Constant performance in balance and proprioception tests across the menstrual cycle – a pilot study in well trained female ice hockey players on hormonal contraception

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Abstract
Introduction: It has yet to be explained why female athletes appear to suffer more often from non-contact ligament injuries during the first half of the menstrual cycle. Fluctuations in balance, caused by impaired proprioception due to increased fluid retention, may be relevant factors contributing to this anomaly in distribution. The aim of this study was therefore to uncover relationships between dynamic stability, proprioception and fluid retention in association with the menstrual cycle as a possible explanation for the observed changes in injury rates.

Methods: Nine healthy, female, well trained ice hockey players on hormonal contraception with regular cycles were tested at least twelve times during one menstrual cycle. Bioimpedance analysis was applied to investigate body composition and fluid distribution. A joint position sense test was performed to characterize changes in proprioception, while unexpected perturbations on a balance board were used to obtain measures for dynamic postural control.

Results: No distinct changes in proprioception, dynamic stability and fluid retention were identified across the menstrual cycle in this population. Weak correlations were found between parameters for dynamic stability and proprioception.

Conclusion: Variations in proprioception, dynamic stability and fluid retention seem unlikely to be major contributors to injury risk peaks, at least in this population of healthy trained athletes using hormonal contraception.

KEYWORDS
female athlete, injury risk, balance, body composition, biomechanics, hormones

1 | INTRODUCTION

The general physiological function and associated hormonal changes during the female menstrual cycle are known for decades and in medical text books well documented. However, its specific effects in the context of physical activity and sports performance are still debated. Some studies have shown small effects on athletic performance, as e.g. a reduction in VO₂max in the luteal compared to the follicular phase, or a higher heart rate during sub-maximal exercise in the luteal compared to the follicular phase, while others found no difference. Furthermore, the trainability appears to change during the menstrual cycle, with strength training being more effective, resulting in a higher increase in muscle diameter and maximum strength, when performed in the follicular compared to the luteal phase. This has been explained with the specific hormonal status in the follicular phase.

Female sex hormones have, amongst other factors, been suspected to negatively affect structure and mechanical properties of ligaments, making them more prone to injury, since the incidence of anterior cruciate ligament (ACL) injuries is also in general higher in women than in men. This is why numerous studies have tried to link...
a specific phase of the menstrual cycle associated with specific levels of estrogen and progesterone to the ACL injury risk.\textsuperscript{12-18} However, results from studies differ widely, and even reviews vary in their conclusions.\textsuperscript{19-21} While one review stated that definite conclusions are not warranted regarding the association between menstrual cycle and ACL injury risk,\textsuperscript{21} another review identified a predisposition to injury in the pre-ovulatory phase\textsuperscript{20} and yet another one suggested that the injury risk was increased during the ovulatory phase.\textsuperscript{19}

Serum estradiol concentrations peak in the ovulatory phase, which is prevented by using oral contraception, leading to constant level of serum estradiol concentrations.\textsuperscript{22} However, despite a more constant hormonal profile when using hormonal contraception fluctuations in ACL injury prevalence appear to persist,\textsuperscript{23} although the overall risk has been reported to decrease when using oral contraceptives.\textsuperscript{24,25}

While to our knowledge a reliable explanation for the mechanisms underpinning the observed fluctuations in injury risk with and without the use of hormonal contraception along the menstrual cycle has not been given yet, several factors have been discussed. For example, significant associations between menstrual cycle phase and knee laxity have been observed, with increases during the ovulatory or luteal phases of the cycle in women not using hormonal contraception,\textsuperscript{26} suggesting a change in ligament compliance. However, this could at least be partly due to the ovulation associated rise in body temperature when not using hormonal contraception rather than to changes of the intrinsic properties of the ligament, since menstrual cycle associated differences in muscle and tendon flexibility disappear when the tested leg is warmed to 38 °C.\textsuperscript{27} In contrast, measures for knee laxity such as ACL elasticity, force to flex the knee and knee flexion-extension hysteresis have been shown to be constant in women using oral contraceptives, while differences between contraceptive users and non-users in force to flex the knee and knee flexion-extension hysteresis disappear after warming the leg to 38°.\textsuperscript{22}

More importantly, the adaptation of tendons to training is usually only measurable after a training duration of at least two months.\textsuperscript{26,29} This suggests that significant changes of the morphological properties of this type of tissue seem rather unlikely to occur within a couple of days during a specific phase of the cycle. Thus, changes in the morphological properties of tendons and ligaments along the female cycle due to the changes in the hormonal milieu are unlikely to cause the observed variations in injury rates.

Another factor which could change more rapidly while affecting injury risk is postural control. This has been shown to change during the menstrual cycle, with lateral sway being greater just before and after menses (day 25 and 5 of the cycle) in cycles without using hormonal contraception.\textsuperscript{30} Fluctuations in postural control also occur in women using hormonal contraception.\textsuperscript{31} In fact one study even suggests that postural stability is reduced in contraceptive users compared to non-users.\textsuperscript{32} The mechanism behind this change in postural control is also unclear. However, it might be related to a change in proprioception, as a decrease in knee joint position sense has been reported to occur at the same time, at menses.\textsuperscript{33,34} Again, this change in proprioception might not be directly linked to specific hormone levels, but to accessory phenomenon such as e.g. menses associated fluid retention, affecting sensory feedback from the mechanoreceptors in the muscle. Whilst there was no significant association between estradiol or progesterone levels, self-reported fluid retention scores also peaked at the onset of menses.\textsuperscript{35}

The aim of this study was therefore to identify possible relationships between postural control, proprioception and fluid retention and to observe variations along the menstrual cycle in female athletes on hormonal contraception. If these relationships can be identified, they might provide a better explanation for the observed changes in injury rates along the menstrual cycle. In turn, this might help female athletes and their coaches to address these issues by adjusting their training accordingly.

As subtle changes in performance are both more relevant to as well as easier to identify in elite athletes than in the normal population, the study was conducted with a homogenous group of well-trained female ice hockey players on hormonal contraception. We hypothesized, that total body water would increase around menses, being accompanied by a reduction in joint position sense and measures of dynamic postural control.

2 | METHODS

The study was performed with nine healthy female well-trained (national level) ice hockey players (age: 22 ± 7 years; height: 168 ± 5 cm; weight: 61 ± 6 kg). They were tested during the general preparation period between May and July, while training 10 ± 2 hours per week. The athletes continued with their normal training regime during the study. All players were using hormonal contraception containing a combination of two active substances, estrogen and progesterone. All contraceptives contained Ethinylestradiol as first active substance. When orally taken the concentration of Ethinylestradiol was 0.02-0.03 mg per pill (eight players) and 2.7 mg per vaginal ring (one player). The second active substance was Levonorgestrel (0.1-0.15 mg per pill; 5 players), Chlormadinone (2 mg per pill, two players), Dienogest (2 mg per pill, one player) or Etonogestrel (11.7 mg per vaginal ring, one player).

Local institutional review board (Humboldt University Berlin, Faculty of Humanities and Social Sciences) approval was obtained. Written, informed consent was given, and the study was performed in compliance with the Declaration of Helsinki.

After two pre sessions, which were used to familiarize the subjects with the dynamic stability test to minimize training effects, the subjects were tested twelve to fourteen times during one full menstrual cycle for bioimpedance measures, dynamic stability and sense of joint position. The participants entered the study at a random point in their menstrual cycle, to exclude sequence effects. The first day of the last menses, the next menses and if necessary the second next menses were recorded. The test days were retrospectively allocated to a day in the menstrual cycle, ranging from day 1 as the onset of menses to day 28 as the day before onset of menses.

2.1 | Bioimpedance measurement

For bioimpedance measurements the subjects stood bare feet and lightly dressed on the scale (InBody 720, Biospace Co., Korea). The subjects adopted an upright posture, with each foot being placed on two foot electrodes (heel and forefoot), each hand being in
contact with two hand electrodes (fingers and thumb) and the arms held in an ~15° angle to the upper body. The bioelectrical impedance analysis was performed according to the manufacturer’s instructions. Briefly, 30 impedance measurements per subject were performed using 6 different frequencies, from which segmental resistance was calculated. The following output parameters are reported as absolute values: body mass [BM, kg], skeletal muscle mass [SMM, kg], fat mass [FM, kg], total body water [TBW, l], extracellular water [ECW, l] and intracellular water [ICW, l].

In terms of accuracy of the eight-point tactile-electrode impedance method, the coefficient of variation for segmental resistance has been reported to be ≤ 2.8 %, calculated from three measurements performed on five consecutive days in 50 men and women. Measuring six women five times within one day the coefficients of variation have been calculated to be 0.1 ± 0.2% for BM, 0.4 ± 0.2% for SMM, 1.0 ± 0.4% for TBW, 0.3 ± 0.2% for ICW and 0.4 ± 0.2% for ECW.

2.2 | Proprioception

In order to obtain a quantifiable measure for proprioception, a joint position sense (JPS) test was performed, which records the awareness of the joints static location in space. A JPS test for the upper limb was developed, allowing marker-less assessment of three angles by image recorded angulation. The subjects were asked to adopt the following target position with closed eyes: standing straight (0° deviation from gravitational axis), arms held at the side with a 90° angle between upper body and upper arm, and 90° angle between forearm and upper arm (Figure 1, A-D). A digital image of the subject's frontal plane was taken under standardized conditions, with fixed positions of subject and camera.

Quantitative objective image analysis was performed using Matlab (R2015a, 64 Bit, The Mathworks, Natick, USA), to obtain the angles between forearm and upper arm (α), upper arm and longitudinal body axis (β) and longitudinal body axis and gravitational axis (γ). For image analysis the subject was isolated from the background by converting the original red-green-blue (RGB) image (Figure 1A) to grayscale. The edges of the subject were found using the Canny method (Figure 1B). The longitudinal body axis was determined by two points, the midpoint of the upper part of the body in waist height and the midpoint between both thighs close to the knees. The orientations of the arm segments were calculated by regression lines out of the midpoints between the outer edges of the forearm and the upper arm. Left and right half of the body were analyzed separately.
2.3 Dynamic postural control

In order to quantify dynamic postural control, the balance platform 
Posturomed (Haider Bioswing GmBH, Germany) was used. The 
Posturomed is widely applied as both training device\textsuperscript{38} and diagnostic 
tool.\textsuperscript{39} A good inter- and intra-day reliability has been reported for the 
dynamic balance test on the Posturomed with intra-class correlation 
coefficients ranging between 0.713 and 0.970.\textsuperscript{40} This vertically 
suspended, unstable balance platform, which is mounted to eight 
springs and has been described in detail elsewhere,\textsuperscript{41} was equipped 
with a lever-based provocation unit allowing unexpected horizontal 
perturbations to be applied in a standardized manner. The Posturomed 
was further fitted with the associated accelerometer to record oscilla-
tions, using the provided software (MicroSwing 5.0, Haider Bioswing 
GmBH, Germany). For the measurements, the subjects were asked to 
stand bare feet on one leg in the center of the platform, with the knee 
of the supporting leg slightly bend, the thigh of the free leg being 
parallel to the supporting leg, and the hands placed on the hips, looking 
straight ahead. Two tests per leg were executed with the perturbations 
occurring once from medial and once from lateral, and the mean was 
calculated for each leg. The unexpected perturbation was triggered 
randomly within the first 5 seconds of each test. After perturbation 
the subjects were asked to regain balance and to stop the platform 
from oscillating as quickly as possible without using their arms. The 
subjects then continued to stand steadily on the platform until the 
end of the test period 20 seconds from perturbation. Time dependent 
data were recorded for platform movements in the x and y axis at a 
frequency of 50 Hz, analyzed with Matlab (R2015a, 64 Bit), from which 
four parameters were derived: 1) threshold, defined as maximum 
excursion in x direction during the regained balance phase [undeter-
dined units], was determined individually for each subject within a 
time period of 7 seconds to the end of the test period. This threshold 
represents postural sway during balance; 2) time, defined as the time 
passed between the start of perturbation and reaching the threshold 
value in x direction [s]; 3) distance, defined as the length of the curve 
between the perturbation and the threshold in x or y direction 
[undetermined units] and 4) area, defined as the integral between the 
perturbation and the threshold in x or y direction [undetermined units] 
(Figure 1E).

2.4 Statistical analysis

A linear mixed-effects model (LMM) was applied to detect statistically 
significant changes in the investigated parameters over the course of 
the menstrual cycle using Matlab (R2016a, 64 Bit, The Mathworks, 
Natick, USA). The LMM considers fixed effects ($\beta_k$) describing population 
parameters, and random effects ($b_{ik}$) which are associated with intrasubject differences over time.\textsuperscript{42} A biquadratic LMM which also 
includes a constant, linear, squared and cubic term was created using the 
function fitlmematrix. Taking into account the small sample size the 
function REML (restricted maximum likelihood method) was used 
to estimate the variance components. The model coefficients were 
tested combined using the hypothesis test coefTest with $H_0$: $\beta_1 = \beta_2$ 
$= \beta_3 = \beta_4 = 0$. If the coefficients $\beta_k$, $k \in \{1,...,4\}$ are equal to zero the 
model is reduced to the constant term. The reduction to the constant 
term indicates an even distribution of the data with no time dependent 
effect.

$$
\gamma_{im} = \beta_0 + b_{0i} + \beta_{i1} m + \beta_{i2} t_{im} + \beta_{i3} t_{im}^2 + \beta_{i4} t_{im}^3 + \beta_{i5} t_{im}^4 + \beta_{i6} t_{im}^5 + \beta_{i7} t_{im}^6 + \beta_{i8} t_{im}^7 + \beta_{i9} t_{im}^8
$$

\begin{table}[h]
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Constant & $\beta_0$ & $\beta_{i1}$ & $\beta_{i2}$ \\
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To establish relationships between parameters describing postural 
control, proprioception and body water independent of the menstrual 
phase, the Pearson correlation coefficient was calculated. For all statisti-
tical tests, significance was established at $P \leq 0.05$.

3 RESULTS

3.1 Body composition

The athletes gained on average 0.37 kg skeletal muscle mass and lost 
0.31 kg body fat during the test period, while their mean body mass, 
skeletal muscle mass and fat mass did not significantly change across the 
menstrual cycle (BM: $P=0.44$; SMM: $P=0.66$; FM: $P=0.46$; Figure 2A). Over all athletes, the mean deviation from the individual 
mean body mass was 0.7 ± 0.5 %, with a maximum individual deviation 
of 2.9 % or 1.5 kg, the mean deviation from individual mean fat mass 
was 3.8 ± 3.1 %, and the mean deviation from individual mean skeletal 
muscle mass was 1.3 ± 1.0 %. The mean relative skeletal muscle mass 
and mean relative fat mass were 44.4 ± 4.4 % and 20.3 ± 3.2 % relative 
to total body mass.

Regarding water distribution along the menstrual cycle the vari-
able total body water and intracellular water did not vary (TBW: 
$P=0.13$; ICW: $P=0.75$), with the mean deviation from individual total 
body water being 1.2 ± 0.9 %, and the mean deviation from individual total 
intracellular water being 1.2 ± 1.0 %. Significant changes were 
detected for extracellular water ($P=0.01$; Figure 2B) with the mean 
deviation from individual extracellular water being 1.5 ± 1.3 %. 
Assessing the fixed effects coefficients $\beta_k$, $k \in \{0,...,4\}$ separately a 
tendency ($P=0.09$) for a linear trend was observed, with an incline of 
0.01 l/day, while the constant model was still the best fit to describe the 
data ($P=0.001$).

3.2 Proprioception

The deviation from the anticipated target joint position was largest for 
the angle between upper arm and longitudinal body axis ($\beta$) with a 
mean deviation of 13.0 ± 6.1°, followed by the angle between forearm
3.3 | Dynamic postural control

Since there were no significant differences between the dominant and the non-dominant leg for all measured parameters, the mean of both legs was calculated. The mean values for distance, time, area and threshold during regained balance did not vary significantly along the menstrual cycle (area: P=0.46; distance: P=0.51; threshold: P=0.46; time: P=0.35; Figure 4).

3.4 | Relationships between postural control, proprioception and body water

No correlations were detected between body water and the parameters for proprioception or dynamic postural control. In terms of postural control and proprioception, weak but significant correlations were found between the parameter time and the angle $\beta$ ($r=-0.25; P=0.012$), and between the parameter threshold and the angle $\gamma$ ($r=0.22; P=0.023$).

4 | DISCUSSION

In contrast to our hypothesis, no significant variations in body water, proprioception or dynamic stability were detected along the
hormonally controlled menstrual cycle. Body water, proprioception and dynamic stability do not appear to be in close relationship to each other. Although two significant correlations were detected, the strength of these were weak. This suggests that a variety of other variables affects this relationship.

Fluid retention is one of the most common side effects linked to the menstrual cycle, with edema being localized not only in the breasts but also in upper and lower limbs. Fluid retention is one of the most common side effects linked to the menstrual cycle, with edema being localized not only in the breasts but also in upper and lower limbs.43 Since it is only possible to measure the water content of different parts of the human body indirectly, several techniques have been applied to assess it using volume44 and circumference43 measurements, bioelectrical impedance analysis45 or self-reported rated impressions.35 It is a common finding that values increase during the second half of the cycle,43-45 peaking at the onset of menses,35 while the exact amount of the additional fluid is difficult to specify. For the breast an additional 100 ml have been measured44 and ~500 ml for total body water,45 with self-reported fluid retention peaking at 0.9 on a 0 (none) - 4 (very intense) scale.35 First, this indicates that the effect might be small, and could thus be missed in smaller populations. Secondly, fluid retention can be reduced44 or completely abolished45 through oral contraceptive intake. In our study a linear trend for an increase in extra-cellular water along the menstrual cycle was detected. If this trend was real it might not be physiologically relevant as the incline was just 10 ml/day. Since the participants in our study were on hormonal contraception it is likely that at least some of them were not affected by fluid retention at all. Furthermore, exercise has been shown to reduce edema,46 thus the training in itself could have led to a reduction in fluid retention, thereby overriding the effects of the menstrual cycle. This could especially be the case for female athletes following a highly systematic and frequent training routine – as it was the case for the participants in our study. While the manufacturer of the bioimpedance scale recommends to measure women outside of menses, the remarkably constant body composition of our participants suggests that in a population such as ours muscle and fat mass can be reliably measured independent of the day in the menstrual cycle. Except for the individual variation in fat mass, which was with 3.8 % slightly higher than in the other parameters, mean individual variations were between 0.7 % and 1.5 %, thereby ranging above the intra-day coefficient of variation calculated by ourselves and below the reported inter-day coefficient of variation reported to be 2.8 %.36 These 2.8 % might however not entirely represent measurement error, as the intra-day variation in that study was measured on five consecutive days, which is a timeframe in which physiological changes in body composition can occur.

Regarding proprioception contradictory results have been reported. While Fouladi et al.33 reported a decreased knee joint position sense accuracy at menses, Hertel et al.47 found no differences across the menstrual cycle. Both studies analyzed a similar sized group of young physically active women not using hormonal contraception, although with slight differences in the testing procedure. To our best knowledge, there are no studies on the effect of hormonal contraception on the joint position sense. The subjects in the above mentioned studies were regularly exercising or even competing, so a specific training regime or a competition prior to testing could have resulted in muscle fatigue, thereby influencing the joint position sense. The effect of a concomitant training on proprioception was reduced in our study, as the participants followed a stable training routine over the course of the data collection period that they were familiar with. In addition, the use of hormonal contraception in our study might also have stabilized the joint position sense.

Similar to proprioception, contradictory results have also been reported for postural stability. An increase in lateral sway just before and after menses compared to the rest of the cycle suggests postural...
stability to be affected by the menstrual cycle. However, several other studies could not detect any significant variations in postural stability across the menstrual cycle. Interestingly, this appears to change when women suffer from premenstrual symptoms. Postural sway is greater and kinaesthesia reduced in women with premenstrual symptoms compared to women without premenstrual symptoms.

Postural sway is also greater in the luteal compared to the follicular and ovulatory phase in women suffering from premenstrual symptoms. Since aerobic exercise has been reported to reduce premenstrual symptoms, including fluid retention, it is conceivable that female athletes, as in our study, are in general less affected by menstrual cycle associated changes in postural control. Also oral contraceptives have been reported to reduce premenstrual symptoms, thereby possibly also reducing negative effects on postural control. However, if premenstrual symptoms still persist in spite of oral contraceptive intake the postural sway area in one-legged stance has shown to be elevated during the active phase of treatment, while it remained constant in oral contraceptive users without premenstrual symptoms.

Overall it was noticeable, that previous studies did not detect direct correlations between hormone levels and measures of postural control, proprioception or fluid retention. Similarly, tendon stiffness and knee laxity were not correlated to hormone levels. Thus it might be too simplistic to assume a direct relationship between those variables and menstrual cycle associated hormone levels. This might indicate that factors secondary to hormone levels or a combination of several factors are responsible for the sometimes observed fluctuations in postural control, proprioception or fluid retention during different phases of the menstrual cycle. Likewise, the observed fluctuations in injury risk might be affected by multiple variables. While the mechanical properties of the musculoskeletal system, balance and proprioception are likely candidates to be amongst those variables,

FIGURE 4  Quantification of dynamic stability along the menstrual cycle by using unexpected perturbations on a balance platform. Analysed parameters were: A) distance, B) area, C) time and D) threshold. Grey symbols represent individual values. Black symbols represent mean values over all subjects within two days of the menstrual cycle.
other contributing factors might not have been identified or investigated yet. E.g. behavioural changes, which have been reported to be associated with certain phases of the menstrual cycle, might as well have an effect on the injury risk.

One limitation of the study is that it was not possible to differentiate between oral contraceptive users and non-users, as we were not able to recruit enough non-contraceptive users. This also means that the results of this study are predominantly applicable to athletes using a hormonal method of contraception. Also, hormone levels were not determined, and thus no correlations between hormone levels and the investigated parameters were analyzed. However, previous studies already failed to show these correlations as described above, so we refrained from investigating them. Furthermore, changes in the investigated variables still might have been occurred, being too small to be detected with the small sample size and by measuring only one menstrual cycle.

5 | PERSPECTIVE

Our ice hockey players constant performances in the executed tests suggests that most healthy young women on hormonal contraception are unlikely to be affected by major fluctuations in proprioception, dynamic stability and fluid retention along the menstrual cycle. It appears that the effect of hormonal changes along the menstrual cycle, often given as an explanation why a study population is restricted to men, might have been overestimated in general.

In conclusion, regarding the parameters investigated in our study, one should be able to include healthy young women on hormonal contraception in a study population, without having to Fear hormone associated fluctuations. We furthermore conclude that fluctuations in balance or proprioception within the menstrual cycle are either nonexistent or so small, that they should not have to be taken into account by athletes and their coaches when planning training or competition.

However, fluctuations might still occur in individual cases or in association with other factors, such as e.g. the premenstrual symptom. The challenge remains to identify the specific ones at risk to suffer from menstrual cycle associated performance impairment or injury. Simple cause and effect relations failed so far to provide a sufficient explanation for menstrual cycle phase associated injuries. Thus, future studies might have to apply complex analytic methods including multiple independent variables to uncover the factors responsible for fluctuations in injury rate and performance.

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DISCLOSURE STATEMENT

In accordance with ethical obligations as researcher, the authors report no conflicts of interest that may affect the research reported in the enclosed paper. We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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