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Effect of 17β Estradiol & Foot Strike Patterns on Physiological & Biomechanical Changes in Runners

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in conjunction with the
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Effect of 17β Estradiol & Foot Strike Patterns on Physiological &
Biomechanical Changes in Runners

by

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A Dissertation submitted in partial satisfaction of
The requirements for the degree
Doctor of Science in Physical Therapy

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Science.

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DEDICATION

To my family,
in gratefulness for your confidence in me.

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ABBREVIATIONS

ACL	Anterior cruciate ligament
AP	Active Peak
AVLR	Average Vertical Loading Rate
BW	Body Weight
BW/S	Body Weight per sec
CNS	Central nervous system
CNS	Central nervous system
E	17β-Estradiol
EMG	Electromyography
EMG	Electromyography
FFS	Forefoot Strike
GAS	Gastrocnemius
GRF	Ground Reaction Force
H/Q	Hamstring quadriceps ratio
IEMG	Integrated Electromyography
IEMG	Integrated Electromyography
IP	Impact Peak
IVLR	Instantenous Vertical Loading Rate
KJL	Knee joint laxity
LH	Lateral Hamstring
MFS	Mid-foot strike
MH	Medial Hamstring

ML	Medial to lateral
MVC	Maximum voluntary contraction
MVC	Maximum Isometric Voluntary Contraction
OC	Oral contraceptive
PA	Preactivation
PO	Push off
QH	Quadriceps hamstring
RFS	Rear Foot Strike
SBR	Simulated Barefoot Running
TA	Tibialis Anterior
VIP	Impact Peak
VL	Vastus lateralis
VM	Vastus medialis
WA	Weight acceptance

ABSTRACT OF THE DISSERTATION

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Doctor of Science, Graduate Program in Physical Therapy
Loma Linda University, September 2014
Dr. Jerrold Petrofsky, Chairperson

It is well established that female runners are at a significantly increased risk of incurring injuries when compared to their male counterparts. Gender-specific factors such as anatomical, hormonal, and altered neuromuscular activation patterns have been implicated as causative factors. An association have been observed between hormonal fluctuation and ACL injury risk indicating potentially hormonal effect on both passive and dynamic knee stabilizer.

A growing contingency believes that we were designed with all we need in our feet to be able to run with minimal shoes that mimic barefoot running striking pattern. Habitual barefoot runners tend to FFS, compared to habitually shod populations who tend to RFS. Reduced collision forces generated with FFS patterns relative to RFS account for the reduced injuries.

The purpose of this study was to assess the effect of 6 weeks of a transition program of SBR on the pattern of muscle activation, spatiotemporal variables, and stance phase kinetics. These running parameters were compared and contrasted during the menstrual cycle to assess whether estrogen fluctuation has an effect on the pattern of muscle activation, and laxness of ACL.

Twenty four females runner were divided into two groups. First group was tested twice across a menstrual cycle for serum levels of E, KJL and EMG activity of the quadriceps and hamstrings muscles. Second group gradually experienced SBR over 6 weeks. Kinetic analysis of running was performed during shod running, habituated SBR conditions.

The results showed an observed increased in KJL in response to peak E during the ovulatory phase, which was associated with increased preactivity of the hamstring muscle. A consistent pattern was observed in the firing of the quadriceps muscle recruitment pattern throughout the follicular phase. The results of the second group indicated a significant decrease in the EMG activity of TA in the habituated SBR. A significant increase was observed in the preactivation of GAS between shod running, and habituated SBR.

In conclusion, changes in KJL in response to 17β-Estradiol fluctuations changes the neuromuscular control around the knee. Changes in motor patterns in previously habitually shod runners are possible and can be accomplished within 6 weeks.

CHAPTER ONE

INTRODUCTION

A female athlete's increased risk for non-contact anterior cruciate ligament (ACL) injury has been well documented. Women are two to eight times more likely to injure their ACL when compared to men in comparable sporting activities (Wojtys et al., 1998). The discrepancy in ACL injury risk between sexes has been attributed to multiple factors including differences in anatomical, hormonal, biomechanical, and neuromuscular characteristics (Ireland and Ott, 2004).

The normal menstrual cycle produces low serum levels of estrogen and progesterone in the early follicular phase (day 1–6), estrogen is elevated in the late follicular phase (day 7–14), and progesterone is elevated during the luteal phase (day 15–28) while estrogen remains elevated and slowly returns to baseline levels (Constantini et al., 2005, Beynnon and Fleming, 1998). The link between ACL injury and fluctuations of the ovarian sex hormones during the female menstrual cycle is controversial. Some investigators have reported an increase in ACL injuries in the late follicular phase (Deie et al., 2002, Wojtys et al., 1998, Wojtys et al., 2002). Other investigators have reported similar phenomena during the luteal phase (Deie et al., 2002, Shultz et al., 2004b) and during the early follicular phase (Slauterbeck and Hardy, 2001). These contradictory results fail to explain the role of sex hormones in ACL injury risk to one phase of the menstrual cycle.

Ovarian sex hormone fluctuations have been associated with tissue alterations and an increased incidence of noncontact ACL injuries (Slauterbeck et al., 2002, Wojtys et al., 2002). Estrogen and progesterone receptors have been detected within the ACL (Liu et al., 1996). Several studies have demonstrated a relationship between peaks in estrogen serum concentration and increased laxity in the ACL (Deie et al., 2002, Shultz et al., 2004a, Slauterbeck and Hardy, 2001). This associated change in tissue tolerance may predispose the ACL to failure at lower tensile loads and/or alter the protective muscle reflex actions associated with ACL tissue receptor stimulation (Raunest et al., 1996).

The muscular system serves a protective role in limiting the external forces and moments created through the knee joints motions that ultimately result in tension loading of the ACL. Estrogen alpha and beta receptors have been reported in skeletal muscle thereby providing a plausible tissue-based mechanism for altering neuromuscular control and myofascial force transmission pathways during the menstrual cycle (Huijing and Jaspers, 2005, Lemoine et al., 2003, Zazulak et al., 2006). In addition, research has not fully described the influence of sex hormone receptors in skeletal muscle on tissue mechanisms that can alter neuromuscular control. However, estrogen both directly and indirectly affects the female neuromuscular system (Rozzi et al., 1999). Sarwar & colleagues reported quadriceps strength increases and a significant slowing of muscle relaxation occurs during the ovulatory phase of the menstrual cycle (Sarwar et al., 1996). Serum estrogen concentrations fluctuate radically throughout the cycle and estrogen has measurable effects on muscle function and tendon and ligament strength (Rozzi et al., 1999, Lebrun and Rumball, 2001). These data indicate that estrogen may have effects on neuromuscular function which may facilitate the potential for neuromuscular imbalances to develop in female athletes.

Decreased neuromuscular control of the joint may place increased stress on the passive ligament structures that exceed the failure strength of the ligament. Neuromuscular recruitment patterns that compromise active joint restraints subject passive joint restraints to greater load, decrease dynamic knee stability, and increase risk of ACL injury (Li et al., 1999, Besier et al., 2001b).

The neuromuscular components of dynamic joint stability in healthy and ACL deficient subjects have yet to be delineated. Preparatory and reflexive muscle activation are two neuromuscular processes that determine muscle activity and contribute to functional joint stability. The muscle spindle contributes to muscle tension through a feedback reflexive stretch mechanism, but the process most likely to benefit the joint dynamically is through information from the feedback loop associated with the muscle spindle (Bergenheim et al., 1995) . This feed forward system may be the best approach to preventive strategies.

Theoretically, protection can be accomplished through generation of muscular tension as the result of efferent information from the descending drive and afferent information from cutaneous and articular mechanoreceptors (Johansson et al., 1991). The information provided from the muscle spindle is modulated by the tension within the muscle spindle, which affects the α motoneurone. This modulation updates the muscle tension and may protect the joint through dynamic stability and ‘‘self’’ regulation.

Tension is crucial to this feed forward paradigm of the muscle spindle modifying the neural signal back to the muscle (Johansson and Sojka, 1991). The tension of the muscle spindle may be determined by both intrinsic and extrinsic modes. The intrinsic mode is a function of the viscoelastic soft tissue properties measured by the resistance to length changes (Huston and Wojtys, 1996).The extrinsic factor is the previously mentioned adjustment resulting from the afferent input(Johansson et al., 1991). Each of these factors may influence the sensitivity and therefore the protective ability of the system. The tension produced by preactivation of a muscle before a stressing experience may improve regulation of muscle activity, thereby increasing its ability to protect the joint. In addition, a learned task will prepare the joint for increased stress by a preparatory muscle contraction based on an experienced expectation, through an increase in the sensitivity of the muscle spindle.

Estrogen also has effects on the central nervous system including the higher motor centers, where it binds to membrane-bound receptors and influences transmitter systems in the brain (Friden et al., 2003). Hence, during the menstrual cycle as the endogenous levels of estrogen undergo dynamic regulation; it stands to reason that their effect (s) on the CNS and thus neuromuscular control will also change (Darlington et al., 2001, Woolley, 1999). In support, a recent study by Dedrick & colleagues (2008) found that eumenorrheic women use a different neuromuscular control pattern when performing a 50 cm drop-jump sequence when estrogen levels are high (luteal phase) compared to when they are low (early follicular phase).

Female athletes display different neuromuscular strategies from male athletes (Myer et al., 2005a). These sex differences in muscle recruitment and timing of muscle activation may affect dynamic knee stability. Neuromuscular preplanning allows feed forward recruitment of the musculature that controls knee joint positioning during landing and pivoting maneuvers (Besier et al., 2001a). Imbalanced or ineffectively timed neuromuscular firing may lead to limb positioning during athletic maneuvers that puts the female ACL under increased strain and risk of injury (Myer et al., 2005a). In addition, fine motor activity and reaction time performance have been reported to fluctuate over the course of the menstrual cycle (Posthuma et al., 1987), with more consistent performance in women using oral contraceptives (OC). Fride´n et al., discovered an increase in postural sway (Friden et al., 2005) during single limb stance and threshold for detection of passive knee motion (Friden et al., 2003) in the mid-luteal phase of the menstrual cycle. Improved neuromuscular coordination may occur in women taking OC with a reduced number of premenstrual symptoms (Ruedl et al., 2009). A previous study (Dedrick et al., 2008) reported modified co-contraction patterns of the gluteus maximus and semitendinosus at different stages of the menstrual cycle, with increased synchronicity of the contraction between the two muscle groups at ovulation. The authors theorized that this may have implications for valgus stability of the knee due to the influence of these two muscles working in conjunction to control torsional movement of the tibio-femoral complex.

Men and women demonstrate similar neuromuscular control strategies during different athletic activities until puberty (Hewett et al., 2004). A link between hormonal fluctuations and changes in neuromuscular control may exist, since alterations in hormonal levels constitute a primary change in development during and after puberty. Neuromuscular control strategies incorporated during athletic movement appear to change in females after puberty, where increased knee valgus alignment places the ACL at greater risk for injury (Hewett et al., 2004). Several theories have been proposed to define the mechanisms for gender differences in ACL injury rates. These theories include gender differences, decreased knee ligament strength due to female sex hormones and neuromuscular imbalances in females (Myer et al., 2005a). Another proposed theory related to neuromuscular imbalances in females is the relatively low knee flexor to extensor recruitment (H/Q ratio). Hewett et al. reported that males demonstrated knee flexor moments, measured using inverse dynamics, that were threefold higher than females when decelerating from a landing (Huston and Wojtys, 1996). This same group of females also demonstrated decreased isokinetically measured H/Q ratio and increased knee abduction (valgus) moments compared to male subjects during landing. The increased dynamic knee valgus significantly correlated to the peak impact forces during landing (Hewett et al., 1996). Also, female subjects show greater dynamic lower extremity valgus (hip adduction and internal rotation, knee abduction, tibial external rotation, and possibly forefoot pronation) (Hewett et al., 2005a). The increased incidence of serious knee injuries in female athletes is well established, however the underlying neuromuscular mechanisms related to the elevated ACL injury rate that occurs after the onset of puberty in females has not been delineated.

Remarkably, an observed association between hormonal fluctuation and ACL injury risk indicates that there were effects of hormonal fluctuation (and potentially hormone stabilization) on either passive or dynamic knee stability (Hewett et al., 2007). The effects of the menstrual cycle may be on the active restraints (neuromuscular in nature) rather than the passive restraints (ligament) of knee stability, because the menstrual cycle has effects on motor control and muscle strength (Posthuma et al., 1987, Sarwar et al., 1996). Some reports suggest that more emphasis should be placed on investigation of neuromuscular factors that may be related to increased ACL injuries in female athletes (Warden et al., 2006).

Circumstantial evidence exists supporting a link between hormonal fluctuations during the menstrual cycle and altered neuromuscular control during selected athletic movements. To date, few studies have focused on changes in neuromuscular control mechanisms over the course of the menstrual cycle and its impact on running activity. Therefore, the purpose of the study was to investigate the effects of 17β-Estradiol across phases of menstrual cycle on lower extremity neuromuscular control patterns and ACL laxity during running. We hypothesized that lower extremity muscle activation patterns and laxness of the ACL will be altered during the periods of high level of serum estrogen concentration compared to the early follicular phase, when estrogen is lowest in young healthy females' runners.

According to the authors of a 2004 article published in Nature, humans were born to run (Bramble and Lieberman, 2004). Bramble and Lieberman have suggested that our body structure was significantly influenced by the fact that we needed to run for survival (Bramble and Lieberman, 2004). A growing contingency believes that we were designed with all we need in our feet to be able to run sans shoes or with minimal shoes that mimic barefoot running striking pattern. In fact, there has been a suggestion that running without the assistance of modern running shoes might lead to a reduction in the incidence of running injuries (Lieberman et al., 2010).

In recent years there has been resurgence in barefoot running as well as running in light, minimalistic shoes (Rothschild, 2012). Research into the foot-strike patterns and lower limb kinematics of barefoot and shod populations has also proliferated (De Wit et al., 2000, Lieberman et al., 2010, Perl et al., 2012, Squadrone and Gallozzi, 2009). Habitual barefoot runners such as adolescents in Kenya's Rift Valley tend to fore-foot or mid-foot strike when barefoot, compared to habitually shod populations who tend to rear-foot strike (Lieberman et al., 2010). Reduced collision forces generated with fore-foot (FFS) or mid-foot strike (MFS) patterns relative to a rear-foot strike (RFS) may account for anecdotal reports or reduced injuries in barefoot populations (Robbins and Hanna, 1987).

Runners who adopt a FFS or MFS pattern while shod also have reported improved performances (Hasegawa et al., 2007) and reduced injuries (Daoud et al., 2012, Diebal et al., 2012) (Goss and Gross, 2012) , spurring industry and researchers to examine foot strike patterns (FSP) more closely and to question the design of standard modern cushioned running shoes, which encourage RFS.

Running kinematics and ground reaction forces during simulating barefoot running (SBR) are reportedly similar to the barefoot condition of habitual barefoot runners (Squadrone and Gallozzi, 2009, Lohman et al., 2011). There are several studies of kinematic and FSP differences between barefoot and various shod conditions but findings vary according to the population under investigation. At velocities typical of endurance running (3.33–4.5 m/s − 1), habitually shod runners tend to land with a dorsiflexed ankle and RFS pattern, both when running shod and (to a lesser degree) when barefoot (Bonacci et al., 2009, Bonacci et al., 2013) .

In contrast, at similar velocities, habitual barefoot runners reported FFS or MFS patterns, with a more plantarflexed ankle at foot strike, than habitually shod populations, both when assessed barefoot and shod (Perl et al., 2012). Therefore, motor patterns laid down over years of training will influence FSP and running kinematics more than the shoe, or lack of, worn on a specific testing occasion. If a FFS/MFS pattern is sought by an athlete, simply changing footwear may not be sufficient to alter these patterns. Despite this, most studies have evaluated the acute effects of shod and barefoot running on kinematics, kinetics, spatiotemporal variables or oxygen cost of running, with minimal or no opportunity beforehand for the participant to habituate to any alternative footwear condition (Bonacci et al., 2013, Braunstein et al., 2010, Hasegawa et al., 2007). This is understandable, as the amount of time required to habituate to another foot strike pattern is not established, and transition to barefoot, SBR or FFS running could, by itself, involve risk of injury (Goss and Gross, 2012, Ridge et al., 2013).

A major limitation of all those prior works was that the subject's recruited were not particularly experience in barefoot running. Runners not accustomed to running barefoot could have their natural foot structure weakened by long term foot wear use and their proprioceptive sensitivity reduced (Divert et al., 2005). So they could be less effective in adopting their running style when running in this condition. Therefore, the subjects of our study were trained regularly for six weeks for a safe transition of simulated barefoot running.

Neuromuscular control during running is influenced by the landing pattern and type of shoes (Wakeling et al., 2001, Nigg et al., 1999). In RFS running pattern, the tibialis anterior muscle is considered to be the one muscle of specific interest. Around the time of heel-strike, the tibialis anterior muscle has two major functions: it positions the foot in dorsiflexion before heel-strike and it reduces the plantar-flexion moment of the foot due to the heel-strike (Inman, 1969, Winter and Yack, 1987). These two functions are quite different in their demands. The positioning of the foot is a slow event whereas the reduction of the foot slapping movement is associated with fast forces and movements. Thus, one can hypothesize that the tibialis anterior muscle activity should be significantly different in the pre- and post-heel-strike phase and altered when running barefoot or with shoes. One should expect that wearing shoes requires (compared to the barefoot situation) higher muscle activity before heel-strike to prevent the forefoot from touching the ground too early. Additionally, sport shoes are known to modify the force development during the impact (Gruber et al., 1998, Nigg et al., 1999). The cushioning effect of the shoe is therefore expected to alter the post-heel-strike muscle activity as measured by the electromyographic signal.

Ground Reaction Force (GRF) is an important factor in the study of the kinetics of the lower-extremities during running. Muscle activity in the leg is tuned in response to ground reaction forces. During running, the human body reacts to input from its external environment. One such input is the ground reaction force, which occurs during the ground contact phase of each stride (Wakeling et al., 2001). One possible reaction is the modification of the muscle activity patterns in response to that force (Nigg and Wakeling, 2001). It has been speculated that there is a requirement for the muscles to control and, thus, minimize soft tissue vibrations during locomotion (Nigg and Wakeling, 2001) and, thus, that there will be a change in muscle activity patterns in response to different vibration loadings on the lower extremity.

Impact forces in heel-toe running are forces resulting from the collision of the heel with the ground, reaching their maximum (the impact peak) earlier than 50 ms after first contact (Nigg and Wakeling, 2001). The rate at which the impact peak is reached is termed the loading rate and is a correlate of the major frequency of the impact peak. Impact forces have frequency contents in the range 10–20 Hz and should be expected to produce vibrations of the soft tissues of the body. Changes in the myoelectric patterns of the lower extremities of muscle activities have been shown to respond to frequencies of applied continuous vibrations of different impact forces.

A thorough search of the current scientific literature revealed that there is no published research investigating differences in habituated and non-habituated subjects, as most studies have used initial responses or habitually barefoot runners for their investigations (Lieberman et al., 2010, Lohman et al., 2011, Squadrone and Gallozzi, 2009). Although, it has been reported that habitually barefoot runners run differently from habitually shod runners (Bonacci et al., 2013, De Wit et al., 2000, Lieberman et al., 2010, Squadrone and Gallozzi, 2009). Still, it remains unclear as to whether adults who have grown up running in shoes will run with "barefoot" kinetics following a habituation period, or how long that habituation period should be.

Previous studies attempting to influence running motor patterns or kinematics through plyometric, strength or neuromuscular interventions have typically been 6–9 weeks in duration (Bonacci et al., 2009, Diebal et al., 2012, Snyder et al., 2009). A 6-week program was chosen for this study to allow initial adaptation of musculoskeletal structures to new forces, with the purpose of reducing the risk from too rapid a transition (Goss and Gross, 2012, Ridge et al., 2013, Salzler et al., 2012). Thereafter higher SBR training loads could be gradually introduced to elicit a training effect. Thus, the current study investigated the effects of a 6 week transition program of SBR on the stance-phase kinetics in habitually shod adult female recreational runners when compared with the same group in a non-habituated state and as such investigate the acute and the chronic changes of this group.

In the present study, two series of experiment were conducted to examine the effect of 17β estradiol fluctuation and different patterns of foot strike on running biomechanics. In series 1 (chapter 1) we investigate the effects of 17β-Estradiol across phases of menstrual cycle on lower extremity neuromuscular control patterns and ACL laxity during running. In series 2 (chapter 3) we investigate the effects of 6 week habituation of simulated barefoot running on the stance-phase kinetics in habitually shod adult female recreational runners.

CHAPTER TWO

17B-ESTRADIOL INDUCED EFFECTS ON ACL LAXNESS AND NEUROMUSCULAR ACTIVATION PATTERNS IN FEMALE RUNNERS

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Abbreviation

E	17β-Estradiol
ACL	Anterior cruciate ligament
CNS	Central nervous system
EMG	Electromyography
H/Q	Hamstring quadriceps ratio
IEMG	Integrated Electromyography
KJL	Knee joint laxity
LH	Lateral Hamstring
MVC	Maximum voluntary contraction
MH	Medial Hamstring
ML	Medial to lateral
OC	Oral contraceptive
PA	Preactivation
PO	Push off
QH	Quadriceps hamstring
VL	Vastus lateralis
VM	Vastus medialis
WA	Weight acceptance

Abstract

Purpose: To investigate the effects of 17β-Estradiol across phases of menstrual cycle on the anterior cruciate ligament (ACL) laxness and neuromuscular control patterns around the knee joint in female runners**.**

Methods: Twelve healthy female runners, who reported normal menstrual cycles for the previous 6 months were tested twice across one complete menstrual cycle for serum levels of 17β Estradiol **(**E), and knee joint laxity (KJL). Electromyographic (EMG) activity of the quadriceps and hamstrings muscles was also recorded during running on a treadmill. The changes in the EMG activity, KJL, and hormonal concentrations were recorded for each subject during the follicular and the ovulatory phases across the menstrual cycle.

Results: An observed increase in KJL in response to peak E during the ovulatory phase, was associated with increased preactivity of the hamstring muscle before foot impact ($p < 0.001$). A consistent pattern was also observed in the firing of the quadriceps muscle recruitment pattern throughout the follicular phase associated with decreased hamstring recruitment pattern during weight acceptance phase of running ($p = 0.02$). Additionally, low ratio of medial to lateral quadriceps recruitment was associated with a significant reduction of the quadriceps to hamstring cocontraction ratio during the follicular phase.

Conclusions: Changes in KJL during the menstrual cycle in response to 17β-Estradiol fluctuations changes the neuromuscular control around the knee during running. Female runners utilize different neuromuscular control strategies during different phases of the menstrual cycle which may contribute to increase ACL injury risk.

Keywords: 17β-Estradiol, ACL injury; knee joint laxity (KJL); EMG; neuromuscular control.

Introduction

A female athlete's increased risk for non-contact anterior cruciate ligament (ACL) injury has been well documented (Dedrick et al., 2008, Ireland and Ott, 2004). Women are two to eight times more likely to injure their ACL when compared to men in comparable sporting activities (Wojtys et al., 1998). The discrepancy in ACL injury risk between sexes has been attributed to multiple factors including differences in anatomical, hormonal, biomechanical, and neuromuscular characteristics (Ireland and Ott, 2004).

Hormone Effects on Injury Risk

The normal menstrual cycle produces low serum levels of estrogen and progesterone in the early follicular phase (day 1–6), estrogen is elevated in the late follicular phase (day 7–14), and progesterone is elevated during the luteal phase (day 15–28) while estrogen remains elevated and slowly returns to baseline levels (Constantini et al., 2005, Beynnon and Fleming, 1998). The link between ACL injury and fluctuations of the ovarian sex hormones during the female menstrual cycle is controversial. Some investigators have reported an increase in ACL injuries in the late follicular phase (Deie et al., 2002, Wojtys et al., 1998, Wojtys et al., 2002). Other investigators have reported similar phenomena during the luteal phase (Deie et al., 2002, Shultz et al., 2004b) and during the early follicular phase (Slauterbeck and Hardy, 2001). These contradictory results fail to explain the role of sex hormones in ACL injury risk to one phase of the menstrual cycle.

Hormone Effects on the ACL

Ovarian sex hormone fluctuations have been associated with tissue alterations and an increased incidence of noncontact ACL injuries (Slauterbeck et al., 2002, Wojtys et al., 2002). Estrogen and progesterone receptors have been detected within the ACL (Liu et al., 1996). Several studies have demonstrated a relationship between peaks in estrogen serum concentration and increased laxity in the ACL (Deie et al., 2002, Shultz et al., 2004a, Slauterbeck and Hardy, 2001). This associated change in tissue tolerance may predispose the ACL to failure at lower tensile loads and/or alter the protective muscle reflex actions associated with ACL tissue receptor stimulation (Raunest et al., 1996).

Hormone Effects on Tissue

The muscular system serves a protective role in limiting the external forces and moments created through the knee joints motions that ultimately result in tension loading of the ACL. Estrogen alpha and beta receptors have been reported in skeletal muscle thereby providing a plausible tissue-based mechanism for altering neuromuscular control and myofascial force transmission pathways during the menstrual cycle (Huijing and Jaspers, 2005, Lemoine et al., 2003, Zazulak et al., 2006). In addition, research has not fully described the influence of sex hormone receptors in skeletal muscle on tissue mechanisms that can alter neuromuscular control. However, estrogen both directly and indirectly affects the female neuromuscular system (Rozzi et al., 1999). Sarwar & colleagues reported quadriceps strength increases and a significant slowing of muscle relaxation occurs during the ovulatory phase of the menstrual cycle (Sarwar et al., 1996). Serum estrogen concentrations fluctuate radically throughout the cycle and estrogen has measurable effects on muscle function and tendon and ligament strength (Rozzi et al., 1999, Lebrun and Rumball, 2001). These data indicate that estrogen may have effects on neuromuscular function which may facilitate the potential for neuromuscular imbalances to develop in female athletes.

Hormonal Effect on the Central Nervous System

Estrogen also has effects on the central nervous system including the higher motor centers, where it binds to membrane-bound receptors and influences transmitter systems in the brain (Friden et al., 2003). Hence, during the menstrual cycle as the endogenous levels of estrogen undergo dynamic regulation; it stands to reason that their effects on the CNS and thus neuromuscular control will also change (Darlington et al., 2001, Woolley, 1999). In support, a study by Dedrick & colleagues (2008) found that eumenorrheic women use a different neuromuscular control pattern when performing a 50 cm drop-jump sequence when estrogen levels are high (luteal phase) compared to when they are low (early follicular phase).

Hormonal Effects on Neuromuscular Control

Female athletes display different neuromuscular strategies from male athletes (Myer et al., 2005a). These sex differences in muscle recruitment and timing of muscle activation may affect dynamic knee stability. Neuromuscular preplanning allows feed forward recruitment of the musculature that controls knee joint positioning during landing and pivoting maneuvers (Besier et al., 2001). Imbalanced or ineffectively timed neuromuscular firing may lead to limb positioning during athletic maneuvers that puts the female ACL under increased strain and risk of injury (Myer et al., 2005a). In addition, fine motor activity and reaction time performance have been reported to fluctuate over the course of the menstrual cycle (Posthuma et al., 1987), with more consistent performance in women using oral contraceptives (OC). Fride´n et al., discovered an increase in postural sway (Friden et al., 2005) during single limb stance and threshold for detection of passive knee motion (Friden et al., 2003) in the mid-luteal phase of the menstrual cycle. Improved neuromuscular coordination may occur in women taking OC with a reduced number of premenstrual symptoms (Ruedl et al., 2009). A previous study (Dedrick et al., 2008) reported modified co-contraction patterns of the gluteus maximus and semitendinosus at different stages of the menstrual cycle, with increased synchronicity of the contraction between the two muscle groups at ovulation. The authors theorized that this may have implications for valgus stability of the knee due to the influence of these two muscles working in conjunction to control torsional movement of the tibio-femoral complex.

Neuromuscular Control Differences between Sexes

Men and women demonstrate similar neuromuscular control strategies during different athletic activities until puberty (Hewett et al., 2004). A link between hormonal fluctuations and changes in neuromuscular control may exist, since alterations in hormonal levels constitute a primary change in development during and after puberty. Neuromuscular control strategies incorporated during athletic movement appear to change in females after puberty, where increased knee valgus alignment places the ACL at greater risk for injury (Hewett et al., 2004). Several theories have been proposed to define the mechanisms for gender differences in ACL injury rates. These theories include gender differences, decreased knee ligament strength due to female sex hormones and neuromuscular imbalances in females (Myer et al., 2005a). Another proposed theory related to neuromuscular imbalances in females is the relatively low knee flexor to extensor recruitment (H/Q ratio). Hewett et al. reported that males demonstrated knee flexor moments, measured using inverse dynamics, that were threefold higher than females when decelerating from a landing (Huston and Wojtys, 1996). This same group of females also demonstrated decreased isokinetically measured H/Q ratio and increased knee abduction (valgus) moments compared to male subjects during landing. The increased dynamic knee valgus significantly correlated to the peak impact forces during landing (Hewett et al., 1996). Also, female subjects show greater dynamic lower extremity valgus (hip adduction and internal rotation, knee abduction, tibial external rotation, and possibly forefoot pronation) (Hewett et al., 2005). The increased incidence of serious knee injuries in female athletes is well established, however the underlying neuromuscular mechanisms related to the elevated ACL injury rate that occurs after the onset of puberty in females has not been delineated.

Remarkably, an observed association between hormonal fluctuation and ACL injury risk indicates that there were effects of hormonal fluctuation (and potentially hormone stabilization) on either passive or dynamic knee stability (Hewett et al., 2007). The effects of the menstrual cycle may be on the active restraints (neuromuscular in nature) rather than the passive restraints (ligament) of knee stability, because the menstrual cycle has effects on motor control and muscle strength (Posthuma et al., 1987, Sarwar et al., 1996). Some reports suggest that more emphasis should be placed on investigation of neuromuscular factors that may be related to increased ACL injuries in female athletes (Warden et al., 2006).

Circumstantial evidence exists supporting a link between hormonal fluctuations during the menstrual cycle and altered neuromuscular control during selected athletic movements. To date, few studies have focused on changes in neuromuscular control mechanisms over the course of the menstrual cycle and its impact on running activity. Therefore, the purpose of the study was to investigate the effects of 17β-Estradiol across phases of menstrual cycle on lower extremity neuromuscular control patterns and ACL laxity during running. We hypothesized that lower extremity muscle activation patterns and laxness of the ACL will be altered during the periods of high level of serum estrogen concentration compared to the early follicular phase, when estrogen is lowest in young healthy females' runners.

Material and Methods

Subjects

A total of 12 female runners mean (±SD) age was 25.6 (±3.7) years, with regular menstrual cycles volunteered to participate in the study. All subjects were currently running not more than 20 km per week. Inclusion criteria were no history of pregnancy, no use of oral contraceptives or other hormone-stimulating medications for 6 months, nonsmoking behavior, two healthy knees with no prior history of joint injury or surgery, no medical conditions affecting the connective tissue, and physical activity was limited to 7 hours or less per week to reduce the likelihood of irregular or an ovulatory menstrual cycles that can occur with high volume or high intensity training. All subjects were heel strikers free of any obvious mal alignment or injuries at the time of data collection. The demographic characteristics of the subjects are displayed in table 1. All subjects had regular menstrual cycles of a mean interval of 28 (±2) days. All participants gave their written informed consent prior to entering the study. All procedures and protocols were approved by Institutional Review Board of Loma Linda University.

Table 1. Demographic Data of Participants

	Mean	**SD**
Age (year)	25.6	3.7
Height (Cm)	56.8	8.6
Weight (Kg)	160.2	8.2
BMI (Kg/m²)	22.0	2.2
Cycle Length (D)	28.8	1.1
Length of Menstruation (D)	6.3	1.2
Average Running Distance (Km/week)	18.00	2.1

SD Standard Deviation

Hormonal Assessment

All subjects came to the laboratory prior to data collection for a precollection session to familiarize them with the study protocol. Subjects reported for neuromuscular testing and blood assay during each of the follicular and ovulatory phase of the menstrual cycle during a month period. The first measurement (follicular phase) was taken during days 1 to 2 at the beginning of the menstrual cycle, when estrogen levels were expected to be low (Slauterbeck et al., 1999, Shultz et al., 2004b). The second data collection coincided with ovulation and occurred 24 to 48 hours after the estrogen surge detected by an ovulation predictor kit (Clearblue, Procter & Gamble, OH,USA) with 99% accuracy(Robinson et al., 2007).

The subjects were given an ovulation predictor kit for home use and were instructed when to employ the predictor kit based on their menstrual history. For example, for a subject with an average 28-day menstrual cycle tested on days 13 to 15 of her cycle, ovulation testing was done at the same time of day. The procedure involved holding a test stick in the urine stream for 5 seconds or collecting the urine in a paper cup and dipping the test stick into the cup for 20 seconds. When a positive result occurred, as indicated by a smiley face on the test stick, the subject contacted the primary investigator to schedule data collection within the subsequent 24 hours.

Estradiol Serum Concentration

Estradiol serum concentration was analyzed using a Cobas e-602 (Roche/Hitachi ,Tokyo, Japan). On each day of testing, 5-7 cc of venous blood were drawn to assay serum levels of estradiol. The blood sample was obtained from the antecubital vein with a 21 gauge needle to yield a minimum of 500ul of plasma which was centrifuged at 1500 rpm for 2 minutes and 3000 rpm for 4 minutes. The centrifugation took place within the Roche MPA module. Specimens were stored in 2-8 degrees Celsius. The methodology was competition principle and the total duration of assay was 18 minutes. The mean intraassay concentration was 100.0 and the mean percentage of coefficient of variation (CV %) ranged from 3.4% to 3.7%. The mean (SD) of interassay was 100.0 and the mean percentage of CV ranged from 3.8 % to 7.4%. Assay sensitivity for the estradiol was 5 pg/ml.

Electromyography

Electromygraphy (EMG) activity was measured for the vastus medialis (VM), vastus lateralis (VL), medial hamstring (MH), and lateral hamstring (biceps femoris) (LH) of the thigh, of the dominant leg. Prior to electrode placement, the skin was lightly abraded, and cleaned with alcohol. Circular pre-gelled 20 mm bipolar Ag–AgCl surface electrodes (EL503; Biopac Systems, Inc., Goleta, CA) were placed in parallel on the belly of each muscle in alignment with the direction of the muscle fibers and the distal tendon of each muscle with an inter-electrode distance of 20mm (according to standards provided by Seniam.org). The EMG electrodes were attached approximately parallel to pennation of muscle fibers half way between muscle insertion tendon and muscle belly to the vastus medialis and vastus lateralis. Electrode placement for the vastus medialis bisected the muscle anteroposteriorly, and was at a point distal from the motor point of the muscle half way to the insertion of the quadriceps tendon. The VL electrode location was centrally in a mediolateral fashion and distal from the midpoint of the belly to the tendinous junction. The MH electrodes were placed over the muscle belly half way between the ischial tuberosity and the tibial insertion point, at least 5 cm proximal to the musculotendinous junction. The LH electrodes were placed over the biceps femoris muscle halfway between the ischial tuberosity and the fibular insertion site, and a minimum of 5 cm proximal to the musculotendinous junction. A reference electrode for the EMG system was placed over the tibia. All electrodes were placed by a single experimenter to insure consistency thorough the study. Electrodes and telemetry amplifiers were secured to the skin using medical tape to minimize movement artifacts and to prevent the electrodes from losing surface contact due to sweating. Maximum voluntary contraction test were conducted for each subject. The MVC test for the vastus lateralis and vastus medialis muscles were performed while the subjects was in a sitting position with the knee flexed at 90 degrees. The MVC test for the biceps femoris and medial hamstring muscles were performed while the subject was in a prone position with the knee flexed at 30. During the MVC tests, the subject was instructed to perform three 5 second maximum voluntary isometric contractions for each selected muscle against the resistance of the same tester and was given verbal encouragement whilst doing so. The middle two seconds of the MVCs of each contraction were analyzed. A 3 min rest period was allocated between each contraction. Surface EMG was recorded using Biopac Inc., Goleta, CA. Acknowledge 4.3.1. The electromyography was recorded using a sampling rate of 2000 Hz through a 24 bit A/D converter. The raw data were processed using a band-pass filter (15-150 Hz). The EMG was integrated then divided by the maxmuim voluntary contraction (MVC) to normalize the EMG activity of every participant. Muscle activities were analyzed by the method described by Besier et al (Besier et al., 2003) , in the following conditions: (A) the preactivation phase: 50 ms before foot landing till foot landing; (B) the weight acceptance and (C) the peak push-off phase (Fig.1). The EMG activity of the selected group of muscles were synchronized with High Frame Rate Camera (CAM-HFR-A) SVHS Sony video camera (Basler, Biopac Systems, Inc., Goleta, CA) to capture the running phases as series of videos at 100 FPS (640*480 resolution). The camera was mounted on a tripod placed 2 m from the treadmill and aligned so the plane of the camera was parallel to the treadmill. The camera was leveled using the bubble level attached to the tripod and set to the height of the subject knee during running.

Fig 1. Phases of running during each EMG activity of the quadriceps and hamstring were analyzed.

Assessment of Knee Laxity

To quantify knee joint laxity, we utilized the KT-2000 (MEDmetric1 Corporation, San Diego, CA) instrumented knee arthrometer to measure anterior tibial translation (ATT) during the application of 133 N (30-Ib) anterior displacement force. Subjects are tested in the supine position in 30 degrees of knee flexion with 15 to 25 degrees of external rotation while the femur and tibia are supported by leg holders. The device was then placed on the anterior aspect of the leg and secured in place with circumferential straps. A strain gauge bridge arranged in a load cell was used to measure the force necessary to generate an anterior glide of the proximal end of the tibia on the femoral condyles. This generated a force versus displacement curve for the anterior cruciate ligament. The process was accomplished by supporting both limbs with a firm, comfortable platform placed proximal to the popliteal space to keep the subject’s knee flexion angle constant for the duration of the test (fig.2). Along with this device, a foot support accessory supplied with the ARTHROMETER® positioned the feet symmetrically allowing the leg position to be optimal for the test while reducing external rotation. For the most comfortable position during the flexion angle test, knee flexion angle was initially at 25 degrees and the only movement was the tibia in relation to the patella. A thigh strap controlled hip external rotation while offering support to help relax the subject. Force used for the experiment was applied at 30 lbs (133 N). The force displacement data were plotted on an *X–Y* plotter. The vernier caliper was used to measure anterior tibial translation (ATT) on the graph. The reliability of the KT 2000 has been has been established by, Van Lunen et al., reported an intraclass correlation coefficient of r = 0.92 (p = 0.001) (Van Lunen et al., 2003).

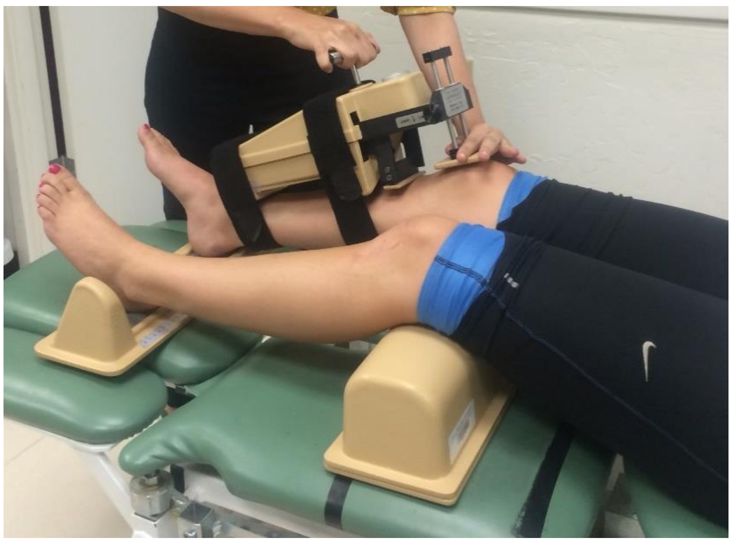

Fig 2. Experimental setup for measuring the ALC Laxity.

Procedures

Subjects were instructed to begin using an ovulation Predictor kit (Clearblue, Procter & Gamble, OH,USA) with 99% accuracy (Robinson et al., 2007) on day 13 to 15 of their menstrual cycle, and were asked to report to the research study coordinator the day the test became positive. The day of ovulation was confirmed to insure an ovulatory menstrual cycle had occurred; provide a common reference point by which to counterbalance participants and to mark the beginning and ending of data collection; and to provide indirect confirmation that female subjects were not pregnant. Hormone assays, neuromuscular testing, and knee joint testing were performed at Loma Linda University. Subjects were tested twice across one complete menstrual cycle, undergoing the same data collection procedures on each day of testing. Testing was performed in the morning (8:00 A.M.–12:00 P.M.) to obtain the most stable concentrations (Licinio et al., 1998) and to control for diurnal fluctuations in hormone levels. Within this window, every attempt was made to bring subjects in at the same time each day. However, some flexibility was needed to accommodate participant's class and work schedules given the daily data collection requirements. Subjects were counterbalanced to begin and end data collection either at ovulation (ovulation kit detecting the estrogen hormone surge), or the onset of menses (self-report of the first day of menstrual bleeding). Although each subjects was familiar with treadmill running, each had adequate time to become accustomed to treadmill running prior to the introduction of the experimental measurments. Subjects then were asked to complete a standardized 6 min running session on a Zebris FDM-T instrumented treadmill (zebris Medical GmbH, ISny Germany) with 0 inclination at 10Km/h with heel strike pattern. The treadmill had an embedded pressure mat containing more than 15,000 pressure sensors, from which data were integrated to produce the vertical ground reaction force to measure the ground reaction force. Once the runners demonstrated a stable running pattern, data were sampled at 200 Hz for 10 seconds. Lastly the ACL laxity was measured by the KT2000.

Data Analysis

A power analysis was conducted for expected outcomes with a type I error probability of 0.05 and a power of 0.8.This analysis indicated that n = 12 would provide a statistical power of ~80 % (G*Power v3.0.10 free software).The means and the standard deviations of the hormonal concentration, normalized EMG and laxness of the ACL were determined for each subject during each phase. Normality was confirmed for the data using Kolmogorov–Smirnov test. Paired T tests were conducted to compare the changes in mean estradiol serum concentration, ACL laxity and EMG activity at different phases of menstrual cycle. Paired T tests were conducted to determine whether the hormonal cycle affects knee joint mechanics and whether changes in knee joint laxity affect knee joint mechanics during running. Statistical analysis was performed using SPSS for windows version 20. The level of significance was set at alpha level $\alpha \leq 0.05$.

Results

The data of one subject was discarded from the analyses after examining her hormonal assays because of a significant deviation from the normal expected hormonal profile for enumenorrhic women, with hormonal profile irregularities of low estrogen level < 5Pg/ml (Wojtys et al., 1998).

Hormonal Profile

Descriptive data about the menstrual phases indicated typical values (Wojtys et al., 1998) including days between cycles (28.8±1.1) length of menstruation (6.3±1.2). Descriptive data for blood assay verified the menstrual cycle phases indicated that all subjects included in the statistical analysis were in the correct phase of the menstrual cycle. 17β estradiol serum concentration was significantly higher in the ovulatory compared with the follicular phase (P<0.001). The lowest estradiol concentration was found during menstruation (34.14 ± 15.47 pg/ml) and the highest estradiol concentration was found during ovulation (207.74 ± 53.42 pg/ml) Table 2 Fig.3a.

Table 2. Mean ± SD of 17β Estradiol serum concentration and ATT during early follicular phase and ovulatory phase.

	Early Follicular Phase Mean ± SD	**Ovulatory Phase Mean ± SD**	**P Value***
Anterior Tibial Translation (mm)	4.18 ± 0.27	5.75 ± 0.47	< 0.001[a]
17β Estradiol (pg/ml)	34.17 ± 15.47	207.74 ± 53.42	< 0.001[a]

Estradiol was typically low in the follicular phase (day1-3). Estradiol peaks during the ovulatory phase (day 13-15). All minimum and peak hormone concentrations were within normal ranges.

**Paired T test*

[a]Significant difference

SD Standard Deviation

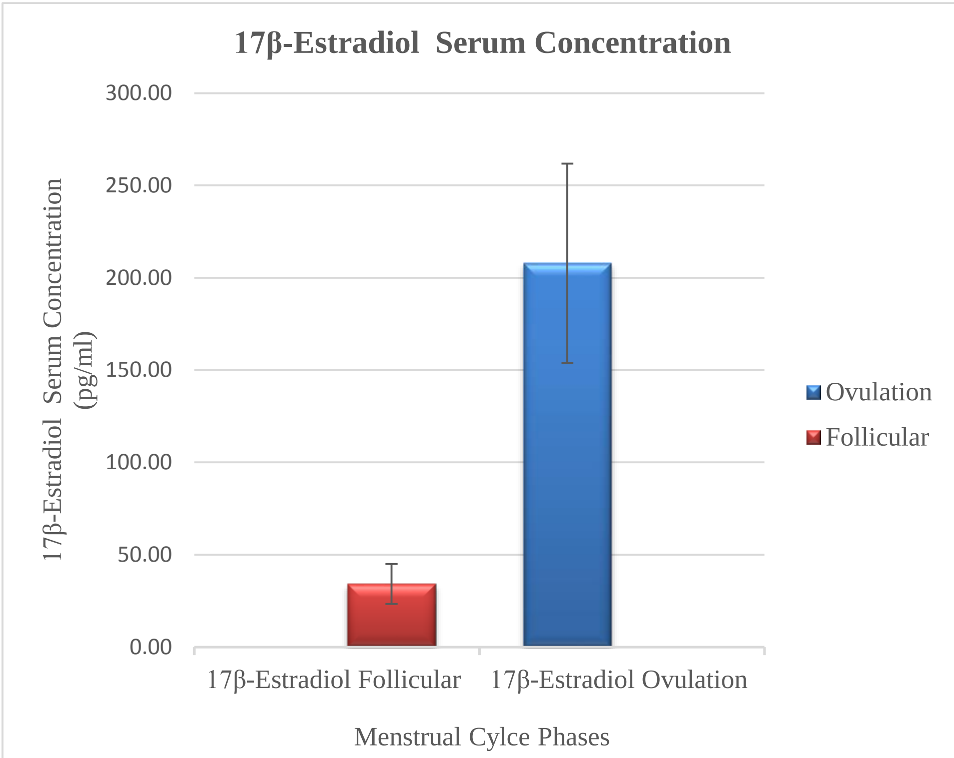

Fig 3a. Mean ± SD of 17β-Estradiol serum concentration during follicular and ovulatory phases

Anterior Cruciate Ligament Laxity

Laxity of the anterior knee ligament was measured by the anterior tibial translation (ATT). There was significant difference in the ATT between the follicular phases and the ovulatory phase of the menstrual cycle ($p<0.01$). The greatest ATT was found during ovulation (4.18 ± 0.27) and the least ATT was found (5.75 ± 0.47) during follicular phase (Table 2; Fig.3b)

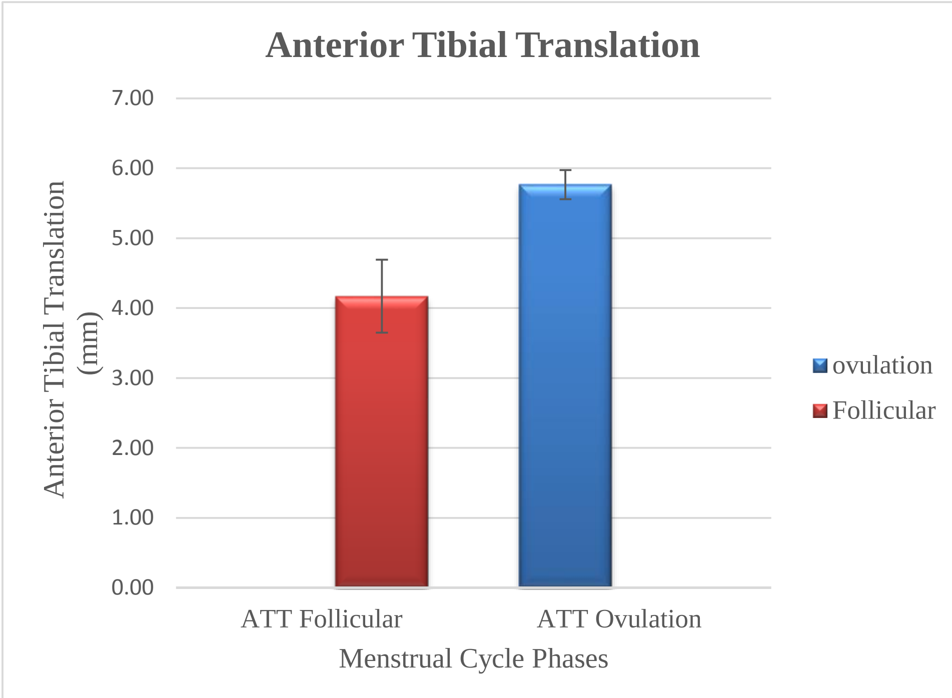

Fig 3b. Mean ± SD of Anterior tibial translation (ATT) during follicular and ovulatory phases

Neuromuscular Control variables

The results of this study demonstrate differences in muscle activation strategies during different phases of menstrual cycle. A summary of the activation values are presented in Table 3.

Quadriceps Muscle Activity

The quadriceps muscle exhibited increased activity during the early follicular phase compare to the ovulatory phase in the precontact and weight acceptance phase of running ($p = 0.02, 0.04$ respectively) Table 3, Fig 5B. The lateral and medial quadriceps were analyzed separately. For the lateral quadriceps, a significant increase was observed during the follicular phase compared with ovulatory phase ($p=0.014$) (Table 3; Fig. 5A). Remarkably, females subjects demonstrated a significant decrease in medial to lateral quadriceps ratios during follicular phase compared to ovulatory phase ($p < 0.001$) during weight acceptance phase (Table 3; Fig. 3). Fig. 4a shows typical data of increased IEMG activity of the VL and VM muscles of a single subject during the follicular phase and fig 4b showed the decreased activity of the two vasti during the ovulatory phase. As shown in the figure, the raw EMG muscle activity was greater in the follicular phase than the ovulatory phase. Below each raw EMG is the integrated EMG showing the same phenomena.

Hamstring Muscle Activity

The ovulatory phase altered the hamstring muscle preactivity before impact. The average peak hamstring activity during the precontact and weight acceptance phase was significantly increased during ovulation compared with the early follicular phase (Fig. 5B; Table 3). Specifically the medial hamstring showed increased activity before impact during g the ovulatory phase compare to the follicular phase ($p <0.001$) (Fig. 5A; Table 3). The increased activity of the hamstring was also observed during weigh acceptance with decreased EMG amplitude ($p < 0.001$). Moreover the quadriceps hamstring cocontraction was significantly higher compared to the follicular phase ($p<0.001$) (Fig. 5C; Table 3).

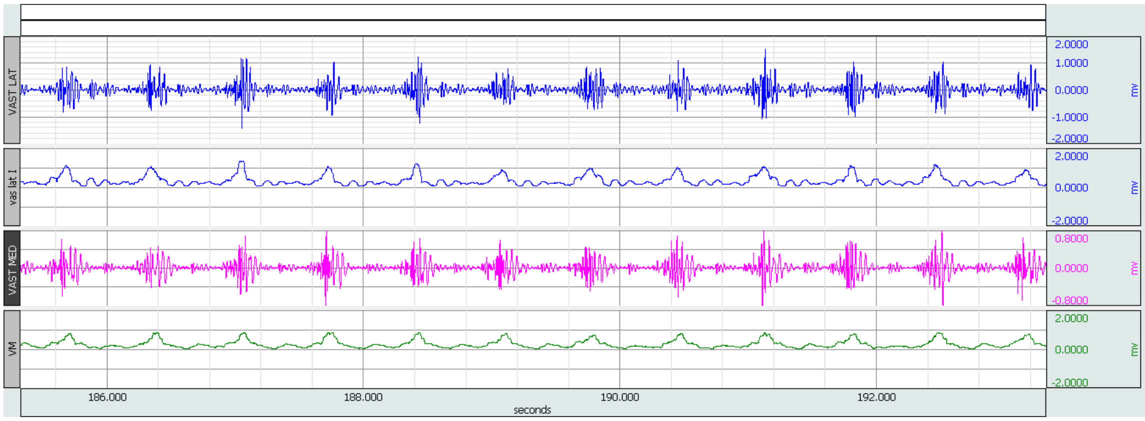

Figure 4 a. Raw EMG and IEMG activity of Quadriceps muscle during follicular phase

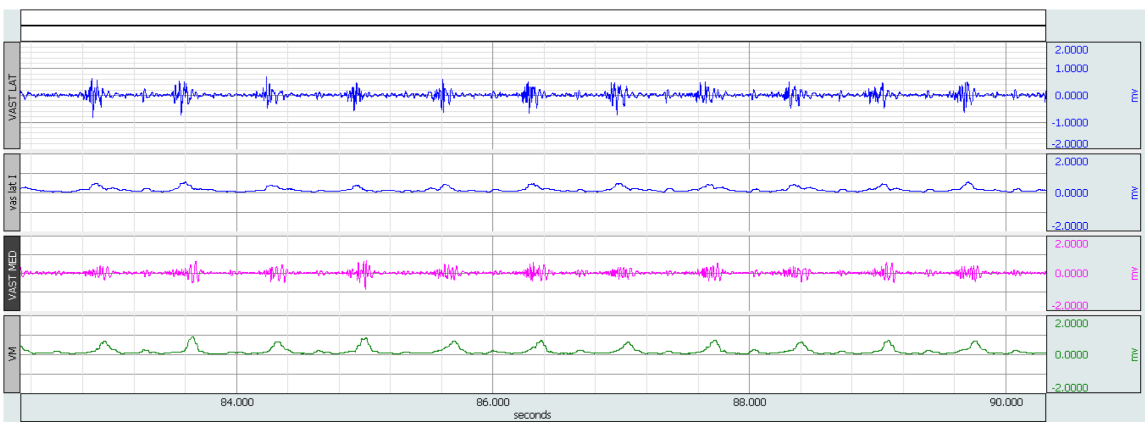

Fig 4b. Raw EMG and IEMG activity of Quadriceps muscle during ovulatory phase

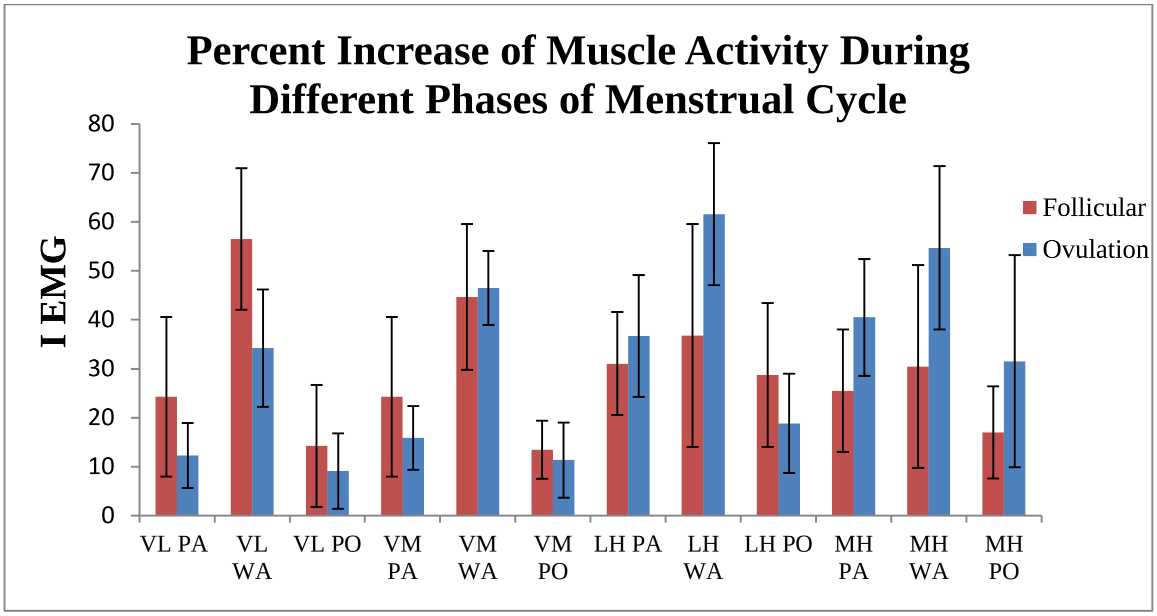

Fig5A. Mean (SD) of the Integrated EMG (IEMG) of the Vastus lateralis (VL), Vatsus medialis (VM), Lateral hamstring (LH), Medial hamstring (MH) during Preactivtaion (PA), weight acceptance (WA) and push off (PO) phase in the follicular phase compared to the ovulatory phase.

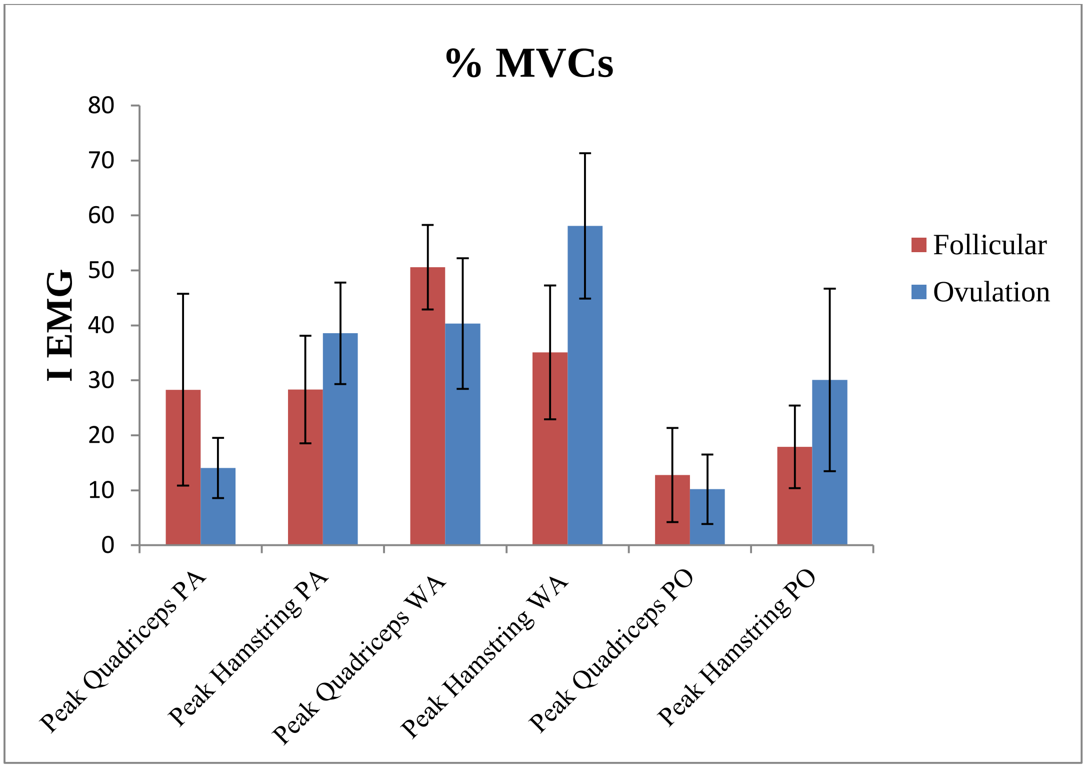

Fig 5B. Mean (SD) of the peak quadriceps and hamstring muscles IEMG activities during (PA), (WA), (PO) phases in both follicular and ovulatory phases.
IEMG integrated EMG
% MVCs percent of maximum voluntary contraction

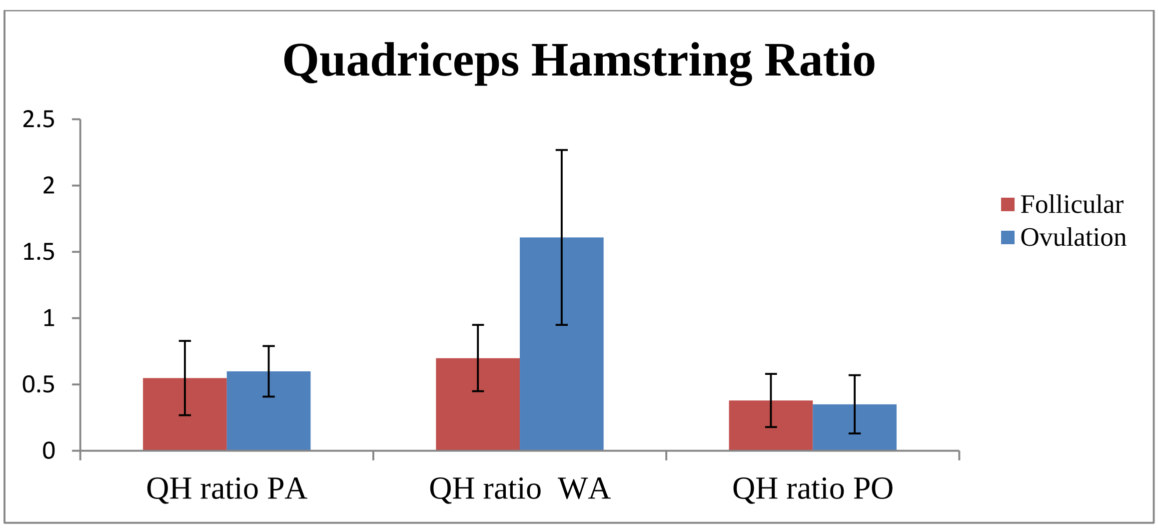

Fig5C. Mean (SD) of the quadriceps to hamstring ratio QH ratio during the follicular phase compared to the ovulatory phases in the PA, WA and PO phases of running.
PA preactivation, WA weight acceptance, PO push off

Table 3. Summary of neuromuscular control variables during different phases of menstrual cycle.

	Follicular Phase	Ovulatory Phase	P Value *
	Mean ± SD	Mean ± SD	
VL PA	24.26±16.27	12.25 ± 6.63	0.042[a]
VL WA	56.47 ± 14.43	34.19 ± 11.96	<0.001[a]
VL PPO	14.22± 12.44	9.07± 7.72	0.281
VM PA	24.26 ± 16.27	15.84 ± 6.50	0.131
VM WA	44.68 ± 14.89	46.51 ± 7.58	0.73
VM PPO	13.45 ± 5.93	11.33 ± 7.65	0.351
LH PA	31. ± 10.51	36.67 ± 12.43	0.035[a]
LH WA	36.76 ± 22.76	61.52 ± 14.52	0.014[a]
LH PO	28.65 ± 14.68	18.82 ± 10.17	0.104
MH PA	25.47 ± 12.50	40.45 ± 11.93	0.004[a]
MH WA	30.43 ± 20.70	54.68 ± 16.69	0.002[a]
MH PPO	16.96 ± 9.4	31.50 ± 21.67	0.101
Peak Quadriceps PA	28.30 ± 17.43	14.05 ± 5.46	0.024[a]
Peak Hamstring PA	28.32 ± 9.80	38.56 ± 9.25	<0.001[a]
Peak Hamstring WA	35.09 ± 12.16	58.10 ± 13.23	<0.001[a]
Peak Quadriceps WA	50.58 ± 7.68	40.35 ± 11.90	0.019 [a]
Peak Quadriceps PO	12.78 ± 8.56	10.20 ± 6.32	0.280
Peak Hamstring PO	17.89 ±7.52	30.07± 16.62	0.068
QH ratio PA	0.55 ± 0.28	0.60 ±0.19	0.667
QH ratio WA	0.70 ± 0.25	1.61 ± 0.66	<0.001[a]
QH ratio PO	0.38 ± 0.20	0.35 ± 0.22	0.712
ML Quadriceps Ratio PA	1.56 ± 0.75	1.51 ± 0.85	0.89
ML Hamstring Ratio PA	0.8 ± 0.36	1.2 ± 0.55	0.05[a]
ML Quadriceps Ratio WA	0.85 ± 0.31	1.40 ± 0.46	0.004[a]
ML Hamstring Ratio WA	0.60 ± 0.37	0.91 ± 0.25	0.008[a]

*****Paired T test
[a] significant difference
VL vastus lateralis VM vastus medialis LH lateral hamstring MH medial hamstring
PA Preactivation, WA weight acceptance ,PO Push Off
QH ratio Quadriceps hamstring ratio
ML medial to lateral ratio

Discussion

The physical disability and long rehabilitation process associated with anterior cruciate ligament (ACL) injury can be both psychologically and financially devastating to the individual, ultimately resulting in a decreased quality of life. Female athletes have a higher rate of ACL injury than do men, and many of these injuries require extensive surgical and rehabilitative interventions, with a financial burden to the American healthcare system estimated to approach $650 million annually (Zazulak et al., 2006). Bearing that in mind, it is imperative to understand the mechanisms leading to such an injury in an effort to prevent its occurrence and its subsequent sequelae. Although both men and women are susceptible, the literature states that women have a 4 to 6 fold increased incidence of ACL injury (Hewett et al., 2007, Hewett et al., 2006).

While the increased incidence of serious knee injuries in female athletes is well established, the underlying neuromuscular mechanisms related to the elevated ACL injury rate has yet to be delineated. Maintenance of joint congruency is important in prevention of injury. Both the ligamentous structures and the muscular system contribute (Hertel et al., 2006) .The role of the muscular system is particularly important when the static restraints are jeopardized and therefore not providing restraint to abnormal motion within the joint.

Our study supports the previous studies which have reported a greater knee laxity during ovulation when estrogen levels are high (Shultz et al., 2004a, Eiling et al., 2007). Conversely, women who experienced high plasma concentration of estrogen experienced a marked increase in joint laxity behavior following peak ovulatory levels. However other studies (Carcia et al., 2004, Van Lunen et al., 2003) found that knee ligament laxity doesn’t differ by menstrual cycle day. Interestingly to note that in these studies, that did not identify changes in knee laxity across select days of the menstrual cycle. The average estradiol levels were near the upper limits of normal ranges at menses (56 and 73 pg/mL) and considerably below the normal ranges postovulation (137 and 120 pg/mL) using similar hormone assay.

The results of our study reveal differences in muscle activation strategies during different phases of the menstrual cycle. Our results showed that women place greater reliance on their quadriceps during the follicular phase to modulate the torsional joint stiffness about the knee joint during running. The increased quadriceps activity observed during the follicular phase was associated with decreased hamstrings activity. We speculate that the observed differences in neuromuscular recruitment strategies may have implications for the greater incidence of non-contact ACL injures observed in women.

A consequence of differences in neuromuscular activation patterns might be injury susceptibility. Markolf & colleagues, found that muscle activation about the knee increased valgus stability threefold, highlighting the influence of the muscular system on knee stability (Markolf et al., 2003). Previous investigations of neuromuscular control (Cowling and Steele, 2001, Wojtys et al., 2003); have not considered muscle activation patterns during running. The present study discovered that the quadriceps and hamstring cocontraction ratios decreased during the early follicular phase compared to ovulatory phase. This suggests a different co-contraction (onset timing of agonist/antagonist around a joint) mechanism between these muscles. This alteration in neuromuscular control may explain the non-significant knee valgus variable since the quadriceps and hamstring work together to control torsional motions of the femur and tibia that may contribute to valgus alignment of the knee (McLean et al., 2005). This co-contractive mechanism suggests a different neuromuscular control pattern when estrogen levels are low; however, more investigation is necessary.

While we are unable to find another published study that evaluated neuromuscular activation patterns in healthy females runners with non-pathological knee elasticity, our findings are surprisingly consistent with those demonstrated in ACL deficient individuals. Alkjaer & colleagues, reported a marked increase in hamstring coactivation towards more extended joint positions in ACL deficient subjects (Alkjaer et al., 2012). Notably, this progressive rise in coactivation may reflect a compensatory strategy to provide stability to the knee joint in the anterior–posterior plane during knee extension. In agreement, our investigation showed that participants with increased knee joint laxity during ovulation demonstrated increased levels of muscle preactivity in the hamstring muscles before impact as well as during weight acceptance phase. Coactivation of the hamstring muscles during dynamic knee extension may compensate for increased knee joint laxity in anterior cruciate ligament. Increased coactivation of the hamstring muscles has been suggested to provide a compensatory strategy to reduce Anterior tibial translation (ATT) in functional conditions that include knee extension (More et al., 1993) (Yanagawa et al., 2002). Several studies have shown that the hamstring muscles are active during submaximal and maximal quadriceps agonist contraction (Aalbersberg et al., 2009) and that coactivation of the antagonist hamstring muscles during knee extension effectively reduces the amount of ATT (Yanagawa et al., 2002, Liu and Maitland, 2000).

The primary purpose of the current study was to investigate whether estradiol fluctuation during the menstrual cycle has an influence on the neuromuscular control around knee joint mechanics during running. Previous studies investigating the relationship between knee joint mechanics and the menstrual cycle found significant changes in biomechanical (Dedrick et al., 2008) or neuromuscular (Shultz et al., 2004a) characteristics corresponding to changes in hormonal levels during the menstrual cycle. The present study also found significant changes in the neuromuscular control around knee joint between the different phases of the menstrual cycle. As a result, ACL injury in female athletes may not be explained simply by the hormonal cycle but is likely influenced by a more complicated and indirect injury mechanism incorporating hormonal fluctuations and dynamic knee joint function that may be individual specific.

Although, we are currently unaware of any other study in the literature that investigated the neuromuscular control variables presented here in females' runners. Previous studies had evaluated gender differences in neuromuscular control. Our study showed that females' runners use a different neuromuscular strategy during different phases of menstrual cycle. Subjects demonstrated a decreased ratio of medial quadriceps to lateral quadriceps recruitment. A preactivation difference did exist for the lateral quadriceps between the follicular and ovulatory phase. The decreased ratio of the medial quadriceps musculature recruitment may be related to decreased control of coronal plane forces at the knee (Markolf et al., 1995).

In addition to low ratio of medial to lateral quadriceps recruitment combined with increased lateral hamstring firing may compress the lateral joint, open the medial joint and increase and increase shear force, which directly loads AC. This disproportional recruitment of the quadriceps musculature increases anterior shear force at the low knee flexion angles that occur during landing. The quadriceps, through the anterior pull of the patellar tendon on the tibia, contributes to ACL loading when knee flexion is less than 30 degrees (Markolf et al., 1995). Of interest is that our participants demonstrated increased activity of quadriceps muscles during the follicular phase compared to the ovulatory phase, which is thought to maximize axial compression, joint congruency and frictional forces to effectively limit joint displacement. Muscular co-contraction compresses the joint, due in part to the concavity of the medial tibial plateau, which may protect the ACL against anterior drawer. However, Zazulak & colleagues reported greater peak quadriceps activity in female than male subjects (Zazulak et al., 2005). Decreased balance in strength and recruitment of the flexor relative to the extensor musculature may put the ACL at greater risk (Hewett et al., 1996). Adequate cocontraction of the knee flexors is needed to balance contraction of the quadriceps, compress the joint, and control high knee extension and abduction torques (Hewett et al., 1996). Appropriate hamstrings recruitment may prevent the critical loading necessary to rupture the ACL during maneuvers that place the athlete at risk of an injury. Female subjects may display a longer latency period that is, electromechanical delay between preparatory and reactive muscle activation. Preparatory muscle activity can stiffen joints before unexpected perturbations. Neuromuscular training that reproduces loads similar to those encountered during competitive sports may assist in the development of both feed forward and reactive muscle activation strategies that protect the knee joint from excessive load (Winter and Brookes, 1991, Lebrun, 1994, Wojtys and Huston, 1994) .

Our findings support the previous studies which have reported a decreased neuromuscular response and/or control around the time of menstruation (Friden et al., 2006, Shultz et al., 2004a). Despite these observations, our findings of decreased neuromuscular control around the knee which may be a potential mechanism for increased risk of injury at this stage of the cycle, there is no consensus as to whether injury risk is also elevated during menstruation (Hewett et al., 2007). These conflicting results could be due to the difficulty in performing a prospective study to assess injury risk, with the majority of protocols consisting of retrospective assessment (Hewett et al., 2007). These results can also be confounded by participation levels during each phase of the cycle, because women who do not take the oral contraceptive pills are significantly less inclined to participate in physical activity during menstruation (Adachi et al., 2008). Taken together, these results suggest that during menstruation the performance of the neuromuscular system is compromised, which may limit both participation and intensity of activity in sporting events and therefor counterbalance the increased risk of injury due to an impaired motor control strategy.

Decreased neuromuscular control of the lower extremity during menstruation may increase the potential for valgus lower extremity position and increased ACL injury risk. Identification of these neuromuscular imbalances has potential for both screening of high risk athletes and targeting interventions to specific deficits. Dynamic neuromuscular training can increase active knee stabilization and decrease the incidence of ACL injury in the female athletic population (Myklebust et al., 2003, Myer et al., 2005b). Training may facilitate neuromuscular adaptations that provide increased joint stabilization and muscular preactivation and reactive patterns that protect the female athlete’s ACL from increased loading. Neuromuscular training will help female athletes adopt muscular recruitment strategies that decrease joint motion and protect the athletes ACL from the high impulse loading while also improving their measures of performance. More investigation is necessary to determine if the neuromuscular control changes occur due to alterations in force transmission properties of passive tissues, levels of feedback from ligamentous and dynamic tissues, levels of feedback from ligamentous and dynamic tissues or centrally driven feed forward mechanism.

Limitations

Utilization of a relatively simple task (running) may not adequately stress the neuromuscular system at the level of athletic population, thus limiting the generality of the results to athletes. Also, the current protocol didn’t measure the muscle activation at high speeds which may limit the applicability of our findings to conditions of higher speed joint loading. In addition, we examined only the quadriceps and hamstring and we didn’t measure other lower extremity musculature that may influence knee joint mechanics. Finally shoe wear was not measured and may have influenced knee kinematics.

Conclusions

This study is the first to examine the influence of the menstrual cycle on knee joint laxity and neuromuscular control around the knee during constant velocity running. Our results suggested that decreased knee joint laxity during the menstrual cycle leads to decreased neuromuscular control during running. A consistent pattern was observed in the firing of the quadriceps muscle recruitment pattern throughout the menstrual cycles associated with unbalanced hamstring recruitment. In addition to low ratio of medial to lateral quadriceps recruitment combined with increased lateral hamstring firing which may compress the lateral joint, open the medial joint and increase and increase shear force, which directly loads ACL. This disproportional recruitment of the vastus musculature increases anterior shear force at the low knee flexion angles which may increase the potential for valgus lower extremity position and possibly increased risk of ACL injury.

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6 WEEKS HABITUATION OF SIMULATED BAREFOOT RUNNING INDUCES NEUROMUSCULAR ADAPTATIONS AND CHANGES IN FOOT-STRIKE PATTERNS IN FEMALE RUNNERS

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Abbreviations

AP	Active Peak
AVLR	Average Vertical Loading Rate
BW	Body Weight
BW/S	Body Weight per sec
CNS	Central nervous system
EMG	Electromyography
FFS	Forefoot Strike
GAS	Gastrocnemius
GRF	Ground Reaction Force
IEMG	Integrated Electromyography
IP	Impact Peak
IVLR	Instantenous Vertical Loading Rate
MFS	Mid-foot strike
MVC	Maximum Isometric Voluntary Contraction
RFS	Rear Foot Strike
SBR	Simulated Barefoot Running
VIP	Impact Peak
TA	Tibialis Anterior

Abstract

Purpose: To investigate the effects of 6 week transition program of simulated barefoot running (SBR) on running kinetics in habitually shod female recreational runners.

Methods: 12 female runners age 25.7± 3 yrs. without SBR experience gradually increased running distance in Vibram Five fingers over 6 weeks. Kinetic analysis of treadmill running at 10Km/h was performed pre and post intervention in shod running, non-habituated SBR and habituated SBR conditions. Spatiotemporal parameters, ground reaction force components and Electromyography were measured in all conditions.

Results: Post intervention data indicated a significant decrease across time in the habituation SBR for EMG activity of the TA in the preactivation and absorptive phase of running ($P<0.001$). A significant increase was denoted in the preactivation amplitude of the GAS between the shod running, unhabituated SBR and Habituated SBR. A 6 weeks of SBR was associated with a significant decrease in the loading rates and impact forces. Additionally, SBR significantly decrease the stride length, step duration, flight time whilst stride frequency was significantly higher compared to shod running.

Conclusion: The findings of this study indicate that changes in motor patterns in previously habitually shod runners are possible and can be accomplished within 6 weeks. Non habituation SBR didn't show a significant neuromuscular adaptation in the EMG activity of TA & GAS as manifested after 6 weeks of habituated SBR. Differences in spatiotemporal variables occurred within a single running session, irrespectable of habituation period.

Keywords: SBR, EMG, GRF, shod running, foot strike pattern.

Introduction

Humans were born to run (Bramble and Lieberman, 2004). Bramble and Lieberman have suggested that our body structure was significantly influenced by the fact that we needed to run for survival (Bramble and Lieberman, 2004). A growing contingency believes that we were designed with all we need in our feet to be able to run sans shoes or with minimal shoes that mimic barefoot running striking pattern. In fact, there has been a suggestion that running without the assistance of modern running shoes might lead to a reduction in the incidence of running injuries (Lieberman et al., 2010).

In recent years there has been resurgence in barefoot running as well as running in light, minimalistic shoes (Rothschild, 2012). Research into the foot-strike patterns and lower limb kinematics of barefoot and shod populations has also proliferated (De Wit et al., 2000, Lieberman et al., 2010, Perl et al., 2012, Squadrone and Gallozzi, 2009). Habitual barefoot runners such as adolescents in Kenya's Rift Valley tend to fore-foot or mid-foot strike when barefoot, compared to habitually shod populations who tend to rear-foot strike (Lieberman et al., 2010). Reduced collision forces generated with fore-foot (FFS) or mid-foot strike (MFS) patterns relative to a rear-foot strike (RFS) may account for anecdotal reports or reduced injuries in barefoot populations (Robbins and Hanna, 1987).

Runners who adopt a FFS or MFS pattern while shod also have reported improved performances (Hasegawa et al., 2007) and reduced injuries (Daoud et al., 2012, Diebal et al., 2012) (Goss and Gross, 2012) , spurring industry and researchers to examine foot strike patterns (FSP) more closely and to question the design of standard modern cushioned running shoes, which encourage RFS.

Running kinematics and ground reaction forces during simulating barefoot running (SBR) are reportedly similar to the barefoot condition of habitual barefoot runners (Squadrone and Gallozzi, 2009, Lohman et al., 2011). There are several studies of kinematic and FSP differences between barefoot and various shod conditions but findings vary according to the population under investigation. At velocities typical of endurance running (3.33–4.5 m/s − 1), habitually shod runners tend to land with a dorsiflexed ankle and RFS pattern, both when running shod and (to a lesser degree) when barefoot (Bonacci et al., 2009, Bonacci et al., 2013) .

In contrast, at similar velocities, habitual barefoot runners reported FFS or MFS patterns, with a more plantarflexed ankle at foot strike, than habitually shod populations, both when assessed barefoot and shod (Perl et al., 2012). Therefore, motor patterns laid down over years of training will influence FSP and running kinematics more than the shoe, or lack of, worn on a specific testing occasion. If a FFS/MFS pattern is sought by an athlete, simply changing footwear may not be sufficient to alter these patterns. Despite this, most studies have evaluated the acute effects of shod and barefoot running on kinematics, kinetics, spatiotemporal variables or oxygen cost of running, with minimal or no opportunity beforehand for the participant to habituate to any alternative footwear condition (Bonacci et al., 2013, Braunstein et al., 2010, Hasegawa et al., 2007). This is understandable, as the amount of time required to habituate to another foot strike pattern is not established, and transition to barefoot, SBR or FFS running could, by itself, involve risk of injury (Goss and Gross, 2012, Ridge et al., 2013).

A major limitation of all those prior works was that the subject's recruited were not particularly experience in barefoot running. Runners not accustomed to running barefoot could have their natural foot structure weakened by long term foot wear use and their proprioceptive sensitivity reduced (Divert et al., 2005b). So they could be less effective in adopting their running style when running in this condition. Therefore, the subjects of our study were trained regularly for six weeks for a safe transition of simulated barefoot running.

Neuromuscular control during running is influenced by landing pattern and type of shoes (Wakeling et al., 2001, Nigg et al., 1999). In RFS running pattern, tibialis anterior muscle is considered to be the one muscle of specific interest. Around the time of heel-strike, the tibialis anterior muscle has two major functions: it positions the foot in dorsiflexion before heel-strike and it reduces the plantar-flexion moment of the foot due to the heel-strike (Inman, 1969, Winter and Yack, 1987). These two functions are quite different in their demands. The positioning of the foot is a slow event whereas the reduction of the foot slapping movement is associated with fast forces and movements. Thus, one can hypothesize that the tibialis anterior muscle activity should be significantly different in the pre- and post-heel-strike phase and altered when running barefoot or with shoes. One should expect that wearing shoes requires (compared to the barefoot situation) higher muscle activity before heel-strike to prevent the forefoot from touching the ground too early. Additionally, sport shoes are known to modify the force development during the impact (Gruber et al., 1998, Nigg et al., 1999). The cushioning effect of the shoe is therefore expected to alter the post-heel-strike muscle activity as measured by the electromyographic signal.

Ground Reaction Force (GRF) is an important factor in the study of the kinetics of the lower-extremities during running. Muscle activity in the leg is tuned in response to ground reaction forces. During running, the human body reacts to input from its external environment. One such input is the ground reaction force, which occurs during the ground contact phase of each stride (Wakeling et al., 2001). One possible reaction is the modification of the muscle activity patterns in response to that force (Nigg and Wakeling, 2001). It has been speculated that there is a requirement for the muscles to control and, thus, minimize soft tissue vibrations during locomotion (Nigg and Wakeling, 2001) and, thus, that there will be a change in muscle activity patterns in response to different vibration loadings on the lower extremity.

Impact forces in heel-toe running are forces resulting from the collision of the heel with the ground, reaching their maximum (the impact peak) earlier than 50 ms after first contact (Nigg and Wakeling, 2001). The rate at which the impact peak is reached is termed the loading rate and is a correlate of the major frequency of the impact peak. Impact forces have frequency contents in the range 10–20 Hz and should be expected to produce vibrations of the soft tissues of the body. Changes in the myoelectric patterns of the lower extremities of the muscle activities has been shown to respond to frequencies of applied continuous vibrations of different impact forces.

A thorough search of the current scientific literature revealed that there is no published research investigating differences in habituated and non-habituated subjects, as most studies have used initial responses or habitually barefoot runners for their investigations (Lieberman et al., 2010, Lohman et al., 2011, Squadrone and Gallozzi, 2009). Although, it has been reported that habitually barefoot runners run differently from habitually shod runners (Bonacci et al., 2013, De Wit et al., 2000, Lieberman et al., 2010, Squadrone and Gallozzi, 2009). Still, it remains unclear as to whether adults who have grown up running in shoes will run with “barefoot” kinetics following a habituation period, or how long that habituation period should be.

Previous studies attempting to influence running motor patterns or kinematics through plyometric, strength or neuromuscular interventions have typically been 6–9 weeks in duration (Bonacci et al., 2009, Diebal et al., 2012, Snyder et al., 2009). A 6-week program was chosen for this study to allow initial adaptation of musculoskeletal structures to new forces, with the purpose of reducing the risk from too rapid a transition (Goss and Gross, 2012, Ridge et al., 2013, Salzler et al., 2012). Thereafter higher SBR training loads could be gradually introduced to elicit a training effect. Thus, the current study investigated the effects of 6 week transition program of SBR on the stance-phase kinetics in habitually shod adult female recreational runners when compared with the same group in a non-habituated state and as such investigate the acute and the chronic changes of this group.

Material and Methods

Subjects

A total of 12 female runners mean (±SD) age was 25.7 ± 3.4 years; height was 162.2 ± 7.7 cm; body weight was 59.4 ± 6.9 Kg, and body mass index was 22.5 ± 1.2 Kg/m² volunteered to participate in the study. All subjects were running in standard cushioned shoes prior to study enrolment, which included neutral, stability, and anti-pronation type models. All subjects were running 3-5 days per week, average 25 km per week for at least the previous 6 weeks, with the intention of continuing at a similar intensity for the following 6 weeks. Participants were excluded if they had any lower limb injuries that had prevented them from running in the last 6 months; or following a lower limb rehabilitation program; or had ever ran in minimalist or SBR footwear. The study was approved by the ethical Committee of Loma Linda University. Prior written consent was obtained from all subjects.

Electromyography

Electromyographic (EMG) activity was measured from the tibialis anterior (TA) and lateral gatstroceniums (GAS). These muscles were selected for their synergistic action. Prior to electrode placement, the skin was lightly abraded, and cleaned with alcohol. Circular pre-gelled 10 mm bipolar Ag–AgCl surface electrodes (EL503; Biopac Systems, Inc., Goleta, CA) were placed in parallel on the belly of each muscle in alignment with the direction of the muscle fibers and the distal tendon of each muscle with an inter-electrode distance of 20 mm (according to standards provided by Seniam.org). For the TA muscle, the electrodes were placed on the upper third of the muscle about 15 cm below the center of the kneecap (von Tscharner et al., 2003b). The GAS electrode location was centrally placed in a lateral fashion distal from the midpoint of the belly to the tendinous junction. A reference electrode for the EMG system was placed over the tibia. All electrodes were placed by a single experimenter to insure consistency thorough the study. Electrodes and telemetry amplifiers were secured to the skin using medical tape to minimize movement artifacts and to prevent the electrodes from losing surface contact due to sweating. Maximum voluntary contraction test (MVC) was conducted for each subject. The MVC tests for the TA and LG muscles were performed while the subjects were in a sitting position with the knee flexed at 90 degrees. The subject was instructed to perform three 5 second maximum voluntary isometric contractions for each selected muscle against the resistance of the same tester and was given verbal encouragement whilst doing so. The middle two seconds of the MVCs of each contraction were analyzed. A 3 min rest period was allocated between each contraction. Surface EMG was recorded using Biopac Inc., Goleta, CA. Acknowledge 4.3.1. The electromyography was recorded using a sampling rate of 2000 Hz through a 24 bit A/D converter. The raw data were processed using a band-pass filter (15-150 Hz). The EMG was integrated then divided by the maximum voluntary contraction (MVC) to normalize the EMG activity of every participant. Muscle activity was analyzed by the by the method described by shih et al. (Shih et al., 2013), in the following conditions: (A) the preactivation phase: 50 ms before foot landing till foot landing; (B) the impact phase and (C) the peak push-off phase (Fig.1a & Fig.2b). The EMG activity of the selected group of muscles were synchronized with High Frame Rate Camera (CAM-HFR-A) SVHS Sony video camera (Basler, Biopac Systems, Inc., Goleta, CA) to capture the running phases as series of videos at 100 FPS (640*480 resolution). The camera was mounted on a tripod placed 2 m from the treadmill and aligned so the plane of the camera was parallel to the treadmill. The camera was leveled using the bubble level attached to the tripod and set to the height of the subject knee during running.

Preactivation Phase | Impact Phase | Push Off Phase

Figure 1a. Phases of running during shod running with RFS pattern.

Preactivation Phase | Impact Phase | Push Off Phase

Figure 1b. Phases of running during simulated barefoot running with FFS pattern.

Ground Reaction Force

Runners ran on an instrumented treadmill (Zebris FDM; Zebris Medical GmbH, Allgäu, Germany) at 10 km/h. The treadmill had an embedded pressure mat containing more than 15, 000 pressure sensors, from which data were integrated to produce the vertical ground reaction force. Once the runners demonstrated a stable running pattern, data were sampled at 100 Hz for 60 seconds. The variables of interest: vertical impact peak (IP), active peak (AP), vertical instantaneous loading rate (VILR), vertical average loading rate (VALR), were extracted from the processed data and were obtained by the method described by Crowell and Davis (Crowell and Davis, 2011). These early impact variables were chosen for their demonstrated association with various running injuries (Willson and Davis, 2009, Davis and Powers, 2010, Hunt et al., 2010). The IP was the local maximum between foot strike and maximum force on the vertical ground reaction force curve. It usually occurred within the first 50 ms of stance phase (Fig. 2). The VILR was the maximum slope of the vertical ground reaction force curve between successive data points in the region from 20% of the VIP to 80% of the VIP (Fig. 2). This was the most linear portion of the curve in the early part of stance. The VALR was the slope of the line through the 20% point and the 80% point. Therefore, all variables were associated with the impact phase of running. The data were processed and averaged for each subject. All stance phases were extracted from each data and transferred to Matlab for processing using a custom-written MATLAB program (V8.3 R2014a, Math Works, Inc., Natick, MA, USA) customized programs. Temporal information for heel-toe latency was used to compute the gait attributes (e.g., IP, AP, VALR, and VILR.) for each stance phases and finally the averages were computed for the data analysis. To visualize, the GRF data were normalized to 0-100% of the stance phase (Fig.2).

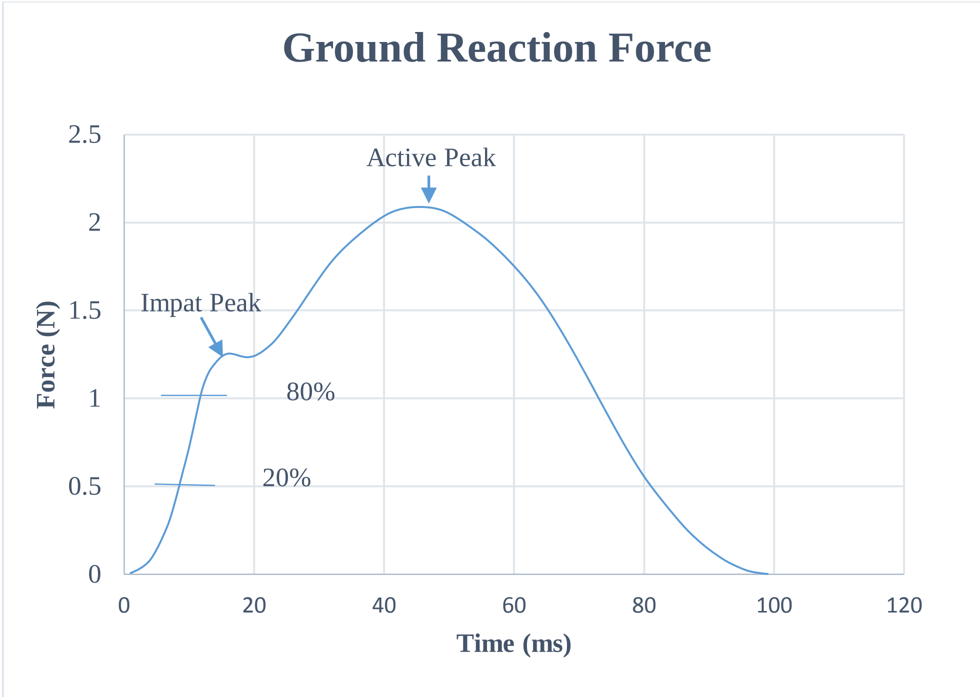

Fig 2. Ground reaction force curve showing the variables of interest: IP, AP, VILR and VALR. Note that both vertical loading rates (VILR and VALR) were calculated in the region between 20% to 80% before the impact peak.

Procedures

Subjects were assessed pre- and post-intervention running at (10 km/h) on a conventional instrumented treadmill (Zebris FDM; Zebris Medical GmbH, Allgäu, Germany) in both barefoot and shod conditions. This velocity was chosen in order to be representative of a comfortable running pace for a recreational running population, and to allow comparison with the findings of other authors who previously assessed barefoot and shod running kinematics at similar velocities (De Wit et al., 2000, Perl et al., 2012, Squadrone and Gallozzi, 2009). All subjects came to our laboratory for three identical testing sessions separated with by the 6 weeks habituation period in addition to the training sessions. Test conditions were identical on all occasions and took place indoors in a temperature-controlled room with artificial lighting. Participants avoided strenuous exercise in the 24 h pre-test and warmed-up according to their usual routines. All participants wore standard, neutral cushioned shoes for the shod trial. Following placement of EMG electrodes in each condition, participants ran at a self-selected velocity for at least 4 min to become comfortable running on a treadmill. After 4 min, treadmill velocity was increased to 10 Km/h before a data collection epoch (duration 60-s). Data was collected for 60 s at the 5th minute of running, allowing enough time above the 4 min that has been suggested to be required to optimize leg stiffness and running technique depending on surface and shoe hardness(Divert et al., 2005a). Given that endurance running involves repetitive impacts, a long sample period of 60 s was selected to more adequately represent average loading over a longer period of time. Stride frequency was calculated by the number of steps that occurred on the right foot during the 60 s. The entire testing protocol was repeated again after a single training session in non-habituated SBR condition and following the 6-week habituation period of SBR (posttests). During the posttests, subjects were reminded before testing commenced to concentrate on running technique, but were given no feedback while running in order to maintain technical consistency. All participants expressed comfort with treadmill running with EMG electrodes attached before data acquisition and were not aware of when kinematic data was being captured.

Interventions

The intervention is this study was instruction and training to adopt a forefoot strike running technique. Familiarization took place in Vibram "Five Finger" Bikila LS (VFF; Vibram®, MA, USA) footwear. Immediately after pretests, each subject was provided with a structured progression of SBR over 6-week habituation period and relevant injury prevention exercises. Running technique guidelines were also provided based on current findings in the literature (Table 1). Both the technique changes and exercises were fully demonstrated (Table 2). The program incorporated SBR running into the subject's normal training routines (increasing from ∼10% to ∼25%)(Warne and Warrington, 2014), where it was required that the SBR running took place at the beginning of any training session, and then subjects were allowed to continue their normal training load in their own preferred conventional running footwear. Thus subjects would gradually increase exposure to SBR during this period, while also maintaining the remainder of their training schedule in conventional running shoes. Each subject was provided with detailed guidelines including a structured progression of SBR over the 6-week habituation period (Table 1 & 2). The program include visual feedback and instruction on technique, simply asked subjects to run in the simulated barefoot condition at a comfortable velocity and to include specific training drills and exercises designed to teach forefoot striking consisted of weight shifting, falling forward, foot tapping, and high hopping, are described previously (Diebal et al., 2012, Romanov and Fletcher, 2007). Additional emphasis included using the hamstrings muscle group to pull the foot from the ground versus push the foot off the ground using the gastrocnemius and soleus muscles (Romanov and Fletcher, 2007). The subjects also practiced running barefoot and were provided with verbal cueing to ‘‘run quietly’’ to eliminate the tendency to heel strike upon ground contact. A video camera was used to record individual running form to demonstrate forefoot technique running errors (ie, heel striking, over striding). Exercise instruction was conducted 3 times per week for approximately 25 minutes each session for the first week. A typical training session during the first week consisted of approximately 15 to 20 minutes of the specific training drills followed by forefoot running practice for distances of 0.25 km. The verbal cueing, and video camera were used during the running practice time. The rationale for adopting this approach was to prepare the lower extremity for safe transition for a forefootfoot stike pattern because most of the typical deficits encountered were weak calf, reduced subtatlar joint dorsiflexion and inhibition/ weakness of foot intrinsic muscles.

Table 1. Running Technique Guidelines.

Running Technique Guidelines
Keep stride short and increase cadence (Divert et al., 2005b, Lieberman et al., 2010)
Land as light and as quiet as possible (Crowell and Davis, 2011)
Land on the forefoot, allowing heel to contact immediately afterwards (Squadrone and Gallozzi, 2011)
Keep hips forward and head up (Lieberman et al., 2010)

Table 2. Exercise program.

Exercise program	
Running form drills	Forefoot Striking
	Increase cadence
	Shorter step length
Proprioceptive Exercise	Single leg stance
Flexibility Exercise	Calf Stretching against wall
	Calf stretching off the edge of a step
Strengthening Exercises	Foot intrinsic
	Doming and hoping drill
	Toe grabs
	Single leg raises (calf raises)
Polymeric activities	Hops (single leg forward hops)
	Squat jumps

Data Analysis

Descriptive statistics for variables and measures of central tendency for continuous variables were calculated to summarize the data. A kolmogrovo-Smirnov normality test proved all variables to be normally distributed. Repeated measures ANOVA was used to evaluate the primary outcome variables of the EMG activities. Post hoc Bonferoni test was used to analyze differences between pre intervention, non-habituated SBR and after 6 weeks habituation SBR. A paired T test was used to evaluate the difference in the biomechanical variables (stride length, stride frequency, vertical GRF, and rates of loading) between the shod and habituated simulated barefoot running conditions. The level of significance was set at P level of .05. All statistical analyses were performed using SPSS statistics software (Version 20).

Results

Spatiotemporal Variables

A significant difference of the spatiotemporal variables (stride length, stride frequency, step time, contact time and flight time) was detected between shod and habituated SBR conditions. The stride length was significantly lower during SBR compared to shod condition (1.59 ± 0.21 vs 1.76 ± 0.19 m, $P < 0.001$). The stride frequency in habituated SBR was significantly greater than shod running (2.80 ± 0.10 vs 2.69 ± 0.13 steps/sec , $P < 0.001$). As a consequence, step time was significantly decreased when running barefoot.

Ground Reaction Force

A comparison of the pre intervention and post intervention results revealed that both VALR and VILR was significantly reduced during habituated SBR running compared to shod running. The rate of loading is calculated from 20 to 80 % of the impact transient (when present) or to (3 to 12%) of stance phase when impact transient is absent (Milner et al., 2006). The average vertical loading rate for habituated SBR runners was (24.27±4.09) body weights per second which was significantly lower than that of shod runners (38.33±5.01, P< 0.001) Table 3; Fig 3. Magnitude of impact force was significantly lower during SBR running compared to shod running. The impact force was (0.60±0.14) body weight in barefoot runners which was significantly lower than shod runners (1.39±0.47, P < 0.001) body weights (Table 3). Figure 4 shows a typical curve of the vertical GRF for one subject during different pattern of running.

Table 3. Means and standard deviation of the spatiotemporal and kinetic variables.

	Shod Running Mean ± SD	Habituated SBR Mean ± SD	P Value *
Vertical Average Loading rate (BW/S)	38.33 ± 5.01	24.27 ± 4.09	< 0.001[a]
Vertical Instantaneous Loading Rate (BW/S)	61.20 ± 9.46	40.11 ± 7.43	< 0.001[a]
Impact Peak (BW)	1.39 ±0.47	0.60 ±0.14	< 0.001[a]
Active Peak (BW)	1.94 ± 0.18	1.98 ± 0.20	0.452
Stride length (m)	1.76±0.19	1.59 ±0.21	<0.001[a]
Stride Frequency (steps/sec)	2.69 ±0.13	2.80±0.10	<0.001[a]
Step Time (s)	0.38± 0.02	0.35± 0.02	0.053[a]
Contact Time (%)	56.14 ± 4.33	40.85 ±5.40	0.021[a]
Flight Time (%)	43.77 ±4.30	59.05 ±5.26	0.042[a]

* Paired T test ;[a] Significant Difference, (AVLR) Average vertical loading rate, (VILR) Vertical instantaneous loading rate, (BW) body weight, (BW/S) body weight per second, second (s)

Fig 3. Average vertical loading rate (AVLR) and Instatenous vertical loading rate (IVLR) during pre-intervention shod running and post intervention 6 weeks of simulated barefoot running (SBR).

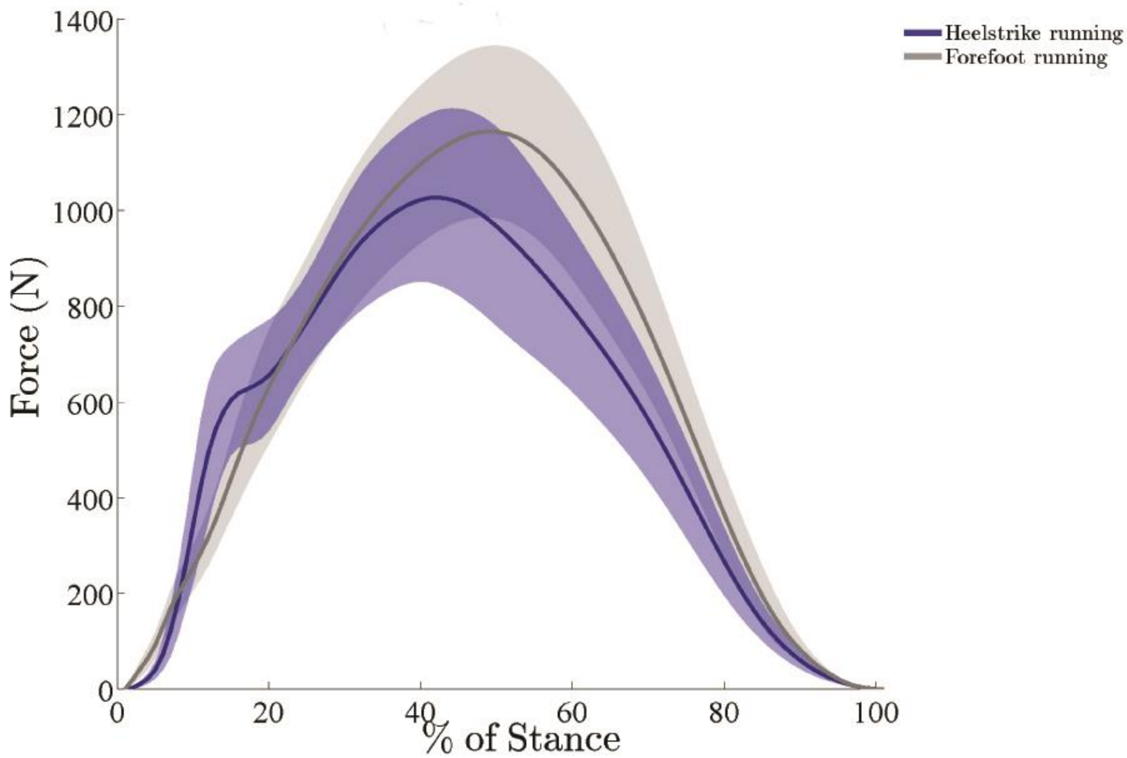

Fig 4. Vertical ground reaction force amplitude variation over time for one representative subject in both conditions of running.

Electromyography

EMG amplitudes of tibialis anterior and gastrocnemius during the preactivation phase showed a significant difference between foot striking pattern in both shod running and habituated SBR (Table 4; Fig. 5). Amplitudes of the GAS in the preactivation and stance phases showed significant higher activity in habituated SBR compared to shod condition (60.02 ± 11.32 vs 18.63 ± 4.70) respectively. No statistical difference was observed concerning the TA muscles for the preactivation amplitudes between the shod condition and non-habituated SBR. However, a significant difference was detected in the preactivation amplitude of the GAS between the shod condition and non-habituated SBR.

Moreover, concerning the stance and push off phases amplitudes, no significant statistical difference between the two conditions of running was reported for both group of muscles (Table 4; Fig 5).

Table 4. Means and standard deviations of the IEMG of TA and GAS.

	Shod Running Mean ± SD	Non Habituated SBR Mean ± SD	Habituated SBR Mean ± SD
Preactivation TA	55.37±10.25 [a]	54.14 ±7.69	15.52 ± 3.77
Preactivation GAS	18.63 ± 4.70 [a]	26.05 ± 8.89 *	60.02 ±11.32
Stance phase TA	27.15 ±10.22 [a]	13.36 ± 4.97	18.68 ± 6.26
Stance phase GAS	46.40 ±16.07 [a]	42.25 ±7.98	77.42 ± 8.57
Push off of TA	13.12 ±6.25	11.35 ± 5.27	11.80 ± 5.66
Push off of GAS	68.31 ± 13.76 [a]	64.37 ± 8.95	53.92 ± 4.51

TA tibialis anterior, GAS gastrocenimus, SBR simulated barefoot running

[a] Significant different from habituated SBR; $P< 0.05$. * Significant different from non-habituated SBR; $P<0.05$.

SD standard deviation

Fig 5. Integrated EMG of the tibialis anterior (TA) and gastrocnemius (GAS) muscles during different phases of running during shod running, non-habituated simulated barefoot running (SBR) and habituated simulated barefoot running.

Discussion

To the best of our knowledge, until now few authors studied barefoot running but none of them, reported a biomechanical and muscular analysis of the differences between shod running and barefoot running after habituation. This is the first study to investigate the effect of 6 weeks habituation of SBR training on running kinetics. The results of the current study highlight that 6-weeks intervention of controlled SBR training was sufficient to elicit significant changes in lower limb kinetics during barefoot running.

The neuromuscular system has the capability to adapt to training, not unlike the cardiorespiratory system. Studies over the past two decades have provided strong evidence that continued practice of a task (i.e. training) facilitates neuromuscular adaptations, which are characterized by more skilled control of movement and muscle recruitment patterns (Burdet et al., 2001, Thoroughman and Shadmehr, 1999).Training induced adaptations of descending motor commands reflect learning within the CNS and can be represented by changes in muscle electromyography (EMG) function (motor recruitment) (Thoroughman and Shadmehr, 1999). Like training, passive interventions such as shoes and in-shoe orthoses (Hertel et al., 2005) (Nigg et al., 2003) have been shown to induce acute adaptations in motor recruitment.

With respect to the neurophysiologic aspect, it is known that the innervation activity during running cycle consists of a pre-activation phase, starting before the ground contact, an activation phase during the weight-acceptance phase, and an innervation phase occurring during the push-off phase. The literature also lacks information concerning the influence of different types of landing pattern on the neuromuscular activation pattern. According to Komi et al. (Komi et al., 1987), differences in EMG activation between conditions of shod are slight and non-systematic whereas compared to barefoot condition differences in EMG activation were found for one subject (Komi et al., 1987).

The main finding in the present study is that 6 weeks habituation of SBR induces neuromuscular changes in the tibialis anterior and gatsrocnemius muscles activation pattern during different phases of running. The TA muscle showed significant differences between shod and habituated SBR conditions during the pre-activation time period. The Integrated EMG value during the 50 ms prior to foot strike was the greatest during pre-activation shod running and the smallest during habituated SBR. The EMG activity of the TA was also found to be significantly more activated during the stance phase of shod condition when compared to the habituated SBR condition. Additionally, a significant increase of pre-activation of gastrocnemius muscle was observed during habituated SBR compared to shod running which further support the reduction of heel impact observed by switching to forefoot technique.

The EMG recruitment patterns for simulated barefoot running are less documented in the literature. Only three studies compared EMG signals between barefoot and shod running (von Tscharner et al., 2003a, Shih et al., 2013, Divert et al., 2005b). Our findings support the results of the previous literature that reported a greater recruitment of GA in the pre-activation phase in the shod condition when compared to non-habituated barefoot running condition (Divert et al., 2005b). However, our results didn't find a significant difference in the EMG activities of the TA for the pre-activation, stance and push off phases between the non-habituated SBR and shod conditions. No statistical difference for the TA muscle between the two conditions of running was reported during different phases of running. In agreement with our findings, Divert et al. reported no significant difference in pre-activation levels of the tibialis anterior when comparing non habituated barefoot and shod running (Divert et al., 2005b).

EMG intensity patterns of the tibialis anterior indicated a high recruitment of muscular activity at specific times before and after heel strike. Tibialis anterior EMG intensities for shod running showed a greater activation 50 ms before impact compared to the weight acceptance phase of shod running. The activity before heel strike is responsible (a) for keeping the forefoot up and (b) tune the muscle for the expected impact. This muscular activity must be released rapidly at impact to release the forefoot. In analogy to chain reactions where the accumulation and degradation of an intermediate component are linked, fast muscle fibers are more likely to allow a fast release than slow ones. The fact that the muscle force has to decay quickly may require that shortly before impact more fast muscle fibers have to be activated. During the stance phase the tibialis anterior showed an activity coinciding with the onset of the activity of the gastrocnemius muscle (von Tscharner et al., 2003b) thus stiffening the ankle joint before progressing to toe-off. The release of the tibialis anterior activation could then greatly contribute to the power available for the movement at the ankle joint. This general activity pattern of the tibialis anterior muscle was observed for shod conditions. The shoes do not change the general pattern. However, they change the fine structure of this pattern in time, frequency and intensity (von Tscharner et al., 2003b).

Indeed EMG activity before heel strike is pre-programmed based on the expected impact shock. Shortly before ground contact, muscle activity has the major goal of preparing the locomotor system for the landing and the subsequent ground contact (Nigg and Wakeling, 2001) . Then the necessary protection from repeated shock of the muscular skeletal structure could lead to a higher pre-activation of plantar flexor muscles. It is also worth noticing that higher active prestretch levels (Aura and Komi, 1986), as well as the reduction of contact time (Bosco and Rusko, 1983) observed here in barefoot condition, could enhance the stretch shortening cycle behavior of the plantar flexor muscles and thus possibly allow a better storage and restitution of elastic energy (Bosco et al., 1982, Bosco et al., 1981) compared to shod condition. Although further studies are necessary to confirm this hypothesis, the higher energy cost of shod running could not be only due to the simple additional mass effect associated to the shoe as hypothesized by

Burkett et al. but also to a worse elastic energy storage and restitution of shod running (Burkett et al., 1985).

Although human feet are designed to run barefoot, it is questionable whether it is appropriate for people accustomed to wearing shoes to abruptly convert to barefoot running. The weakened muscles and heel strike pattern may cause certain unpredictable injuries when habitually shod runners run barefoot. With the increasing trend of barefoot running, clinics have started seeing an increase in the number of injuries caused by barefoot running(Giuliani et al., 2011). To explain the discrepancy of the results reported in the literature, we hypothesized that slight modifications in mechanical characteristics of landing pattern are easily and automatically compensated by the neuromuscular control of the runner. In the same manner, Ferris et al. reported a perfect adjustment of mechanical parameters (stiffness of the runner's leg, elevation of the centre of mass) to keep the locomotion mechanics constant whatever the surface stiffness. Therefore changes in the foot strike landing pattern could induce modifications of running mechanics(Ferris et al., 1998).

From a biomechanical viewpoint, the most obvious difference between barefoot and shod running is the landing pattern. Habitually barefoot runners tend to use the forefoot strike pattern, whereas most of the shod runners use the heel strike pattern (Lieberman et al., 2010). Forefoot or midfoot strikes are considered less efficient running patterns because the center of pressure trajectory of these strikes goes backward after landing and subsequently goes forward, whereas the center of pressure trajectory of the heel strike goes forward directly after landing (Cavanagh and Lafortune, 1980). On the other hand, the forefoot strike resulted in decreased collision force during running, which is believed to reduce injury rates for runners (Lieberman et al., 2010). Our study showed that all participants ran with a RFS pattern during shod running. This agrees with the findings of other authors (De Wit et al., 2000, Lieberman et al., 2010), who reported 88.9–100 % of habitually shod recreational runners to exhibit a RFS pattern. The significant changes across time in the running kinetics and neuromuscular changes of the habituated SBR state supported the carry-over hypothesis from shod gait to SBR.

The most favorable difference between SBR and shod running is the significant reduction in impact transient and the loading rate in the SBR barefoot condition. This is deemed significant because the magnitude of this impact transient has been correlated with the risk of running injuries (Lieberman et al., 2010). In the present experiment, SBR running was characterized by decreased loading rates and impact forces. The significantly lower values of impact forces and loading variables (VILR, VALR, VIP and AP) observed in SBR compared to the shod running conditions are in line with previous studies (Shih et al., 2013, Divert et al., 2005b). Divert et al. reported similar results of significant different amplitudes between barefoot and shod condition (Divert et al., 2005b). In contrast, no significant difference was observed by De Wit et al. for running speed 3.5 and 5.5 m/s and by Dickinson et al. in 6 subjects running across a force plate (De Wit et al., 2000, Dickinson et al., 1985). In both of those works, impact force amplitudes were considerable higher than those recorded in our study. Many methodological differences may explain the divergent results. Firstly, the subjects of the previous works were not accustomed to running barefoot and this could have reduced their ability to dampen the forces elicited at the impact while barefoot. According to Robbins et al. adaptation to barefoot running could take several weeks (Robbins and

Hanna, 1987). Furthermore, the subjects run in a lab runaway and a limited number of steps were analyzed. As suggested by divert el al. it is possible that when data are collected on limited number of steps, runners are able to sustain and then maintain high impact forces (Divert et al., 2005b). Differently, habituation period as those experienced by our subjects would lead the runners to adopt strategies to reduce stress under the heel.

A new paradigm for impact forces during running proposes that impact forces are input signals that produce muscle tuning shortly before the next contact with the ground to minimize soft tissue vibration and/or reduce joint and tendon loading. Another new paradigm for movement control for the lower extremities proposes that forces acting on the foot during the stance phase act as an input signal producing a muscle reaction. The two proposed paradigms suggest that the locomotor system use a similar strategy for "impact" and "movement control." In both cases the locomotor system keeps the general kinematic and kinetic situations similar for a given task. The proposed muscle tuning reaction to impact loading affects the muscle activation *before* ground contact. The proposed muscle adaptation to provide a constant joint movement pattern affects the muscle activation before and *during* ground contact (Nigg and Wakeling, 2001).

When running barefoot, step duration, stride length, and flight time were significantly shorter, and stride frequency significantly higher, than the shod conditions. Stride frequency is inversely proportional to step duration multiplied by velocity, and velocity was controlled in this study. Thus, it would be expected that if one variable increased, the other should decrease, and vice versa; as was recorded in the present study. An increased contact time when shod might be may be partially attributable to the mass of the traditional shoe (Divert et al., 2005b). Although shoe mass was not recorded in the present study, it is likely that the minimalist shoes weighed significantly less than the traditional shoes. Therefore, this could possibly explain the increased contact time recorded when running shod than when running at the same velocity in minimalist footwear. Stride frequency when running barefoot has been compared with running in traditional shoes by Divert et al. and Squadrone and Gallozzi (Divert et al., 2008, Squadrone and Gallozzi, 2009). Our study and that by Squadrone and Gallozzi both reported significantly higher stride frequencies in the barefoot condition (Squadrone and Gallozzi, 2009). However, in runners with no barefoot running experience, stride frequencies similar to the current results have been reported as well (Divert et al., 2008).

We hypothesized that the biomechanical adjustment observed in stride kinematics could help to limit the larger impact forces that should be absorbed by the muscular skeletal system at each step. However, the direction of significant changes in stride kinematics in the SBR across time was towards that observed during the kinematics of non-habituated barefoot participants in other studies. The hypothesis that further changes would occur in barefoot kinetics was supported by significantly changes in the EMG activities of the TA and GAS after 6 weeks of SBR.

Evidence that barefoot and minimally shod runners avoid RFS strikes with high impact collisions may have public implications. The average runners strikes the ground 600 times per kilometer making runners prone to repetitive stress injuries (Milner et al., 2006, van Gent et al., 2007). The incidence of such injuries has remained considerable for 30 years despite technological advancements that provide more cushioning and motion control in shoes designed for heel–toe running (van Mechelen, 1992). Although cushioned, high-heeled running shoes are comfortable, they limit proprioception and make it easier for runners to land on their heels. Furthermore, many running shoes have arch supports and stiffened soles that may lead to weaker foot muscles, reducing arch strength. This weakness contributes to excessive pronation and places greater demands on the plantar fascia, which may cause plantar fasciitis. Although there are anecdotal reports of reduced injuries in barefoot populations (Robbins and Hanna, 1987), controlled prospective studies are needed to test the hypothesis that individuals who do not predominantly RFS either barefoot or in minimal footwear, as the foot apparently evolved to do, have reduced injury rates.

Of particular note is that the validity of the previous body of work is compromised by the lack of evaluation after habituation, or re-training, of previously shod rearfoot-striking runners to barefoot forefoot-striking running styles. While future research may seek to standardize the transition protocol, this should be done cautiously since individual progress differently, and higher injury rates may ensue from enforcing progression. The current study was delimited to recreational female athletes. Therefore, one should be wary of extrapolating the results to highly trained athletes, whose running mechanics may be highly consistent (Bonacci et al., 2013, Perl et al., 2012, Chapman et al., 2008). Care should also be taken when comparing kinetic data from this study with that of studies in which participants were tested running at higher velocities (Bonacci et al., 2013, Lieberman et al., 2010) (Braunstein et al., 2010). The prospective research would have higher validity were the biomechanical effects of habituating to barefoot running fully examined alongside an evaluation of prevention of repetitive use injury.

Limitations

We acknowledge limitations of the present study. Treadmill gait and over-ground gait are not identical (Lieberman et al., 2010, Leitch et al., 2011). However, treadmill usage allowed participants to adapt to each condition and reach a steady state in their stride pattern before data sampling occurred. Not knowing when data would be acquired also had other potential benefits when compared with over-ground testing and use of force-plates (Belli et al., 2001). The lack of standardization of the traditional shoes used may have impacted the results in the shod condition. Participants were tested in their regular training shoes, in order to most accurately represent the normal running mechanics exhibited in training. For the transition program, SBR sessions were prescribed based on the total training volume to simplify the protocol and encourage compliance. This, however, makes it more difficult to quantify the "dose" of the intervention received by each participant or the amount of SBR training as a proportion of total training volume for each individual.

Conclusion

The findings of this study indicate that changes in motor patterns in previously habitually shod runners are possible and can be accomplished within 6 weeks. There is emerging evidence that a FFS pattern such as developed over time by SBR could have performance benefits and perhaps lead to lower injury rates or the potential to treat existing injuries. 6 weeks of habituated SBR led to significantly decrease activity of tibialis anterior in the preactivation and absorptive phase of stance and may reduce higher risk of running injuries (e.g chronic exertional compartment syndrome). Habitual shod runners did not automatically alter their landing patterns from heel strike to a non-heel strike pattern during early exposure to barefoot running. During non-habituated SBR, subjects did not experienced neuromuscular adaptations they experienced after 6 weeks of habituation. However, the neuromuscular adaptation was influenced by the habituation period. Therefore a gradual transitioning program with real time kinetic feedback and evaluation is recommended. The simplicity and relatively low cost of the intervention makes it accessible to both recreational and competitive athletes who may wish to develop more “barefoot-like” running pattern, regardless of preferred footwear condition.

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CHAPTER FOUR

DISCUSSION

The physical disability and long rehabilitation process associated with anterior cruciate ligament (ACL) injury can be both psychologically and financially devastating to the individual, ultimately resulting in a decreased quality of life. Female athletes have a higher rate of ACL injury than do men, and many of these injuries require extensive surgical and rehabilitative interventions, with a financial burden to the American healthcare system estimated to approach $650 million annually (Zazulak et al., 2006). Bearing that in mind, it is imperative to understand the mechanisms leading to such an injury in an effort to prevent its occurrence and its subsequent sequelae. Although both men and women are susceptible, the literature states that women have a 4 to 6 fold increased incidence of ACL injury (Hewett et al., 2007, Hewett et al., 2006).

While the increased incidence of serious knee injuries in female athletes is well established, the underlying neuromuscular mechanisms related to the elevated ACL injury rate has yet to be delineated. Maintenance of joint congruency is important in prevention of injury. Both the ligamentous structures and the muscular system contribute (Hertel et al., 2006) .The role of the muscular system is particularly important when the static restraints are jeopardized and therefore not providing restraint to abnormal motion within the joint.

Our study supports the previous studies which have reported a greater knee laxity during ovulation when estrogen levels are high (Shultz et al., 2004a, Eiling et al., 2007).

Conversely, women who experienced high plasma concentration of estrogen experienced a marked increase in joint laxity behavior following peak ovulatory levels. However other studies (Carcia et al., 2004, Van Lunen et al., 2003) found that knee ligament laxity doesn't differ by the menstrual cycle day. Interestingly to note that in these studies, that did not identify changes in knee laxity across select days of the menstrual cycle. The average estradiol levels were near the upper limits of normal ranges at menses (56 and 73 pg/mL) and considerably below the normal ranges postovulation (137 and 120 pg/mL) using similar hormone assay.

The results of our study reveal differences in muscle activation strategies during different phases of the menstrual cycle. Our results showed that women place greater reliance on their quadriceps during the follicular phase to modulate the torsional joint stiffness about the knee joint during running. The increased quadriceps activity observed during the follicular phase was associated with decreased hamstrings activity. We speculate that the observed differences in neuromuscular recruitment strategies may have implications for the greater incidence of non-contact ACL injures observed in women.

A consequence of differences in neuromuscular activation patterns might be injury susceptibility. Markolf & colleagues, found that muscle activation about the knee increased valgus stability threefold, highlighting the influence of the muscular system on knee stability (Markolf et al., 2003). Previous investigations of neuromuscular control (Cowling and Steele, 2001, Wojtys et al., 2003); have not considered muscle activation patterns during running. The present study discovered that the quadriceps and hamstring cocontraction ratios decreased during the early follicular phase compared to ovulatory phase. This suggests a different co-contraction (onset timing of agonist/antagonist around a joint) mechanism between these muscles. This alteration in neuromuscular control may explain the non-significant knee valgus variable since the quadriceps and hamstring work together to control torsional motions of the femur and tibia that may contribute to valgus alignment of the knee (McLean et al., 2005). This co-contractive mechanism suggests a different neuromuscular control pattern when estrogen levels are low; however, more investigation is necessary.

While we are unable to find another published study that evaluated neuromuscular activation patterns in healthy females runners with non-pathological knee elasticity, our findings are surprisingly consistent with those demonstrated in ACL deficient individuals. Alkjaer & colleagues, reported a marked increase in hamstring coactivation towards more extended joint positions in ACL deficient subjects (Alkjaer et al., 2012). Notably, this progressive rise in coactivation may reflect a compensatory strategy to provide stability to the knee joint in the anterior–posterior plane during knee extension. In agreement, our investigation showed that participants with increased knee joint laxity during ovulation demonstrated increased levels of muscle preactivity in the hamstring muscles before impact as well as during weight acceptance phase. Coactivation of the hamstring muscles during dynamic knee extension may compensate for increased knee joint laxity in anterior cruciate ligament. Increased coactivation of the hamstring muscles has been suggested to provide a compensatory strategy to reduce Anterior tibial translation (ATT) in functional conditions that include knee extension (More et al., 1993) (Yanagawa et al., 2002). Several studies have shown that the hamstring muscles are active during submaximal and maximal quadriceps agonist contraction (Aalbersberg et al., 2009) and that coactivation of the antagonist hamstring muscles during knee extension effectively reduces the amount of ATT (Yanagawa et al., 2002, Liu and Maitland, 2000).

The primary purpose of the current study was to investigate whether estradiol fluctuation during the menstrual cycle has an influence on the neuromuscular control of knee joint mechanics during running. Previous studies investigating the relationship between knee joint mechanics and the menstrual cycle found significant changes in biomechanical (Dedrick et al., 2008) or neuromuscular (Shultz et al., 2004a) characteristics corresponding to changes in hormonal levels during the menstrual cycle. The present study also found significant changes in the neuromuscular control around the knee joint between the different phases of the menstrual cycle. As a result, ACL injury in female athletes may not be explained simply by the hormonal cycle but is likely influenced by a more complicated and indirect injury mechanisms incorporating hormonal fluctuations and dynamic knee joint function that may be individually specific.

Although, we are currently unaware of any other study in the literature that investigated the neuromuscular control variables presented here in female runners. Previous studies had evaluated gender differences in neuromuscular control. Our study showed that female runners use a different neuromuscular strategy during different phases of menstrual cycle. Subjects demonstrated a decreased ratio of medial quadriceps to lateral quadriceps recruitment. A preactivation difference did exist for the lateral quadriceps between the follicular and ovulatory phase. The decreased ratio of the medial quadriceps musculature recruitment may be related to decreased control of coronal plane forces at the knee (Markolf et al., 1995).

In addition to low ratio of medial to lateral quadriceps recruitment combined with increased lateral hamstring firing may compress the lateral joint, open the medial joint and increase and increase shear force, which directly loads AC. This disproportional recruitment of the quadriceps musculature increases anterior shear force at low knee flexion angles that occur during landing. The quadriceps, through the anterior pull of the patellar tendon on the tibia, contributes to ACL loading when knee flexion is less than 30 degrees (Markolf et al., 1995). Of interest is that our participants demonstrated increased activity of the quadriceps muscles during the follicular phase compared to the ovulatory phase, which is thought to maximize axial compression, joint congruency and frictional forces to effectively limit joint displacement. Muscular co-contraction compresses the joint, due in part to the concavity of the medial tibial plateau, which may protect the ACL against anterior drawer. However, Zazulak & colleagues reported greater peak quadriceps activity in female than male subjects (Zazulak et al., 2005). Decreased balance in strength and recruitment of the flexor relative to the extensor musculature may put the ACL at greater risk (Hewett et al., 1996). Adequate cocontraction of the knee flexors is needed to balance contraction of the quadriceps, compress the joint, and control high knee extension and abduction torques (Hewett et al., 1996). Appropriate hamstrings recruitment may prevent the critical loading necessary to rupture the ACL during maneuvers that place the athlete at risk of an injury. Female subjects may display a longer latency period that is, electromechanical delay between preparatory and reactive muscle activation. Preparatory muscle activity can stiffen joints before unexpected perturbations. Neuromuscular training that reproduces loads similar to those encountered during competitive sports may assist in the development of both feed forward and reactive muscle activation strategies that protect the knee joint from excessive load (Winter and Brookes, 1991, Lebrun, 1994, Wojtys and Huston, 1994) .

Our findings support the previous studies which have reported a decreased neuromuscular response and/or control around the time of menstruation (Friden et al., 2006, Shultz et al., 2004a). Despite these observations, our findings of decreased neuromuscular control around the knee which may be a potential mechanism for increased risk of injury at this stage of the cycle, there is no consensus as to whether injury risk is also elevated during menstruation (Hewett et al., 2007). These conflicting results could be due to the difficulty in performing a prospective study to assess injury risk, with the majority of protocols consisting of retrospective assessment (Hewett et al., 2007). These results can also be confounded by participation levels during each phase of the cycle, because women who do not take the oral contraceptive pills are significantly less inclined to participate in physical activity during menstruation (Adachi et al., 2008). Taken together, these results suggest that during menstruation the performance of the neuromuscular system is compromised, which may limit both participation and intensity of activity in sporting events and therefor counterbalance the increased risk of injury due to an impaired motor control strategy.

Decreased neuromuscular control of the lower extremity during menstruation may increase the potential for valgus lower extremity position and increased ACL injury risk. Identification of these neuromuscular imbalances has potential for both screening of high risk athletes and targeting interventions to specific deficits. Dynamic neuromuscular training can increase active knee stabilization and decrease the incidence of ACL injury in the female athletic population (Myklebust et al., 2003, Myer et al., 2005b). Training may facilitate neuromuscular adaptations that provide increased joint stabilization and muscular preactivation and reactive patterns that protect the female athlete's ACL from increased loading. Neuromuscular training will help female athletes adopt muscular recruitment strategies that decrease joint motion and protect the athletes ACL from the high impulse loading while also improving their measures of performance. More investigation is necessary to determine if the neuromuscular control changes occur due to alterations in force transmission properties of passive tissues, levels of feedback from ligamentous and dynamic tissues, levels of feedback from ligamentous and dynamic tissues or centrally driven feed forward mechanism.

To the best of our knowledge, until now few authors studied barefoot running but none of them, reported a biomechanical and muscular analysis of the differences between shod running and barefoot running after habituation. This is the first study to investigate the effect of 6 weeks habituation of SBR training on running kinetics. The results of the current study highlight that 6-weeks intervention of controlled SBR training was sufficient to elicit significant changes in lower limb kinetics during barefoot running.

The neuromuscular system has the capability to adapt to training, not unlike the cardiorespiratory system. Studies over the past two decades have provided strong evidence that continued practice of a task (i.e. training) facilitates neuromuscular adaptations, which are characterized by more skilled control of movement and muscle recruitment patterns (Burdet et al., 2001, Thoroughman and Shadmehr, 1999).Training induced adaptations of descending motor commands reflect learning within the CNS and can be represented by changes in muscle electromyography (EMG) function (motor recruitment) (Thoroughman and Shadmehr, 1999). Like training, passive interventions such as shoes and in-shoe orthoses (Hertel et al., 2005) (Nigg et al., 2003) have been shown to induce acute adaptations in motor recruitment.

With respect to the neurophysiologic aspect, it is known that the innervation activity during running cycle consists of a pre-activation phase, starting before the ground contact, an activation phase during the weight-acceptance phase, and an innervation phase occurring during the push-off phase. The literature also lacks information concerning the influence of different types of landing pattern on the neuromuscular activation pattern. According to Komi et al. (Komi et al., 1987), differences in EMG activation between conditions of shod are slight and non-systematic whereas compared to barefoot condition differences in EMG activation were found for one subject (Komi et al., 1987).

The main finding in the present study is that 6 weeks habituation of SBR induces neuromuscular changes in the tibialis anterior and gatsrocnemius muscles activation pattern during different phases of running. The TA muscle showed significant differences between shod and habituated SBR conditions during the pre-activation time period. The Integrated EMG value during the 50 ms prior to foot strike was the greatest during pre-activation shod running and the smallest during habituated SBR. The EMG activity of the TA was also found to be significantly more activated during the stance phase of shod condition when compared to the habituated SBR condition. Additionally, a significant increase of pre-activation of gastrocnemius muscle was observed during habituated SBR compared to shod running which further support the reduction of heel impact observed by switching to forefoot technique.

The EMG recruitment patterns for simulated barefoot running are less documented in the literature. Only three studies compared EMG signals between barefoot and shod running (von Tscharner et al., 2003b, Shih et al., 2013, Divert et al., 2005). Our findings support the results of the previous literature that reported a greater recruitment of GA in the pre-activation phase in the shod condition when compared to non-habituated barefoot running condition (Divert et al., 2005). However, our results didn't find a significant difference in the EMG activities of the TA for the pre-activation, stance and push off phases between the non-habituated SBR and shod conditions. No statistical difference for the TA muscle between the two conditions of running was reported during different phases of running. In agreement with our findings, Divert et al. reported no significant difference in pre-activation levels of the tibialis anterior when comparing non habituated barefoot and shod running (Divert et al., 2005).

EMG intensity patterns of the tibialis anterior indicated a high recruitment of muscular activity at specific times before and after heel strike. Tibialis anterior EMG intensities for shod running showed a greater activation 50 ms before impact compared to the weight acceptance phase of shod running. The activity before heel strike is responsible (a) for keeping the forefoot up and (b) tune the muscle for the expected impact. This muscular activity must be released rapidly at impact to release the forefoot. In analogy to chain reactions where the accumulation and degradation of an intermediate component are linked, fast muscle fibers are more likely to allow a fast release than slow ones. The fact that the muscle force has to decay quickly may require that shortly before impact more fast muscle fibers have to be activated. During the stance phase the tibialis anterior showed an activity coinciding with the onset of the activity of the gastrocnemius muscle (von Tscharner et al., 2003a) thus stiffening the ankle joint before progressing to toe-off. The release of the tibialis anterior activation could then greatly contribute to the power available for the movement at the ankle joint. This general activity pattern of the tibialis anterior muscle was observed for shod conditions. The shoes do not change the general pattern. However, they change the fine structure of this pattern in time, frequency and intensity (von Tscharner et al., 2003a).

Indeed EMG activity before heel strike is pre-programmed based on the expected impact shock. Shortly before ground contact, muscle activity has the major goal of preparing the locomotor system for the landing and the subsequent ground contact (Nigg and Wakeling, 2001). Then the necessary protection from repeated shock of the muscular skeletal structure could lead to a higher pre-activation of plantar flexor muscles. It is also worth noticing that higher active prestretch levels (Aura and Komi, 1986), as well as the reduction of contact time (Bosco and Rusko, 1983) observed here in barefoot condition, could enhance the stretch shortening cycle behavior of the plantar flexor muscles and thus possibly allow a better storage and restitution of elastic energy (Bosco et al., 1982, Bosco et al., 1981) compared to shod condition. Although further studies are necessary to confirm this hypothesis, the higher energy cost of shod running could not be only due to the simple additional mass effect associated to the shoe as hypothesized by Burkett et al. but also to a worse elastic energy storage and restitution of shod running (Burkett et al., 1985).

Although human feet are designed to run barefoot, it is questionable whether it is appropriate for people accustomed to wearing shoes to abruptly convert to barefoot running. The weakened muscles and heel strike pattern may cause certain unpredictable injuries when habitually shod runners run barefoot. With the increasing trend of barefoot running, clinics have started seeing an increase in the number of injuries caused by barefoot running (Giuliani et al., 2011). To explain the discrepancy of the results reported in the literature, we hypothesized that slight modifications in mechanical characteristics of the landing pattern are easily and automatically compensated by the neuromuscular control of the runner. In the same manner, Ferris et al. reported a perfect adjustment of mechanical parameters (stiffness of the runner's leg, elevation of the centre of mass) in order to keep the locomotion mechanics constant whatever the surface stiffness. Therefore changes in the foot strike landing pattern could induce modifications of running mechanics (Ferris et al., 1998).

From a biomechanical viewpoint, the most obvious difference between barefoot and shod running is the landing pattern. Habitually barefoot runners tend to use the forefoot strike pattern, whereas most of the shod runners use the heel strike pattern (Lieberman et al., 2010). Forefoot or midfoot strikes are considered less efficient running patterns because the center of pressure trajectory of these strikes goes backward after landing and subsequently goes forward, whereas the center of pressure trajectory of the heel strike goes forward directly after landing (Cavanagh and Lafortune, 1980). On the other hand, the forefoot strike resulted in decreased collision force during running, which is believed to reduce injury rates for runners (Lieberman et al., 2010). Our study showed that all participants ran with a RFS pattern during shod running. This agrees with the findings of other authors (De Wit et al., 2000, Lieberman et al., 2010), who reported 88.9–100 % of habitually shod recreational runners to exhibit a RFS pattern. The significant changes across time in the running kinetics and neuromuscular changes of the habituated SBR state supported the carry-over hypothesis from shod gait to SBR.

The most favorable difference between SBR and shod running is the significant reduction in impact transient and the loading rate in the SBR barefoot condition. This is deemed significant because the magnitude of this impact transient has been correlated with the risk of running injuries (Lieberman et al., 2010). In the present experiment, SBR running was characterized by decreased loading rates and impact forces. The significant lower values of impact forces and loading variables (VILR, VALR, VIP and AP) observed in SBR compared to the shod running conditions are in line with previous studies (Shih et al., 2013, Divert et al., 2005). Divert et al. reported similar results of significant different amplitudes between barefoot and shod condition (Divert et al., 2005). In contrast, no significant difference was observed by De Wit et al. for running speed 3.5 and 5.5 m/s and by Dickinson et al. in 6 subjects running across a force plate (De Wit et al., 2000, Dickinson et al., 1985). In both of those works, impact force amplitudes were considerable higher than those recorded in our study. Many methodological differences may explain the divergent results. Firstly, the subjects of the previous works were not accustomed to running barefoot and this could have reduced their ability to dampen the forces elicited at the impact while barefoot. According to Robbins et al. adaptation to barefoot running could take several weeks (Robbins and Hanna, 1987). Furthermore, the subjects ran in a lab runaway and a limited number of steps were analyzed. As suggested by divert el al. it is possible that when data are collected on a limited number of steps, runners are able to sustain and then maintain high impact forces (Divert et al., 2005).

Differently, habituation period as those experienced by our subjects would lead the runners to adopt strategies to reduce stress under the heel.

A new paradigm for impact forces during running proposes that impact forces are input signals that produce muscle tuning shortly before the next contact with the ground to minimize soft tissue vibration and/or reduce joint and tendon loading. Another new paradigm for movement control for the lower extremities proposes that forces acting on the foot during the stance phase act as an input signal producing a muscle reaction. The two proposed paradigms suggest that the locomotor system uses a similar strategy for "impact" and "movement control." In both cases the locomotor system keeps the general kinematic and kinetic situations similar for a given task. The proposed muscle tuning reaction to impact loading affects the muscle activation *before* ground contact. The proposed muscle adaptation provides a constant joint movement pattern that affects the muscle activation before and *during* ground contact (Nigg and Wakeling, 2001).

When running barefoot, step duration, stride length, and flight time were significantly shorter, and stride frequency significantly higher, than the shod conditions. Stride frequency is inversely proportional to step duration multiplied by velocity, and velocity was controlled in this study. Thus, it would be expected that if one variable increased, the other should decrease, and vice versa*;* as was recorded in the present study. An increased contact time when shod might be may be partially attributable to the mass of the traditional shoe (Divert et al., 2005). Although shoe mass was not recorded in the present study, it is likely that the minimalist shoes weighed significantly less than the traditional shoes. Therefore, this could possibly explain the increased contact time recorded when running shod than when running at the same velocity in minimalist footwear. Stride frequency when running barefoot has been compared with running in traditional shoes by Divert et al. and Squadrone and Gallozzi (Divert et al., 2008, Squadrone and Gallozzi, 2009). Our study and that by Squadrone and Gallozzi both reported significantly higher stride frequencies in the barefoot condition (Squadrone and Gallozzi, 2009). However, in runners with no barefoot running experience, stride frequencies similar to the current results have been reported as well (Divert et al., 2008).

We hypothesized that the biomechanical adjustment observed in stride kinematics could help to limit the larger impact forces that should be absorbed by the muscular skeletal system at each step. However, the direction of significant changes in stride kinematics in the SBR across time was towards that observed during the kinematics of non-habituated barefoot participants in other studies. The hypothesis that further changes would occur in barefoot kinetics was supported by significant changes in the EMG activities of the TA and GAS after 6 weeks of SBR.

Evidence that barefoot and minimally shod runners avoid RFS strikes with high impact collisions may have public implications. The average runners strikes the ground 600 times per kilometer making runners prone to repetitive stress injuries (Milner et al., 2006, van Gent et al., 2007). The incidence of such injuries has remained considerable for 30 years despite technological advancements that provide more cushioning and motion control in shoes designed for heel–toe running (van Mechelen, 1992). Although cushioned, high-heeled running shoes are comfortable, they limit proprioception and make it easier for runners to land on their heels. Furthermore, many running shoes have arch supports and stiffened soles that may lead to weaker foot muscles, reducing arch strength. This weakness contributes to excessive pronation and places greater demands on the plantar fascia, which may cause plantar fasciitis. Although there are anecdotal reports of reduced injuries in barefoot populations (Robbins and Hanna, 1987), controlled prospective studies are needed to test the hypothesis that individuals who do not predominantly RFS either barefoot or in minimal footwear, as the foot apparently evolved to do, have reduced injury rates.

Of particular note is that the validity of the previous body of work is compromised by the lack of evaluation after habituation, or re-training, of previously shod rearfoot-striking runners to barefoot forefoot-striking running styles. While future research may seek to standardize the transition protocol, this should be done cautiously since individual progress differently, and higher injury rates may ensue from enforcing progression. The current study was delimited to recreational female athletes. Therefore, one should be wary of extrapolating the results to highly trained athletes, whose running mechanics may be highly consistent (Bonacci et al., 2013, Perl et al., 2012, Chapman et al., 2008). Care should also be taken when comparing kinetic data from this study with that of studies in which participants were tested running at higher velocities (Bonacci et al., 2013, Lieberman et al., 2010) (Braunstein et al., 2010). The prospective research would have higher validity were the biomechanical effects of habituating to barefoot running fully examined alongside an evaluation of prevention of repetitive use injury.

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APPENDIX A

INFORMED CONSENT FORM

LOMA LINDA UNIVERSITY
School of Allied Health Professions

EFFECT OF FOREFOOT STRIKE PATTERNS ON PHYSIOLOGICAL ADAPTATIONS AND BIOMECHANICAL CHANGES IN RUNNERS DURING THE MENSTRUAL CYCLE

Informed Consent to Participate in Research

This study is being conducted by Iman Khowailed, for graduate academic credit in the Physical Therapy Department. You are invited to take part in a research study. Your participation in this research study is strictly voluntary, meaning that you may or may not choose to take part. Before you agree, you need to take time to carefully read and understand what your participation would involve. To decide whether or not you want to be part of this research, the purpose, procedures, risks, and possible benefits of the study are described in this form so that you can make an informed decision.

PURPOSE OF THE STUDY AND STUDY PROCEDURES

You are invited to participate in a study to see how your body changes running pattern when running with regular shoes versus running with sports barefoot shoes. In addition, we want to follow you during full a menstrual cycle; to see how your running changes during different phases of your menstrual cycle. If you choose to participate, you will need to be female between 20 and 40 years old and in good health. You need to be a recreational runner, running at least once a week with a regular normal menstrual cycle and should not be taking oral contraceptive pills.

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 4/29/14 Void after 12/25/2014
#5130372 Chair R L Rugsegger

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PROCEDURES

If you qualify for the study, you will participate in one of two groups. Subjects in the first group will participate for one month and subjects in the second group will participate for two months. In both groups, you will be tested during each of the three phases of the menstrual cycle during a month period. Once between days 1 to 7 after the onset of menstruation; once between days 12 to 18 days after the onset of menstruation; and once between 21 to 28 days after the onset of menstruation. You will be asked to report the days of your cycle at the beginning of the study. The first group will undergo pretesting measurements over three testing sessions in one month. The second group will participate for six weeks of training and post testing measurements in one testing session. At each session, we will measure the electrical activity from muscles in your legs with small devices called electrodes which are the size of quarter and adhere above yopur muscles. We will also take videos of your running for 10 minutes. We will also measure the laxness of your knee ligament while you are lying down. Finally, we will collect 4 ml of your blood (1 teaspoon) to measure the level of estrogen in your blood. We will need 45 minutes of your time at each of these testing sessions to measure joint angular excursion, activity of your muscles, and laxity of your knee ligament.

Research study protocol summary:

If you are in the first group

During each testing session, the following measurements will be taken

- Video of your running for 10 minutes
- Electrical activity of your muscles
- Laxity of your knee ligament
- Blood draw at medical center of Loma Linda university

If you are in the second group

6 weeks (Training)

This month consists of one month of forefoot running training to learn how to run with forefoot strike pattern instead of heel strike pattern.

- Subjects will receive a pair of barefoot sports shoes
- Subjects will be instructed on how to safely and successfully transition to running with these shoes.
- Subjects have to come to the lab three times a week to ensure proper training during running.
- Each subject will be provided with a structured progression of running with barefoot sports shoes over the 6-week familiarization period and relevant injury prevention exercises. Forefoot running technique guidelines will be also provided. Both the technique changes and exercises will be fully demonstrated.

Second month

During each testing session, the following measurements will be taken

- Video of your running for 10 minutes
- Electrical activity of your muscles
- Laxity of your knee ligament
- Blood draw at medical center of Loma Linda university

POSSIBLE RISKS AND DISCOMFORTS

The risk of participation in this study is the risk of running injury, which is minimal and inherent to running and transitioning to running with Barefoot Sports shoes. Possible bruising from blood draw, and possible injury to the lower legs from running. If you experience any of these symptoms, you must notify the study investigator immediately.

BENEFITS

You will not benefit from participating in this study, but it will give us a better understanding of differences and safety between forefoot strikers and heel strikers' runners. In addition we hope to learn the effect of menstrual cycle on the pattern of muscle activation, and laxness of knee ligament.

PARTICIPANTS RIGHTS

Participation is voluntary. You may leave the study at any time. If at any time during a procedure you experience tiredness or discomfort beyond what you are willing to endure, just tell the person conducting the procedure you want to stop. This decision will NOT affect your standing with those conducting the study or loss of benefits that you are entitled to.

- You are free to withdraw from this study at any time. If you decide to withdraw from this study you should notify the research team immediately. The research team may also end your participation in this study if you do not follow instructions, miss scheduled visits, or if your safety and welfare are at risk.
- If you withdraw or are removed from the study, the researcher may ask you to return for a final close-out visit or complete an exit telephone interview.
- Likewise, your participation in the study may be stopped by the study staff/investigator for any reason without your agreement.

RESEARCH RELATED INJURY

Researchers will be monitoring your condition throughout the study, and precautions will be taken to minimize the risks to you from participating. If you are injured or become ill while taking part in this study:

- o If the situation is a medical emergency **call 911** or go to the nearest emergency room. Then, notify the study doctor as soon as you can.
- o For a non-emergency injury or illness, notify your study doctor as soon as you can.
- o To contact Dr Jerrold Petrofsky during regular business hours at 909-558-7274, during daytime hours (8:00 am-5:00 pm.

Appropriate medical treatment will be made available to you. However, you and your insurance company will be billed at the usual charge for the treatment of any research-related injuries, illnesses, or complications. You might still be asked to pay whatever your insurance does not pay. Also, no funds have been set aside, nor any plans made to compensate you for time lost for work, disability, pain or other discomforts resulting from your participation in this research. You do not give up any of your legal rights by participating in the study,

CONFIDENTIALITY

All records will be confidential and stored in a locked cabinet in a locked room. We will not disclose your participation without your written permission. Any publication resulting from this study will refer to you by ID number and not by your name. The video tapes aren't for photographic uses, only for motion analysis purposes. Your privacy rights are described in the attached Authorization for Use of Protected Health Information.

COSTS/COMPENSATION

There is no cost for participating in these studies. You will receive a free pair of Barefoot Sports shoes. Additionally, $50 will be given to thank you for your contribution to the study. You may be asked to provide your home address and/or your Social Security number.

IMPARTIAL THIRD PARTY

If you wish to contact a third party not associated with the study for any questions or a complaint, you may contact the Office of Patient Relations at Loma Linda University, Loma Linda University Medical Center, Loma Linda, California 92354. Phone (909) 558-4647. patientrelations@llu.edu

INFORMED CONSENT STATEMENT

I have read the contents of the consent form and have listened to the verbal explanation given by the investigator. My questions regarding the study have been answered to my satisfaction. I hereby give voluntary consent to participate in the study described here. Signing this form does not waive my rights nor does it release the responsibilities of the principal investigator, Jerrold Petrofsky Ph. D. or Loma Linda University of their responsibilities. I may call Dr. Jerrold Petrofsky during routine office hours at (909) 558-4300 ex 82186 or leave a voice mail message at this number during non- office hours.

Signature of subject

Date

Investigator's Statement

I have reviewed the contents of the consent form with the person signing above. I attest that the requirements for informed consent for the medical research project described in this form have been satisfied – that the subject has been provided with a copy of the California Experimental Subject's Bill of Rights, that I have discussed the research project with the subject and explained to him or her in non-technical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the subject to ask questions and that all questions asked were answered. I will provide the subject with a signed and dated copy of this consent form.

Signature of investigator ___________________________________

Phone Number ______________________________ Date _____________________

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 4/29/14 Void after 12/25/2014
#5130372 Chair R L Rigsby MD

APPENDIX B

AUTHORIZATION OF USE OF PROTECTED HEALTH INFORMATION FORM

OSR#

INSTITUTIONAL REVIEW BOARD
Authorization for Use of Protected Health Information (PHI)

Per 45 CFR §164.508(b)

OFFICE OF SPONSORED RESEARCH
Loma Linda University • 11188 Anderson Street • Loma Linda, CA 92350
(909) 558-4531 (voice) / (909) 558-0131 (fax)

TITLE OF STUDY:	**EFFECT OF FOREFOOT STRIKE PATTERNS ON PHYSIOLGICAL ADAPTTAIONS AND BIOMECHANCAL CHANGES IN RUNNERS DURING THE MENSTRUAL CYCLE"**
PRINCIPAL INVESTIGATOR:	Jerrold Petrofsky, Ph.D, JD
Others who will use, collect, or share PHI:	Iman Khowailed , MPT

The study named above may be performed only by using personal information relating to your health. National and international data protection regulations give you the right to control the use of your medical information. Therefore, by signing this form, you specifically authorize your medical information to be used or shared as described below.

The following personal information, considered "Protected Health Information" (PHI) is needed to conduct this study and may include, but is not limited to: name, address, telephone number, date of birth

The individual(s) listed above will use or share this PHI in the course of this study with the Institutional Review Board (IRB) and the Office of Research Affairs of Loma Linda University.

The main reason for sharing this information is to be able to conduct the study as described earlier in the consent form. In addition, it is shared to ensure that the study meets legal, institutional, and accreditation standards. Information may also be shared to report adverse events or situations that may help prevent placing other individuals at risk.

All reasonable efforts will be used to protect the confidentiality of your PHI, which may be shared with others to support this study, to carry out their responsibilities, to conduct public health reporting and to comply with the law as applicable. Those who receive the PHI may share with others if they are required by law, and they may share it with others who may not need to follow the federal privacy rule.

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/6/14 Void after 12/25/2014
5130372 Chair R L Ringleymd

OSR 1/6/2014

Subject to any legal limitations, you have the right to access any protected health information created during this study. You may request this information from the Principal Investigator named above but it will only become available after the study analyses are complete.

- The authorization expires upon the conclusion of this research study.

You may change your mind about this authorization at any time. If this happens, you must withdraw your permission in writing. Beginning on the date you withdraw your permission, no new personal health information will be used for this study. However, study personnel may continue to use the health information that was provided before you withdrew your permission. If you sign this form and enter the study, but later change your mind and withdraw your permission, you will be removed from the study at that time. To withdraw your permission, please contact the Principal Investigator or study personnel at (909) 558 4300 ext. 82186 or e-mail him at jpetrofsky@llu.edu

You may refuse to sign this authorization. Refusing to sign will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are entitled. However, if you do not sign this authorization form, you will not be able to take part in the study for which you are being considered. You will receive a copy of this signed and dated authorization prior to your participation in this study.

I agree that my personal health information may be used for the study purposes described in this form.

Signature of Patient *or* Patient's Legal Representative	Date
Printed Name of Legal Representative (if any)	Representative's Authority to Act for Patient
Signature of Investigator Obtaining Authorization	Date

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 1/6/14 **Void after** 12/25/2014
5130372 **Chair** R L Rigsby

OSR 1/6/2014

APPENDIX C

CALIFORNIA EXPERIMENTAL SUBJECT’S BILL OF RIGHTS

California Experimental Subject's Bill Of Rights

You have been asked to participate as a subject in an experimental clinical procedure. Before you decide whether you want to participate in the experimental procedure, you have a right to:

1. Be informed of the nature and purpose of the experiment.

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.

3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.

4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.

5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.

6. Be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise.

7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.

8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.

9. Be given a copy of any signed and dated written consent form used in relation to the experiment.

10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

I have carefully read the information contained above in the "California Experimental Subject's Bill of Rights" and I understand fully my rights as a potential subject in a medical experiment involving people as subjects.

_________________________	___________________________________
Date	Patient
_________________________	___________________________________
For inpatient studies, add: Time	Parent/Legal Guardian

If signed by other than the patient, indicate relationship:

_________________________	___________________________________
Relationship	Witness