



**HAL**  
open science

## Exploring the effect of the menstrual cycle or oral contraception on elite athletes' training responses when workload is not objectively quantifiable: the MILS approach and findings from female Olympians

Quentin de Laroche Lambert, Imad Hamri, Tom Chassard, Alice Meignié, Florent Storme, Marine Dupuit, Allison Diry, Jean-François Toussaint, Pierre-Yves Louis, Nicolas Coulmy, et al.

### ► To cite this version:

Quentin de Laroche Lambert, Imad Hamri, Tom Chassard, Alice Meignié, Florent Storme, et al.. Exploring the effect of the menstrual cycle or oral contraception on elite athletes' training responses when workload is not objectively quantifiable: the MILS approach and findings from female Olympians. *BMJ Open Sport and Exercise Medicine*, 2024, 10 (2), pp.e001810. 10.1136/bmjsem-2023-001810 . hal-04621045

**HAL Id: hal-04621045**




**<https://hal.science/hal-04621045v1>**

Submitted on 23 Jun 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Exploring the effect of the menstrual cycle or oral contraception on elite athletes' training responses when workload is not objectively quantifiable: the MILS approach and findings from female Olympians

Quentin De Laroche Lambert <sup>1,2,3,4</sup> Imad Hamri,<sup>1</sup> Tom Chassard,<sup>1</sup> Alice Meignié <sup>1</sup>, Florent Storme,<sup>1</sup> Marine Dupuit,<sup>1</sup> Allison Diry,<sup>2</sup> Jean-François Toussaint,<sup>1,5</sup> Pierre Yves Louis,<sup>4,6</sup> Nicolas Coulmy,<sup>3</sup> Juliana da Silva Antero <sup>1</sup>

**To cite:** De Laroche Lambert Q, Hamri I, Chassard T, *et al.* Exploring the effect of the menstrual cycle or oral contraception on elite athletes' training responses when workload is not objectively quantifiable: the MILS approach and findings from female Olympians. *BMJ Open Sport & Exercise Medicine* 2024;**10**:e001810. doi:10.1136/bmjsem-2023-001810

► Additional supplemental material is published online only. To view, please visit the journal online (<https://doi.org/10.1136/bmjsem-2023-001810>).

Accepted 21 May 2024



© Author(s) (or their employer(s)) 2024. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

## Correspondence to

Dr Quentin De Laroche Lambert; Quentin.DELAROCHELAMBERT@insep.fr

## ABSTRACT

**Objectives** Develop the Markov Index Load State (MILS) model, based on hidden Markov chains, to assess athletes' workload responses and investigate the effects of menstrual cycle (MC)/oral contraception (OC), sex steroids hormones and wellness on elite athletes' training.

**Methods** On a 7-month longitudinal follow-up, daily training (volume and perceived effort, n=2200) and wellness (reported sleep quality and quantity, fitness, mood, menstrual symptoms, n=2509) data were collected from 24 female rowers and skiers preparing for the Olympics. 51 MC and 54 OC full cycles relying on 214 salivary hormone samples were analysed. MC/OC cycles were normalised, converted in % from 0% (first bleeding/pill withdrawal day) to 100% (end).

**Results** MILS identified three chronic workload response states: 'easy', 'moderate' and 'hard'. A cyclic training response linked to MC or OC (95% CI) was observed, primarily related to progesterone level (p=8.23e-03 and 5.72e-03 for the easy and hard state, respectively). MC athletes predominantly exhibited the 'easy' state during the cycle's first half (8%–53%), transitioning to the 'hard' state post-estimated ovulation (63%–96%). OC users had an increased 'hard' state (4%–32%) during pill withdrawal, transitioning to 'easy' (50%–60%) when on the pill. Wellness metrics influenced the training load response: better sleep quality (p=5.20e-04), mood (p=8.94e-06) and fitness (p=6.29e-03) increased the likelihood of the 'easy' state. Menstrual symptoms increased the 'hard' state probability (p=5.92e-02).

**Conclusion** The MILS model, leveraging hidden Markov chains, effectively analyses cumulative training load responses. The model identified cyclic training responses linked to MC/OC in elite female athletes.

## INTRODUCTION

Sex hormones steroids, namely oestrogens, progesterone and testosterone may impact female athletes' training and wellness.<sup>1 2</sup>

## WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Athletes' workload quantification aims a better training periodisation to improve performance and prevent injuries.
- ⇒ Robust models capturing the stochastic responses of elite athletes to workload, particularly in sports whose load is not objectively quantifiable are currently lacking in the sports science context.
- ⇒ The menstrual cycle (MC) and hormonal contraception phases may influence the response to workload in female athletes.

## WHAT THIS STUDY ADDS

- ⇒ We developed a novel algorithm based on robust mathematical methodology, that is a pioneering approach to identify states of fatigue responses to workload (even when such load is not objectively quantifiable).
- ⇒ A cyclical pattern in training responses, associated with the MC or oral contraception (OC), reveals an infradian rhythm in elite female athletes.
- ⇒ The progesterone concentration is the sex-steroid hormone associated with the diminished adaptative reserve state of workload response identified by Markov Index Load State.
- ⇒ Wellness indicators, such as a better self-reported sleep quality, mood and fitness, influence positively the workload response while the menstrual symptoms influence negatively such responses.

These hormones fluctuate along the natural menstrual cycle (MC) and during hormonal phases of combined oral contraceptive pills (OC).<sup>1 3</sup> However, studies investigating the effect of MC or OC on elite athletes training responses and wellness remain scarce<sup>4</sup> and face several methodological issues.<sup>5</sup> Many debates have been held to properly define the

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ The developed model offers a novel perspective for studying athletes' workload responses, considering complex parameters such as the MC and OC.
- ⇒ Sport staff can use the algorithm to optimise and anticipate training responses paving the way for more personalised training programmes.
- ⇒ Considering athletes' hormonal cycles and wellness in training periodisation might be informative, especially when planning heavy training loads at critical moments.

MC phases.<sup>6</sup> Yet, considering the cycle as a continuum of hormonal fluctuations, where every single day (or time point) would be worth testing, has been preconised in medicine<sup>7</sup> but has not yet been done in sports setting.

Studies relying on objective data measurements such as inertial data,<sup>8–10</sup> which captures movement and acceleration, have shown significant impact of MC on training or competition load parameters. Yet, most sports, such as skiing or rowing, face the challenge of quantifying workload through subjective measures, making even more difficult to estimate the complex effect of MC or OC on athletes' daily responses to training. In order to disentangle complex hormones-performance interactions among a network of determinants, it has been suggested to search for specific relationship patterns under a complex system model.<sup>7</sup> Hence, a longitudinal monitoring in situ (ie, measurements are made in the field, directly within the context of interest) seems to be the most relevant design to investigate training responses considering the continuum of hormonal fluctuations during MC (and OC).

Historically, various mathematical models like TRIMP, Foster, ACWR, EWMA and REDI have been employed to gauge workload among elite athletes.<sup>11–17</sup> However, these indicators quantify the workload of a training session, or of a chronic or acute phase of workload,<sup>13</sup> but cannot estimate the response to this load. In a real-world process evolving over time, the relationships between relevant variables evolve, requiring different inference models for each state of the workload responses. The possible states that athletes go through when adapting to training loads may be considered as part of a stochastic process. A hidden Markov model (HMM) fulfils this dynamic requirement, expressing trends as a latent variable.<sup>18</sup> While HMMs have found applications in diverse fields,<sup>19–22</sup> their use in analysing athlete workload responses remains uncharted.

This study aims to:

1. Develop and implement a model of cumulative workload indicators based on an HMM to estimate latent states representing workload responses.
2. Investigate the continuous impact of MC/OC, sex steroid hormones and wellness on the workload responses of elite athletes in preparation for the Olympic and Paralympic Games, through a longitudinal monitoring in situ.

## METHODS

### Design of study

A longitudinal prospective study was conducted to follow-up daily multiparametric data of elite athletes through assessments on the field during their final preparation for the Summer and Winter Games.

### Participants

We asked the French Federation of skiing and rowing to propose to participate in the study the female athletes in preparation to the Olympic and Paralympic Games of Tokyo in July 2021 and the Winter Games in February 2022. All 16 skiers (biathlon, alpine skiing and cross-country skiing) and 12 rowers (out of 14), including 3 Paralympic athletes, volunteered to this study. The a posteriori inclusion criterium was naturally menstruating women with regular cycles, defined as a cycle length comprised between 22 and 35 days<sup>23</sup> and a length variation lower than 7 days during the entire study follow-up, without any diagnosed cycles disorders; or users of oral combined pills ( $\geq 3$  months prior to recruitment) implying a phase of pill taking (active pill phase) and pill pause (non-active phase)<sup>23</sup> (ie, excluding continuous hormonal contraception). The athletes were asked to fill in a preliminary questionnaire that collected general information (age, body mass index, training volume) and gynaecological history (eg, cycle regularity, contraceptive methods) to identify any prior contraindications to participate in the study.

### Data collection

The longitudinal follow-up (ie, daily monitoring of multiparametric data) of the included participants started with the beginning of the Olympic season final preparation; rowers were included in February 2021 and skiers in August 2021. Both follow-ups lasted up to 7 months. During the entire follow-up time, the athletes were asked to complete a questionnaire every morning, and after every training session, using an online questionnaire created for this purpose. This questionnaire, as well as the entire protocol, was presented and explained to the athletes beforehand.<sup>1</sup> The questionnaire included items related to the beginning/end of menses or the pills start/withdrawal; menstrual symptoms (eg, stomach cramps, mood changes, headaches, as listed before<sup>24</sup>), and used Likert rating scales<sup>25 26</sup> to quantify reported wellness (sleep quality, fitness, mood), and declared sleep duration. After each training session, the rated perceived exertion (RPE),<sup>27 28</sup> and the training volume (minutes) were collected. A researcher monitored daily data of all athletes, promptly addressing any inconsistencies. Abnormal or suspicious data (eg, symptoms identified as menstrual but occurring non-cyclically across different MC phases, or concomitantly to a disease) were discussed with participants, and adjustments or exclusions were made if needed to ensure reliable data collection.

## Sex hormones measurements

The first athletes to start the longitudinal follow-up in February 2021 were the rowers in preparation for the Summer Olympics and Paralympics Games. Fasting salivary samples for hormones including 17 $\beta$ -oestradiol, progesterone and free testosterone were collected immediately on waking (at 07:47 $\pm$ 01:13) on days 8, 14 and 24 $\pm$ 2 of the MC for the natural MC. The first cycle among MC group was used to individually determine the exact days of hormone sample testing. Then, this sampling was repeated over the subsequent 4 months of follow-up, to capture the largest differences in hormone levels between the mid and late follicular phases and to identify increased progesterone levels during the mid-luteal phase. Hence, we aimed 12 hormonal tests in the MC group per athlete. In the OC group the regimen was similar but only for two full cycles, aiming six tests at total per OC athlete.

The skiers commenced the follow-up in August 2021 during their final preparation for the Winter Games. Fasting salivary hormone samples were collected under similar conditions of rowers, that is, just after waking up (08:48 $\pm$ 00:40), but following a different regimen: on days 3, 8 and every 2 days thereafter for MC group. This was done to obtain a more precise definition of hormonal fluctuations across a cycle, following the observed consistency in hormone dynamics collected among rowers across different cycles. Hence, we aimed 12 hormonal tests in the MC group per athlete. In the OC group five hormonal tests equally spaced during a 28 days period were performed.

The salivary samples were analysed through the luminescence Immunoassay method by a commercial lab and followed their procedures of storage and transportation. More details are provided in online supplemental file.

## Cycles' continuum

The first day of the cycle was determined as the first bleeding day in the MC group or the first day of pills pause in the OC group.

To consider the cycles a continuum phenomenon we transformed the cycle length into a proportion of advancement in the cycle to consider the cycles' continuum.<sup>7</sup> Let  $T = \{t_1, \dots, t_n\}$  be the set of times available for cycle  $i$ , we calculate the normalised time  $t'_i = \frac{t_i}{t_n}$  with  $t_i$  on the  $i$ th day and on the last day of the cycle. The same procedure was done for the OC cycles, ranging from day 1 to day 28.

To locate the phases of OC pills in the continuum analysis they were represented as the non-active pill phase and active pill phase<sup>29</sup> and it was not expected to observe large hormonal fluctuations between the active and non-active pill's phases.

To locate the schematic subphases used in previous studies<sup>30</sup> within this cycles' continuum, the follicular (preovulatory) and luteal (postovulatory) phases were schematically divided in three subphases: early, mid and late, as detailed previously.<sup>1</sup> An estimate, hence, not a validation of the MC group ovulation relied on a linear

regression model based on the cycle length of more than 30 000 women with confirmed ovulation.<sup>31</sup>

The early follicular phase corresponds to the days of menstrual bleeding when hormone levels are theoretically expected to be at their lowest. The mid-follicular phase occurs between menstruation and the late follicular phase when oestrogen levels are expected to peak. The mid-luteal phase is characterised by peaks in both oestrogen and progesterone levels, which diminish during the premenstrual phase. The early luteal phase encompasses the days between estimated ovulation and the mid-luteal phase.

These hormones dynamics distribution were analysed to properly characterise such schematic phases' estimation within our cycles' continuum for both groups. We therefore carried out an additional analysis by calculating the quantiles of order 0.25, 0.5 and 0.75 of the levels of each hormone according to the estimated phases of the cycle. To identify whether these hormones differed depending on the calendar-based phases in MC and active/inactive pills' phases in OC, we performed a Kruskal-Wallis test. If the test was significant, we performed a post hoc test with a Bonferoni adjustment to identify the differences between each phase. The significance threshold was set at 0.05.

## Markov Index Load State

In order to develop a cumulative load indicator representing a proxy for the athletes' response to the workload we created the Markov Index Load State model (MILS). This is an area-divided cumulative load indicator based on hidden Markov chain (HMM). MILS is a mathematical algorithm allowing to identify the athletes' responses to workload. This response is classified into three states. The algorithm is based on probabilities of transitioning between these three states each day following the workload regimen. The mathematical theory of the MILS algorithm is detailed in online supplemental materials.

## Model features

### Observed variable

The observed variable of the model is the workload  $C$ . Let  $I_i(t)$  and  $D_i(t)$  be the RPE and the training duration of the individual  $i$  at time  $t$ . We quantified the daily workload, meaning the training or competition load by the RPEsession (defined as RPE $\times$ training duration)<sup>14</sup> for each individual  $i$  and each day  $t$  defined by  $C_i(t)$ :

$$C_i(t) = I_i(t) \cdot D_i(t)$$

with  $I_i(t)$  and  $D_i(t)$  the RPE and the duration of the D-Day training.

The distribution of these two parameters being variable according to the individual's perception, readiness and training programme, we normalised the workload for each individual  $i$ :

$$C'_i(t) = \frac{C_i(t) - \mu(C_i)}{\sigma(C_i)}$$



with  $\mu(C_i)$  and  $\sigma(C_i)$  being respectively the mean and the SD of all workloads  $C$  for individual  $i$ .

### Calculation of state probabilities

At each time  $t$ , each individual displays a probability of being in each of the three state of training load response. The sum of the probabilities is equal to 1. Each day a probability for a given athlete to be in each of these hidden states is calculated considering their previous state (hence, their total cumulative pathway) using the forward backward algorithm<sup>32 33</sup> based on the transition matrix, the emission matrix and the initial probability vector previously estimated. The expectation-maximisation algorithm is used to estimate the model parameters.<sup>34</sup>

### Number of hidden states

The selection of the number of hidden states in a hidden Markov chain model is complex,<sup>35</sup> especially when the states are concretely interpretable. Indeed, the usual criteria such as Akaike information criterion and Bayesian information criterion tend to select the model with many of hidden states.<sup>36</sup> We have chosen to estimate three hidden states in the MILS algorithm to be able to identify a highest (hard) and lowest (easy) level and an intermediate state, in order to have interpretable states of physiological states as in General Adaptation Syndrome model.<sup>37</sup> We consider  $S = \{S_1, S_2, S_3\}$  the  $N = 3$  states of the model.

### Relationship to the studied variables

#### Fitness questionnaire/hormonal variables

For each wellness variable (ie, sleep quantity, quality, mood, fitness and symptoms) we used three mixed linear models for each state to account for inter-individual variability.

To estimate the association among the hormonal variables (ie, 17 $\beta$ -estradiol, progesterone and free testosterone) and the MILS, we adjusted the models by cycle phases, as hormone levels are cycles' phase-dependent. We added a random effect related to the cycle phase to isolate the influence of each hormone.

The fixed coefficient indicates if the hidden state positively or negatively correlates with the explained variable. A t-test was used to test if the coefficient is different from 0. We set the type I error rate at 0.1 (\*), 0.05 (\*\*), and 0.01 (\*\*\*).

### Workload responses during the MC or OC

For each of the states  $S_1$  and  $S_3$ , a non-parametric Loess regression<sup>38</sup> was performed to explain the progress of the cycle (cycles' continuum) as a function of the hidden state. We then calculated the average value estimated by the model at each instant of the cycle and as its CI.

In the OC group, only the cycles under the most common type of pill was used for this analysis for more homogeneity.

## RESULTS

### Participants

After 7 months of longitudinal daily monitoring, four athletes were excluded: one has stopped the pills during the study and three with natural cycles presented at least one irregular cycle during the follow-up. Then, data of 24 athletes, 24.6 $\pm$ 5.21 years old (12 skiers; 12 rowers (3 Paralympic)) followed for 7.1 months $\pm$ 2.6 on average were analysed. A total of 105 full cycles were studied including 51 naturally menstruating and 54 cycles under combined contraceptive pills for 21 days with a 7-day break. Regarding the OC group, there were six monophasic, two biphasic and two triphasic pill types. Hence, only monophasic pills were used for the workload responses analyses across the OC cycles. At total, 2509 wellness questionnaires and 2200 training and competition data were collected. The compliance rate, related with double questionnaires everyday was of 0.54 (ranging from 0.17 to 0.92), indicating that all athletes answered these questionnaires, on average, every 2 days for about 7 months (or at least one questionnaire everyday), which we consider to be a high compliance rate. The missing answers to the questionnaires were mostly due to random forgetfulness. Therefore, we consider that the data are missing at random and therefore do not interfere with the reliability of the results of the study. A total of 214 hormonal salivary samples were performed and validated by the commercial laboratory.

### MILS model features

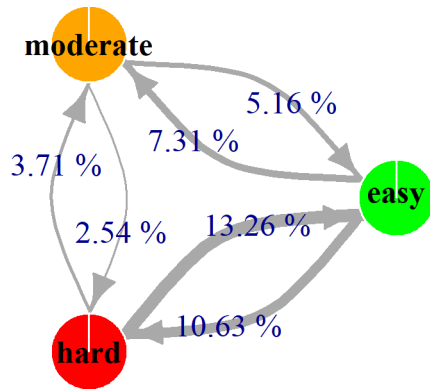
For a detailed theoretical background on the HMM used in this study, refer to online supplemental materials.

### Hidden state

We estimated the initial probability vector  $\pi(1) = \begin{bmatrix} 0.32 & 0 & 0.68 \end{bmatrix}$  containing the probabilities of being in each state at time  $t_1$ . Gaussian conditional density means are estimated by -0.74, -0.01, 0.79 and the SD are estimated by 0.52, 0.75, 1.1 for states  $S_1$ ,  $S_2$  and  $S_3$ , respectively.

This means that regardless of the state probabilities of time  $t - 1$ , the greater the load of day  $C_i$ , the greater the probability of being in  $S_3$  state. Conversely, the smaller  $C_i$ , the greater the probability of being in  $S_1$  state. State  $S_2$  appears as an intermediate state between  $S_1$  and  $S_3$ . The  $S_1$  state is associated with a low cumulative load suggesting an appropriated adaptative reserve state, while the  $S_3$  state is associated with a higher cumulative load suggesting a state with diminished adaptative reserve or RPE fatigue related. The  $S_2$  state appears as an intermediate area of increased workload to which the body may adapt. Knowing the emission probabilities, we renamed the states as: ( $S_1$ ) 'easy', ( $S_2$ ) 'moderate', ( $S_3$ ) 'hard'.

The transition matrix  $A = \begin{bmatrix} 0.82 & 0.07 & 0.11 \\ 0.05 & 0.92 & 0.03 \\ 0.13 & 0.04 & 0.83 \end{bmatrix}$  is shown in figure 1. For example, the probability of going

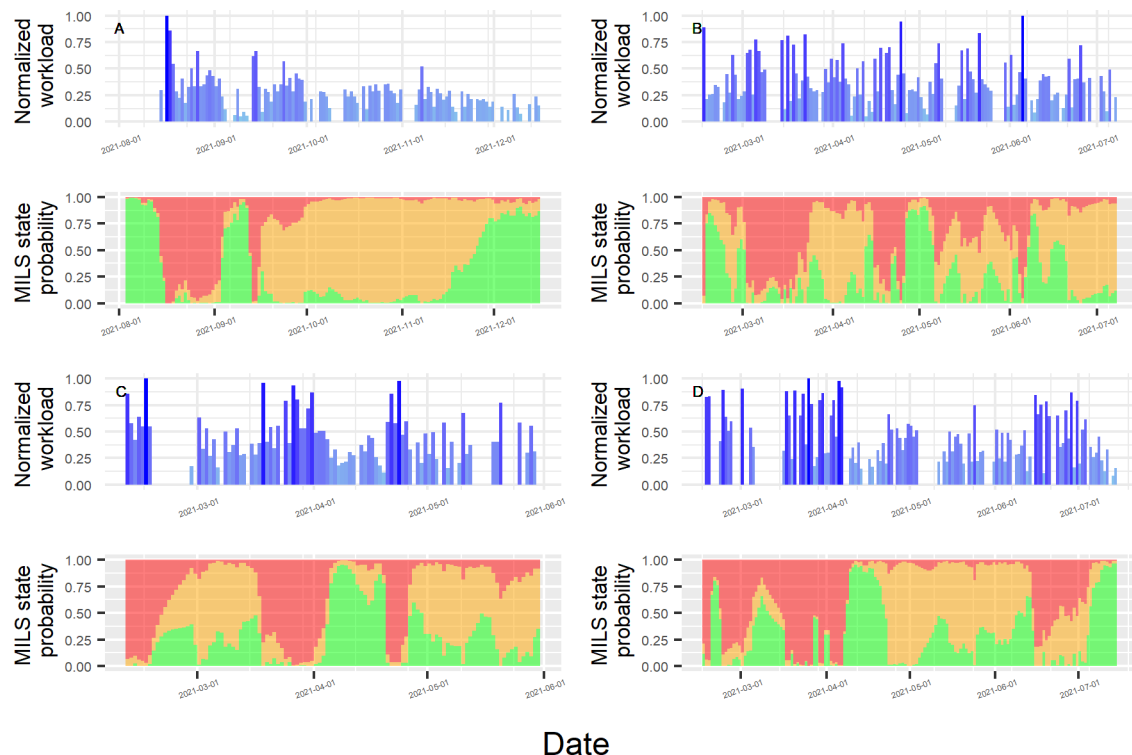


**Figure 1** A transition matrix of the Markov Index Load State algorithm showing the three states identified: red—hard, orange—moderate and green—easy. The percentages between the states represent the probability of passing from a state  $S_i$  of workload response to a state  $S_j$  from an instant  $t$  to an instant  $t + 1$  without considering the transmission probabilities.

from ‘easy’ state to ‘moderate’ state from time  $t$  to time  $t + 1$  is 7.31%, while the probability of going to ‘hard’ state is 10.63%.

**Individual examples**

Figure 2 displays an outcome from the MILS algorithm for four athletes. It presents the RPEsession over the follow-up time and represents the results of the MILS



**Figure 2** Individual example of the workload quantified through RPEsession, represented each day by a blue column and the result of the Markov Index Load State (MILS) algorithm representing the three states of workload response, easy (green), moderate (orange) and hard (red) along the follow-up time. For each individual, the graph above represents the charge  $C$  for the day  $t$ . The figure below shows the probabilities of being in each state on each day  $t$ . Each day, the sum of the probabilities equals 1 in the MILS model.

algorithm for each individual. For each day, we obtain, for each individual a probability  $P(S_1)$  of being in ‘easy’ state, a probability  $P(S_2)$  of being in ‘moderate’ state and a probability  $P(S_3)$  of being in ‘hard’ state, with  $\sum_{i=1}^3 P(S_i) = 1$ .

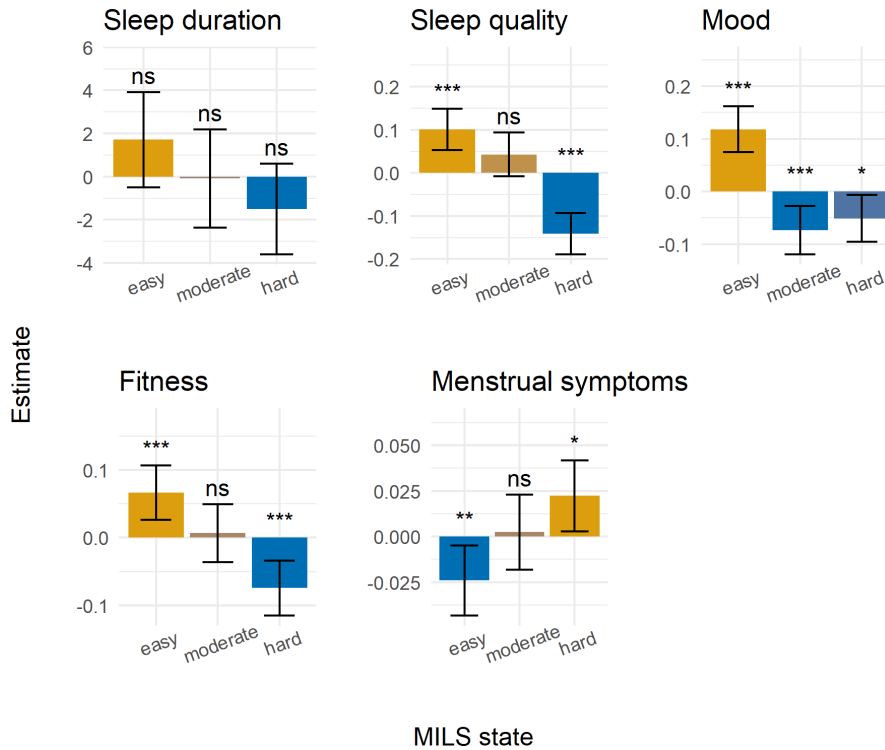
**Relationship to the studied variables**

**Fitness questionnaire**

We found that the ‘easy’ state was positively and significantly correlated with sleep quality ( $p=5.20e-04$ ), but not sleep duration, mood ( $p=8.94e-06$ ) and fitness ( $p=6.29e-03$ ) (figure 3) and negatively correlated with the menstrual symptoms ( $p=3.87e-02$ ). That is, the better the athletes evaluated their sleep quality, mood and fitness, and the less they declared menstrual symptoms, the higher the probability to be in the ‘easy’ state. The ‘hard’ state is negatively and significantly correlated with sleep quality ( $p=1.22e-06$ ), mood ( $p=5.73e-02$ ), fitness ( $p=2.63e-03$ ) and is positively correlated with the number of menstrual symptoms ( $p=5.92e-02$ ). The ‘moderate’ state is negatively and significantly correlated with the mood ( $p=5.73e-02$ ).

**Hormonal variations**

The ‘easy’ state is significantly and negatively associated with the progesterone levels considering it isolated from the cycle phases ( $p=8.23e-03$ ), and the ‘hard’ state is positively correlated with progesterone ( $p=5.72e-03$ ). That is, the greater the progesterone

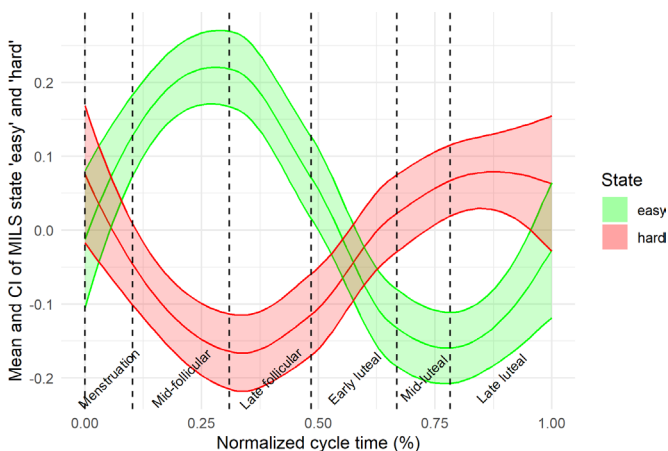


**Figure 3** Estimate of the parameters of the mixed model to explain the variables of the daily questionnaire by the states of the Markov Index Load State (MILS) (easy, moderate, hard). The higher the estimate (in gold), the more the variable is positively related to the MILS. The lower the estimate (in blue), the more the variable is negatively related to the state of the MILS studied. ns, no significant; \* $p < 0.1$ ; \*\* $p < 0.05$ ; \*\*\* $p < 0.01$ .

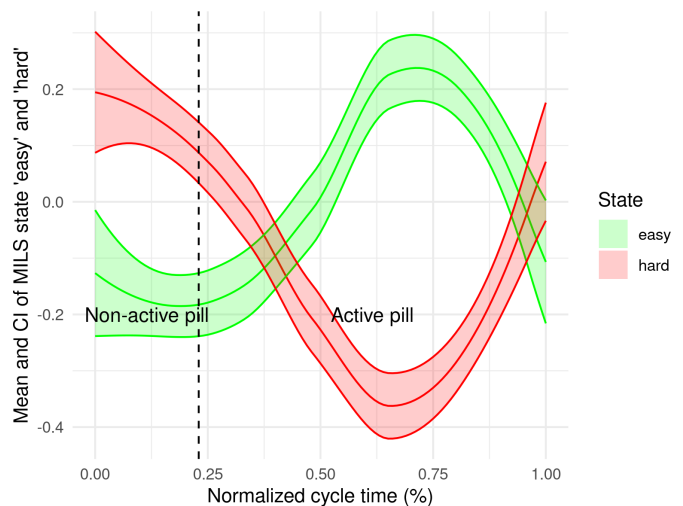
level, the lower the probability for an athlete to be in the ‘easy’ state and the higher the probability to be in the ‘hard’ state (figure 4). No significant correlations between MILS and free testosterone or 17 $\beta$ -estradiol levels were found.

**Workload responses through the cycles’ continuum**

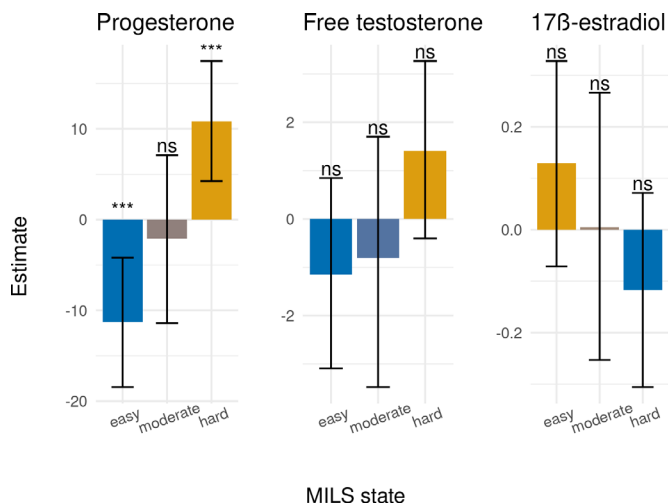
We observe an inverse cyclicity between the ‘easy’ and ‘hard’ state across the MC continuum (figure 5). The gap between the 95% CIs reveals that the ‘easy’ state is significantly higher in the first half of the cycle from 8% to 53% of the cycle, corresponding to the schematic



**Figure 4** Estimate of the parameters of the mixed model to explain hormone levels by the states of the Markov Index Load State (MILS) (easy, moderate, hard). The higher the estimate, the more the hormonal variable is positively correlated to the MILS. The lower the estimate, the more the hormonal variable is negatively related to the MILS. ns, no significant; \* $p < 0.1$ ; \*\* $p < 0.05$ ; \*\*\* $p < 0.01$ .



**Figure 5** Mean and estimated CIs of the Markov Index Load State (MILS) ‘hard’ and ‘easy’ states of workload responses across the menstrual cycle continuum normalised among athletes according to the percentage of the cycle advancement. The dotted lines represent the schematic subphases division of early, mid and late follicular and luteal phases.



**Figure 6** Mean and estimated CIs of the Markov Index Load State (MILS) ‘hard’ and ‘easy’ states of workload responses across the hormonal cycle continuum normalised among athletes according to the percentage of the cycle advancement. The dotted lines represent the schematic subphases division of non-active pill and active pill.

division of the mid and late follicular phases. The ‘hard’ state is higher from 63% up to 96% of the cycle duration corresponding to the mid and late luteal phase. The beginning of the cycles (0%–8%), associated with the menstruation phase and the end (96%–100%) associated with the premenstrual phase, do not show significant differences between both states.

In the OC group, only the six athletes using monophasic pills were analysed. Across the hormonal phases associated with the pills withdrawal we observed a cyclicity between the ‘easy’ and ‘hard’ state (figure 6). The ‘easy’ state is higher from 50% up to 92%, in the middle of the pill active phase. The ‘hard’ state is higher from 4% to 32%, correspondingly mostly to the phase of non-active pill.

The description of the hormonal milieu observed according to each schematic phase is detailed in online supplemental files.

## DISCUSSION

We developed MILS, the first model capable of measuring the workload and the athlete’s cumulated response to this load. This model is particularly useful to monitor training load among athletes in sports where objective measurements are not available, and to investigate complex and latent variables such as hormonal fluctuations. This is the first study based on this model that provides evidence of an infradian rhythm based on a cyclic response to training workload associated with the MC or with hormonal contraception use. Such cyclicity was most driven by the progesterone concentration. We also found that athletes’ wellness influences the training load response, independently of the cycle phase, with greater probability to be in the easy state when reporting better sleep quality, mood and fitness. Declared menstrual symptoms and low

wellness evaluation increase the probability of an athlete to be in the hard state.

## MILS model

The MILS mathematical features, allows to categorise the workload response into multiple hidden states and therefore provide an enhanced approach for studying complex variables. The response to the workload better addresses the complexity associated with variables inherent to performance.<sup>39</sup> The International Civil Aviation Organisation defines fatigue as ‘a physiological state of reduced mental or physical performance capability resulting from sleep loss or extended wakefulness, circadian phase, or workload (mental and/or physical activity) that can impair a crew member’s alertness and ability to safely operate an aircraft or perform safety related duties’.<sup>40</sup> The MILS algorithm allows to take into account proxies of cumulated fatigue that are RPE-related, and categorised into states linked to the workload responses. The model distinguished a hard state associated with increased fatigue and an opposite easy state, reflecting a lower cumulated fatigue. An intermediate state was also identified allowing to quantify the transitions between these three different states across the follow-up. The three hidden states identified by the MILS algorithm may be interpreted as: (a) the ‘easy’ state qualifying a state with greater margins of adaptation in which the athlete may possibly better adapt to increased workload, (b) a ‘moderate’ state in which the adaptive capacities persist, suggesting that the athlete absorbs the workload and continues to adapt to it and (c) a ‘hard’ state in which the load is higher than the adaptive capacity of the athlete.

This categorisation allows to consider the complexity of the workload response, in contrast with previous models such as REDI<sup>17</sup> or EWMA.<sup>16</sup> The advantage of REDI lies in its robustness to missing data by replacing for the missing day, the value of the REDI of the day before.<sup>17</sup> Yet, the MILS response to missing data seems more correct, propagating the latent state distribution using only the transition matrix across the missing days with emission probabilities set in an equiprobable manner. This also allows a fairer comparison between MC/OC phases across different training periods. In summary, we estimate the most likely workload response probabilities based only on the transitions between state passages. Like Foster’s model, we are based on variables of perceived effort and duration.<sup>10</sup> These variables have already shown their usefulness for quantifying the intensity of a session by comparing it to the time spent in the different heart rate zones,<sup>14</sup> which is easier than gathering heart rate data from the TRIMP model available.<sup>11</sup> In addition, Foster’s model makes it possible to quantify the load of a training session or a competition, but the methods proposed to relate the indicators of monotony, stress and fitness with fatigue are not robust enough.<sup>14</sup> All the models proposed so far are descriptive and calculate mean and SD of the training load. Thus, they are good models to quantify the cumulative load, but not to estimate the response to this





load. Beyond the simple load quantification of current models, which follow a hierarchy from low to high load levels, MILS calculates the probability of response (states) to the workload. Thus, it is possible for an athlete to be in the 'easy' state and, depending on the circumstances (eg, higher load, low fitness, luteal phase) move directly to a 'hard' state. For the population studied, this probability was estimated at 10.63%. Inversely, the probability to move from a 'hard' state to 'easy' state (eg, lower load, better sleep quality, follicular phase), without going through a mandatory 'moderate' state, was 13.26%. These transitions between states are more complex because it estimates the physiological responses to the applied load. MILS is the first unsupervised parameter-based inference model optimised specifically for a sample via likelihood. Therefore, the estimated parameters and the results are specific to the population studied. By estimating a latent variable using an emission and transition matrix, this model is therefore the first to quantify the response to load. Moreover, the Markov chains have already shown their effectiveness in modelling the alternation between an active and an inactive state.<sup>41</sup>

### Infradian rhythm

Previous studies have highlighted the need of robust research to investigate the influence of MC among athletes.<sup>23 42</sup> The recommendations rely primarily on methods to properly classify and distinguish each cycle phase designed to capture the most different hormonal milieu.<sup>3</sup> Although extremely necessary, especially to identify anovulatory cycles, or luteal insufficiency<sup>3</sup> such methods are mostly designed for testing single time points in the cycle. Here, we used a method to consider each athletes' cycles under a continuum lenses to capture its dynamics. The MC and the OC phases were conceptualised as a nonlinear function of time<sup>43</sup> and modelled using a continuous 'cycle day' variable, such as previously recommended.<sup>7 28</sup>

The demonstrated cyclicity shows the relevance of understanding the hormonal transitions through a physiological perspective to understand the continuous succession of states, rather than only isolating specific time points. Among the MC group, we observed that the transition from the predominant 'easy' state to the predominant 'hard' state occurs nearly together with the transition from the follicular to luteal phase, that is, prior to and after an estimated ovulation.

Also, the largest differences occur between the most different hormonal milieu<sup>3 7</sup> (ie, mid-follicular phase vs mid-luteal phase), where most studies have observed an impact of the MC on performance proxies.<sup>4 30</sup> Finally, such differences are equivalently mirrored from follicular to luteal phase, with an observed continuity from the premenstrual phase to menstrual phase, reinforcing the robustness of the model.

The circadian rhythm<sup>44</sup> and temperature (ie, associated with a seasons rhythm)<sup>45</sup> have been shown to influence performance. A recent study has shown evidence

that the woman's ovarian cycle is driven by an internal circamonthly timing system.<sup>46</sup> The dynamics found here highlight a cyclicity characterising an infradian rhythm affecting female elite athlete responses to workload. The results showed a significant relation between the progesterone and the workload responses states, where progesterone, mostly predominant on mid-luteal phase is positively correlated with the 'hard' state and negatively correlated with the 'easy' state. Previous studies have shown an impaired postexercise recovery during the mid-luteal phase with an impaired ventilatory efficiency.<sup>2</sup> Correspondingly, other studies have shown greater workload capacity during football competitions, measured through inertial devices during late follicular phase in comparison with mid-luteal and early luteal phases, but without further distinction in between phases.<sup>8</sup> We showed similar results based on daily measurements *in the field*. The cyclic dynamics evidenced here are associated with better workload responses on the follicular phase, that is, the first half of the MC in comparison with the luteal phase.

Yet it is not possible to draw any causal relation between the response changes according to the cycle phases and sex hormones fluctuations. The mechanisms underlying are complex, may be much larger than just the hormones fluctuations and may present an asynchronous nature of cycle pathways.

Interestingly, we also observed a cyclicity among the OC group (monophasic pills only), that a simple hormonal/non-hormonal phase division could not capture.<sup>29</sup> However, it is important to exercise caution in interpreting these findings due to the small sample size of this subgroup. This finding, although significant, they rely on a very small group, and any interpretation should be taken with extreme caution. One potential explanation for this unexpected cyclicity could be attributed to residual endogenous hormone production despite the exogenous administration of hormones to suppress ovulation. It could also be related to an internal circamonthly timing system<sup>46</sup> despite of the OC use. Nonetheless, the relationship between these cyclical patterns and exogenous hormone levels remains largely unexplained, underscoring the need for further research involving larger cohorts of individuals.

Menses are usually highlighted by the athletes as a performance impairing phase.<sup>47 48</sup> In accordance, we show a strong relation between the presence of menstrual symptoms and the 'hard' states, reinforcing the documented experience of athletes. However, we have not observed a significantly greater probability to be in the 'hard' state during menses, suggesting that difficulties faced in training during menses may be more symptom-related, than attributable to hormones fluctuation. In addition, previous studies have also shown that menstrual symptoms impact the athletes reported wellness.<sup>1</sup> Here, we highlight that a reported lower sleep quality, lower mood, fitness and greater symptoms are associated with a greater probability to be in the 'hard' state, independently

of the cycle phase they are in. These are commonly used measures in elite athletes monitoring<sup>46</sup> that can be easily integrated in training settings to evaluate readiness in light of the current results. In addition, these results suggest that considering the athletes' cycles and wellness in training periodisation may be informative, especially when planning heavy training loads in critical moments.

### Limits and strengths

We acknowledge an important limit regarding our restrained sample size. Studies including larger groups of athletes would help diminish the influence of individual variability and provide more robust results. However, all rowers and skiers in preparation to the Games and fulfilling the requirement of the study were included, excepting two athletes. Hence, they are representative of female rowers and skiers at the Olympic level, where the impact of hormonal phases may be more relevant.

We also acknowledge the impossibility in the current study to ensure ovulatory cycles and absence of luteal phase deficiency among every cycle included in the MC group, since we could not perform ovulatory tests or hormonal tests in every cycle, which may affect results in MC research.<sup>23</sup> But we monitored the cycles' regularity over 7 months and confirmed our capacity to detect distinct hormonal milieus across the MC. Hence, it is reasonable to suppose that if the inclusion of anovulatory or cycle with luteal phase deficiency occurred, this may have contributed to an increased variability and larger CIs, rather than change the direction of the results.

It is worth noting that our primary objective was not to delineate distinct phases for comparison, but rather to conceptualise the MC as a continuous spectrum and investigate potential infradian rhythms. From both physiological and mathematical perspectives, treating physiological phenomena as continuous variables rather than categorical ones offers a more comprehensive understanding.<sup>49</sup> For instance, even when precisely defining the onset of menses, variations may gradually occur from day 1 to consecutive days, underscoring the dynamic nature of the cycle. Despite these considerations, we opted to schematically display the common phases into this continuum analysis to facilitate comparability with previous studies. Additionally, incorporating information on hormone variations across the cycle demonstrates that each schematic subphase aligns with expected hormone levels.

Another limit is that hormonal testing was based on salivary samples instead of serum samples that are gold-standard.<sup>23</sup> Nonetheless, previous studies have suggested a high correlation between salivary and serum levels of progesterone and 17 $\beta$ -estradiol.<sup>47 48</sup>

The strengths of this paper rely on repeated measures with multi-daily assessments directly within the context of interest, a robust approach to cycle research.<sup>7</sup>

Our study shows that MILS is a useful proxy to characterise the responses to training load and is closely related

to fatigue RPE-related. It would be interesting to explore other fields of elite sport such as injury and performance.

### CONCLUSION

We developed MILS, an algorithmic model for estimating workload response among elite athletes that captured three distinct states of training responses: the 'easy', the 'moderate' and the 'hard' state related with the athletes' adaptative response to workload. This model allowed to highlight an infradian rhythm with a cyclic response, in both MC and OC users. Among MC the athletes present greater probability to display adaptative responses to workload during the first half of the cycle (follicular phase), and greater probability to be in the 'hard' state in the second half (luteal phase). Among monophasic OC users, they display greater probability to be in the 'hard' state during the pills withdraw and greater probability to be in the 'easy' state during the pills taking. Such cyclicity was mainly associated with the progesterone levels. Independently of the MC or OC, a better wellness indicator, with greater sleep quality, better mood and fitness, and less menstrual symptoms is associated with greater probability to be in the 'easy' state.

### Patient and public involvement

This study was designed to answer the coach and athletes' questions regarding the effect of the MC or the contraceptive hormones in their training monitoring. We cocreated the protocol in order to adapt to their training set. At the end of the follow-up every athlete received a report and explanations regarding their personal data and the coaches received a report of their group of athletes.

### Statement on equity, diversity and inclusion

Our research team is composed of female and male researchers from a single country, ranging from university students to senior experts. The study focused exclusively on female elite and Paralympic athletes given our specific aim of examining the relationship between the MC and athletic performance. The results are therefore generalisable to this population.

### Author affiliations

<sup>1</sup>Institut de Recherche bioMédicale et d'Epidémiologie du Sport (IRMES), Institut National du Sport de l'Expertise et de la Performance (INSEP), Paris, France

<sup>2</sup>French Rowing Federation, Nogent-sur-Marne, France

<sup>3</sup>Scientific Department, Fédération Française de Ski, Annecy, France

<sup>4</sup>Institut de Mathématiques de Bourgogne, UMR 5584, CNRS & Université de Bourgogne, F-21000 Dijon, France, Dijon, France

<sup>5</sup>CIMS, Hôtel-Dieu, AP-HP, Paris, France

<sup>6</sup>Université Bourgogne Franche-Comté, Institut Agro, Université de Bourgogne, INRAE, UMR PAM 1517, 21000 Dijon, France

**Acknowledgements** We would like to thank both INSEP and ANS institutions for their full support, notably the technical support for data collection and security. We also would like to thank all the athletes that participated in this study, as well as all the staff from the French Rowing and skiing Federation.

**Contributors** JdSA was responsible for the overall study design. TC, MD, FS made the inclusions and collected data. QDL analysed all data with IH. QDL and JdSA wrote the first drafts of the paper. TC, MD, JdSA, PYL, NC, IH, FS and J-FT carried

out the successive versions of the manuscript. All authors contributed to the manuscript revisions and approved the submitted version. DQL is responsible for the overall content as the guarantor.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and the conducted investigations adhered to the code of ethics outlined by the World Medical Association (Declaration of Helsinki), and received approval from the Institutional Ethics Committee (IRB00012476-2022-03-11-206). The data collection process complied with the General Data Protection Regulation (2016/679) implemented in the European Union and received a certificate of compliance from the Commission Nationale Informatique et Libertés (CNIL - 2221532 v0). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data may be obtained from a third party and are not publicly available.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Quentin De Larochelambert <http://orcid.org/0000-0001-8356-4028>

Alice Meignié <http://orcid.org/0000-0003-1037-990X>

Juliana da Silva Antero <http://orcid.org/0000-0001-6894-6693>

#### REFERENCES

- Antero J, Golovkine S, Niffoi L, et al. Menstrual cycle and hormonal contraceptive phases' effect on elite rowers' training, performance and wellness. *Front Physiol* 2023;14:1110526.
- Benito PJ, Alfaro-Magallanes VM, Rael B, et al. Effect of Menstrual cycle phase on the recovery process of high-intensity interval Exercisea cross-sectional observational study. *Int J Environ Res Public Health* 2023;20:3266.
- Janse DE, Jonge X, Thompson B, Han A. Methodological recommendations for Menstrual cycle research in sports and exercise. *Med Sci Sports Exerc* 2019;51:2610–7.
- Meignié A, Duclos M, Carling C, et al. The effects of Menstrual cycle phase on elite athlete performance: A critical and systematic review. *Front Physiol* 2021;12:654585.
- McNulty KL, Elliott-Sale KJ, Dolan E, et al. The effects of Menstrual cycle phase on exercise performance in Eumenorrhic women: A systematic review and meta-analysis. *Sports Med* 2020;50:1813–27.
- Igonin P-H, Rogowski I, Boisseau N, et al. Impact of the Menstrual cycle phases on the movement patterns of sub-elite women Soccer players during competitive matches. *Int J Environ Res Public Health* 2022;19:4465.
- Bittencourt NFN, Meeuwisse WH, Mendonça LD, et al. Complex systems approach for sports injuries: moving from risk factor identification to injury pattern recognition-narrative review and new concept. *Br J Sports Med* 2016;50:1309–14.
- Calvert TW, Banister EW, Savage MV, et al. A systems model of the effects of training on physical performance. *IEEE Trans Syst, Man, Cybern* 1976;SMC-6:94–102.
- Foster C, Hector LL, Welsh R, et al. Effects of specific versus cross-training on running performance. *Eur J Appl Physiol Occup Physiol* 1995;70:367–72.
- Foster C, Daines E, Hector L, et al. Athletic performance in relation to training load. *Wis Med J* 1996;95:370–4.
- Foster C. Monitoring training in athletes with reference to Overtraining syndrome. *Med Sci Sports Exerc* 1998;30:1164–8.
- Gabbett TJ, Hulin BT, Blanch P, et al. High training workloads alone do not cause sports injuries: how you get there is the real issue. *Br J Sports Med* 2016;50:444–5.
- Hunter JS. The Exponentially weighted moving average. *J Qual Technol* 1986;18:203–10.
- Moussa I, Leroy A, Sauliere G, et al. Robust exponential decreasing index (REDI): adaptive and robust method for computing Cumulated workload. *BMJ Open Sport Exerc Med* 2019;5:e000573.
- Puerto-Santana C, Larranaga P, Bielza C. Autoregressive asymmetric linear Gaussian hidden Markov models. *IEEE Trans Pattern Anal Mach Intell* 2022;44:4642–58.
- Lüken M, Kucharský Š, Visser I. Characterising eye movement events with an Unsupervised hidden Markov model. *J Eye Mov Res* 2022;15.
- Du J, Wang C, Wang L, et al. Automatic block-wise genotype-phenotype Association training in athletes with reference to Overtraining syndrome. *Med Sci Sports Exerc* 1998;30:1164–8.
- Rao K, Speier W, Meng Y, et al. Machine learning approaches to classify self-reported rheumatoid arthritis health scores using activity Tracker data. *JMIR Form Res* 2023;7:e43107.
- Wang Z, Bi C, You S, et al. Hidden Markov model-based Video recognition for sports. *Advan Mathemat Phys* 2021;2021:1–12.
- Elliott-Sale KJ, Minahan CL, de Jonge XAKJ, et al. Methodological considerations for studies in sport and exercise science with women as participants: A working guide for standards of practice for research on women. *Sports Med* 2021;51:843–61.
- Bruinvels G, Goldsmith E, Blagrove R, et al. Prevalence and frequency of Menstrual cycle symptoms are associated with availability to train and compete: a study of 6812 exercising women recruited using the Strava exercise App. *Br J Sports Med* 2021;55:438–43.
- Haddad M, Chaouachi A, Wong DP, et al. Influence of fatigue, stress, muscle soreness and sleep on perceived exertion during Submaximal effort. *Physiol Behav* 2013;119:185–9.
- Claudio JG, J Gabbett T, de Sá Souza H, et al. Which parameters to use for sleep quality monitoring in team sport athletes? A systematic review and meta-analysis. *BMJ Open Sport Exerc Med* 2019;5:bmjsem-2018.
- Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377.
- Arney BE, Glover R, Fusco A, et al. Comparison of RPE (rating of perceived exertion) scales for session RPE. *Int J Sports Physiol Perform* 2019;14:994–6.
- Carmichael MA, Thomson RL, Moran LJ, et al. The impact of Menstrual cycle phase on athletes' performance: A narrative review. *Int J Environ Res Public Health* 2021;18:1667.
- Soumpasis I, Grace B, Johnson S. Real-life insights on Menstrual cycles and ovulation using big data. *Hum Reprod Open* 2020;2020:hoaa011.
- Barba-Moreno L, Cupeiro R, Romero-Parra N, et al. Cardiorespiratory responses to endurance exercise over the Menstrual cycle and with oral contraceptive use. *J Strength Cond Res* 2022;36:392–9.
- Baum LE, Petrie T. Statistical inference for probabilistic functions of finite state Markov chains. *Ann Math Statist* 1966;37:1554–63.
- Lystig TC, Hughes JP. Exact computation of the observed information matrix for hidden Markov models. *J Computat Graph Statist* 2002;11:678–89.
- Visser I, Speekenbrink M. Depmix4: an R package for hidden Markov models. *J Stat Softw* 2010;36:1–21.
- Celeux G, Durand J-B. Selecting hidden Markov model state number with cross-validated likelihood. *Comput Stat* 2008;23:541–64.
- Pohle J, Langrock R, van Beest FM, et al. Selecting the number of States in hidden Markov models: pragmatic solutions illustrated using animal movement. *JABES* 2017;22:270–93.
- SELYE H. The general-adaptation-syndrome. *Annu Rev Med* 1951;2:327–42.
- Jacoby WG. Loess: a Nonparametric, graphical tool for depicting relationships between variables. *Elect Stud* 2000;19:577–613.
- ICAO. Manual for the oversight of fatigue management approaches (Doc 9966). 2020. Available: <https://store.icao.int/en/manual-for-the-oversight-of-fatigue-management-approaches-doc-9966>
- Ren B, Barnett I. Combining mixed effects hidden Markov models with latent alternating recurrent event processes to model diurnal active-rest cycles. *Biometrics* 2023;79:3402–17.
- Colenso-Semple LM, D'Souza AC, Elliott-Sale KJ, et al. Current evidence shows no influence of women's Menstrual cycle phase on

- acute strength performance or adaptations to resistance exercise training. *Front Sports Act Living* 2023;5:1054542.
- 39 Singer JD, Willett JB. Applied longitudinal data analysis. In: *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. New York, NY, US: Oxford University Press 2003 Available: <https://academic.oup.com/book/41753>
- 40 Schmalenberger KM, Tauseef HA, Barone JC, *et al.* How to study the Menstrual cycle: practical tools and recommendations. *Psychoneuroendocrinology* 2021;123:S0306-4530(20)30318-8.
- 41 Pierson E, Althoff T, Thomas D, *et al.* Daily, weekly, seasonal and Menstrual cycles in women's mood, behaviour and vital signs. *Nat Hum Behav* 2021;5:716–25.
- 42 Thun E, Bjorvatn B, Flo E, *et al.* Sleep, circadian rhythms, and athletic performance. *Sleep Med Rev* 2015;23:1–9.
- 43 Haida A, Coulmy N, Dor F, *et al.* Return to sport among French Alpine skiers after an anterior Cruciate ligament rupture: results from 1980 to 2013. *Am J Sports Med* 2016;44:324–30.
- 44 Read P, Mehta R, Rosenbloom C, *et al.* Elite female football players' perception of the impact of their Menstrual cycle stages on their football performance. A semi-structured interview-based study. *Sci Med Footb* 2022;6:616–25.
- 45 Findlay RJ, Macrae EHR, Whyte IY, *et al.* How the Menstrual cycle and menstruation affect sporting performance: experiences and perceptions of elite female Rugby players. *Br J Sports Med* 2020;54:1108–13.
- 46 Gabbett TJ, Nassis GP, Oetter E, *et al.* The athlete monitoring cycle: a practical guide to interpreting and applying training monitoring data. *Br J Sports Med* 2017;51:1451–2.
- 47 Choe JK, Khan-Dawood FS, Dawood MY. Progesterone and estradiol in the saliva and plasma during the menstrual cycle. *Am J Obstet Gynecol* 1983;147:557–62.
- 48 Fiers T, Dielen C, Somers S, *et al.* Salivary estradiol as a surrogate marker for serum estradiol in assisted reproduction treatment. *Clin Biochem* 2017;50:145–9.
- 49 Naggara O, Raymond J, Guilbert F, *et al.* Analysis by categorizing or dichotomizing continuous variables is inadvisable: an example from the natural history of unruptured aneurysms. *AJNR Am J Neuroradiol* 2011;32:437–40.



## Supplementary materials

### Markov Index Workload Ratio (MILS)

The HMM can model a stochastic process by a succession of states over time. Such a process is classified as Markovian, where all the information used to predict the future is included in the present state. In a classical Markov chain, the prediction of the future state is based on a transition matrix, containing the probabilities of passing from one state to another from an instant  $t$  to an instant  $t + 1$ . In a hidden Markov model, the states of a feature are unknown, but other parameters correlated to those states are known. For instance, using a Markov model to determine the athlete's response to workload, hidden states may be characterized by known load parameters such as Rating of Perceived Exertion (RPE), athletes' fitness or training duration and intensity. We assume a Gaussian distribution of the observed variable.

In addition to the transition matrix, state predictions also rely on an emission matrix, which contains the probabilities of observing a given value of a known variable, given the hidden states. A hidden Markov chain is defined by:

- $S = \{S_1, \dots, S_n\}$  represents the discrete set of  $n$  states of the model.  $\pi(t) = \{\pi_1(t), \dots, \pi_n(t)\}$  is the probability vector of being in state  $S_i$  at time  $t$ .  $O(t)$  denotes the value of the observed variable at time  $t$ , with  $t \in \{1, \dots, T\}$  being the set of discrete times studied.
- The initial probability vector is given by  $\pi(1) = \{\pi_1(1), \dots, \pi_n(1)\}$ .  $\forall i \in \{1, \dots, n\}$ ,  $\pi_i(1) = P(S(1) = S_i)$  contains the probabilities of being in state  $S_i$  at time  $t = 1$ .
- $a_{ij} = P(S(t+1) = S_j | S(t) = S_i)$  represents the transition matrix of moving from state  $S_i$  to state  $S_j$  from time  $t$  to time  $t + 1$ .
- $b_j = f(O | S = S_j)$  is a vector of conditional gaussian densities of observations  $O$  associated with latent states  $S_j$ .

The classic properties of an HMM are:

- $\forall S_i \in S, \sum_{i=1}^n \pi_i(t) = 1$ : The sum of the probabilities of belonging to a state at each time  $t$  is equal to 1.
- $\forall i \in \{1, \dots, n\}, \sum_{j=1}^n a_{ij} = 1$ : The sum of the probabilities of the transitions starting from a state  $S_i$  is equal to 1.

### Saliva Test Details

#### Salivary test and Luminescent immunoassay method

17 $\beta$ -estradiol, progesterone and free testosterone concentrations were measured using a saliva collection kit (SaliCap Set, IBL International GmbH, Germany). Athletes were instructed to collect a saliva sample on waking, fasting and at least 30 minutes after drinking. They were also advised to rinse the oral cavity with water and not to chew chewing gum just before saliva

collection. A straw in polypropylene could be used to salivate in the SaliCap. Once the tube is half-filled with saliva, all sampling vials were properly labeled (patient name, date, time). Athletes were instructed to send their saliva sample within 48 hours storing tubes in their fridge (between 2 and 8 °C) until the expedition. Saliva samples can be stored at temperatures between 2 and 8 °C for up to 4 weeks without affecting the validity of the test. If saliva samples are stored at -20 °C, they can be kept for longer.

The BIOAVENIR laboratory (Metz, France) carries out the test analysis procedure in accordance with the appropriate protocol recommendations, using reagents from the German company Tecan, IBL International. The luminescent immunoassay (IBL, Hamburg, Germany) used is based on the competition principle. An unknown amount of antigen presents in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After addition of the luminescence substrate solution the intensity of the luminescence measured is inversely proportional to the amount of the antigen in the sample. The luminescence was measured on a Berthold Luminometer, and a four-parameter logistic was used for curve fitting.

#### Salivary estradiol

The calibration was established by use of 17 $\beta$ -estradiol ranging from 0 to 64 pg/mL. Cross reactivities (Abraham method) of the antiserum were measured against various compounds: Estrone –14%; Deoxycortisol – 0.58%; Estriol – 0.5%; Fulvestrant – 0.42%; Estrone-3-sulfate – 0.26%; and other substances <0.05. Analytical sensitivity was calculated from the mean of the relative luminescence units of the zero calibrator minus 3 standard deviations of 30 replicate analyses. The lowest detectable level that could be distinguished from the zero standard is 0.3 pg/mL. Functional sensitivity was determined from the inter-assay variation coefficient of very low concentrations in saliva samples. The lowest 17 $\beta$ -estradiol concentration that could be measured with a coefficient of variation below 20% is 0.9 pg/mL. The intra-assay variation, the inter-assay variation and the inter-lot variation were determined by repeated measurements of control samples. The mean intra-assay precision of twenty runs with a panel of 3 human saliva samples in the mean range 1.6 – 39.6 pg/mL was found to be 10%, and a range of 7.2 - 13.3 %. The mean inter-assay precision of 5 runs with a panel of 10 human saliva samples in the mean range 1.82 - 36.16 pg/mL was found to be 6.4 %, and a range of 4.0 - 11.6 %. The mean inter-lot precision of three saliva samples in the mean range 1.88 – 40.00 pg/mL was found to be 12.8 %, and a range of 8.1 - 25.1 %.

#### Salivary progesterone

The calibration was established by use of progesterone ranging from 2.6 to 1000 pg/mL. Cross reactivities (Abraham method) of the antiserum were measured against various compounds: 17 $\alpha$ -OH-Progesterone – 1.84%; 6 $\alpha$ -Methyl-17 $\alpha$ -OH-Progesterone – 1.41%; Pregnenolone – 0.41%; Desoxy-Corticosterone– 0.28%; Androsterone Sulfate– 0.25%; Androstenedione – 0.20% ; Androsterone - 0.20% ; DHEA-S – 0.11% and Corticosterone – 0.06. Analytical sensitivity was calculated from the mean of the relative luminescence units of the zero calibrator minus standard deviations of 30 replicate analyses. The lowest detectable level that could be distinguished from the zero standard is 2.4 pg/mL. Functional sensitivity was determined from the inter-assay variation coefficient of very low concentrations in saliva samples. The lowest progesterone concentration that could be measured with a coefficient of variation below 20% is 8.9 pg/mL. The Intra-Assay and Inter-Assay study was conducted during 20 days using one reagent lot. Two runs were performed per day with high and low kit controls

and with a panel of 10 human saliva samples. The mean intra-assay precision of twenty runs with a panel of 3 human saliva samples in the mean range 1.6 – 39.6 pg/mL was found to be 10%, and a range of 7.2 -13.3 %. The mean inter-assay precision of 5 runs with a panel of 10 human saliva samples in the mean range 1.82 - 36.16 pg/mL was found to be 6.4 %, and a range of 4.0 - 11.6 %. The mean inter-lot precision of three saliva samples in the mean range 1.88 – 40.00 pg/mL was found to be 12.8 %, and a range of 8.1 - 25.1 %. The mean precision of 10 saliva samples was found to be 7.7% (a range of 4.3% - 14.2%) and 9.5% (a range of 6.0% - 15.2%) for intra-assay and inter-assay variation.

#### Salivary free testosterone

The calibration was established by use of free testosterone ranging from 0 to 760 pg/mL. The detection range of the luminescent immunoassay encompasses the physiological range of testosterone concentrations in saliva and in 1:40 diluted serum samples. Cross reactivities (Abraham method) of the antiserum were measured against various compounds: 11 $\beta$ -hydroxytestosterone – 8.7%; 11 $\alpha$ -hydroxytestosterone – 3.2%; 5 $\alpha$ -dihydrotestosterone – 1.9%; androstendione – 0.8%; methyltestosterone – 0.44%; DHEAS – 0.07%; testosterone sulfate : 0.04%, progesterone : 0.03% and other steroids <0.01. Analytical sensitivity was calculated from the mean of the relative luminescence units of the zero calibrator minus 2 standard deviations of 20 replicate analyses. The lowest detectable level that could be distinguished from the zero standard is 1.8 pg/mL. Functional sensitivity was determined from the inter-assay variation coefficient of very low concentrations in saliva samples. The lowest free testosterone concentration that could be measured with a coefficient of variation below 20% is 3.9 pg/mL. The intra-assay variation, the inter-assay variation and the inter-lot variation were determined by repeated measurements of control samples. The mean intra-assay precision of three saliva samples in the range 12.8 – 223.9 pg/mL was found to be 9.1%, and a range of 4.0 - 16.7 %. The mean inter-assay precision of ten saliva samples in the range 5.54 - 557.3 pg/mL was found to be 4.7 %, and a range of 2.90 - 6.96 %. The mean inter-lot precision of three saliva samples in the range 12.8 – 223.9 pg/mL was found to be 9.2%, and a range of 0.0 - 16.0 %.

#### Hormonal variation

Hormonal data is based on a total of 214 tests performed. The description of the hormones levels of progesterone, free testosterone and 17 $\beta$ -estradiol according to the estimated phases are detailed in Figures 1 for naturally menstruating athletes and Figure 2 for athletes under oral contraception use.

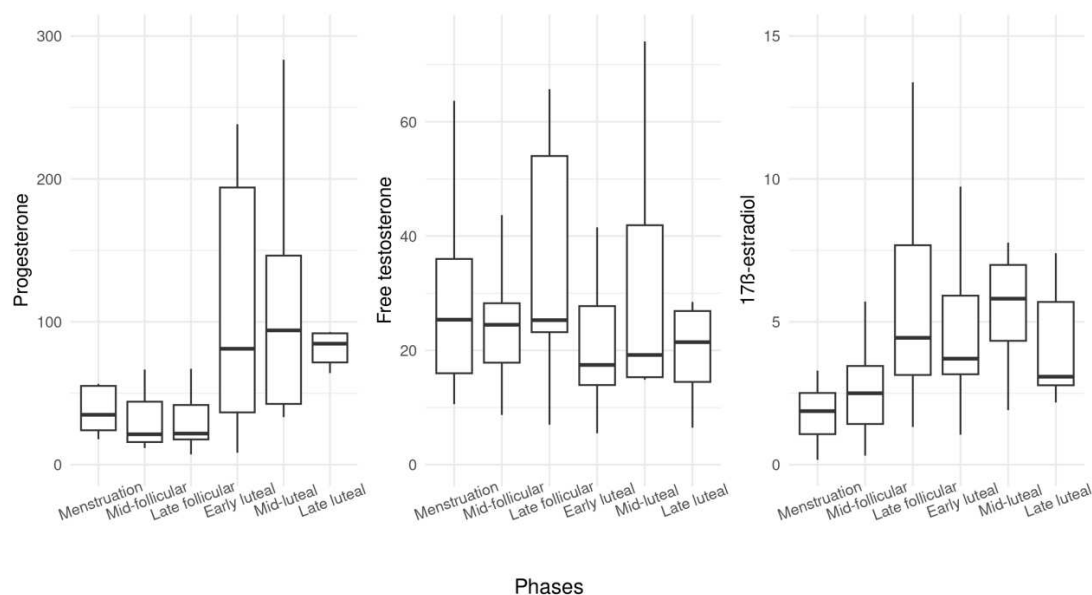


Figure 1: Description of progesterone, free testosterone and 17β-estradiol levels according to the phases for naturally menstruating athletes.

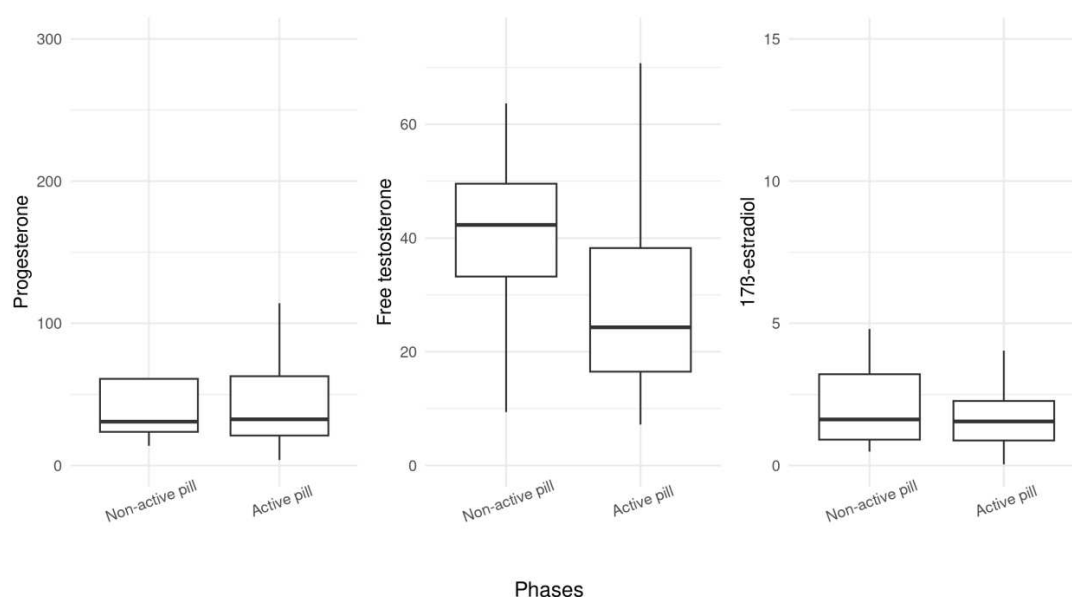


Figure 2: Description of progesterone, free testosterone and 17β-estradiol according to the phases for athletes under oral contraception use.

We observed significant higher levels of progesterone in the mid-luteal phase compared to the other mid-follicular ( $p=0.001$ ) and late follicular phase ( $p = 0.004$ ). We also observed higher levels of 17β-Estradiol in the mid-luteal phase compared to menstruation ( $p=0.04$ ) and mid-follicular phase ( $0.003$ ). No significant differences in free testosterone were observed across the MC estimated phases. Regarding individuals on the OC group, we observe a significant difference in free testosterone between non-active pill phase and active pill phase ( $p=0.03$ ).