Continuous moderate intensity versus discontinuous high intensity treadmill running on anterior cruciate ligament laxity and hamstrings flexibility in eumenorrheic women

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Objective: To differentiate running intensity and menstrual phase effects on knee stability before and after exercise.

Methods: Ten eumenorrheic aerobically trained females were recruited to determine effects of a randomized crossover design of exercise intensity (85%HRR vs 42.5%HRR) on anterior cruciate ligament laxity (APLAX) and hamstrings flexibility (HF). A KT-2000 arthrometer measured APLAX and a 90-90 supine knee extension (MKE) and sit-and-reach test (SRD) measured HF in follicular (FP) and luteal (LP) menstrual cycle phases.

Results: Significant difference pre-exercise was observed for both 90N and 120N APLAX in LP compared to FP. Exercise increased APLAX at 90N and 120N in...
both FP and LP with LP exhibiting larger changes than
FP. MKE and SRD increased significantly following
exercise but were not different across menstrual phases
or between exercise intensities.

Conclusion: $A_{PLAX}$ taken together with increased HF
post-exercise demonstrates a less stable knee joint in the
LP before and following exercise.

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KEY WORDS: aerobic exercise, anterior cruciate
ligament, joint range of motion, menstrual cycle,
hormone

Introduction
Anterior cruciate ligament (ACL) tears have been dem-
onstrated to be approximately three times more likely
in populations of female athletes compared to male ath-
letes.1 However, the underlying causes of ACL tears re-
main unclear.2 Sport-related collision with other players
accounts for about 30% of cases with the remaining 70%-
being non-collision.3 In non-collision ACL injuries inher-
ent anatomical differences in tibia and thigh length, pel-
vis width, and femoral notch width have been examined
as contributing factors.2,3 Additionally, hormone medi-
ated changes to connective tissues, hamstrings flexibility
(HF), and anterior-posterior knee laxity ($A_{PLAX}$) have all
been cited as risk factors.4

Clinical evidence in females indicates that for every
1.3 mm increase in $A_{PLAX}$ the risk of injury increas-
es fourfold4 and high $A_{PLAX}$ (+1 SD) showed 2.7 times
greater risk for ACL tear over a four year study period5.
Among athletes who have sustained non-collision ACL
injury, there was a higher level of HF compared with con-
trols.6,7 The higher level of HF is thought to reduce the
passive protection of the ACL by the hamstrings during
actions such as deceleration and landing.6 It has long been
thought that the hamstrings act as a load regulator on the
ACL during anterior tibial displacement.7 Using a cadav-
er knee model, More et al.8 simulated squatting using an
Oxford rig and demonstrated that by adding tension to the
hamstrings there was a significant decrease in anterior tib-

augmenter l’$A_{PLAX}$ à 90° et à 120° autant dans la PF que
dans la PL, les variations étant plus importantes dans la
PL que dans la PF. MKE et SRD augmentaient de façon
significative après l’exercice physique mais n’étaient pas
différents durant les phases du cycle menstruel ou entre
les intensités de l’exercice physique.

Conclusion : L’$A_{PLAX}$ en association avec la hausse de
la SI post-exercice prouve une moins grande stabilité du
genou durant la PL avant et après l’exercice physique.

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MOTS CLÉS : exercice aérobie, ligament croisé
antérieur, amplitude du mouvement de l’articulation,
cycle menstruel, hormone
et al.\textsuperscript{17} compared somewhat similar intensities at strikingly different volumes. They compared males undertaking a 20-minute treadmill run (7km/hr) to those competing in a long-distance aerobic event (variable speed during the 135 km bike and 42 km run) and concluded that $A_{PLAX}$ increased by about 30% following either condition. Whereas discontinuous exercise, such as a 90-minute soccer specific practice increased $A_{PLAX}$\textsuperscript{16}; however, was not compared to continuous running. Comparisons between discontinuous running and continuous running have yielded mixed results.\textsuperscript{11,15} Shuttle runs of various intensities, when compared to a continuous treadmill run in males, resulted in significant $A_{PLAX}$ increases in the discontinuous running group.\textsuperscript{11} Whereas a 90 minute vigorous basketball practice that included shuttle-runs was not dissimilar from a 10 km road run.\textsuperscript{15} Few studies describe exercise activities such as warm-ups, cool downs, and the degree of multi-directional running. Further, none of these studies that incorporated female participants controlled for menstrual cycle phase.

Few studies examining knee laxity extensively tracked or controlled for menstrual cycle phases for the female participants and many used mixed-sex groups, further complicating interpretation.\textsuperscript{18} Although the data on how laxity varies in the menstrual cycle have been contradictory in the most recent systematic reviews, most observational studies agree there is increased laxity around ovulation and during the LP\textsuperscript{18,19}, making high estrogen levels a likely underlying cause of increased female ALC injury rates\textsuperscript{3,18}. Some possible explanations for cycle-dependent laxity changes involve estrogen receptors localized on the ACL\textsuperscript{10,20} and body temperature changes\textsuperscript{21}. The increase of estrogen levels before ovulation and during LP may change the tensile strength of the ACL by decreasing procollagen synthesis thereby making it more susceptible to injury.\textsuperscript{3} The female menstrual cycle presents a complicated set of variables to control, which is the most cited reason for the lack of a clear consensus on cyclic laxity variation. Recent studies have used differing approaches for determining menstrual cycle.\textsuperscript{18} Some investigations assumed stages solely based on reported days since menses, which is inconsistent even among females with regular 28 day cycles, others have measured hormones such as estrogen or progesterone to directly compare hormone concentrations at different cyclic stages.\textsuperscript{22}

Currently, there are no studies that have compared the effects of work-matched moderate and high intensity aerobic exercise on HF and $A_{PLAX}$ in females while also measuring hormone concentrations across cycle phases. Therefore, the purpose of this study is to determine whether moderate intensity continuous versus high intensity discontinuous treadmill running has an influence on $A_{PLAX}$ and HF in eumenorrheic women across LP and follicular phases (FP).

Methods

Subjects
Ten eumenorrheic women were recruited from a university population through flyers, online message boards, and word of mouth to participate in this study. No participant had in the past or were currently taking any form of oral contraceptive or hormonal therapy. Participants with prior knee injury, a body mass index $\geq 30$ kg m\textsuperscript{-2}, or known cardiovascular or pulmonary disease were excluded from the study. Participants were aerobically trained, undertaking $\geq 3$ days and $\geq 150$ min/week of aerobic exercise at a combination of moderate ($\sim 5$-6 METS) to high ($\sim 10$-11 METS) intensities, VO\textsubscript{2peak} above the median for age ($>37.8$ ml kg\textsuperscript{-1} min\textsuperscript{-1}).\textsuperscript{23} The experimental procedures were explained to all participants and informed consent was obtained before testing as submitted to and approved by the Institutional Review Board which are in accordance with the Helsinki Declaration.

Study design
Study participants reported to the lab on six separate occasions (Figure 1). The first ($V_1$) was baseline testing to determine anthropometric and body composition values and peak aerobic capacity. Following this, the participants went through a four-week period where they tracked menstrual cycle and body temperature through a mobile application to determine ovulation. After the control period ended, participants returned to the lab to repeat baseline testing ($V_2$) to determine changes to anthropometrics or fitness over the four weeks between $V_1$ and $V_2$. After the first full menstrual cycle was tracked, the phase (either LP or FP) was identified based on when menstruation and ovulation occurred. Using daily temperature readings, experimenters identified the post-ovulation temperature spike marking the beginning of the LP.\textsuperscript{3} Participants con-
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Continued tracking their menstrual cycle while they completed the experimental phase of the protocol.

Beginning the experimental phase, participants were randomized to one of two exercise conditions using a spreadsheet randomization function (Excel 2016, Microsoft Corp., CA, USA). Conditions were either a high (HIIT; 85%HRR; 30 min; 1 min:1 min run to rest) or a moderate (MOD; 42.5%HRR; 30 min; continuous run) intensity protocol before crossing over to the other condition four days later within the same phase (V_3 & V_4, respectively). During the next menstrual cycle phase, participants were randomized to one of the two exercise conditions again before completing the other four days later (V_5 & V_6, respectively).

Upon arrival to the lab on experimental days, participants sat in a quiet room while a researcher confirmed cycle phase data. A sterile conical vial was given to the participant for unstimulated saliva collection which was immediately frozen for future estrogen concentration analysis. Following this, participants underwent AP_LAX and HF testing. Testing was performed by the same trained investigator who was blinded to the menstrual phase of the participants. Participants then completed the assigned exercise protocol for the visit. Within five minutes of exercise completion, participants underwent another round of AP_LAX and HF testing.

Anthropometric and body composition assessment
Height was measured using a stadiometer (402LB, Health-o-meter, Toledo, OH, USA) and recorded to the nearest 0.5cm. The Prodigy densitometer (GE Healthcare, Madison, WI) was used to assess percent body fat through dual-energy X-ray absorptiometry and was recorded to 0.1%.

Peak aerobic capacity
Aerobic capacity was assessed using the standardized Bruce treadmill protocol. A test was considered successful if three of the following four criteria were met: (1) a plateau (∆VO_2 < 2 mL/min at VO_2_{peak} and the closest neighboring data point) in VO_2, (2) maximal respiratory exchange ratio (RER) > 1.1, and (3) sustained peak heart rate within 10 b/min of the age-predicted maximum (220 – age) for > 1 minute (4) a rating of perceived exertion ≥17. Heart rate was recorded continuously during the protocol and a minimum of four minutes into recovery using a Polar Heart Rate Monitor (Polar Electro Inc., Woodbury, NY, USA). Expired gases were analyzed using a Care Fusion Vmax Encore (Vyaire Medical, Yorba Linda, CA, USA) breath-by-breath metabolic system and was smoothed as 10 second averages.

Menstrual cycle tracking
During the four week period between V_1 and V_2, subjects

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Figure 1.
Schedule of visits.
were provided thermometers to track their daily basal body temperature and menstrual cycle using a mobile ovulation tracking application (Fertility Friend, Tamtris Web Services Inc.). They would record their daily values in the application and then respond to weekly update requests from researchers regarding cycle stages.

**Saliva collection**
Recommendations for improving uniformity of acquisition and analysis of saliva were followed. Briefly, participants were given a sterile 15 milliliter conical tube (Corning, Inc.; Corning, NY) and instructed to passively drool into it for five minutes. Once that time had elapsed, participants capped the tube and gave it to the investigator. Saliva tubes were immediately frozen at -20°C until analysis. Samples were acquired between 7:00-8:00 am for all participants.

**Hormone analysis**
Analysis of salivary estrogen concentration was completed within 90 days of collection via commercially available enzyme-linked immunosorbent assay (ELISA) kit (Eagle Biosciences, sensitivity: 0.5 pg/ml). Samples were done in triplicate according to the manufacturer’s instructions. Intra-assay coefficient of variation was 3.6%.

**Anterior cruciate ligament laxity**
APLAX was measured using a KT 2000 arthrometer (MEDmetric Corp., San Diego, CA). Per device instructions, participants laid supine on a test table with feet positioned in the provided U-shaped foot rest to prevent rotation. A platform was placed under the knee to keep the flexion angle of 25° consistent for the duration of the trials and across participants. Participants were instructed to relax while pulling force was applied and the device’s gauge displayed the anterior displacement of the tibia on the femoral condyles (mm). Tones emitted from the device marked 90N and 120N and an investigator recorded the displacement measurement at each. The same trained researcher performed three trials on the right (arbitrarily chosen) leg (Figure 2).

**Hamstrings flexibility**
Flexibility of the right (arbitrarily chosen) hamstrings was measured before and after each exercise intervention using the following two tests. First, for the 90-90 knee extension test (MKE), the lateral malleolus, lateral epicondyle of the femur, and greater trochanter of the right leg were located and marked with a felt tipped pen for goniometric measurement. Each participant was positioned supine with the right hip and knee flexed to 90°, confirmed with a goniometer. One researcher then held the thigh in place while the participant attempted maximal active knee extension and a second researcher measured the amount of extension with a goniometer. The end point of knee extension (degrees) was recorded as the participants’ maximum active knee extension (Figure 3).

Additionally, a sit-and-reach test (SRD) was performed while the participant was not wearing shoes. The participant sat on the ground with the soles of her feet against a standard sit-and-reach box with a top-mounted ruler. With hands overlapped, palms down, and middle fingers even, the participant stretched forward sliding their hands along the box ruler as far as possible. The fingertip position on...
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the ruler at maximal stretch was recorded as the sit-and-reach distance (cm).

Statistical analysis
Data were analyzed using paired t-tests and a 2 x 2 x 2 [phase x intensity x exercise (pre vs post)] within-participants MANOVA with repeated measures carried out with a least significant difference correction. If significant interactions were detected, a univariate ANOVA was conducted to determine significant changes in the dependent variables. Further analyses were performed using ANCOVA to isolate the individual effects of exercise, menstrual cycle phase, and baseline when values were held constant. A priori significance was set at α≤0.05, and data are reported as mean ± SEM.

Results
There were no significant differences in any demographic characteristic between V₁ and V₂. Results were then collapsed into an average (Table 1).

There was a significantly higher concentration of estradiol in the LP than there was in the FP [p=0.046] with no significant differences within each phase; therefore, the two testing days within the LP (80.27 ± 8.59 pg/mL) were consistent in estradiol concentration as were the two within FP (68.84 ± 6.97 pg/mL).

Pre-exercise AP LAX was significantly greater during LP than FP at 90N [p=0.019] and 120N [p=0.014]. Regardless of phase, both intensities induced a significant AP LAX increase in 90N [p=0.032] and 120N [p=0.022], but not differently between intensities. When controlling for pre-exercise values and exercise intensity, AP LAX in LP was greater than FP at both 90N [p=0.020] and 120N [p=0.011] following exercise.

There was a significant increase in MKE [p<0.001] and SRD [p<0.001] from the pre-exercise to the post-exercise condition after controlling for the effects of menstrual phase and exercise intensity (Table 2).

Discussion
Non-collision exercise and menstrual cycle phase as causes of increased AP LAX and HF are of interest because they are commonly cited ACL-tear risk factors.4-6 Two elements of knee stability that we examined were HF and AP LAX under continuous moderate intensity and discontinuous high intensity treadmill running across both LP and FP. The primary findings of this study were that a baseline difference of AP LAX was evident for both 90N and 120N with the LP displaying greater laxity before exercise. Exercise also increased AP LAX at 90N and 120N in both FP and LP with LP exhibiting larger changes com-

Table 1.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.82±2.72</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.16±5.31</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.81±9.89</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>32.46±5.32</td>
</tr>
</tbody>
</table>
pared to FP. Both MKE and SRD increased significantly following exercise, but not differently across menstrual cycle phases or exercise intensities.

The increase in HF with exercise may be related to the biomechanics of the hamstring muscles during repetitive linear aerobic exercise. As observed in flexibility training, dynamic stretching, in which the muscle is cyclically taken through its range of motion (ROM), has been found to increase skeletal muscle flexibility to a greater degree than static stretching.26 During running, the hamstrings are taken through about 60% of their ROM27,28 and it is possible this movement acts similarly to dynamic stretching, which may explain the similar increases to ROM regardless of exercise intensity. Schache et al.27 were able to find a difference in the magnitude of hamstrings stretch at different intensities in a study comparing short-term, high intensity 110 m sprints at speeds ranging from 7 to 20 mph. At a higher speed, the stride length increase caused the hamstrings muscle to lengthen more. It is possible that if we increased the difference between our intensities, or used relative running speeds as opposed to %HRR, the change in magnitude of the stretch-per-stride would cause a differential effect on the increase in HF.

In a similar way, the increase of AP LAX with exercise may have to do with the loading of the ACL itself. Both running and walking produce anterior/posterior translation of the knee joint29 and therefore ACL loading. Markolf et al.30 used cadaveric knees to quantify the loads on the ACL through the knee’s range of motion and found that anterior tibial loads at full extension and hyperextension produced the highest force on the ACL. Therefore, during our exercise interventions, where the lead leg was near fully extended, the ACL may have undergone “creep,” in which laxity of a ligament increases with cyclic loading.31 Besier et al.32 found that during cutting and side-stepping movements, the ACL experiences even more load than it does during linear running; therefore, these types of exercise may produce a larger change due to loading in different planes. Moreover, the knee may not be stable in all planes of motion in all people.33 ACL tears are often accompanied by injury to other ligaments such as the medial collateral ligament and anterolateral ligament that are essential to planting a foot and rotating while cutting which modifies the forces the knee must resist.33,34 While the ACL provides knee stability near full extension during cutting and tibial rotation, it appears that other structures like the anterolateral ligament take on a greater stabilizing role as the knee flexes.35 The inclusion of varied running patterns may explain the differential findings in field studies that evaluate live play and drilling which may challenge knee stability differently than linear running.16 Future studies may want to restrict movements so that the role of the ACL can be isolated from other ligaments that may be providing stability to the knee joint.

Running-based exercise undertaken as a warm-up and in competition has shown effects on ACL laxity,11,15-17 but

| Table 2  
Flexibility and Laxity. * LP significantly greater than FP pre exercise (p<0.05); b LP significantly greater than FP post exercise controlling for intensity or baseline AP LAX (p<0.05); c Pre-post exercise increase (p<0.05) regardless of cycle phase; d Pre-post exercise increase (p<0.001) controlling for intensity and cycle phase. All data mean±SEM. MOD, moderate continuous; HIIT, high discontinuous; LP, luteal phase; FP, follicular phase; AP LAX, Anterior-posterior laxity; MKE, maximum active 90-90 knee extension; SRD, sit-and-reach score. |
| MOD | HIIT |
| LP | FP | LP | FP |
| AP LAX 90N (mm, pre/post) | 0.77±0.08*/1.09±0.13bc | 0.60±0.03/0.78±0.04c | 0.72±0.08/1.11±0.09bc | 0.66±0.07/0.85±0.08c |
| AP LAX 120N (mm, pre/post) | 1.06±0.11*/1.32±0.17bc | 0.88±0.04/1.09±0.12c | 1.03±0.12/1.43±0.11bc | 0.98±0.07/1.26±0.12c |
| MKE (Degrees, pre/post) | 148.60±7.08/153.8±7.36d | 145.55±6.92/151.55±6.19d | 144.44±8.78/153±7.37d | 143.25±5.96/149.55±7.04d |
| SRD (cm, pre/post) | 19.61±2.96/21.67±2.77d | 20.30±3.17/21.76±2.89d | 20.34±2.97/21.82±2.91d | 17.97±3.08/20.03±3.23d |
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Few studies have clearly identified the durations and intensities undertaken by study participants. Although previous comparisons were not work-controlled, Nawata et al. hypothesized that above a certain intensity threshold, \( A_{PLAX} \) will not increase differentially. Our results agree that it is possible there is a maximum laxity that each individual ACL will reach with exercise based on the structural characteristics of the ACL at the time. Some participants in our study had MOD conditions where the treadmill speed was below the absolute intensity of the participants in our study had MOD conditions where the treadmill speed was below the absolute intensity of the Nawata et al. study which suggests that there may be a minimum that is lower than previously appreciated. More work-controlled studies should be conducted in order to explore this possibility for both males and females. In females, our study found that laxity may have had a different maximum depending on the menstrual cycle phase. Other studies that have added baseline ACL laxity tests during ovulation have agreed that ACL laxity has a significant increase at ovulation and likely in the LP after. We saw non-significant variation in baseline \( A_{PLAX} \) within phases which agrees with some of the better controlled examples. This suggests researchers should at least note menses and the ovulation temperature spike to estimate phase.

This is the first female only, work-equated treadmill study to test whether exercise intensity affects \( A_{PLAX} \) across the phases of the menstrual cycle. We found significant differences in percent \( A_{PLAX} \) increase between LP and FP. While the mean percent change in \( A_{PLAX} \) with exercise was 25.53% at 90N with variability between subjects and trials, when we split the data into phases and co-varied for intensity and baseline laxity, the adjusted mean percent change at 90N for the LP was 56.2% while FP was 12.2%. The menstrual phase did significantly affect the baseline and exercise induced increase in \( A_{PLAX} \) with higher values before and after exercise in LP. This indicates \( A_{PLAX} \) is likely constrained by a certain increase resulting from exercise, but the influence of LP expands that constraint.

Our study had several strengths. Overall, our methods of predicting the phase of the menstrual cycle were confirmed with our quantification of salivary estradiol. Therefore, as we analyzed changes among the two phases, we can confirm that it is truly a LP vs. FP comparison. However, the hormones of the menstrual cycle change even within a phase, so this study does not aim to isolate the effects of the hormones, but rather to analyze the overall effect of a particular phase. The present study employed a cross-over design with a four day break between conditions to avoid connective tissue and muscular changes that might have arisen if we had attempted to test different exercise intensities more frequently. Finally, the recruitment of participants that had never taken any form of oral contraceptive or hormonal therapy was a strength. These therapies can inhibit estrogen surges which may alter the ACL tissue’s responsiveness to changes in the hormone which may reduce ACL tear risk for users by nearly 20%.

The use of a treadmill had a positive outcome on our ability to tightly control running speeds to ensure %HRR goals were being met. However, dissimilarities between treadmill and normal running were a limitation. Participants had to straddle the treadmill belt to begin and end exercise which might have created jarring acceleration and an absence of deceleration at the beginning and end of the exercise bouts. We were limited by the lack of an uphill running condition. Uphill running appears to be of interest as the greater degree of involvement from the hamstrings may have implications for \( A_{PLAX} \). Knee ligament laxity has been shown to increase in the first 15 minutes of moderate exercise and an increased engagement and fatigue of the hamstrings may decrease the passive protection the hamstrings provide the ACL. Our study only investigated posterior to anterior directional laxity of the ACL, which occurs in the sagittal plane. However, often during ACL rupture other ligaments such as the medial collateral ligament and anterolateral ligament are compromised. Future studies should incorporate measures to quantify laxity changes in the frontal plane and knee rotation to better understand risks to injury.

Conclusion
This is the first study to examine equated aerobic exercise workloads in eumenorrheic women across hormone-confirmed menstrual cycle phases. We describe a significant change in baseline \( A_{PLAX} \) between phases and both \( A_{PLAX} \) and HF measures that increase with exercise, regardless of condition. Our findings suggest that engaging in exercise during the LP results in the greatest perturbation to knee stability. The degree to which this change in knee stability increases the chances of knee injury remains to be explored.
References:


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