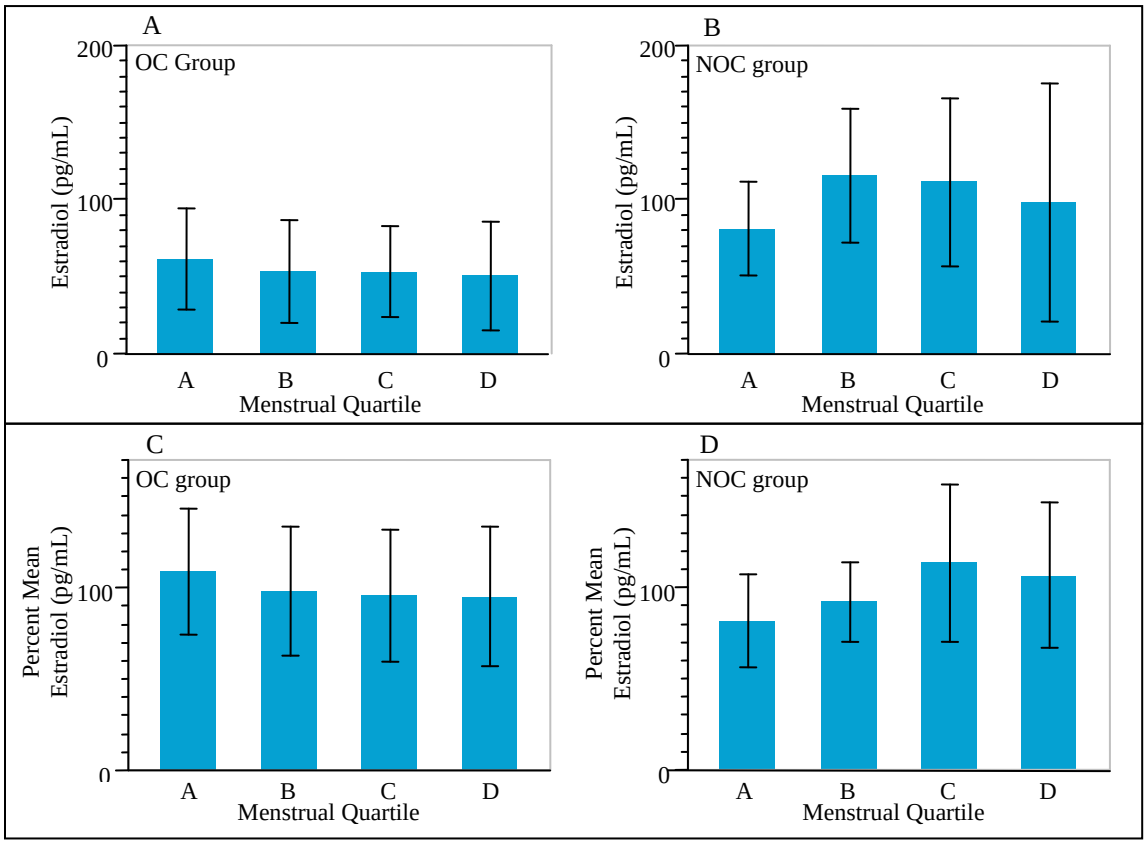

Figure 7. Estradiol levels at four menstrual quartiles (A=menstruation, B=follicular/ovulation, C=early luteal, D=late luteal) for the oral contraceptive group (A) and the non oral contraceptive group (B). Standardized estradiol levels at four menstrual quartiles for the OC group (C) and the NOC group (D). Columns represent means and error bars are 1 standard deviation.

Fatigue. Lactic acid levels were assessed before and after exercise to ensure fatigue. Post-fatigue lactic acid levels were significantly higher than pre-fatigue lactic acid levels ($p<0.01$). Pre-fatigue levels averaged 1.42 ± 0.76 ranging from 0.7 to 2.8 mmol/L. Post-fatigue levels averaged 7.93±2.52, ranging from 2.9 to 16.7 mmol/L. Lactic acid levels were higher after exercise for every subject at each visit. Ratings of perceived exertion ranged from 11-19 with an average rating of 15.45. Athletes participated in about 4-5 exercises (games, practices, training) per week. On average, athletes ran the stairs at 6 minutes and 15 seconds and reached past level 11-3 (level-jumps within the level) of the beep test.

Stride Characteristics. In a subset of individuals, velocity, stride length, and step lengths were compared before and after exercise. Velocity, stride length, and step length did not change after exercise ($p=0.37$, $p=0.65$, $p=0.87$, respectively).

Ankle and MP1 Laxity. A repeated measures ANOVA revealed that females had significantly higher ankle laxity than males ($p<0.01$, Figure 8A). Ankle laxity did not increase after the exercise protocol in males or females ($p=0.53$). There was no interaction between sex differences and exercise on ankle laxity ($p=0.18$). Individual differences accounted for 41.19% of the variation. First metatarsophalangeal (MP1) joint laxity is represented by the MP1 dorsiflexion ROM. Females had significantly higher MP1 joint laxity than males ($p=0.02$, Figure 8B). MP1 joint laxity significantly increased after exercise in both males and females ($p=0.03$). There was no interaction of sex differences and exercise on MP1 joint laxity ($p=0.62$). Individual differences accounted for 71.45% of the variation.

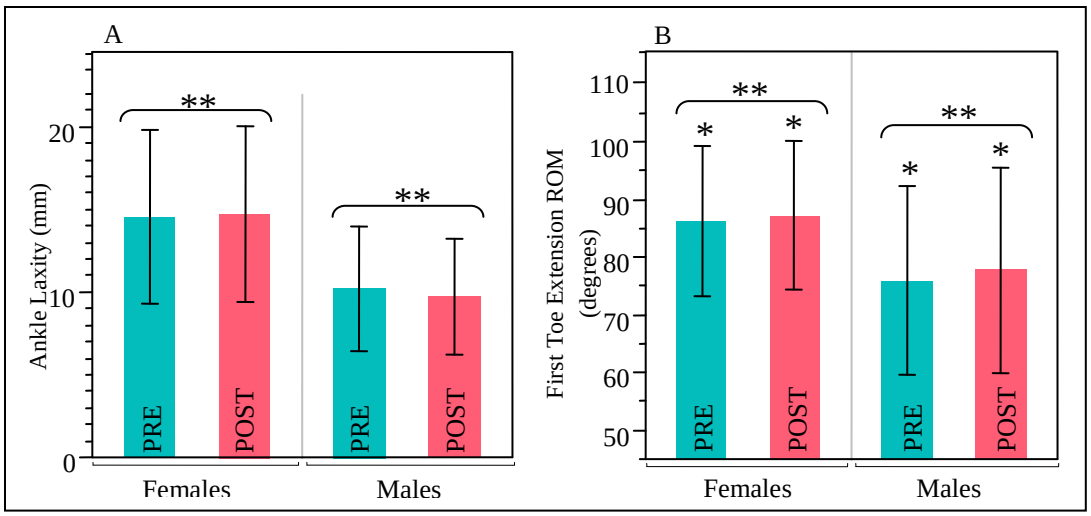

Figure 8. A) Ankle laxity between sexes before (PRE) and after (POST) exercise. B) MP1 joint dorsiflexion ROM between sexes before and after exercise. Columns represent means and error bars are 1 standard deviation. *Statistically significant difference between pre and post; **Statistically significant difference between sexes.

To compare ankle and MP1 joint laxity in females on OC and NOC, a repeated measures ANOVA was performed. There were no differences between OC and NOC groups on ankle laxity (p=0.21, Figure 9A). There was no interaction between of OC/NOC groups and exercise on ankle laxity (p=0.32). Inter-individual variation represented 39.83% of the variation. MP1 joint laxity was also not different between OC and NOC groups (p=0.43, Figure 9B). There was no interaction between OC use and exercise in MP1 joint laxity (p=0.45). Individual differences accounted for 63.85% of the variation.

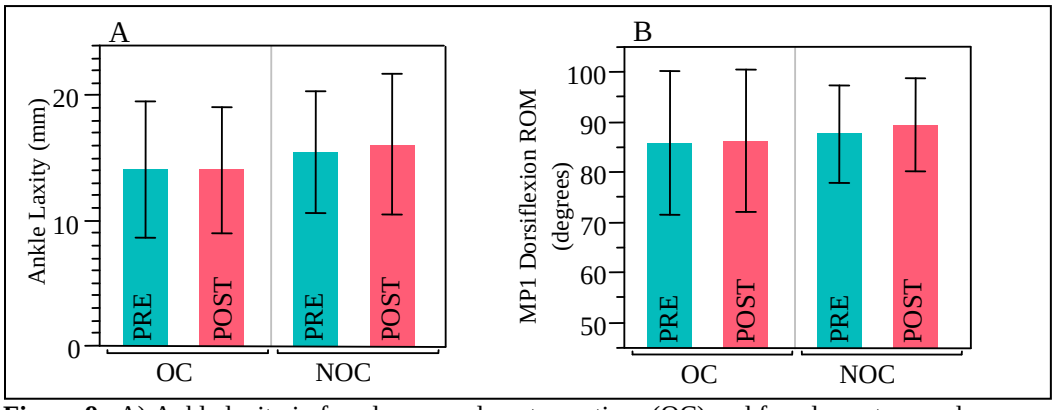

Figure 9. A) Ankle laxity in females on oral contraceptives (OC) and females not on oral contraceptives (NOC) before (PRE) and after (POST) exercise. B) MP1 joint dorsiflexion ROM in females on OC and females NOC before and after exercise. Columns represent means and error bars are 1 standard deviation.

No differences in ankle laxity were found between menstrual quartiles (p=0.64, Figure 10A). There were no interactions between menstrual quartiles and exercise (p=0.95). Individual differences accounted for 41.5% of the variation. There were no changes in MP1 joint laxity between menstrual quartiles (p=0.91, Figure 10B). There were no interaction effects of menstrual quartile and exercise on MP1 joint laxity (p=0.54). Individual differences accounted for 62.83% of the variation.

To determine if laxity was different between OC and NOC groups in certain menstrual phases, a repeated measures MANOVA was run. Ankle and MP1 joint laxities did not differ between OC and NOC groups in any of the quartiles (Figure 11).

Least squares regression demonstrated no relationship between estradiol and ankle laxity (R^2=0.02, y=13.63 + 0.02xFigure 12A). There was also no relationship between estradiol levels and MP1 joint laxity values before or after exercise (R^2=0.003, y= 87.47 - 0.02x, Figure 12B). To determine if change in estradiol and change in laxity values correlated, the percent of lowest estradiol and joint laxity values were regressed. There was no relationship between percent of lowest estradiol and ankle laxity (R^2=0.023, y=161.80+0.06x) or MP1 joint laxity (R^2=0.002, y=113.51+0.005x).

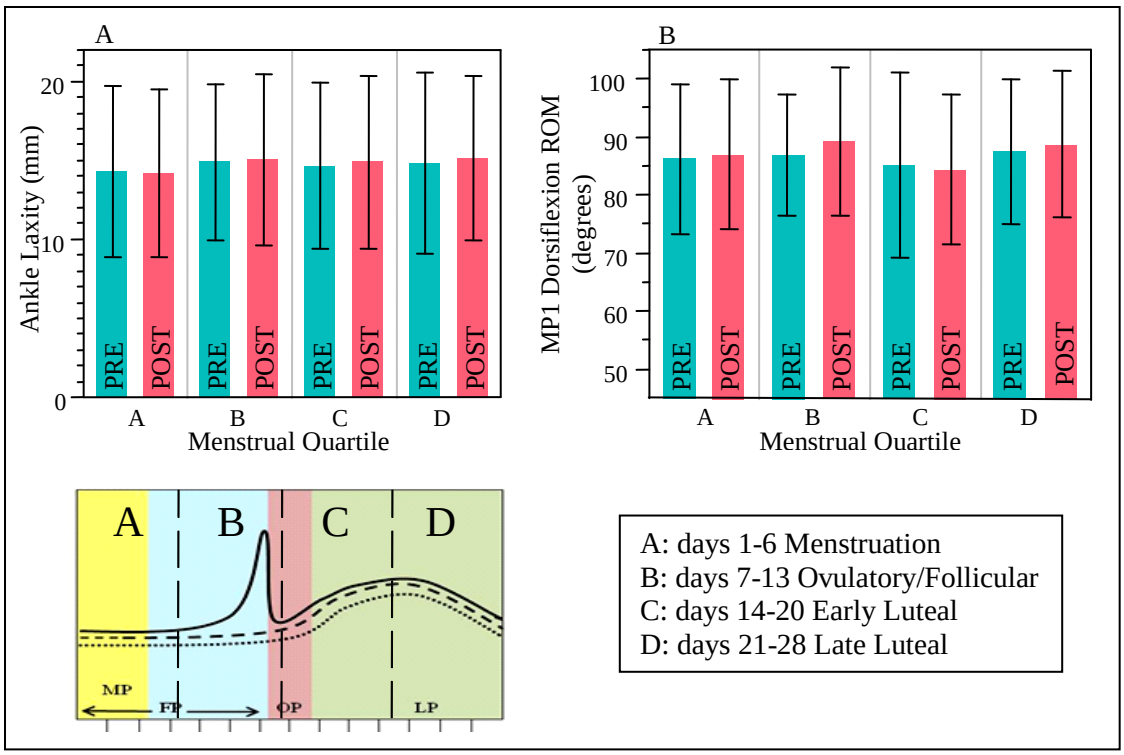

Figure 10. A) Ankle laxity across menstrual quartiles before (PRE) and after (POST) exercise. B) MP1 joint dorsiflexion ROM across menstrual quartiles before and after exercise. Quartile denotation and associated menstrual phase listed below with graph of hormone levels across menstrual cycle based on 28 day cycle. Columns represent means and error bars are 1 standard deviation.

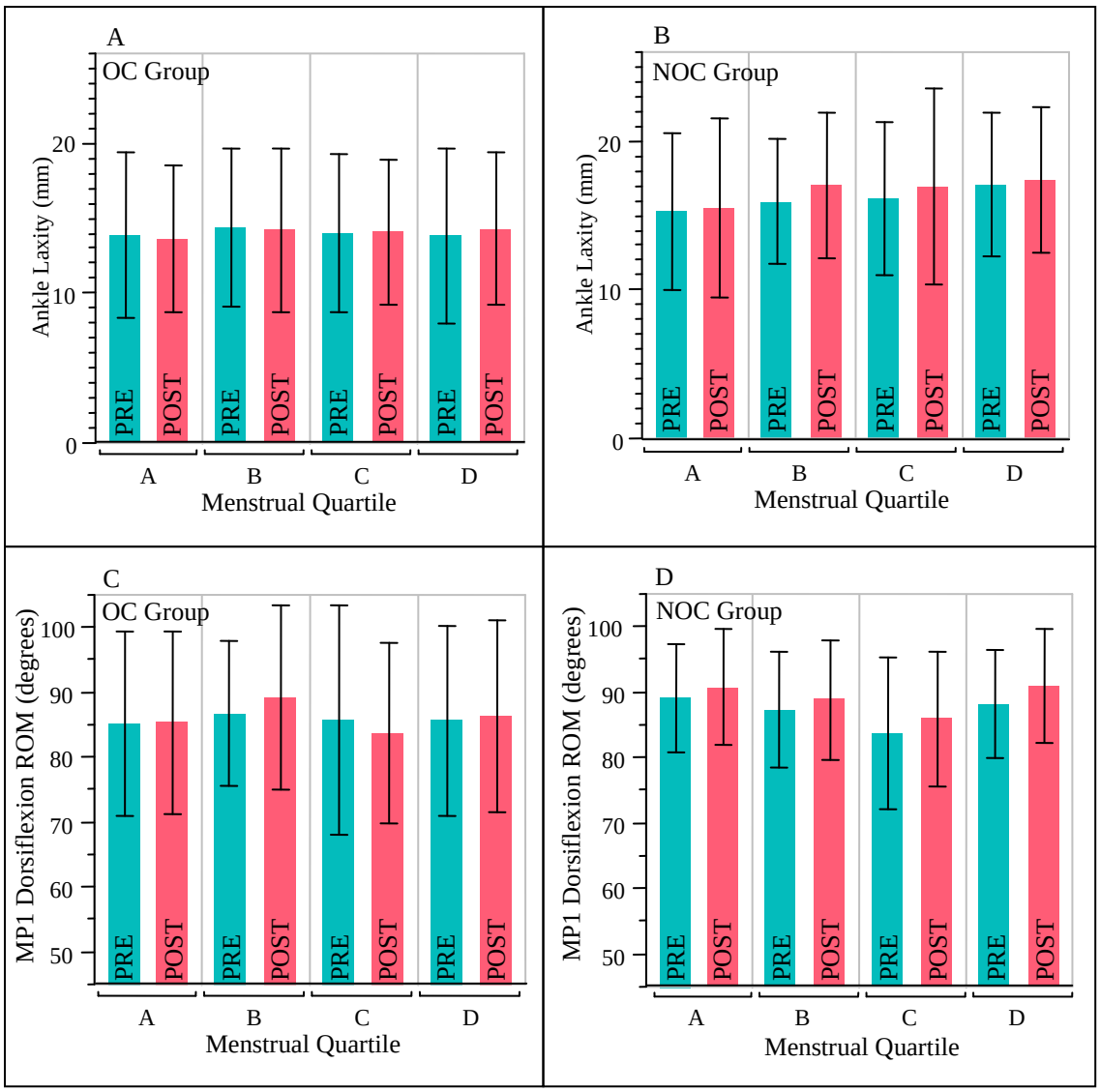

Figure 11. A) Ankle laxity in OC group before (PRE) and after (POST) exercise across menstrual quartiles (A=menses, B=follicular/ovulation, C=early luteal, D=late luteal). B) Ankle laxity in NOC group before and after exercise across the menstrual quartiles. C) MP1 joint dorsiflexion ROM in OC group before and after exercise across menstrual quartiles. D) MP1 joint dorsiflexion ROM in the NOC group before and after exercise across the menstrual quartiles. Columns represent means and error bars are 1 standard deviation.

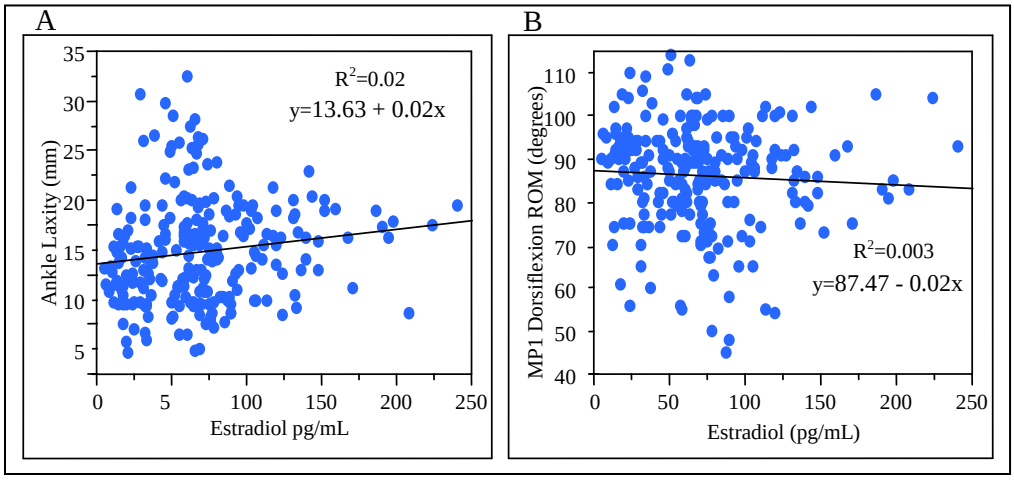

Figure 12. A) Ankle laxity and B) MP1 dorsiflexion ROM regressed with estradiol levels.

Plantar Pressure. Peak pressure under the foot during a typical walk is depicted in Figure 13. The first toe has the most, followed by the second metatarsal and hindfoot. The midfoot regions experience the least peak plantar pressure.

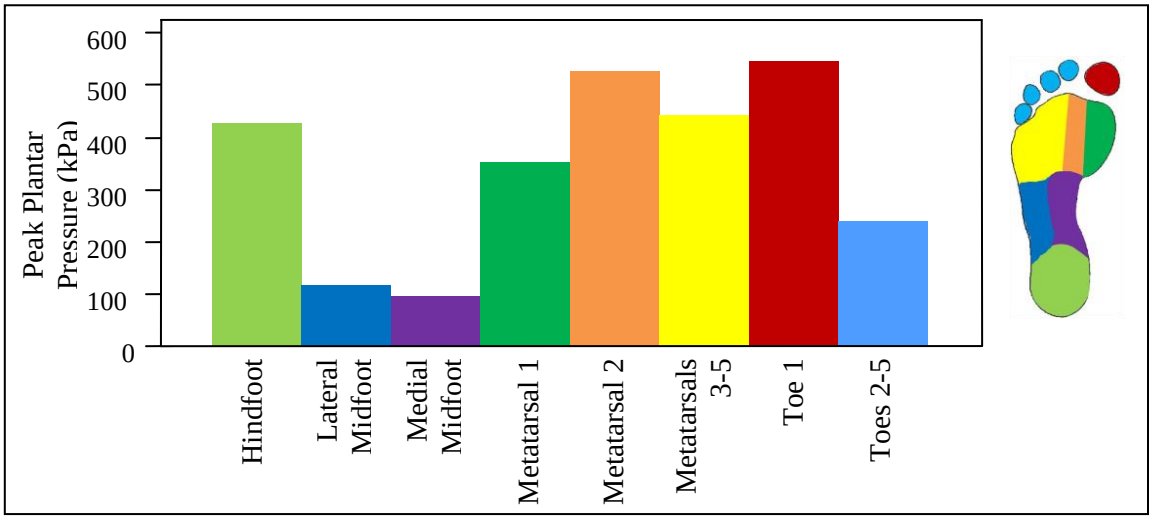

Figure 13. Peak plantar pressures under the foot during a forward walk.

Peak plantar pressure in the first toe was significantly higher in males than females ($p=0.04$, Figure 14A, Table 4). Following exercise, toe 1 peak plantar pressure significantly decreased ($p<0.01$). There were no interaction effects of sex differences and exercise on peak plantar pressure in the first toe. Individual differences were high and accounted for 88.24% of the

variation. The ratio of post-exercise to pre-exercise contact area in the first toe was not different between sexes ($p=0.85$, Table 4). Contact area increased after exercise ($p<0.01$, Table 4). Pressure time integral in the first toe region was higher in males than females ($p=0.01$, Table 4) and decreased after exercise ($p<0.01$). Maximum force in the first toe was not different between males and females ($p=0.05$, Table 4) and decreased after exercise ($p=0.03$).

Peak plantar pressure in toes 2-5 did not significantly differ between males and females ($p=0.11$) but significantly decreased following exercise ($p<0.01$, Figure 14B, Table 4). There was not an interaction between sex differences and exercise ($p=0.35$). Individual differences were high and accounted for 81.01% of the variation. Peak pressure in the medial midfoot was not different across menstrual quartiles ($p=0.87$). Peak plantar pressure did not differ between OC and NOC groups ($p=0.45$). The ratio of post-exercise to pre-exercise contact area in toes 2-5 was not different between sexes ($p=0.31$, Table 4). Contact area decreased after exercise ($p<0.01$, Table 4). Pressure time integral in toes 2-5 did not differ between sexes ($p=0.46$, Table 4) and decreased after exercise ($p<0.01$). Maximum force in toes 2-5 was not different between males than females ($p=0.12$, Table 4) and decreased after exercise ($p<0.01$).

There was no difference between males and females in the peak plantar pressure in the metatarsal 1 region ($p=0.22$, Figure 14C, Table 4). After exercise, peak plantar pressure in the metatarsal 1 region significantly decreased ($p<0.01$). There was no interaction effect between sex differences and exercise on metatarsal 1 peak plantar pressure ($p=0.64$). Individual differences were high and accounted for 78.22% of the variation. Metatarsal 1 peak plantar pressure was not different between menstrual quartiles. Metatarsal 1 peak plantar pressure was higher in the NOC group compared to the OC group ($p=0.02$). The ratio of post-exercise to pre-exercise contact area in the first metatarsal was not different between sexes ($p=0.62$, Table 4). Contact area in the metatarsal 1 region increased after exercise ($p<0.01$, Table 4). Pressure time integral in metatarsal 1 did not differ between sexes ($p=0.76$, Table 4) and decreased after exercise ($p<0.01$).

Maximum force in the metatarsal 1 was not different between males and females ($p=0.54$, Table 4) and did not change after exercise ($p=0.31$).

Metatarsal 2 peak plantar pressure did not differ between males and females ($p=0.22$) but significantly decreased following exercise ($p<0.01$, Figure 14D, Table 4). There was an interaction between sex differences and exercise ($p=0.03$) in the metatarsal 2 peak plantar pressure region: peak pressure increased after exercise in females ($p<0.01$) but not in males, ($p=0.24$, Tukey HSD post-hoc test). Individual differences were high and accounted for 93.86% of the variation. Peak pressure in metatarsal 2 did not differ between menstrual quartiles. Metatarsal 2 peak plantar pressure was higher in the NOC group compared to the OC group ($p=0.04$). The ratio of post-exercise to pre-exercise contact area in the second metatarsal was not different between sexes ($p=0.42$, Table 4). Contact area in the metatarsal 2 region was increased after exercise ($p<0.01$, Table 4). Pressure time integral in the metatarsal 2 did not differ between sexes ($p=0.54$, Table 4) and decreased after exercise ($p<0.01$). Maximum force in the metatarsal 2 was not different between males and females ($p=0.05$, Table 4) and did not change after exercise ($p=0.11$).

When comparing metatarsals 3-5 peak plantar pressures, there were no differences between males and females ($p=0.34$), but metatarsals 3-5 peak plantar pressure significantly increased following exercise ($p<0.01$, Figure 14E, Table 4). There was an interaction between sex differences and exercise ($p=0.02$); females significantly increased after exercise ($p<0.01$), while males did not ($p=0.14$, Tukey HSD post-hoc). Individual differences were high and accounted for 89.62% of the variation. Peak pressure in the medial midfoot was not different across menstrual quartiles ($p=0.81$). Peak plantar pressure did not differ between OC and NOC groups ($p=0.62$). The ratio of post-exercise to pre-exercise contact area in the metatarsal 3-5 region was higher in males than females ($p=0.046$, Table 4). Contact area in the metatarsals 3-5 region did not change after exercise ($p=0.33$, Table 4). Pressure time integral in metatarsals 3-5 did not differ between sexes ($p=0.59$, Table 4) and decreased after exercise ($p<0.01$). Maximum

force in metatarsals 3-5 was not different between males and females (p=0.96, Table 4) and did not change after exercise (p=0.33).

Peak plantar pressure in the medial midfoot did not differ between males and females (p=0.86) but significantly increased after exercise (p<0.01, Figure 14F, Table 4). There was not an interaction between sex differences and exercise on medial midfoot peak plantar pressure, (p=0.24). Individual differences accounted for 91.82% of the variation. Peak pressure in the medial midfoot was not different across menstrual quartiles (p=0.22). Peak plantar pressure did not differ between OC and NOC groups (p=0.82). The ratio of post-exercise to pre-exercise contact area in the medial midfoot was higher in males than females (p<0.01, Table 4). Contact area in the medial midfoot increased after exercise (p<0.01, Table 4). Pressure time integral in the medial midfoot did not differ between sexes (p=0.87) and increased after exercise (p<0.01, Table 4). Maximum force in the medial midfoot increased after exercise (p<0.01) and was higher in males than females (p=0.03, Table 4).

Lateral midfoot peak plantar pressure was not significantly different between sexes (p=0.22) or exercise conditions (p=0.35, Figure 14G, Table 4). There were no interaction of sex differences and exercise on lateral midfoot peak plantar pressure (p=0.17). Individual differences were high and accounted for 93.25% of the variation. Lateral midfoot peak plantar pressure was not different between any menstrual quartile (p=0.41). Peak pressure in the lateral midfoot did not differ between OC and NOC groups (p=0.41). The ratio of post-exercise to pre-exercise contact area in the lateral midfoot was not different between sexes (p=0.80, Table 4). Contact area in the lateral midfoot increased after exercise (p<0.01, Table 4). Pressure time integral in the lateral midfoot did not differ between sexes (p=0.07) and was not different after exercise (p=0.08, Table 4). Maximum force in the lateral midfoot did not change after exercise (p=0.09) and was not different between males than females (p=0.09, Table 4).

No differences were found in heel peak plantar pressure between sexes (p=0.73) or with exercise (p=0.11, Figure 14H, Table 4). There were no interaction effects of sex and exercise on

heel peak plantar pressure (p=0.24). Individual differences were high and accounted for 83.86% of the variation. Hindfoot peak plantar pressure did not differ between any menstrual quartiles (p=0.18). Hindfoot peak plantar pressure did not differ between OC and NOC groups (p=0.78). The ratio of post-exercise to pre-exercise contact area in the first toe was not different between sexes (p=0.95, Table 4). Contact area in the hindfoot increased after exercise (p<0.01, Table 4). Pressure time integral in the hindfoot did not differ between sexes (p=0.65) and did not change after exercise (p=0.23, Table 4). Maximum force in the heel did not differ between males and females (p=0.26, Table 4) and did not change after exercise (p=0.69).

Table 4. Plantar pressure relationships between sex differences and exercise conditions.

		Peak Plantar Pressure		Post:Pre CA	Contact Area	Maximum Force		Pressure Time Integral	
		Sexes	**Fatigue**	**Sexes**	**Fatigue**	**Sexes**	**Fatigue**	**Sexes**	**Fatigue**
Toe 1	p-value	0.04*	<0.01*	0.85	<0.01*	0.05	0.03*	0.01*	<0.01*
	relationship	**M**	↓	-	↑	-	↓	**M**	↓
Toes 2345	p-value	0.51	<0.01*	0.31	<0.01*	0.12	<0.01*	0.46	<0.01*
	relationship	-	↓	-	↓	-	↓	-	↓
Metatarsal 1	p-value	0.25	<0.01*	0.62	<0.01*	0.54	0.31	0.76	<0.01*
	relationship	-	↓	-	↑	-	-	-	↓
Metatarsal 2	p-value	0.19	<0.01*	0.42	<0.01*	0.05	0.11	0.54	<0.01*
	relationship	-	↓	-	↑	-	-	-	↓
Metatarsal 345	p-value	0.40	0.24	0.046*	0.17	0.96	0.33	0.59	<0.01*
	relationship	-	-	**M**	-	-	-	-	↓
Medial Midfoot	p-value	0.91	<0.01*	<0.01*	<0.01*	0.03*	<0.01*	0.87	<0.01*
	relationship	-	↑	**M**	↑	**M**	↑	-	↑
Lateral Midfoot	p-value	0.20	0.35	0.80	<0.01*	0.09	0.09	0.07	0.08
	relationship	-	-	-	↑	-	-	-	-
Hindfoot	p-value	0.81	0.11	0.95	<0.01*	0.26	0.69	0.65	0.23
	relationship	-	-	-	↑	-	-	-	-

F= females had higher values, M=males had higher values; ↑=decreased after exercise, ↓=increased after exercise; the minus sign means there was no change. Post:Pre CA is the ratio of post contact area to pre contact area.

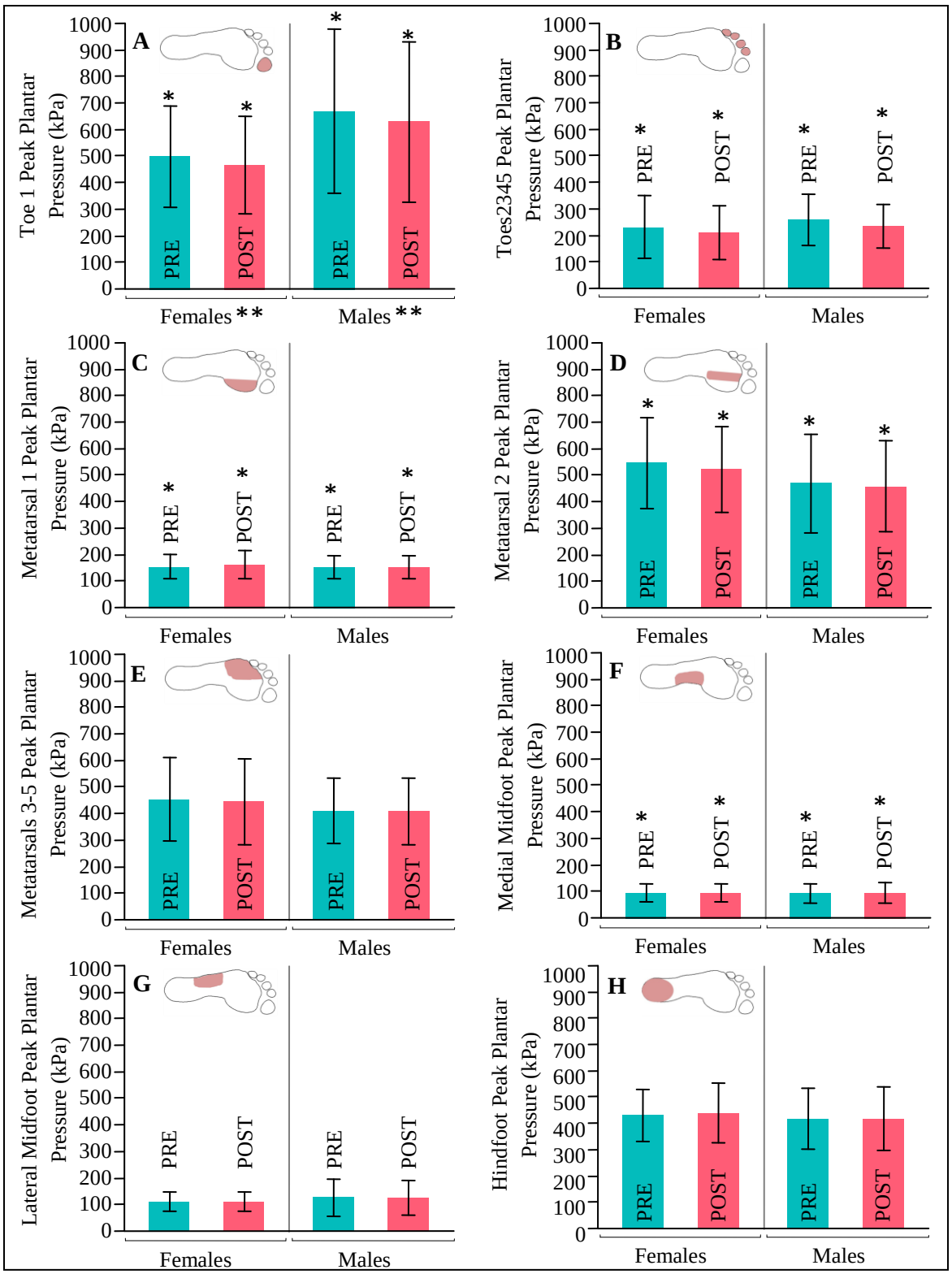

Figure 14. Peak plantar pressure between sexes before and after exercise in toe 1(A), and toes2345 (B), metatarsal 1 (C), metatarsal 2 (D), metatarsal 3-5 (E), medial midfoot (F), lateral midfoot (G), and hindfoot (H) regions. Columns represent means and error bars are 1 standard deviation.*Statistically significant difference between pre and post; **Statistically significant difference between sexes.

Least squares regression demonstrated no association between estradiol and peak plantar pressure in any region of the foot. Metatarsal 2 peak plantar pressure represented the most linear relationship found compared to the other foot regions (Figure 15).

Regression analysis shows no relationship between ankle laxity and peak pressure in any region of the foot with all R^2 values below 0.03. Regression analysis also showed no relationship between MP1 dorsiflexion ROM and peak pressure in any foot region (all R^2 values <0.09).

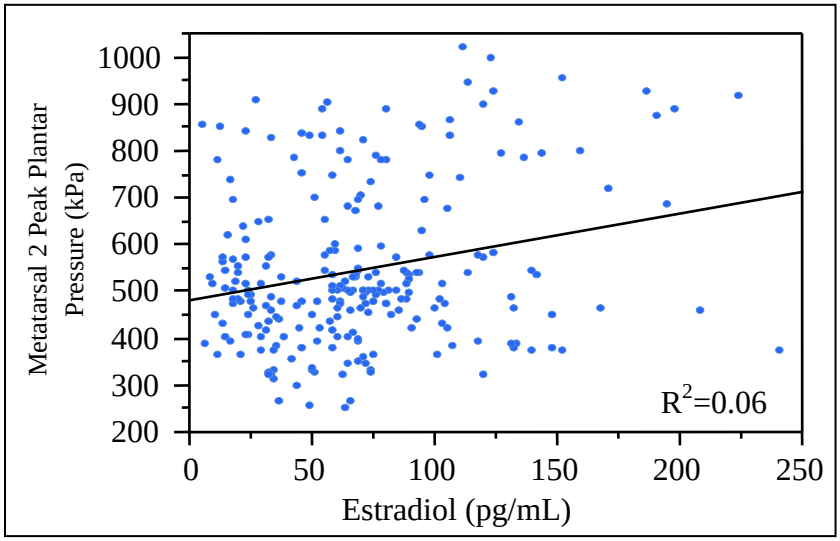

Figure 15. Metatarsal 2 peak plantar pressure regressed with estradiol levels.

Discussion

Sports related injury rates are proposed to be influenced by sex hormones and exercise. The sex hormone estradiol has been associated with increased joint laxity (Beynnon, 2005; Deie, 2002; Park(a), 2009; Shultz, 2010; Wojtys, 2002; Zazulak, 2006) and injury incidence (Adachi, 2008; Arendt, 1999; Myklebust, 2003; Myklebust, 1998; Slauterbeck, 2002; Wojtys, 2002). Risk for injury also increases with repetitive stresses on bones and ligaments during exercise (Bennell, 1999; Hreljac, 2004; Wen, 2007). To our knowledge, this is the first study to investigate the combined effects of sex hormones and repetitive stress from exercise on changes in ankle and MP1 joint laxity and subsequent risk for injury.

We found that females have higher laxity in the ankle and MP1 joint compared to males. Oral contraceptives, menstrual quartiles, and serum hormone levels did not affect ankle and MP1 joint laxity in this study. After an exercise protocol targeting lower leg and foot muscles, MP1 laxity increased while ankle laxity stayed the same. Peak plantar pressure decreased in the forefoot and increased in the medial midfoot after the exercise protocol. In the first toe and metatarsals 1 and 2, contact area increased after exercise. Maximum force did not change in metatarsals 1 and 2 and increased in toe 1 following the exercise protocol. Overall, males had higher first toe peak plantar pressure than females. After the exercise, peak plantar pressure in the second metatarsal decreased in females but not in males. Females on OC had lower metatarsal 2 peak plantar pressures than females NOC. The menstrual cycle and serum hormone levels did not affect peak plantar pressure.

The results of this study demonstrated that females have higher ankle and MP1 joint laxity than males, but these differences were not affected by short-term changes in estradiol levels. In terms of hormonal effects on joint laxity, the sex differences are most likely due to prolonged exposure to estradiol and possibly the other sex hormones, progesterone and relaxin. Over time, estradiol decreases total collagen content within soft tissues (Abubaker, 1996; Fischer, 1973; Liu, 1997) which would cause the tissue to become more distensible. Higher joint laxity

has been associated with an increased risk for ligamentous injuries (Baumhauer, 1995; Beynnon, 2001; Chomiak, 2000; Glick, 1976; Myer, 2008; Nicholas, 1970). Therefore, the higher laxity females have in the ankle in comparison to males may help explain why females sustain more ankle sprains than males (Beynnon, 2005; Chandy, 1985; Douglas, 1999; Hosea, 2000; Ristolainen, 2009). In this study, we also found that an acute bout of exercise repetitively stressing the metatarsophalangeal joints causes an increase in laxity in the soft tissues under the metatarsals. However, this exercise does not alter ankle laxity. Therefore, joints become more distensible as a result of repetitive tension forces on the soft tissue and not in response to metabolic fatigue. High metatarsophalangeal joint laxity and fatigued intrinsic foot/calf muscles in response to the exercise protocol give less support to the metatarsal bones causing a potential increase in bone strain, and there would be an increased risk for a stress fracture (Figure 16). We found that maximum force did not change in the medial metatarsal regions. Therefore the metatarsals are experiencing the same amount of GRF with a lessened ability to dampen forces on the bone which may help explain why metatarsal 1 and 2 are at an increased risk for stress fractures (Arendt, 2003). Lastly, we also found that repeated jumping causing an increase in medial midfoot peak pressures. Therefore, fatiguing foot/calf muscles and stretching out soft-tissues supporting the longitudinal arch increases pronation which may have implications for raising the risk for patellofemoral overuse injury. These conclusions are summarized in Figure 16 and will be further explained in the following sections.

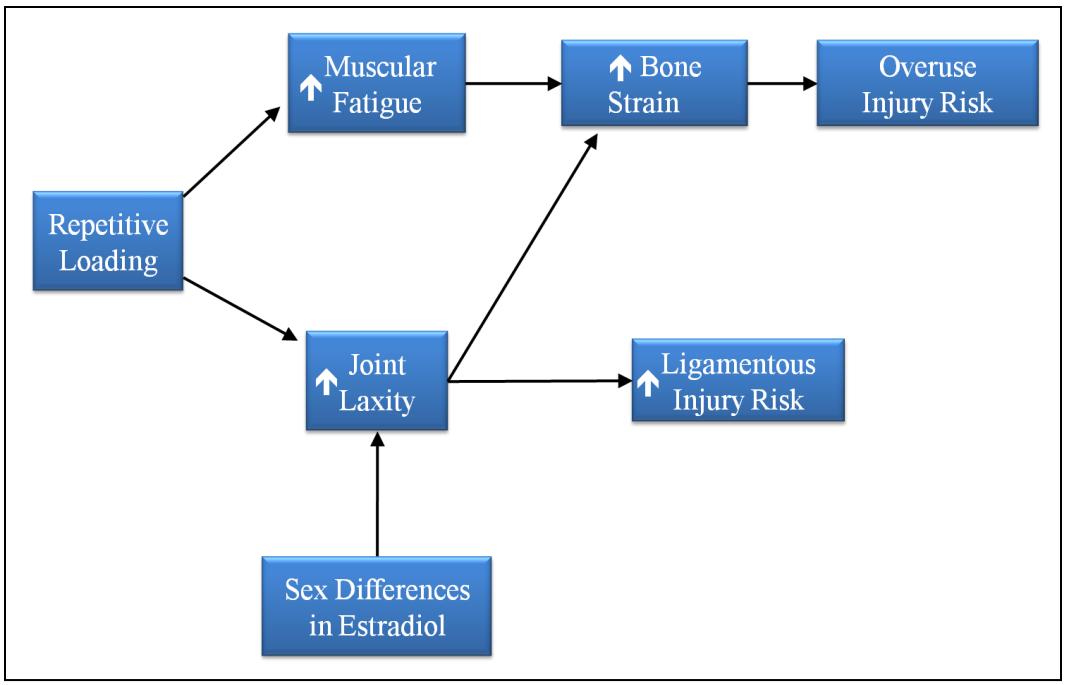

Figure 16. Summary of conclusions.

Etiology of Sex Differences in Ligamentous Injuries

The sex differences found in the ankle and MP1 joint laxity are consistent with previous literature investigating sex differences in knee laxity (Arnold, 2002; Beynnon, 2005; Pollard, 2006; Rozzi 1999; Shultz, 2005). Joint stability is maintained by ligaments and tendons helping to resist joint displacement. In particular, the deltoid, calcaneofibular, talofibular, and talocalcaneal ligaments of the ankle help to limit and resist inversion and eversion. If ankle laxity is high, ankle stability will decrease and the ability to resist inversion/eversion will be reduced. Therefore, more stress is placed directly on tight ligaments during maneuvers involving inversion and eversion which increases the risk for ligament ruptures. Females suffer from more ankle injuries than males (Beynnon, 2005; Cameron, 2010; Chandy, 1985; Hosea, 2000; Messina, 1999; Ristolainen, 2009), specifically in sports that require cutting such as soccer and basketball. Previous literature suggests that high joint laxity might be associated with ligamentous injury risk. For example, Nicholas *et al.* (1970) found in a prospective study that football players with loose ligaments were more likely to rupture their knee ligaments compared to players with tight

ligaments. Similarly, Myer *et al*. (2008) found that increased knee hyperextension and anterior-posterior tibiofemoral translation were predictive of future ACL injuries. In the ankle, higher laxity has been associated with increased ankle sprain injury risk when testing the anterior drawer (Baumhauer, 1995; Chomiak, 2000) and talar tilt (Beynnon, 2001; Chomiak, 2000; Glick, 1976) laxity. In addition, the uses of ankle braces and taping have been shown to lower the risk for reinjuring the ankle (McKay, 2001; Surve, 1994). On the other hand, some studies have demonstrated that laxity has no predictive value for ankle sprains (Barett, 1993; Jackson, 1978). Higher laxity in the joint-supporting soft tissues might help to protect ligamentous sprains by allowing for joint displacement to occur. However, if the joint is too distensible, joint displacement may be excessive resulting in the ligaments stretching past their limits and being ruptured. Most likely, there is an optimal level for how distensible a joint should be in order to protect against ligamentous injury. If a prospective study was conducted relating ranges of laxity values with rates of ankle sprains, I would expect that individuals past a certain laxity value would be more likely to sustain an ankle sprain. It is possible that many females have joint laxity past the optimal level that predisposes them to a higher risk for excessive inversion and subsequent ankle sprains. Therefore higher ankle laxity experienced by females may help explain why females suffer from more ankle injuries than males. Further prospective research examining the spectrum of ankle laxity values and risk for ankle sprains is warranted.

Effects of Sex Hormones on Joint Laxity

The difference in joint laxity between males and females has been attributed to differences in sex hormones. Estradiol is involved with inhibiting collagen synthesis within tendons and ligaments thereby potentially affecting the laxity of the associated joint (Figure 17) (Fischer, 1973; Liu, 1997). However, in this study, joint laxity in the ankle and MP1 joint did not fluctuate according to peaks in estradiol levels throughout the menstrual cycle. These results are consistent with those found by Belanger *et al.* (2004), Eiling *et al.* (2007); Hertel *et al.* (2006),

Karangeanes *et al.* (2000), and Pollard *et al.* (2006) when examining knee laxity. Although there have been relationships found between knee laxity and menstrual cycle phases (Deie, 2002; Heitz 1999; Park 2009; Shultz, 2010; Shultz, 2005), important limitations in these studies suggest a possible type I statistical error resulting from small sample sizes, a lack of clear documentation of menstrual phases, and inconsistent subject criteria (Zazulak, 2006). If there are changes in laxity across the menstrual cycle, they are easily masked by study limitations and likely very small, posing little clinical impact.

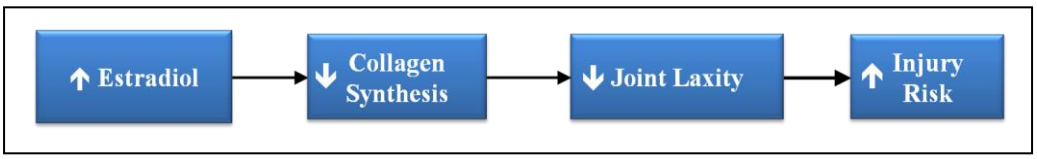

Figure 17. Flow chart demonstrating how estradiol potentially relates to injury risk.

The results of this study are evidence suggests that acute peaks in serum hormone levels during the menstrual cycle do not have cyclic effects on joint laxity. Sex differences in laxity instead may be due to the prolonged exposure to estrogen females experience over time (Arnold, 2002; Karangeanes, 2000; Pollard, 2006). The relatively small and frequent peaks in estradiol might not cause fluctuations in joint laxity because the effect that estradiol has on collagen content is dose and time dependent. Fischer (1973) administered more than ten thousand times that of normal physiological amounts to rats and found an effect of estradiol on collagen as soon as 24 hours later. This demonstrated that high amounts of estradiol will cause a fast response on collagen content. In this study, we found that the female participants had relatively low estradiol levels compared to the levels of the general female population. Therefore, the levels of estradiol experienced by female athletes may not be large enough to elicit a cyclic response on collagen content and subsequent joint laxity. There is also some evidence to suggest that time is a factor in how estradiol influences joint laxity. For example, Shultz *et al.* (2004) suggested that there is a 3-4 day time delay in the action of estrogen on knee laxity. When this time delay was accounted for, the multiple regression analysis of hormone levels accurately predicted changes in knee

laxity. Also, hyperextension increases during the last trimester of pregnancy as well as with successive pregnancies supporting the idea that hormones have cumulative effects (Calguneri, 1982). If it takes multiple weeks for estradiol to inhibit enough collagen synthesis in order for there to be clinically-relevant increases in joint laxity, fluctuations in joint laxity would unlikely be seen across the menstrual cycle.

Effects of Exercise on Joint Laxity

This study showed that joint laxity increases in response to exercise. The data from previous studies indicate that knee laxity tends to increase after exercises that included running, cutting, and jumping (Johannsen, 1989; Rowe, 1999; Sakai, 1992; Skinner, 1986). In our study, we used an exercise protocol that preferentially targeted the metatarsophalangeal (MP) joints with repetitive and highly loaded MP dorsiflexion, while not placing stress on the lateral ankle ligaments. Laxity in the MP1 joint increased in both males and females after exercise while ankle laxity did not change. This demonstrates that repetitive loading rather than metabolic fatigue (increased heart rate and lactate levels) causes joint laxity to increase. Likewise, Belanger *et al*. (2004) used cycling as an exercise protocol instead of lower limb loading and found that knee laxity does not increase after exercise. The continuous loading to ligaments and tendons can cause acute tissue microdamage, reducing the integrity of the soft tissue and increasing laxity. An increase in joint laxity, especially in the metatarsophalangeal joints, may have implications for increasing the risk for metatarsal stress fractures. This will be further explained in the following section.

Etiology of Overuse Injuries

In this study, we found that peak pressure in metatarsals 1 and 2 decreased after a bout of repeated jumping. This is in contrast to the results found in previous studies which show that peak plantar pressure in the metatarsal region increases after exercise (Nagel, 2008; Weist, 2004;

Wu, 2007). An important difference between this study and the studies done by Weist *et al*. (2004) and Wu *et al.* (2007) was that they measured plantar pressure during the exercise while subjects were forced to maintain a constant running speed. In the current protocol, pressure was measured after the exercise was completed and subjects were allowed to choose their own speed while walking over the pad (the speed did not change after exercise). Nagel *et al*. (2008) had a similar protocol to this study in which plantar pressure was measured after a marathon as the participants walked at a self-selected pace. However, they found peak pressure in the toes to decrease while metatarsal peak pressure increased. Therefore, the decreased peak pressure in the toes and medial metatarsal regions is not only attributed to measuring pressure after exercise as opposed to during exercise but also due to the unique exercise protocol that highly fatigued intrinsic foot muscles and stretched out the metatarsophalangeal joints.

To understand biomechanically why peak pressure decreased in the metatarsal 1 and 2 regions after exercise, we compared speed, stride length, and step length before and after exercise. We found that all three parameters remained the same. Decreased peak pressure in the metatarsals 1 and 2 is also not explain by load shifting because peak pressures did not increase in the heel, lateral midfoot, metatarsals 345, or toe regions. Our data also showed that peak pressure in the medial metatarsals and toes decreased because contact area increased and force stayed the same. Spreading the force over a larger area may be a result of increased laxity in the soft tissue surrounding the MP joints including flexor tendons, MP joint capsules, plantar aponeurosis, and fat bodies. The plantar aponeurosis is connected to the MP joint capsule by vertical fibers below the metatarsal heads (Bojsen-Møller and Flagstad, 1976). In between the vertical fibers are fat bodies and the plantar ligaments (Figure 18). As the aponeurosis and vertical fibers become more distensible, the encapsulated fat bodies would spread out resulting in an increase in contact area. Therefore decreased plantar loading after exercise may reflect increased laxity in the tendons, ligaments, and aponeurosis in the ball of the foot.

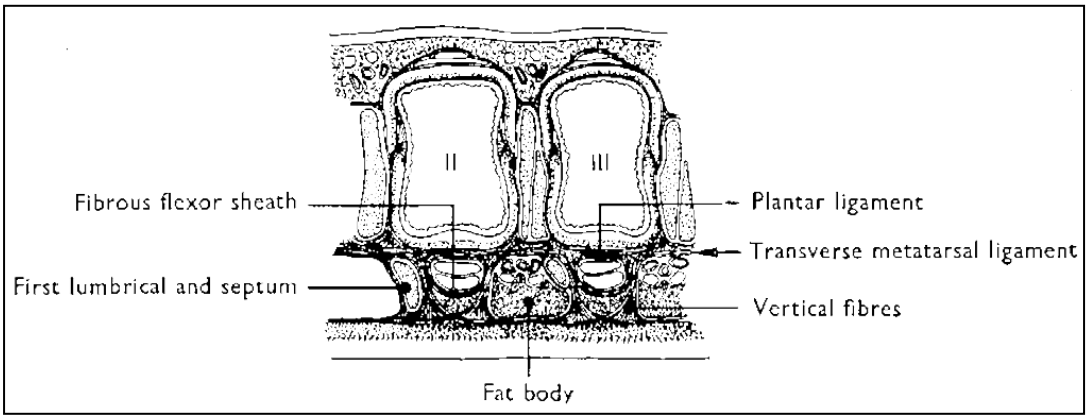

Figure 18. Transverse section of the second and third metatarsal heads from Bojsen-Møller and Flagstad, 1976.

The decrease in metatarsal peak pressure is also likely a result of fatigued muscles supporting the metatarsal heads. Muscles and ligaments crossing and surrounding the metatarsophalangeal joints actively and passively help to reduce bending forces on the metatarsal shaft brought on by high ground reaction forces (GRF). Some of the targeted muscles fatigued in the exercise protocol were those that cross the metatarsals including flexor hallucis longus, flexor hallucis brevis, flexor digitorum longus, and flexor digitorum brevis. After fatiguing these muscles and stretching out MP soft tissues, we found that maximum force remained the same. Therefore, the metatarsals are subjected to the same amount of GRF while muscles and MP soft tissues are less able to dampen the resulting compression forces on dorsal side of the metatarsals. As a result, this increases the risk for metatarsals 1 and 2 stress fractures.

The risk for stress fractures may also be increased in the presence of certain sex hormones. In a study where stress fracture injury rates were compared between females on OC and NOC, Barrow and Saha (1988) found that female runners who had never been exposed to OC were over twice as likely to sustain a stress fracture. Our study demonstrated that plantar loading in the first and second metatarsal is higher in females NOC than females on OC. Since there were no differences between OC and NOC groups in joint laxity, the difference in peak plantar pressure is not attributed to joint laxity. Sex hormones associated with OC use instead may be affecting other factors, such as muscle function, that potentially influence peak pressure. For

instance, slower relaxation rates have been associated with high estradiol levels (Jaskolska, 2007; Sarwar, 1996). Sarwar *et al*. (1996) demonstrated that females NOC had increased fatigability. Also, Phillips *et al.* (1996) observed cyclic changes in maximum voluntary force in females NOC. Sex hormones are suggested to influence force production by altering crossbridge states within a muscle fiber (Sarwar, 1996). Further investigations are warranted that are designed to determine how sex hormones influence muscle properties and function and how this may relate to altered plantar loading in order to further understand how sex hormones influence stress fracture risk.

The increased medial midfoot peak pressure reflected the increased pronation experienced by a fatigued foot and is consistent with previous studies (Weist, 2004; Wu 2007). Higher medial midfoot peak pressure is due to medial arch collapse. One of the functions of the medial arch is to absorb the majority of the shock of impact while walking. The arch is passively supported by plantar aponeurosis, superomedial and inferior calcaneonavicular ligament (Davis, 1996) and is actively supported by tibialis posterior (Kaye and Jahss, 1991). As this muscle becomes fatigued and the ligaments and aponeurosis become more distensible during the stairs/hurdle protocol implemented in this study, the arch is less supported causing pronation. Increased pronation has been associated with shin splints (Messier and Pittala, 1988) and patellofemoral pain (Clement, 1981). Since females are more prone to patellofemoral pain, we predicted that medial midfoot pressure would be higher in females. This however was not supported by our data. It is probable that the exercise protocol was intense enough to cause similar amounts of fatigue to the arch supporting muscles in males as in females. Therefore strengthening the arch-supporting muscles and adding shock-absorbent insoles during conditions of fatigue may help reduce the risk for shin splints (Thacker, 2002) and patellofemoral pain overuse injuries (Almeida, 1999; Fulkerson, 2002; Iwamoto, 2008). In addition, there are other factors that have been associated with patellofemoral pain such as sex differences in genu valgum, q-angle, and hip rotation (Moss; 1992; Ferber, 2003) that need to be taken into account

in future studies to understand more completely the progression of shin splints and patellofemoral pain.

Study Complications and Future Investigation

There is a possibility that limitations to the study affected some of the outcomes of the study. Only 34 females (18 on OCs and 16 NOCs) and 13 males were included in the analysis. Sample size was low relative to the high variation in ankle laxity, MP1 joint laxity, estradiol levels, and peak plantar pressure. For the most part, visits for each subject were at the same time and day each week which helps explain low variability within subjects. However, variation between subjects may be influenced by the time of day at which they were measured. For example, some subjects may have been more active than others prior to the visit by having a practice, work out, or walking long distances to classes earlier in the day. There also may be differences in body temperature according to the time of day and time of year measurements were recorded. In addition, some subjects were in season, while others were not. Therefore the level of activity between weeks for each subject was different which may contribute to inter-individual variation. Future investigation should measure all in-season athletes at the same of time of day and year in order to help reduce variation between individuals.

Another potential problem when comparing OC and NOC groups is that the type of OC and amount of time the subjects had been on OC was unregulated. If consistent oral contraceptives were used for the same long time period, differences between OC and NOC might have been seen in ankle and MP1 joint laxity.

Studies involving the menstrual cycle are difficult and pose several limitations. No clear ovulatory peaks were captured for any subject at any visit, a problem Beynnon *et al.* (2005) also encountered. Therefore effects from the estradiol peak just before ovulation would not be apparent. Additionally, menstruation was self-reported by the subjects and cycle length for each subject was evaluated over the 12 weeks. These were the only parameters used and sometimes

had to be estimated in order to place the visit in the respective menstrual quartile. Consequently, there may have been some error in placing visits into menstrual week, a common issue menstrual cycle studies struggle with (Hewett, 2007). Zazulak *et al*. (2006) reviewed 9 studies and concluded that it is difficult to define a "normal cycle".

The highly variable levels of estradiol and cyclic changes were consistent with what Shultz *et al.* (2010) found. The menstrual cycle can vary substantially between females, particularly in athletes. Such disparities include delayed menarch, amenorrhea, and luteal suppression and occur because training causes a disruption in the endocrine system involved with regulating the menstrual cycle (Loucks, 1990). For example, Beynnon *et al.* (2005) found that a large proportion of the participants had anovulatory cycles. As a result of assorted menstrual discrepancies, the potential changes in laxity due to hormones in the menstrual cycle would be variable. Shultz *et al.* (2006) suggested that variability in hormone levels among female athletes may account for the variability in joint laxity. Therefore any small fluctuation in laxity may be masked and prevented from becoming statistically significant.

Further testing is warranted and should include a larger sample size and a more accurate method for determining menstrual cycle phase. The use of an ovulation kit or measuring morning body temperature may be helpful. It would also be valuable to measure the subjects at the same time within each phase in order to place visits into appropriate phases accurately. Additionally, strict guidelines for subject selection, including extremely regular menstrual cycles and normal fluctuating estradiol levels would likely help decrease the variation.

An important aspect of this study is that the results demonstrate what the body does in response to repeated loading without being forced to maintain a certain performance level. During a sporting event, the athlete's objective is to perform at optimal levels in order to win pushing their bodies past normal physiological limits. It is likely that plantar loading would be different under fatigued conditions during the sporting event. Therefore it would be warranted to investigate how joint laxity and plantar pressure is altered during a sporting event.

It would also important to take into consideration the potential roles that other sex hormones and the combination of sex hormones play on collagen content and subsequent laxity. Progesterone has been shown to have opposite effects by increasing procollagen synthesis, a precursor to collagen (Yu, 2001). Relaxin is known to play a role in preparing the pelvic ligaments for parturition by driving collagen biosynthesis (MacLennan, 1981). Therefore, relaxin likely also has an effect on lower limb joint laxity. Relaxin has been shown to be present in ACL specimens (Galey, 2003) and to decrease gene expression of collagen (Takano, 2009). In addition, Dragoo *et al.* (2009) found that administering relaxin causes ACL strength in a rat model to weaken. Relaxin increases and decreases throughout the luteal phase and has been shown to increase in women taking oral contraceptives (Wreje, 1995). Lack of differences in laxity between our NOC and OC groups and across the menstrual cycle may be a result of a more complicated interplay among sex hormones where relaxin and possibly estradiol increases laxity, while progesterone is decreasing laxity. Further investigation should address the individual and combined effects of sex hormones on collagen content and subsequent alterations in joint laxity.

In addition to soft joint-supporting tissues, muscles play a larger role in helping to decrease the risk of overuse injuries by helping to reduce bending strains on bones. Therefore, further research should investigate how increasing muscle strength over time may alter plantar loading. Since stress fractures occur during long bouts of repeated lower limb loading, it would be important to not only increase muscle strength, but train muscles for endurance by increasing type I muscle fibers. If the muscles crossing the metatarsals have a greater ability to endure fatigue, the compression and tension forces would be reduced for greater duration throughout the exercise. Further investigation on how fiber type differs within muscles supporting commonly stress-fractured bones is warranted in order to incorporate training regimens that would increase endurance in relevant muscles and reduce the risk for metatarsal stress fractures.

Further investigation should also take into consideration other factors that influence the progression of injuries. This includes extrinsic factors such as training techniques, neuromuscular

coordination, and shoe design as well as other intrinsic factors including anatomical and geometrical characteristics, muscle characteristics, and bone properties.

Conclusions

Differences in joint laxity between males and females may be due to differences in sex hormones. However, the effect that estradiol has on ankle and first metatarsophalangeal joint is not due to acute fluctuations and is possibly due to prolonged exposure to estradiol as well as influences from other sex hormones. Laxity induced by exercise is dependent on the type of exercise and the stress placed on the ligaments and tendons. After fatiguing the intrinsic foot and calf muscles and walking at a self-selected relaxed pace, MP1 joint laxity increased and peak pressure decreased in the forefoot region. Peak pressures were decreased by spreading forces over a larger area, which is potentially a result of increased laxity in the soft-tissues under the metatarsal heads. The metatarsals might be a common site for stress fractures because maximum force remains the same after the supporting muscles were fatigued. Fatiguing the arch supporting muscles causes increased medial midfoot pressure which has been linked to increasing the risk for shin splints and patellofemoral overuse injury. Implementing strength and endurance training to the intrinsic calf/foot muscles that support the metatarsals and longitudinal arch as well as optimizing joint laxity would likely help to reduce the risk for metatarsal stress fractures, shin splints, and patellofemoral pain.

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