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RESEARCH ARTICLE

Application of Vagal-Mediated Heart Rate Variability and Subjective Markers to Optimise Training Prescription: An Olympic Athlete Case Report

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Abstract
Purpose: The aim of this case study was to investigate whether the variation in parasympathetic branch activity, in conjunction with subjective data, could optimize the training prescription for an elite athlete during an Olympic season. Methods: During the preparatory phase for the Tokyo Olympic Games, a world-class female 3000m steeplechase runner (Age: 20 years, Height: 168 cm, Weight: 52 kg, VO2max: 67.1 mL·min·kg^{-1}) recorded Heart Rate Variability (HRV), conducted orthostatic tests, and completed subjective evaluations four times a week throughout the different training periods that constitute a pre-Olympic season. The Pearson correlation coefficient and the Shapiro-Wilk test, along with paired T-tests, were used to assess correlations and compare mean differences between variables concerning baseline measurements and each of the other training periods. Results: The natural logarithm of the root mean square of the standard deviation during supine measurement (LnRMSSDsu) exhibited a significant negative correlation with the training load based on Rating of Perceived Exertion (RPE) and sleep quality ($P < 0.05$). Heart rate during supine measurement (HRsu) showed a significant positive correlation with subjective perceived exertion, sleep quality, stress, muscle soreness, and fatigue ($P < 0.01$). Compared to the baseline (rest), LnRMSSDsu, HRsu, RPE, and sleep quality were significantly different during the training, camp, and altitude training camp periods. Conversely, markers of standing Heart Rate Variability (HRV) did not differ during competition periods. Conclusion: Standing Heart Rate Variability, when combined with subjective markers, serves as a relevant monitoring tool for adapting training periods to regulate psychophysiological effects.

Keywords
Training Monitoring, Psychophysiological Stress, Olympic Athlete

INTRODUCTION

Careful planning of an athlete's training regimen is essential to optimize their performance during competitions. Coaches and sports scientists must strike a delicate balance between maintaining sufficient training load to induce adaptation and safeguarding their overall health, with a specific focus on preventing maladaptation and injury (**Morton 1991**). Periodisation is a training method that breaks the season into several phases. This enables the modification of training parameters to achieve performance goals by targeting necessary adaptations (**Bompa 1983; Bompa 1987**) Periodically during specific training phases, elite athletes face periods of intense training, often characterised by multiple sessions in a single day (**Hartman et al. 2007**) and compounded by

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heightened psychological pressures imposed by recovery and nutritional strategies, travel, and media obligations that disrupt an athlete's daily routine (Faustin et al. 2022). As a result, these athletes face a unique combination of psycho-physiological stressors that, if not managed appropriately, may impair vital cognitive functions such as learning, memory, and neuroendocrine function (Kim and Diamond 2002; Kuipers and Keizer 1988) and increase overuse injury risk (Martin and Amos 2021).

During increased physical activity and mental stress, observable changes occur in autonomic nervous system (ANS) activity. A commonly used indirect indicator of ANS is heart rate variability (HRV), which represents the variation in time intervals between successive heartbeats (Malik 1996; Saboul 2013). HRV analysis indicates the contribution of the parasympathetic nervous system, which is primarily regulated by the vagal nerve (Brodal 2004) referred to as vagal-mediated Heart Rate Variability (vmHRV). The analysis of HRV can be performed via multiple methods, with temporal analysis of vmHRV more reliable than spectral indices (Al Haddad et al. 2011)and is frequently used to reflect the activity of the parasympathetic branch (Berntson et al. 1997).

Using time domain parameters of vmHRV to measure training load (Saboul, Pialoux, and Hautier 2013), optimize training intensity (Earnest et al. 2004), monitor psychophysiological stress adaptation (Flatt and Esco 2016) and track recovery in athletes (Abaji et al. 2016), vmHRV is increasingly used as a tool to monitor psychophysiological adaptation to stress and recovery (Brodal 2004). It is now frequently incorporated into athletic training load management to enhance the efficacy of training programs in athletes undergoing strenuous training regimes (Plews et al. 2012; Gordan, Gwathmey, and Xie 2015).

Critically, several studies have reported the measurement if vmHRV in a single position is insufficient for fatigue detection in athletes (Abaji et al. 2016; Fatisson, Oswald, and Lalonde 2016; Massin et al. 2000). Thus, HRV data should be collected in several positions including supine or standing. Incorporating an orthostatic test, transitioning from lying to standing, during vmHRV testing prompts specific changes in vmHRV due to diminished vagal output and heightened sympathetic output. This test provides insight into the dynamic adaptations of the ANS during the transition from lying to standing (Lutfullin and Almetova 2014) and can provide additional information about changes in parasympathetic activity (Tulppo et al. 2001). Additionally, many studies treat vmHRV as a singular marker of the stress response, despite the physiological response despite the complex and systemic nature of the stress (Baumann and Turpin 2010; Charmandari, Tsigos, and Chrousos 2005; Seaward 2006). The intricate relationships between stress and vmHRV can often be hard to interpret based on the nature of the stressor. While vmHRV is a beneficial monitoring tool for understanding the effects of psychophysiological stress during different training phases of a season (competition, training phases), it can also be influenced by non-sport-related stressors (Mosley and Laborde 2022). As such, vmHRV analysis should ideally be coupled with a subjective assessment of self-reported variables. Using subjective measures of psychophysiological stress (RPE, sleep, muscle soreness, fatigue, and stress) can optimise the interpretation of vmHRV analysis by providing both physiological and psychological context to HRV changes (Saboul, Pialoux, and Hautier 2013). The inclusion of self-reported variables in subjective assessments is important, given the association of the parasympathetic nervous system with numerous factors relevant to psychophysiology, including cognitive, affective, social, and health phenomena (Plews 2014; Dobbs et al. 2019; Manresa-Rocamora et al. 2021;Carrasco-Poyatos et al. 2022). Despite the availability of many easy-to-use and non-invasive measurement tools, few studies have investigated changes in vagal system activity across different phases of an athletic season and multiple training periods in elite athletes. And to our knowledge, no study has investigated the changes in vagal system activity associated with the evolution of related self-reported variables in elite athletes during different training periods of a pre-Olympic season.

This case study examines the changes in vmHRV and associated subjective data collected during various training phases of a pre-Olympic season in an elite female athlete. More specifically, the goal was to investigate whether the variation of parasympathetic branch activity with subjective data could optimize the training planning of an elite athlete. The athlete was monitored over 291 days of the investigation and was an international athlete preparing for the 2020 Tokyo Olympic Games. Analyzing the results of vmHRV in combination with subjective data provided feedback to the coach, allowing them to adapt the training program for each training period, fine-tune the planning as the competition neared, and adjust the training content.

MATERIALS AND METHODS

Subject

A Tier 5, World-class female 3000m steeplechase runner (Age: 20 years, Height: 168 cm, weight: 52kg, VO_{2max}: 67.1 $mL·kg·min^{-1}$) (McKay et al. 2022) collected vmHRV data and provided accompanying subject information for 291 days in preparation for Tokyo 2022 Olympic Games. All data presented were collected as part of routine professional practice in collaboration with exercise physiologists and athletic trainers from the French Institute of Sport (INSEP) and the French Athletics Federation, respectively. Year-round support was provided by INSEP, including accommodation (Paris, France), nutrition (advice and provision), physiological testing, and on-site medical treatment. Exercise training was prescribed and monitored by athletic trainers of the French Athletics Federation, with sessions completed on-site and in the immediate surrounding area (2500-acre Public Park). Specifically, the athlete completed regular endurance and track-based running sessions 10 to 12 times weekly, totalling 75-85km/week, with run sessions supplemented by two strength and conditioning and two yoga sessions. Data were collected through 5 distinct periods of the season (described below). The participating athlete was informed about the study protocol, their rights, and the associated risks of participation before providing written informed consent. All procedures were approved by the CERSTAPS ethics committee (Approval No. 2022-A00644-39) and conducted in accordance with the Helsinki Declaration (1964, revised in 2001). Regarding vulnerable and disadvantaged groups, the authors took into account the needs and priorities of the groups/individuals in which the study was conducted, in accordance with Articles 19 and 20 of the WMA Declaration of Helsinki, and the situation that the study could not be carried out outside these groups and individuals was taken into account.“Additional precautions were taken by the investigator(s) to protect the volunteers in this study.”

Periods of the season

The composition of the training programme and the distribution of the different training periods during a season are shown in figures 1 and 2, respectively (Fig. 1 and Tab. 1)

- Baseline (rest): a period of no training
- Competition: reduced training volume whilst intensity is maintained around periods of competition.
- Training: a period during which the athlete trained daily in their usual environment, typically within the French Institute of Sport and surrounding public park.
- Training Camp: a short period during which the athlete trains in a different training environment.
- Altitude Training Camp: All altitude training camps were completed at the National
- Altitude Training Centre, Font Romeu, France (~1800m elevation)

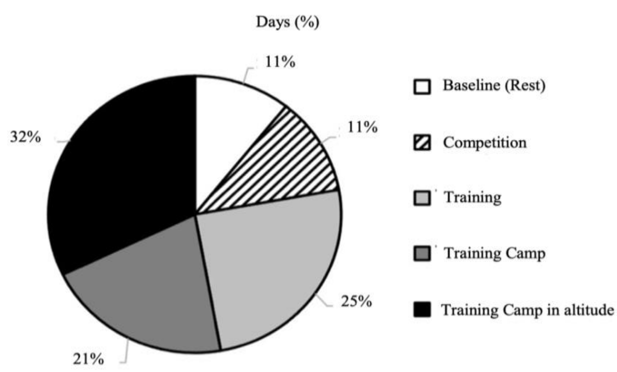

Figure 1. Distribution of the different periods of the season of a high-level athlet

Table 1. Example of a typical training week according to the different periods

		Rest	Competition	Training	Training Camp	Train Camp in altitude
Day 1	Morning	Rest	Rest	Footing + Bike	Footing + strethcing	Bike session + yoga
	Afternoon	Rest	Footing + stretching + yoga	Footing + technical + straight lines	Footing + technical + straight lines	Footing + Musculation
Day 2	Morning	Rest	Footing + Musculation	Track session	Track session	Footing + stretching
	Afternoon	Rest	Rest	Footing + Musculation	Footing	Bike session + stretching
Day 3	Morning	Rest	Track session	Footing	Footing	Rest
	Afternoon	Rest	Rest	Rest	Rest	Bike session + musculation
Day 4	Morning	Rest	Rest	Footing + stretching	Hill session	Track session
	Afternoon	Rest	Rest	Bike session + musculation	Footing + Yoga + stretching	Rest
Day 5	Morning	Rest	Footing + stretching	Track session	Track session	Footing + yoga
	Afternoon	Rest	Rest	Footing	Footing + musculation	Bike session
Day 6	Morning	Rest	Footing + straight lines	Active footing	Active footing	Track session
	Afternoon	Rest	Rest	Rest	Rest	Rest
Day 7	Morning	Rest	Competition	Bike session	Footing + yoga + stretching	Footing
	Afternoon	Rest	Rest	Track session	Rest	Rest

Lying to standing test: Orthostatic test

The orthostatic test, a variant of the tilt test, to collect R-R intervals for 10 minutes was completed 4 times weekly at approximately the same time (~8:50 am) in a darkened room (i.e. curtains drawn) (**Bourdillon et al. 2017**). Before starting the test, the athlete had to go to the toilet before recording their heart rate to avoid sympathetic activation during the recording. Upon waking, the subject attached a Garmin heart rate belt around their chest, maintained a supine position on their bed for 5 minutes, and asked to breathe normally and spontaneously before quickly standing and standing still for 5 minutes. Data were recorded throughout the test via a Garmin Forerunner 245 Music watch connected to the Garmin HRM-Pro heart rate monitor. All data were uploaded to Garmin Connect before exportation for further analysis.

Analysis of vagally mediatedHeart Rate Variability (vmHRV)

After exporting the data, each data file was viewed to correct for artefacts manually and then analysed using specialisedvmHRV analysis software "Kubios HRV Standard" (The Biomedical Signal and Medical Imaging Analysis Group, Department of Applied Physics, University of Kuopio, Finland) (**Tarvainen et al. 2014**). A systematic average correction was applied to all data to reduce the number of artefacts present. A test was considered unusable if the percentage of artefacts was less than 5%. The following vmHRV of the parasympathetic branch indices were calculated and processed:

Time-domain indices

Mean Heart Rate: HR (global activation indicator of the SNA)

Natural logarithm of the RMSSD LnRMSSD: (*Parasympathetic modulation (short-term components of vmHRV*

Subjective assessment of self-reported variables

In addition to collecting vagally mediated vmHRV indices, self-reported subjective markers were collected to provide psychophysiological and behavioural context to facilitate the interpretation of the analyses of vmHRV data. In this context, the athlete provided information on her previous day's activities, including subjective questionnaires, hich the coach routinely considered in the management of training load and recovery. Rating Perceived Exertion (RPE) - Each evening, the perceived difficulty of the day's training was self-reported by the athlete using the Borg Category Report Scale (CR-10) (Borg, Ljunggren, and Ceci 1985). Subjective sleep assessment - Qualitative and quantitative sleep assessmentswere assessed via the Spiegel scale in conjunction with self-reported sleep duration. The Spiegel scale consists of six items that assessed sleep quality in the previous month, including sleep latency, duration, night waking occurrences, sleep depth, night-time dreams, and feeling upon waking up. Each item was scored from 0 to 5, with the final score ranging between 0 and 30 (Ren et al. 2018). Subjective assessment of fatigue, sleep, muscle soreness, and perceived stress (Total Subjective) - Perceived tiredness, sleep, muscle soreness, and stress was assessed using subjective questionnaires based on the Hooper scale (Hooper et al. 1995). Each of the 4 items was scored out of 7. These self-reported data were then summed to produce a single variable.

Statistical analyses

The Pearson correlation coefficient was calculated to estimate the correlation between each pair of variables. The results are presented in a correlation matrix in figure and table form.Levene's tests were performed to verify the homogeneity of variables. Before univariate analysis, the Shapiro-Wilk test was performed to check for normal data distributionbefore comparing each variable as a function of training load and period by one-way ANOVA. Paired T-tests were used to compare mean differences between variables from the baseline and each of the other training periods. All statistical analyses were performed with R (version 3.6.1; The R Foundation for Statistical Computing, Vienna, Austria) and the R-compatible Jamovi software (The jamovi project (2021); Version 1.6.23; Retrieved from https://www.jamovi.org). All data reported as mean ± standard deviationunless otherwise stated. The results were considered significant at $P < 0.05$.

RESULTS

A correlation matrix representing the relationships between the multiplevmHRV indices, and the self-reported subjective markers is summarised in a table (Tab. 2). This type of analysis makes it possible to visualise the intensity (significant or not) and direction (positive or negative) of the relationships between several variables. Among the main observations, LnRMSSDsu significantly correlated with the RPE and Sleep quality (P<0.05). HRsucorrelates significantly with RPE, Sleep quality, Stress and Muscle Soreness (P<0.05). Lastly, RPE significantly correlates with Muscle Soreness and Tiredness (P< 0.01).

Table 2. Correlation matrix (Pearson’s r2) of the different HRV indices and self-reported

The different training periods were compared to the rest period representing the baseline. The vmHRV

	Ln RMSSDsu	Hrsu	LnRMSSDsu - LnRMSSDst	RPE	Spiegel questionnaire	Sleep duration	Sleep quality	Stress	Muscle Soreness	Tiredness	Total Subjective
Ln RMSSDsu	—										
Hrsu	**-0.260** ***	—									
LnRMSSDsu - LnRMSSDst	**0.766** ***	0.001	—								
RPE	-0.120	**0.134** *	**-0.149** *	—							
Spiegel questionnaire	0.011	0.052	-0.014	-0.107	—						
Sleep duration	-0.102	0.110	-0.087	-0.067	**0.343** ***	—					
Sleep quality	**0.122** *	**0.116** *	**0.124** *	0.122	**-0.564** ***	**-0.353** ***	—				
Stress	0.084	**0.164** **	0.064	-0.082	-0.009	-0.002	**0.208** ***	—			
Muscle Soreness	0.029	-0.001	-0.019	**0.202** **	-0.342 ***	**-0.455** ***	**0.436** ***	**0.216** ***	—		
Tiredness	-0.106	**0.122** *	**-0.177** **	**0.290** ***	**-0.145** ***	-0.079	**0.219** ***	0.136	**0.353** ***	—	
Total Subjective	0.033	**0.132** *	-0.022	**0.261** ***	**-0.458** ***	**-0.398** ***	**0.679** ***	**0.490** ***	**0.801** ***	**0.680** ***	—

* P < 0.05, ** P < 0.01, *** P < 0.001

indices and self-reported subjective markers classified according to the training period can be seen in Tab. 3. The evolution of LnRMSSDsu as a function of RPE and training periods during the different training periods is presented in Fig. 2. The comparison of HR, LnRMSSD according to the different training periods of the season is shown in Fig. 3. During competition, only RPE (*P* > 0.05) and Sleep Quality (Spiegel questionnaire) (*P* > 0.01) were significantly different during the competition period compared to the Baseline (Tab. 3).

Table 3. HRV indices and subjective markers classified according to training period

	Baseline (rest)	Competition	Training	Training Camp	Altitude Training Camp
LnRMSSSDsu	1.80 ± 0.14	1.80 ± 0.04	1.55***± 0.30	1.69* ± 0.18	1.82 ± 0.15
HRsu (bpm)	49.87 ± 2.62	50.19 ± 2.31	52.30 ± 2.56	53.5** ± 2.47	54.53*** ± 2.15
RPE (au)	0.00 ± 0.00	3.57* ± 2.65	5.18*** ± 2.04	4.64*** ± 1.44	4.62*** ± 2.09
Spiegel questionnaire (au)	28.00 ± 0.76	26.2** ± 1.6	26.56 ± 1.89	25.89*** ± 1.76	25.48** ± 1.85
Sleep duration (minutes)	530.00 ± 35.86	546.00 ± 57.13	529.85 ± 39.40	535.00 ± 53.39	504.03 ± 59.00
Total subjective (au)	13.00 ± 2.67	12.27 ± 1.06	12.85 ± 1.54	12.29 ± 1.19	13.84 ± 1.63

All data reported as mean ± SD. Significant differences represented by * P < 0.05, ** P < 0.01, *** P < 0,001,

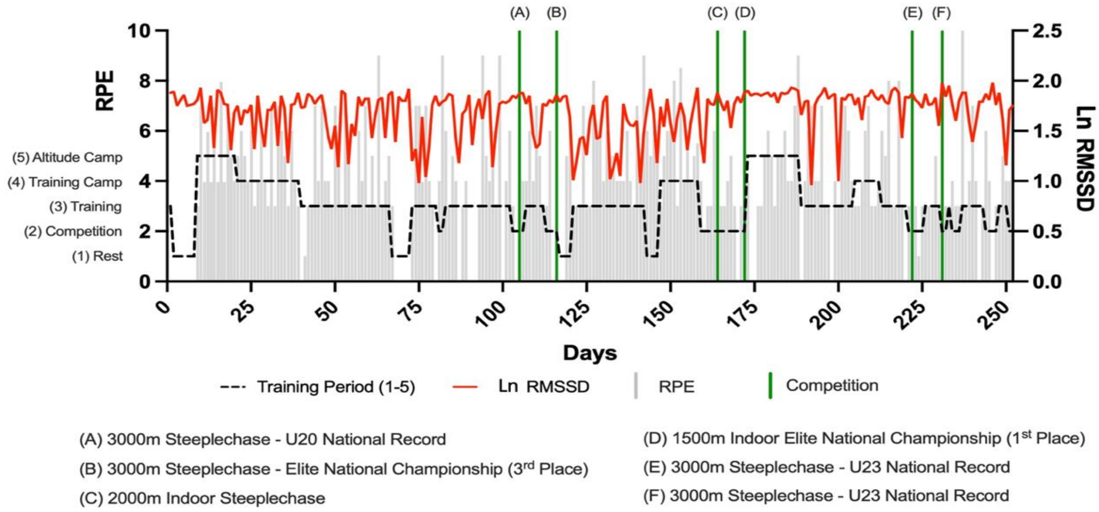

Figure 2. Evolution of the LnRMSSDsu depending of RPE and the training period during a pre-Olympic period

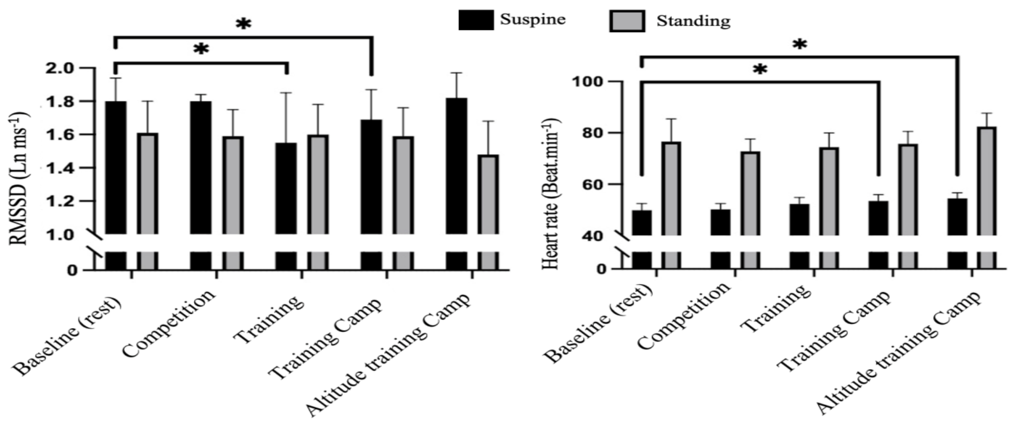

Figure 3. Heart Rate andLnRMSSD responses according to the period of the season

By comparison, during training, LnRMSSDsu were significantly lower (P> 0.001), and the RPE (P > 0.001) was significantly higher in comparison to the training and the Baseline (Tab 3. and Fig. 3). Furthermore, during training camps, LnRMSSDsu and Sleep Quality (Spiegel Questionnaire) were significantly lower (P> 0.05 and P> 0.001). At the same time, the HRsu and RPE were significantly higher (P> 0.5 and P> 0.001) between the Training Period and the Baseline (Tab. 3 and Fig. 3). HRsu and RPE were significantly higher (P > 0.001), and Sleep Quality (Spiegel Questionnaire) was significantly lower (P> 0.01) during altitude training camps in comparison to training camps and the baseline (Tab. 3 and Fig. 3).

DISCUSSION

The primary focus of this study was to assess the utility ofvmHRV combined with subjective markers for optimising training load prescription and management throughout an Olympic season in a high-performance athlete. During the monitoring period, the data generated from vmHRV and subjective reports were used by coaches to manipulate critical training variables, including intensity, duration, and recovery periods based on alterations in ANS activity and psychophysiological stress. This approach represented a data-informed approach and enabled the athlete to excel during critical competitions (Fig. 3).

Tapering is a common approach athletes employ to ensure optimal physiological condition in preparation for competition. Tapering involves a gradual decrease in the training volume with the maintenance of exercise intensity (**Mujika et al. 2000**). The resultant changes in vmHRV activity throughout taperingremain to be resolved. Nevertheless, the present case study observed that LnRMSSDSu and HRsuwere similar during the competition and rest (baseline) (Tab. 3). Additionally, in contrast to previous work by **Iellamo et al.** (**2002**),LnRMSSDsu was elevated following periods of intense training loads during specific preparation phases for competition (Fig. 2 and Fig. 3). This vagal activity change demonstrates that applying a tapering strategy enables a return of vmHRV to its baseline level, even while training continues. In this instance, the athletic support staff used daily vmHRV and subjective data to ensure sufficient tapering preceding competition.

We observed significant correlations between perceived training load, self-reported subjective data, and vmHRV indices, which contrasted the study by **Morales et al**. (**2017**) (Tab. 2). In conjunction with the observed decline in sleep quality, this data emphasises the value of coupling vmHRV with subjective markers for tracking the psychophysiological stress in elite athletes, particularly during competition periods. The performance results from national and international competitions and various national records associated with positive vmHRV adaptations in response to training modificationshighlight the necessity for a data-informedtapering strategy (**Plews et al., 2014**). Therefore, monitoring vmHRV with subjective markers enables training-specific alterations to training prescription for example, by adapting training content or postponing important training sessions and recovery periods increasing recovery time or switching from running to cyclingto ensure optimal performance at competition.

During the Training and Training Camp periods, it was observed that LnRMSSDsu and HRsu values significantly deviated from those during the rest period (Tab. 3). These substantial alterations in vmHRV indices align with periods of peak RPE (Fig. 3). **Baumert et al.**, (**2006**)similarly reported changes in the parasympathetic branch activity during periods of high training load typically occurring during training camp periods. Consequently, monitoring the autonomic nervous system (ANS) using vmHRV is recommended to keep track of individual adaptations to increased training loads **(Manzi et al., 2009**). Effective training planning and programming for endurance sports necessitate an optimal physiological stress level to trigger positive adaptations in response to the training load (**Buchheit et al. 2010**) Critically, when exercise training is mismanaged (characterised by high training load, intensity and inadequate recovery) performance may be impaired or worsened and increased risk of overuse injuries.

In our case study, coupling vmHRV monitoring with subjective markers empowered the coach to tailor his training programs and content to optimise the positive adaptations brought on by the increased psychophysiological stress from the increased training load. This approach to training periods and camps prepared the athlete adequately for competitions (Fig. 2.). Monitoring vmHRV and subjective markers enables personalised training (**Plewset al., 2014**) and training load adjustment (**Pichot et al. 2000**), enhancing different training periods for optimal performance and injury prevention.

During Altitude camps, RPE and HRsuwere significantly increased (Tab. 3 and Fig. 3), which may be partly explained by the increase in relative hypoxia (**Naeije 2010**). Despite the significant rise in HRsu, correlating with the increased training load, it's crucial to mention that LnRMSSDsu didn't decrease substantially. LikeField Schmitt, **Regnard, and Millet** (**2015**), LnRMSSD monitoring during altitude training failed to effectivelymonitor changes in ANS activity induced by training-related stress, which can't be separated from hypoxic stress. Moreover, it's widely acknowledged that altitude significantly impacts both the quality and quantity of sleep (**Bloch et al. 2015**), a hypothesis supported by this case study (Tab. 3). This deterioration in sleep quality can be attributed to the shift in the sleep environment, further exacerbating the effects of hypoxic stress on sleep. Therefore, using vmHRV in combination with subjective markers to monitor ANS activity is crucial to track the effects of psychophysiological stress induced by increased training load, intensified by hypoxic stress.

Practical application

Tracking shifts in ANS activity, particularly the parasympathetic branch, can provide a beneficial instrument for coaches to manage training across varying season periods. However, accounting for the cast range of inter-individual variabilities between athletes is crucial. Therefore, practitioners providing feedback to coaches must consider these personal differences and offer customised training load management to facilitate the stress/adaptation process. It's also vital to understand that elite athletes must impose a sufficiently high-stress level to trigger psycho-physiological adaptations that ultimately enhance performance. Unlike some suggestions, elite athletes might need to train even with reduced vagal tone (parasympathetic activity) to elicit necessary adaptations for performance improvement. As such, tracking changes in ANS activity, particularly via specific vmHRV markers, can furnish valuable insights for coaches about the autonomic adaptations resulting from the psycho-physiological stress applied to the athlete, effectively averting any overload phenomenon (**Bosquet et al. 2007**). In this regard, monitoring the training load and conducting a comprehensive analysis of the training program, including associated indirect factors, is imperative to facilitate long-term modifications to the training schedule (**Mujika 2012**).

However, there remains a necessity for more studies on elite athletes employing this methodology before we can assert that vmHRV, in conjunction with subjective markers, forms an effective tool for training planning.

Conclusion

In conclusion, this case study has reported alterations in ANS activity, specifically focusing on the parasympathetic branch, in conjunction with subjective data during varying phases of an Olympic season for an elite athlete. Combined with self-reported indicators, this approach supplied critical information that allowed practitioners to fine-tune the psycho-physiological stress imposed on their athletes during their decision-making process. Ultimately, this study underscores the sensitivity of vmHRV throughout different stages of an athletic season and pinpoints an imbalance in vagal tone during high training load periods (Training period and Training Camp period), manifested as a reduction in parasympathetic activity. Conversely, during lower training load phases (Competition Period), vmHRV improved, providing indirect feedback for perfecting pre-competition tapering and ensuring appropriate athlete recovery. From a monitoring perspective, vmHRV, along with subjective markers, could be an effective tool to adapt training periods, allowing the regulation of psychophysiological stress effects throughout an Olympic season.

Conflict of interest

No conflict of interest is declared by the authors. In addition, no financial support was received.

Ethics Statement

This study was approved by the CERSTAPS ethics committee (Approval No. 2022-A00644-39) and conducted in accordance with the Helsinki Declaration (1964, revised in 2001).

Author Contributions

Planned by the author: Study Design, Data Collection, Statistical Analysis, Data Interpretation, Manuscript Preparation, Literature

Search. Author have read and agreed to the published version of the manuscript

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