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Effectiveness of endurance training guided by oxidative stress analysis

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Abstract

Introduction: The purpose of this study was to assess the effectiveness of endurance training guided by oxidative stress analysis.

Methods: Twenty-three endurance athletes were randomized into an oxidative stress guided training group (guided, n = 12) and a control predefined training group (control, n = 11). At the end of a baseline week, a performance pre-test was performed before starting a 4-wk protocol followed by a post-test. Sleep duration, O2 score, subjective fatigue, training load and stress score were recorded daily. Heart rate variability (HRV) was measured three times a week. The training adjustment in the guided group was based on individual changes in O2 score values combined with the subjective fatigue. If the O2 score value was higher or lower than the individual recovery zone (calculated by the redox sensor) combined with a high total score of fatigue, a lower training load or rest was prescribed. The control group followed the standard predefined training program.

Results: There was a significant ($F = 2.45$; $P = 0.02$) group x time interaction for O2 score. The average training volume was lower in the guided group in comparison to the control group (w3: $P = 0.029$, w4 $P= 0.008$). O2 scores were in both groups correlated with the training volume ($R = 0.82$, $P < 0.05$), $RMSSD_{SU}$ ($R = 0.69$, $P < 0.05$) and HR_{SU} ($R = -0.91$, $P < 0.001$). However, no performance change was observed in both guided and control groups (pre-to-post- change 0.4 vs. -0.2%).

Conclusion: As expected, daily individualized adjustment of the training loads based on oxidative stress stabilized the O2 score in the guided group while it continued to increase in the control group. Aerobic performance remained unchanged in both groups despite a lower training volume in the guided group. Of interest is the correlation between the O2 score values and the training volume. These two parameters are also related with some HRV parameters. Overall, the O2 score seems sensitive to training volume and direct or indirect markers of fatigue. Therefore, the redox sensor may be a practical tool for monitoring endurance training.

Résumé

Titre en français :

L'efficacité de l'entraînement en endurance, guidé par l'analyse du stress oxydatif

Introduction: Le but de cette étude était d'évaluer l'efficacité de l'entraînement en endurance guidé par l'analyse du stress oxydatif.

Méthodes: Vingt-trois athlètes d'endurance ont été répartis au hasard dans un groupe d'entraînement guidé par le stress oxydatif (guidé, n = 12) et un groupe témoin d'entraînement prédéfini (témoin, n = 11). À la fin d'une semaine de référence ou « baseline », un pré-test de performance a été effectué avant de commencer un protocole de 4 semaines se terminant par un post-test. La durée du sommeil, la valeur O2 score, la fatigue subjective, la charge d'entraînement et le score de stress ont été enregistrés quotidiennement. La variabilité de la fréquence cardiaque (VFC) a été mesurée trois fois par semaine. L'ajustement de l'entraînement dans le groupe guidé était basé sur les changements individuels des valeurs O2 score, combinés à la fatigue subjective. Si la valeur O2 score était supérieure ou inférieure à la zone de récupération individuelle (calculée par l'appareil O2 score) combinée à un score total de fatigue élevé, une diminution de la charge d'entraînement ou du repos était prescrit. Le groupe témoin a suivi le programme d'entraînement standard et prédéfini.

Résultats: Un interaction significative (F = 2,45 ; P = 0,02) groupe x temps pour les valeurs O2 score a été relevée. Le volume moyen d'entraînement était plus faible dans le groupe guidé que dans le groupe témoin (w3 : P = 0,029, w4 P= 0,008). Les valeurs O2 score des deux groupes étaient corrélées au volume d'entraînement (R = 0,82, P < 0,05), $RMSSD_{SU}$ (R = 0,69, P < 0,05) et HR_{SU} (R = -0,91, P < 0,001). Cependant, aucune évolution de la performance n'a été observée dans le groupe guidé et le dans groupe témoin (évolution pré-post 0,4 vs -0,2 %).

Conclusion: Comme attendu, l'ajustement quotidien et individualisé des charges d'entraînement en fonction du stress oxydatif a permis de stabiliser les valeurs O2 score dans le groupe guidé alors qu'elles ont continué à augmenter dans le groupe témoin. Les performances aérobies sont restées inchangées dans les deux groupes malgré un volume d'entraînement plus faible dans le groupe guidé. Il est intéressant de noter la corrélation entre les valeurs O2 score et le volume d'entraînement. Ces deux paramètres sont

également liés à certains paramètres de la VFC. Dans l'ensemble, la valeur O2 score semble sensible au volume d'entraînement et aux marqueurs directs ou indirects de la fatigue. Par conséquent, l'appareil O2 score pourrait être un outil pratique pour monitorer l'entraînement en endurance.

Table of contents

1. Introduction 8
1.1 Endurance factors and training adaptation 8
1.2 Importance of recovery and overtraining risks 9
1.3 Functional fatigue – overreaching – overtraining continuum. 10
1.4 Diagnosis and monitoring methods 13
1.5 Oxidative stress 20
1.6 Interest in the daily adjustment of training by oxidative stress analysis and hypothesis 24
2. Méthodes 25
2.1 Subjects 25
2.1.1 Inclusion critera 25
2.1.2 Exclusion criteria 25
2.1.3 Subject recruitment 25
2.2 Study design 25
2.3 Material 27
2.4 *O2 score* questionnaire 27
2.5 Daily measurements 27
2.6 Edel device *« O2 score »* 28
2.7 Fatigue Questionnaire 29
2.8 HRV 30
2.9 Training load measure 32
2.10 Daily adjustment of training by O2 score analysis 33
2.11 Pre- and post- test 34
2.12 Data analysis and statistics 35
3. Results 36
3.1 HRV and daily measurements 36
3.2 Performance tests (pre and post) 39
3.3 Comparison between groups 39
3.4 Relationships with the training volume 44
3.5 Relationships with the fatigue 47
3.6 Relationship with O2 score values 50
4. Discussion 56
4.1 Effectiveness of training guided by oxidative stress 56
4.2 Correlations with O2 score values 57
4.3 The training volume 57
4.4 The fatigue 58
4.5 O2 score values 58
5. Strengths and limits 59
6. Perspectives 60
7. Conclusion 61
8. Bibliography 62
9. Annexes 67

List of figures

Figure 1 Possible presentation of the different stages of training, OR (OVERREACHING) and OTS (OVERTRAINING SYNDROME) (Meeusen et al. 2013). 13
Figure 2 ROS production during physical exercise by electron leakage in the mitochondria (Pialoux 2010). 22
Figure 3 Study design showing the training load adjustment in the guided group (B), according to the O2 score values obtained daily (A) while no adjustment is made in the control group (C). All the measurements recorded during the study are presented under the O2 score values (A). 36
Figure 4 Time course of O2 score values (nW) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol. 40
Figure 5 Time course of the volume (min) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol. 41
Figure 6 Time course of the fatigue (TSF) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol. 42
Figure 7 Time course of LF (ms^2) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol. 43
Figure 8 Time course of HF (ms^2) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol. 44
Figure 9 Relationship between the volume (min) and $RMSSD_{SU}$ (ms) 45
Figure 10 Relationship between the volume (min) and HR_{SU} (bpm) 46
Figure 11 Relationship between the volume (min) and the fatigue (TSF) 47
Figure 12 Relationship between the fatigue (TSF) and $RMSSD_{SU}$ (ms) 48
Figure 13 Relationship between the fatigue (TSF) and HF_{SU} (ms^2) 49
Figure 14 Relationship between the fatigue (TSF) and LF_{SU} (ms^2) 50
Figure 15 Relationship between the volume (min) and O2 score (nW) 51
Figure 16 Relationship between O2 score (nW) and $RMSSD_{SU}$ (ms) 52
Figure 17 Relationship between O2 score (nW) and HR_{SU} (bpm). 53
Figure 18 Relationship between Δ O2 score (nW) and LF_{SU} (ms2). 54
Figure 19 Relationship between RPE (0-10) and Δ O2 score (nW). 55

List of tables

Table 1 Diagnosis of overtraining syndrom (Meeusen et al. 2013).. 14

Table 2 Summary and evaluation of some methods used to monitor athlete training load and/or responses (Bourdon et al. 2017).. 20

Table 3 Antioxidants and their anti-radical actions (Pialoux 2010).. 22

Table 4 Characteristics of the subjects divided into oxidative stress guided training and control groups.. 26

Table 5 Description of the eight items of the fatigue questionnaire (Chatard, Atlaoui 2003).. 30

Table 6 Selected time-domain measures of HRV (Task Force 1996).. 31

Table 7 Selected frequency domain measures of HRV (Task Force 1996).. 32

Table 8 Scale (CR10) modified from Foster et al. (2001) to rate the perceived level of exertion (RPE).. 32

Table 9 Guide to compare the training zone with other intensity assessment methods. 33

Table 10 Parameters daily measured during the baseline and the protocol (week 1, 2, 3 and 4).. 37

Table 11 Training parameters daily reported during the baseline and the protocol (week 1, 2, 3 and 4).. 38

Table 12 HRV parameters measured in supine (SU) and standing (ST) positions during the baseline and the protocol (week 1, 2, 3 and 4).. 39

1. Introduction

1.1 Endurance factors and training adaptation

Endurance is defined as the ability to maintain a certain level of required intensity over the time. It is the ability to maintain an effort of a given relative intensity for a prolonged period of time.

For endurance sports, three main factors emerge from the literature as being decisive in the production of performance.

Maximum oxygen consumption during exercise is a major indicator of maximum aerobic capacity and is positively correlated with performance (Saltin et al. 1967). Endurance athletes achieve much higher VO_{2max} values than the general population, mainly reflected in high cardiac output.

The fractional utilization of VO_{2max} or lactic threshold is one of the best determinants of an athlete's endurance capacity. The ability to perform intense exercise without accumulating lactate indicates a better tolerance to aerobic exercise since lactate formation contributes to fatigue (Wilmore & Costill 1999). The ability to maintain a high percentage of VO2max determined by the oxidative capacities of the muscle, thus promoting the use of lipids during exercise and sparing that of glycogen. This is directly related to a decrease in lactate accumulation in the blood, i.e. a higher lactic threshold (Holloszy & Coyle 1984).

Energy cost is also a determining factor in endurance performance since it defines the oxygen consumption required to develop a given speed or power. Endurance athletes have more type I fibers, less fatigable, and thus increase their efficiency, (Coyle et al. 1992).

Adaptations of the pulmonary, cardiovascular and neuromuscular systems improve the oxygen supply from atmospheric air to mitochondria and the control of metabolism in muscle cells, (Jones & Carter 2000).

These adaptations can be central and are mainly observed with an increase in maximum aerobic power (VO_{2max}). They are more limited in subjects with a high level of physical fitness at baseline and also depend on genetic factors. Green et al. (1995) concluded a possible increase from 15 to 20%. Adaptations can also be observed at the peripheral level.

They occur within the muscle and induce changes in its structure and energy functioning (i.e. oxidative fibers function, muscle capillarisation, number and size of mitochondria).

In endurance sports such as running, cycling, rowing, swimming or cross-country skiing, the majority of training is aerobic, sub-maximum, continuous and prolonged sessions. Strength training sessions and anaerobic or interval training are also essential to improving performance.

The training intensity distribution in elite endurance athletes has recently been studied by Seiler & Kjerland (2006). They show the emergence of a *polarized*-training model from a limited number of publications (Steinacker 1993; Steinacker et al. 1998; Schumacker & Mueller 2002; Billat et al. 2001; Solli et al. 2017) suggesting that at high-performance levels, athletes generally train below the lactate threshold intensity. About 75% of sessions are performed at low intensity while only 15% of training sessions are above the lactate threshold. In essence, the training intensity distribution is polarized away from the moderately hard intensity range represented by the lactate threshold.

Recent studies (Tonnessen et al. 2014; Sandbakk et al. 2016; Solli and al. 2017) have been conducted on elite cross-country skiers showing that approximately 60% of the total training time is performed during the general preparation. This period typically includes high volumes of LIT (low intensity training) and 50–60% of the endurance training conducted as sport-specific exercise. The remaining 40% of annual training is performed during the specific preparation and competition phase, with decreased total volume, increased amount of HIT (high intensity training) and higher amount of sport-specific activity forms (Sandbakk & Holmberg 2017). To ensure peak performance, a typical tapering approach has been to perform 2–4 weeks of overload training, followed by 1–3 weeks with decreased load (Hellard et al. 2013).

1.2 Importance of recovery and overtraining risks

In endurance sports, following a standardized training program doesn't lead to homogeneous physiological responses. Age, sex, initial fitness level and other genetic factors are known determinants of individual differences in response to endurance training (Bouchard et al. 1999; Bouchard & Rankinen 2001).

The load-performance relationship and the ability to tolerate high training loads are highly individual. For beginners small training loads usually cause large performance improvements, while elite athletes need larger training loads to elicit small performance improvements. To perform at the highest level, athletes must be able to sustain high training loads while avoiding overtraining. The critical point above which 'training' becomes 'overtraining' is individual and difficult to identify (Foster 1996). Overreaching is often utilized by athletes during typical training cycles to enhance performance. The resultant acute fatigue can cause a positive adaptation or improvement in performance provided appropriate recovery is allowed. However, if the balance between appropriate training stress and adequate recovery is disturbed, an abnormal training response may occur and a state of overtraining may develop (Halson & Jeukendrup 2004). Overtraining syndrome is frequently observed in response to sustained high intensity and high volume athletic training, particularly when coupled with other stressors in the athlete's life (Foster 1998). This includes frequent competitions, monotonous training, psychosocial stressors, illness/infection and heavy travel schedules (Halson & Jeukendrup 2004). There are two main reasons for the onset of this syndrome. First, to improve their performance, athletes must gradually increase the training load in order to obtain even greater benefits over the course of his career. Second, when a state of overtraining leads to a decrease in performance, the instinctive response of most athletes, as well of the coach is to further increase the training load of subsequent training session to compensate for the decrease in performance (Foster 1998).

1.3 Functional fatigue – overreaching – overtraining continuum.

A unique and isolated training causes stress to the body, which will lead to so-called "acute responses". Repeated and regular training leads to chronic physiological adaptations specific to the type of stress induced by the repetition of exercises (Wilmore & Costill 1999).

When planning an annual training cycle, coaches apply the concept of periodization, which is based on the interaction between load and recovery.

This model assumes that when a training load exceeds the capacity of the athlete, performance will initially decrease. After sufficient recovery, the capacity of the subject increases beyond the baseline level. This effect is known as "overcompensation" and considered the building of training periodization (Brink et al. 2014).

It's difficult to find an optimal alternation between training stimulus and recovery. The purpose being to benefit from the effects of overcompensation, without falling into persistent negative fatigue (overtraining) or decreased capacity (undertraining) when recovery periods are too long (Turner 2011)
Mesocycle blocks are usually arranged in a 3:1 loading paradigm, whereby the load gradually increases for the first three microcycles (weeks) before an unloading phase in the fourth creating the typical undulating appearance of periodized programs. The unloading phase reduces fatigue, thereby allowing adaptations to take place. It should also be noted that because training adaptations take place during recovery periods, the need to reduce accumulated fatigue cannot be understated (Haff 2004).

Following intense physical exercise, a state of normal fatigue develops. The athlete recovers in a few hours, two days for the main substrates or hormones (Cleare 2003; Doyle et al. 1993). Muscle pain may persist a little longer and muscle recovery may last up to two weeks (Sbriccoli et al. 2001).

If the exercises are repeated, a persistent state of fatigue and reduced performance will develop (Millet 2009). Ideally, subsequent training should not take place until overcompensation has occurred. If a prolonged excessive training takes concurrent with other stressors and insufficient recovery, athletes may experience an unexplainable decrease in performance (Meeusen et al. 2013). Thus can result in « chronic maladaptations » that can lead to the overtraining syndrome.

The magnitude of this decreased performance necessary for the diagnosis of overtraining and the nature of an "appropriate" regeneration period are, however, difficult to define and may vary depending upon the training background of the subjects and the nature of the preceding training. It may or may not be associated with biochemical, hematological, physiological and immunological indicators (Fry et al. 1992).

Because it is speculated that a continuum exists between acute fatigue, overreaching (OR) and overtraining state (OTS) (Fry et al. 1992), many groups tried to work on the definitions of OT & OR (Kreider et al. 1998; Halson & Jeukendrup 2004; Urhausen & Kindermann 2002; Meeusen et al. 2013).

Overreaching consists of an accumulation of training and/or non-training stress resulting in short-term decrement in performance capacity with or without related psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks.

Overtraining consists of an accumulation of training and/or non-training stress resulting in long-term decrement in performance capacity with or without related psychological signs and symptoms of maladaptation in which restoration of performance capacity may take several weeks or months.

However, the difficulty lies in the subtle difference that might exist between extreme overreached athletes and those having an Overtraining Syndrome (Meeusen et al. 2013). Meeusen & Duclot (2006) proposed a continuum in three distinct steps, represented in Figure 1: **1. Functional Overreaching (FO);** this state represents a short-term Overreaching (few days to two weeks approximately) following a period of intensive training limited in time, for example when an athlete attends a training camp. Performance decreases for a few days without any other negative symptoms. This state is reversible if the recovery period is respected and may be accompanied by positive performance effects induced by overcompensation. **2. Non-Functional Overreaching (NOF)**; It represents a state of extreme Overreaching. When this training is prolonged or following too much intensification of this training, the athlete may experience a state of extreme non-functional overreaching which will cause stagnation or even a drop in performance that may last for several weeks or months. However, the athlete will eventually recover fully after an adequate rest period. At this stage, the first signs and symptoms may be present (such as psychological, hormonal, disturbance or other factors such as illness or sleep disorder). **3. Overtraining syndrome (OTS);** The difference between this state and the previous one is difficult to distinguish since the athlete often shows the same symptoms. Moreover, the clinical features are varied from one individual to another, and are non-specific, anecdotal and numerous.
The most obvious expression is clinical and is defined by a 'prolonged maladaptation' of several biological, neurochemical and hormonal regulation mechanisms. This state may take months or possibly years to completely recover, in which time an athlete's career may be seriously compromised (Halson & Jeukendrup 2004).

Figure 1 Presentation of the different stages of training, OR (OVERREACHING) and OTS (OVERTRAINING SYNDROME) (Meeusen et al. 2013)

PROCESS	TRAINING (overload)	INTENSIFIED TRAINING		
OUTCOME	ACUTE FATIGUE	FUNCTIONAL OR (short-term OR)	NON-FUNCTIONAL OVERREACHING (extreme OR)	OVERTRAINING SYNDROME (OTS)
RECOVERY	Day(s)	Days – weeks	Weeks – months	Months - …
PERFORMANCE	INCREASE	Temporary performance decrement (e.g., training camp)	STAGNATION DECREASE	DECREASE

1.4 Diagnosis and monitoring methods

The diagnosis of overtraining detection depends mainly on clinical results, questionnaires and interviews. Many studies such as Meeusen et al. (2013) have demonstrated that psychological indicators are more sensitive and consistent than physiological indicators. Biological examinations only occur following a lasting decrease in the performance of a high fitness' questionnaire score (SFMS) and a preliminary interview (Brun et al. 2009). Urhausen & Kindermann (2002) have highlighted the validity of different tools for diagnosing overtraining. In resting conditions, an impaired mood state can be detected through the Profile of Mood State (POM) as well as other symptoms such as sleep disturbances. The POMS questionnaire is a 5-point Likert scale that calculates a total mood disturbance score by summing the totals for the negative subscales (i.e. tension, depression, fatigue confusion) and then subtracting the totals for positive subscales such as vigor and esteem-related affect (Mc Nair et al. 1971). During high training periods, Meeusen et al. (2013) reported an elevation in negative moods by the athletes and a decrease in the positive mood of vigor. They proposed a "Check List" presented in Table 1, that might help to diagnosis overtraining syndrome (OTS) Over-trained athletes also related a pronounced feeling of muscular soreness. Overtraining detection is also evaluated on maximum performance. It can be reproduced using an ergometer test, which can be expressed as an impaired lactate production and a reduction in the maximum heart rate. This decrease in maximum performance may also be the result of a depletion of muscle glycogen (Urhausen & Kindermann 2002).

Table 1 Diagnosis of overtraining syndrom (Meeusen et al. 2013).

Performance – fatigue
Is the athlete suffering from: - Unexplainable underperformance - Persistent fatigue - Increased sense of effort in training - Sleep disorders - ...
Exclusion Criteria Are there confounding diseases? - Anaemia - Epstein Barr virus - Other infectious diseases - Muscle damage (high CK) - Lyme disease - Endocrinological diseases (diabetes, thyroid, adrenal gland, ...) - Major disorders of eating behaviour - Biological abnormalities (increased erythrocyte sedimentation rate, C-Reactive Protein, creatinine, or liver enzymes, decreased ferritin ...) - Injury (musculoskeletal system) - Cardiological symptoms - Adult-onset asthma - Allergies - ...
Are there training errors? - Training volume increased (>5%) (h/wk, km/wk) - Training intensity increased significantly - Training monotony present - High number of competitions - In endurance athletes: Decreased performance at 'anaerobic' threshold - Exposure to environmental stressors (altitude, heat, cold, ...) - ...
Other confounding factors: - Psychological signs and symptoms (disturbed POMS, RestQ-sport, RPE, ...) - Social factors (family, relationships, financial, work, coach, team, ...) - Recent or multiple time zone travel - ...
Exercise test: - Are there baseline values to compare with? (Performance, Heart Rate, Hormonal, Lactate, ...) - Maximal exercise test performance - Submaximal or sports specific test performance - Multiple performance tests - ...

Several monitoring methods are widely used today by coaches. They allow to control the evolution of the training load, the individual responses of each one and the recovery needs in order to minimize the risks of overtraining, injuries and diseases. While adolescent age is accompanied by many non-training or non-competition related stressors, it is also during this period that an athlete's training loads begin to become significant. It is therefore recommended that young athletes keep a training diary on a regular basis. It allows them to understand their training and competitions loads and to preserve the health of those if in order to guarantee them a prolonged sporting career (Bourdon et al. 2017). Among the wide variety of drive load measurements, there are two categories of loads; internal and external loads. Internal loads characterize the so-called biological stressors. They include both psychological and physiological aspects such as heart rate and it derivatives, blood lactate, oxygen consumption or rating of perceived exertion (RPE). External loads are objective and measurable measurements on the field during training or competitions. In endurance sports, it is mainly speed, power, training time or distance that are most commonly used. Bourdon et al. (2017) present a table with the summary and evaluation of some methods of load's monitoring reported in Table 2. The ideal is to combine the use of external and internal loads. Each individual responds differently and individually to the same training session. Also, there are intra-individual differences when an athlete repeats the same training session. He can maintain the same external loads (duration and distance similar for example) but experiences different internal loads, which will depend on factors such as stress, fatigue, illness or previous trainings (Bourdon et al. 2017). Monitoring the individual responses of athletes to the same training helps to assess the recovery potential and the stress tolerance and to determine the relationship between loads and performance. From this, coaches can adjust the training program of the athletes in concordance to their individual stress/recovery balance (Mujika 2017).

Training duration, distance, speed or intensity are variables used in several studies to control the load responses. The most commonly used methods are heart rate (HR) or perceived self reported variables (RPE) to calculate training load, although the use of questionnaires is also widely used when combined with physiological measurements (Le Meur et al. 2013). These are simple and time-efficient measures that are good indicators for coaches and athletes in endurance sports (Roos et al. 2013). However, psychological measures can be applied and reported more quickly than physiological indicators. RPE is part of this and represents an effort perception scale ranging from 1 to 10 (Borg scale) that can be used as a marker of training intensity. Foster (1998) uses this load quantification method by multiplying the effort perception score by the duration of the training session 30 minutes after the end of the training. The load can be calculated on a daily or weekly training load. He showed that the variability of training (daily mean training load divided by the standard deviation of the load over a period of one week) allows to highlight the "monotony" of training. The product of the weekly training load and the monotony is calculated as "stain". These two factors are linked to negative adaptations to training that can contribute to the genesis of overtraining. Thus, being able to control and reduce these different training indicators such as monotony and stain, helps to prevent the risk of injuries, infections or overtraining (Foster 1998).

Other methods may be used to diagnose overtraining in the field of sport sciences. Le Meur et al. (2013) reported that the blood lactate concentration (BLC) is one of the most reliable variables in the differentiation between normal and overtraining state. Although BLC is an objective and low-cost measurement, it's still a subject to debate when measured during submaximal exercise (Meeusen et al. 2013 cited by Buchheit 2014).

In recent years there has been a growing interest in monitoring the status of the autonomic nervous system via measures of heart rate (HR), including the level and variability of HR at rest (Kiviniemi et al. 2007; Plews et al. 2013; Schmitt et al. 2018; Javaloyes et al. 2019). Changes in cardiac variability depend largely on the autonomous cardiac system that attempts to adapt cardiovascular function. The heart rate variability (HRV) analysis would be likely to detect a state of fatigue (e.g., FO, NOF or OTS) (Schmitt et al. 2015). HRV is regulated by a sympato-vagal balance with two antagonistic components operating simultaneously: sympathetic cardio-accelerating activity and parasympathetic cardio-moderating activity (which dominates at rest). HRV analyses the level of fluctuation of heartbeats. It more precisely evaluates the time variation between 2 heartbeats called "R-R interval". Changes in heart rate can be assessed by several methods. The first method belongs to the time domain and is certainly the simplest measurement to perform. It measures the time between normal R-R intervals and their standard deviation. The values obtained from the R-R intervals are then graphically represented as a function of time on a curve called Tachogram. Temporal analysis is influenced by both sympathetic and parasympathetic activities, but indices calculated from differences between normal R-R intervals are strongly correlated with parasympathetic modulation in relation to ventilation. Temporal variables apply mainly to long periods of time. RMSSD variable expressed in (ms) represents the square root of the mean sum of the square differences between successive R-R intervals and evaluates the parasympathetic tone. This is a recommended parameter because it measures short-term variables and has good statistical properties (Task Force, 1996). The second method, known as the frequency method, is a non-parametric analysis using the Fourier rapid transformation technique. It allows the oscillations between the R-R intervals to be detected and represented as spectral density, as a function of the frequency of these oscillations. The power of the spectral density is measured in (ms^2 Hz) and the spectral power is expressed in (ms^2). There are three main spectral components in a spectrum;

high frequency (HF), low frequency (LF) and very low frequency (VLF). The high frequency power band (HF: 0.15 - 0.4 Hz) reflects a mainly parasympathetic activity related to the respiratory responses of the sinus node, while the low frequency power band (LF: 0.04 - 0.15 Hz) is also controlled by the parasympathetic tone but mainly reflects a sympathetic (and sometimes mixed) tone modulation. VLF is a less well-defined frequency and is therefore not always included in analysis. Measurements of LF and HF power components are usually made in absolute values of power (ms^2), but LF and HF may also be measured in normalized units (nu) which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in normalized units (nu) emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power. Nevertheless n.u. should always be quoted with absolute values of LF and HF power (ms^2) order to describe in total the distribution of power in spectral components. The ratio of low frequency to high-frequency power bands is used to define the sympatho-vagal balance (LF/HF). By calculating the ratio of frequency amplitude to total power of the oscillation spectrum (TP), it is possible to evaluate changes in the sympatho-vagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Buchheit (2014) suggested that the most useful resting HRV indicator would be the time domain index RMSSD measured in supine position. It is an easy and quick recording method with good statistical properties (Task Force, 1996). Saboul et al. (2013) reported that RMSSD has a lower sensibility to breathing pattern than spectral variables and tends to decrease linearly toward NFOR (Buchheit 2014). It allows to detect a global fatigue level using a weekly average of a minimum three measures (Plews et al. 2013). However, RMSSD values from the lying position doesn't provide any information about the interplay between sympathetic and parasympathetic activity and the preserved or altered ability to dynamic control adjustment from supine to standing position (Schmitt et al. 2015). The study of Schmitt et al. (2013) compared HRV spectral differences in a "fatigue" and "non fatigue" state in both supine and standing positions and highlight that HRV variables were fully independent from one position to another and different in "fatigue" vs." no-fatigue" state. In "fatigue state" differences were related in supine position with an increase in HR, LF/HF and LF (nu) and a decrease in LF, HF, TP and HF (nu). In standing position, it's HR that increased, while LF, HF and TP decreased in "fatigue" state.

Despite the practical and effective usefulness of RMSSD in supine position, spectral analysis in both positions allows a more complex evaluation of the different sub-categories of fatigue as well as changes in autonomic modulations attributable to the LF and HF components, (Schmitt et al. 2015).

The main reason of HR measures interest is that they are non-invasive, inexpensive and take little time on a daily basis (Buchheit 2014). HRV analysis using as a measure of cardiac autonomic balance is an important determinant of training adaptation (Vallverdù et al. 2017). In endurance sports, positive adaptations to training would be marked by an increase in vagal-derived indices of HRV while a decrease of the latter one would suggest negative adaptations to training (Plews et al. 2013). Concerning the overtraining monitoring, the study of Portier et al. (2001) demonstrated that autonomic balance would be one of the first to be affected in case of fatigue-induced physiological perturbation. However, although HRV measurements are simple and fast, the interpretation of results remains a limitation of this daily monitoring tool. Some studies seem to show a positive correlation between improved performance and increased resting HRV after a training period in sedentary subjects or amateur athletes (Buchheit et al. 2011; Lee 2003) However, this is not always the case when it comes to elite athletes (Portier et al. 2001; Iellamo et al. 2002). This is important to be aware to the different responses of the HRV variables and a longitudinal monitoring is therefore required to understand the training status in elite athletes (Plews et al. 2013). Although a reduction in HRV is in most cases associated with fatigue or overtraining, each individual has his own unique cardiac autonomic status and HRV relationship (Plews et al. 2013). The HRV measurements remains a tool widely used in endurance sports and allows daily monitoring that may prevent an overreaching or overtraining state. Some authors demonstrated that the daily and individualized adjustment of the training loads based on morning HRV measurements reduced altitude-induced responses (Schmitt et al. 2018). The HRV-guided hypoxic group of elite athletes expressed a reduction in the combination of increase sympathetic and decreased parasympathetic nervous activities.

The study of Kiviniemi et al. (2007) used daily HRV measurements to adjust the timing and the amount of high-intensity exercises at individual level. The HRV guided training has improved cardiorespiratory fitness such as VO2 peak thanks to an individualized periodization and frequency of training. Javaloyes et al. (2019) also showed an improvement in performance in well-trained cyclists using a daily training prescription based on HRV.

These different monitoring methods used in the field would therefore be beneficial in the daily control of the athletes' load. This would allow better regulation of individual responses to training and to detect overtraining or injuries early on (Roos et al. 2013).

Table 2 Summary and evaluation of some methods used to monitor athlete training load and/or responses (Bourdon et al. 2017).

Method	Cost	Hardware needed	Software needed	Ease of use	Valid	Reliable	Used to interpret	Used to prescribe	Variables
Internal Measures									
RPE	L	N	Y/N	H	M–H	M–H	Y	Y	Single variable in AU (time dependent)
Session rating of perceived exertion	L	N	Y/N	H	M–H	M–H	Y	Y	Single variable in AU (time dependent)
TRIMP[4]	L–M	Y	Y	M	M–H	M–H	Y	N	Single variable in AU (time dependent)
Wellness questionnaires*	L	N	Y/N	M–H	M	M–H	Y	Y/N	Ratings, checklists, AU scale measures
Psychological inventories (eg, POMS, Rest-Q-Sport)*	L–M	N	Y/N	M–H	M–H	M–H	Y	Y	Ratings, checklists, AU scale measures
Heart-rate indices	L–M	Y	Y	H	H	M–H	Y	Y	Heart rate, time in zones, HR variability/recovery measures, etc
Oxygen uptake	H	Y	Y	L	H	H	Y	Y	VO_2, metabolic equivalents
Blood lactate	M	Y	Y/N	M	H	H	Y	Y	Concentration
Biochemical/hematological assessments	M–H	Y	Y/N	L	H	M–H	Y	Y	Concentrations, volumes
External Measures									
Time	L	Y	Y/N	H	H	H	Y	Y	Units of time (s, min, h, d, wk, y)
Training frequency	L	N	N	H	H	H	Y	Y	Session count
Distance/mileage	L	Y/N	Y/N	H	H	H	Y	Y	Units of distance (m, km)
Movement repetition counts	L	Y/N	Y/N	M–H	H	M–H	Y	Y	Activity counts (eg, steps, jumps, throws)
Training mode	L	Y/N	N	H	H	H	Y	Y	Weight training, run, cycle, swim, row, etc
Power output	M–H	Y	Y	L–M	H	H	Y	Y	Relative (W/kg) and absolute power (W)
Speed	L–M	Y	Y/N	M–H	H	H	Y	Y	Speed measures (m/s, m/min, km/h)
Acceleration	L–M	Y	Y	L	H	H	Y	Y	Acceleration measures (m/s^2)
Functional neuromuscular tests	L–M	Y	Y/N	M	M–H	H	Y	Y	Countermovement-jump and drop-jump measures
Acute:chronic-workload ratio	L–M	Y/N	Y	M	M–H	M–H	Y	Y	Size of acute training load relative to chronic load
GPS measures	M	Y	Y	M	M–H	M	Y	Y	Velocity, distance, acceleration, time in zones, location
Metabolic power	M	Y	Y	L–M	L–M	M	Y	N	Energy equivalent
Time–motion analysis video (automated)	H	Y	Y	L	M–H	M	Y	Y	Velocity, location, acceleration
Time–motion analysis video (nonautomated)	M–H	Y	Y	L	M–H	M	Y	Y	Velocity, location, acceleration
Accelerometry	M	Y	Y	L–M	M–H	M	Y	N	x-y-z g force
Player load	M	Y	Y	M	M	M	Y	Y	Single variable in AU (time dependent)

Abbreviations: L, low; M, medium; H, high; Y, yes; N, no; AU, arbitrary units.

*Measures of training response.

1.5 Oxidative stress

Oxygen is an essential source of energy for the cell. It is within the mitochondrial respiratory chain that oxygen is reduced to generate ATP molecules and provide the energy necessary for metabolism. This phenomenon is paradoxical since it allows a small percentage of electrons to escape and react with the O2 dissolved in the cytoplasm forming oxygenated free radicals or reactive oxygen species (ROS), such as the peroxyl radical (ROO-). Jackson (2000) reported that 2 to 5% of the oxygen used in the mitochondria forms free radicals.

Free radicals (FR) are chemical species with an unpaired electron (e^-) called single in the outer orbit. This phenomenon gives FRs special characteristics such as paramagnetism, instability and reactivity. If the number of electrons is odd, free radicals will try to bind to other atoms to form new molecules, either by giving them a single e^- (reduction) or by removing an e^- (oxidation). This makes it possible to form a new couple of e^- on the outer orbit and form a new FR. Free radicals are produced permanently in the body and used for physiological purposes such as maintaining good homeostasis (the cellular redox state necessary for cell growth, repair and gene expression). They are also used for their reactivity, by immune cells and involved in the systemic defense responses to pathogens (Pialoux 2010).

In a normal state of oxidation-reduction (also called redox), the cells of aerobic species have a constant basal concentration of free radicals. However, physical activity requires all the more energy and therefore greater oxygen consumption, which modifies the balance of oxidation-reduction. Oxidative phosphorylation thus increases the production of FR. The first of these molecules formed from O2 and a free electron (e-) is the anion superoxide (see Figure 2). These unstable chemical molecules are potentially toxic when their concentration is too high and exceeds the cells' ability to neutralize them, thereby disrupting the redox homeostasis. They are capable of damaging other organic molecules by attacking various cellular components (lipids, proteins, DNA,) causing harmful oxidative changes, aging triggers, pathologies, cell death (Haleng et al. 2007).

In order to counter the oxidative action of free radicals, our body has two main types of antioxidants; Enzymatic antioxidants (endogenous) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) or glutathione reductase (GR) and non-enzymatic antioxidants (mainly brought by food) such as vitamin C, E, flavonoids or micronutrients. Antioxidants and their anti-free radical actions are presented in Table 3.

Figure 2 ROS production during physical exercise by electron leakage in the mitochondria (Pialoux 2010).

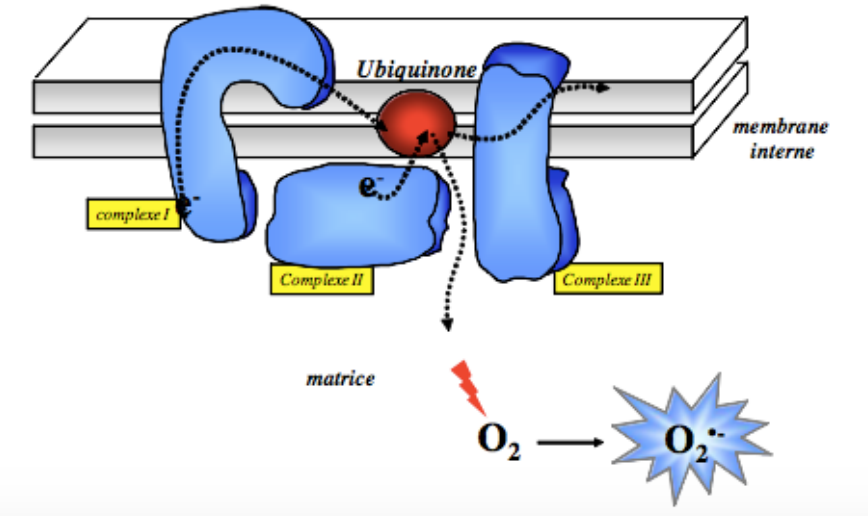


Table 3 Antioxidants and their anti-radical actions (Pialoux 2010).

	Antioxydant	Action
enzymes	SOD	Dismute $O_2^{\bullet-}$ en H_2O_2
	Catalase	Transforme H_2O_2 en H_2O et O_2
	GPX	Réduit H_2O_2 et ROOH
	Glutathion réductase	Recycle GSSG en GSH
Non-enzymatiques	Acide urique	Réduit $OH^{\bullet}$
	Glutathion	Réduit H_2O_2 et ROOH Recycle la vitamine C
	Vitamine C	Réduit $O_2^{\bullet-}$ et $OH^{\bullet}$ Recycle la vitamine E
	Vitamine E	Neutralise $ROO^{\bullet}$ et $RO^{\bullet}$ Limite l'action de la phospholypase A2
	Flavonoïde	Identique à vitamine E
	β-carotène	Piège $^1O_2$ et peut réduire $ROO^{\bullet}$
	Lycopène	Piège $^1O_2$ et peut réduire $ROO^{\bullet}$
	Co-enzyme Q10	Réduit Fe^{3+} - $O_2^{\bullet-}$

Redox balance is importantly influenced by external factors such as physical activity. It can lead to a redox imbalance between antioxidants and free radicals called oxidative stress (Finaud et al. 2006). Debevec et al. (2017) reported that the redox balance modulation is highly dependent on the level of physical activity. Acute and intensive exercise as well as physical inactivity are known to promote oxidative stress. However, chronic and moderate exercise training seems beneficial for restoring the redox balance (Debevec et al. 2017). Other authors have shown that aerobic training would reduce post exercise oxidative stress (Radak et al. 1999; Clarkson, 1995 cited by Finaud et al. 2006). However, the results did not determine whether this reduction in oxidative stress causes a decrease in stress ox production or an increase in antioxidants that can neutralize them. Some authors have shown that in response to a higher production of free radicals during exercise, we would observe a better efficiency of the antioxidant system (Venditti et al. 1997; Selamoglu et al. 2000 cited by Finaud et al. 2006).

Despite the improvement in antioxidant status, recent studies show that an overload of training, particularly in high-performance athletes, could compromise their state of defence (Balakrishnan et al. 1998; Subudhi et al. 2001 cited by Finaud et al. 2006).

Oxidative stress can increase during periods of intensive training. The production of free radicals during exercise plays an important role in the induction of muscle lesions and post-exercise inflammation. Therefore, it may be one of the actors of the overtraining syndrome (MC Kenzie et al. 1999; Petibois et al. 2002). It would be relevant to implement a longitudinal follow-up that includes oxidative stress markers complementary with biological variables because of the possible link between an increase in oxidative stress and an overtraining state

Oxidative stress can be estimated by measuring either the production of ROS or by measuring the concentration of antioxidants activity. ROS production can be calculated using a direct spectroscopic method based on the electronic paramagnetic resonance (ERP). It’s the only technique capable of providing a direct and quantitative free radicals detection in biological samples. A recent study (Mrakic-Sposta et al. 2012) tried to apply reliable rapid and micro-invasive ERP measurement of the instantaneous concentration of ROS directly in human peripheral blood. It permits to investigate the alteration in the redox homeostasis and to assess the fitness of intensively training athletes. Other possibility to measure oxidative stress is to calculate the antioxidant power using a novel redox sensor to assess the reducing species directly in human peripheral blood. Mrakic-Sposta et al. (2015) examined the effects of high intensity discontinuous training exercice on ROS production and on antioxidant capacity in master swimmer using a commercial “EDEL” potentiostat electrochemical analyser (Edel Therapeutics, Switzerland). The study showed an antioxidant adaptive pathway correlated with a decrease in ROS production following acute exercise and training. This method appeared to be a reliable method to evaluate oxidative stress markers ant to detect or prevent an overtraining state.

1.6 Interest in the daily adjustment of training by oxidative stress analysis and hypothesis

At this stage, to our knowledge, oxidative stress analysis (e.g., ROS production, antioxidant capacity) has not been investigated in the context of the daily monitoring of training. The issue of the study was to test the effectiveness of the endurance training adjustment by the oxidative stress analysis, through the "O2 score" redox sensor. This monitoring method is not widely used with endurance athletes compared to other methods such as HRV training guided, already used and recognized in the field (Kiviniemi et al., 2007; Plews et al., 2013; Schmitt et al., 2018; Javaloyes et al., 2019).
The basic idea was to decrease training stimulus when the oxidative stress (indirectly the antioxidant capacity) was not at its rest value (reference value) and to maintain the training stimulus when the values were neither too high nor too low. In parallel, HRV measurements were performed in order to combine a usefulness and actual monitoring method with this recent tool.

We hypothesized that training guided by daily oxidative stress measurements would increase aerobic performance gain when compared to the control group.

We also hypothesized that endurance training guided by oxidative stress would regulate daily obtained values (antioxidant power) and would therefore also influence some HRV parameters associated to fatigue or overreaching.

2. Méthodes

2.1 Subjects

23 healthy subjects practicing an endurance sport took part in the study. 9 middle-distance runners, 9 cross-country skiers and 4 swimmers competing at regional, national and/or international level for some of them have agreed to participate in the study, in agreement with the coach. Among them, 12 men (age 20.2 ± 2.1; weight 66.8 ± 8.6; height 178.3 ± 4.7) and 11 females (age 17.1 ± 1.8; weight 58.3 ± 6.7; height 167.8 ± 5.5).

2.1.1 Inclusion critera

Subjects practicing an endurance sport including regular training sessions (minimum three times a week) such as aerobic sessions, but also more intensive such as interval training sessions as well as specific strength training sessions. The subjects were all competitors at the regional, national and/or international level.

2.1.2 Exclusion criteria

Injured or over-trained subjects were not accepted into the study so as not to bias the variables measured and evaluated during the study.

2.1.3 Subject recruitment

The recruitment of the subjects was initially done through and with the consent of the coaches of various athletes whom I contacted by email or phone. Then a visit to the club training centers took place to present the project and explain the protocol and operation of the equipment to the athletes. Interested subjects have announced themselves and the material has been lent to them for the duration of the study. An electronic file sharing account (online training logbook) has been created between the athlete, the coach and myself. Communication was consistent throughout the study by message and via the sharing folder.

2.2 Study design

The study began with baseline from 1 to 2 weeks at the beginning of the general preparation period (June for cross-country skiers, September for swimmers and

November for middle-distance athletes). Sleep duration, *O2 score* measure, questionnaire of fatigue, training load and stress score were registered daily. HRV measurements were performed three times a week in order to compare a usefulness and actual monitoring method with the *O2 score* tool. During this period and to ensure that subjects started the protocols in good conditions, the training program mainly consisted of basic endurance sessions (< intensity 2) with low intensity and without training camp. The athletes performed the pre-performance test at the end of the baseline under the most optimal conditions possible and without significant pre-fatigue. The 4-weeks protocol began the day after the performance test. From that moment on, the subjects were matched according to their age, sex, level and similar training program. They were then separated and randomly assigned to a control or guided group. The characteristics of the subjects divided in both groups are presented in Table 4. During this protocol, the control group followed the training prescribed by the coach and took the measurements on a daily basis. The guided group took the same daily measurements but adjusted the training according to the *O2 score* values combine with the total score of fatigue (TSF) obtained daily. At the end of the protocol, the subjects again performed a post-performance test, similar to the pre-test, to compare the performance before and after the experimental period.

Table 4 Characteristics of the subjects divided into oxidative stress guided training and control groups

	Guided n = 12	**Control n = 11**
Sex	0.5 F / 0.5M	0.45 F / 0.55M
Age, years	19.5 ± 5.0	17.8 ± 3.0
Weight, kg	64.7 ± 9.4	60.6 ± 7.9
Height, cm	175.1 ± 7.8	171.4 ± 7.2

Data are mean ± SD. (M) men, (F) femal

2.3 Material

- *O2 score* device *Edel for Life* (Edel Therapeutics Switzerland)
- Edel Strips to analyze the blood *Bio-capteurs, Edel for life*
- Single-use sterile lancets *Safety Lancets, My life*
- *O2 score* application to connect the *O2 score* device via Bluetooth
- Training diary on Microsoft Excel 2011 software (14.7.2 version)
- Heart rate watch RS800CX, v800, Garmin Forerunner 935, Suunto Ambit 3: Heart rate and R-R interval measurement
- Heart rate monitor (chest strap)
- Kubios HRV Standard 3.1.0.1 software
- RPE: Borg scale (CR10)
- Fatigue questionnaire: (Atlaoui, Duclos 2004; Chatard, Atlaoui 2003)
- Stress scale (1-10)
- SigmaStat 3.5 software (Systat Software, San Jose, CA, USA).

2.4 *O2 score* questionnaire

This questionnaire was developed by the Edel Company but was not validated (see annex). It was completed by subjects at the beginning of the study to know the diet habits and other informations about the daily life. These informations helped to understand the variations in the values obtained with the *O2 score* device.

2.5 Daily measurements

Each morning of the baseline and the 4 weeks of protocol, the following parameters were recorded: Sleep duration (hour), O2 score measure (Edel), questionnaire of fatigue referred to the perceptions of the previous days. After each training session, the RPE (0-10) was assessed link with the duration of the session (minutes) to quantify the training load (AU). At the end of the day the global stress was calculated using a 1-10 scale. Heart rate variability test was performed three times a week in the morning immediately after waking up and emptying the urinary bladder. All this measurements were annotated in a training diary prescribed at the beginning of the study (see annex).

2.6 Edel device « *O2 score* »

Reducing capacity in blood was measured by a redox sensor in 10 μL of capillary blood.

The electrochemical measurements were performed using a commercial *Edel* potentiostat electrochemical analyser (*Edel* Therapeutics, Switzerland) in a three-electrodes arrangement. The working electrode (WE) was a screen-printed carbon electrode operating in conjunction with a screen-printed counter and a silver/silver-chloride (Ag/AgCl) reference one. This technique is an electrochemical-based method responding to all water-soluble compounds in biological fluids, which can be oxidized within a defined potential range. Blood sample was loaded onto a chip and an increasing potential between 0 and 1.2 V at a scan rate of 100 mV·s^{-1} (versus Ag/AgCl reference electrode) was applied while the resulting current was measured at the working electrode. The result was then pseudo-titrated to account for the most biologically relevant antioxidants. Data are expressed in nW (1 *Edel* = 1nW). *Edel* device measured oxidative stress indirectly by calculating the level of antioxidant present in the blood.

The measurements were taken daily in the morning on an empty stomach. First, the subject introduced an *Edel* strip into the *Edel* analyser. Then, a micro-drop of blood was collected using a sterile single-use lancet. The drop of blood was introduced on the strip sensor. A green color blinked quickly during the measurement and then almost continuously when the analysis was completed. Pressing and holding the device button for a long time would then display a blue light and connect the device via Bluetooth to the *O2 score* application.

This method was simple and fast since the analyser gave a value in less than 30 seconds by connecting via Bluetooth to a smartphone application. After several measurements over a period of about 7 days, the *O2 score* device calculated the subject's energy capacities as a function of time. Despite individual differences, an *O2score* comfort zone has been established, based on age and gender. It represents the ideal conditions for energy recovery when antioxidant level is at its rest value. The Figure 3 shows an example of a value obtained daily with the established comfort zone in green.

Figure 3 Value obtained with the *O2 score* application after the measurement.

If the value is higher than the comfort zone, it indicates that the recovery has not returned to its initial state, with an over-activation of antioxidants trying to neutralize the oxidative stress. If this value persists, there is a risk of overtraining.

When the value is below the comfort zone, it means that the antioxidant defense system is decreased with a higher risk of oxidative stress. In both cases, recovery is poor and carries a higher risk of fatigue, inflammation or injury.

During the protocol, O2 score was measured every morning, as well as the difference between the daily antioxidant level (O2 score) and the antioxidant level at its rest value (reference value). The reference value was established as the median value of the green comfort zone. In figure 2, it would have been 200 (= median value between 180 -220). This allowed to know the delta between these two values and to adjust the daily training according to this value (Δ O2 score).

2.7 Fatigue Questionnaire

The questionnaire has been validated by (Atlaoui, Duclos 2004; Chatard, Atlaoui 2003) and is presented in Table 5.

The eight questions focused on the perception of training, sleep, leg pain, infection, concentration, efficacy, anxiety, irritability, and general stress were assessed on a 7-point scale from very very good (1 point) to very very bad (7 points). The responses to the questions were collated to obtain the total score of fatigue (TSF). All the questions played an important part in the TSF. The lower the score the better the perception of well - being, the higher the score the higher the perception of fatigue.

The questionnaire was completed daily in the morning and the answers referred to the perceptions of the previous days. It was a complementary tool to the *O2 score* device in the daily adjustment of the training. TSF was the second criterion used to validate or not the adjustment of daily training session.

Table 5 Description of the eight items of the fatigue questionnaire (Chatard, Atlaoui 2003).

		Rating Scale						
No.	Item	Very Very Good			Average			Very Very Bad
1	I found training more difficult than usual	1	2	3	4	5	6	7
2	I slept more	1	2	3	4	5	6	7
3	My legs felt heavy	1	2	3	4	5	6	7
4	I caught cold/infection/flu	1	2	3	4	5	6	7
5	My concentration was poorer than usual	1	2	3	4	5	6	7
6	I worked less efficiently than usual	1	2	3	4	5	6	7
7	I felt more anxious or irritable than usual	1	2	3	4	5	6	7
8	I had more stress at home, school, training, work	1	2	3	4	5	6	7

2.8 HRV

The HRV test consisted of recording heartbeats twice a week while lying down for 5 minutes using an HR watch capable of detecting RR intervals. Once a week the "Tilt test" was performed and this time consists of recording the R-R intervals 5 minutes in the lying position followed by 5 minutes in the standing position. This test assesses the responsiveness of the autonomous system to orthostatic change. The recording data was then transferred to a computer capable of downloading the recording via a software application. HRV was calculated using Kubios 3.1.0.1 analysis software. The HRV analysis was performed on the last 4 minutes of the lying test. On the Tilt test the analysis is performed between the 2nd and 5th minute lying down and between the 7th and 10th minutes standing. Measurement errors and artifacts were corrected and eliminated according to signal quality using a function available on the Kubios software.

Many temporal variables presented in Table 6 are used in the overall analysis of the sympatho-vagal balance. For this study, the average R-R interval expressed in (ms) and the average HR heart rate (bpm) were calculated for each measurement. The RMSSD variable expressed in (ms) was analyzed in the time domain. It represents the square root of the mean sum of the square differences between successive R-R intervals and evaluates the parasympathetic tone. RMSSD is a recommended parameter because it measures short-term variables and has good statistical properties (Task Force of the European Society of Cardiology and the North American Society of Pacing and

Electrophysiology, 1996).

Table 6 Selected time-domain measures of HRV (Task Force 1996).

Variable	Units	Description
		Statistical measures
SDNN	ms	Standard deviation of all NN intervals.
SDANN	ms	Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording.
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording.
SDSD	ms	Standard deviation of differences between adjacent NN intervals.
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording. Three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer.
pNN50	%	NN50 count divided by the total number of all NN intervals.

Measurements of LF and HF power components presented in Table 7 are usually made in absolute values of power (ms^2), but LF and HF may also be measured in normalized units (nu) which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in normalized units (nu) emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power. Nevertheless n.u. should always be quoted with absolute values of LF and HF power (ms^2) order to describe in total the distribution of power in spectral components. Therefore both in supine (SU) and in standing (ST) positions, LF and HF were calculated in absolute spectral power units (ms^2) and in normalized units (nu) with LF (nu) = LF/(LF+HF)×100 and HF (nu)= HF/(HF+LF)×100. The total spectral power (TP) was calculated from the sum of LF and HF. (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Table 7 Selected frequency domain measures of HRV (Task Force 1996).

Variable	Units	Description Analysis of short-term recordings (5 min)	Frequency range
5 min total power	ms^2	The variance of NN intervals over the temporal segment	approximately $\leq 0{\cdot}4$ Hz
VLF	ms^2	Power in very low frequency range	$\leq 0{\cdot}04$ Hz
LF	ms^2	Power in low frequency range	0·04–0·15 Hz
LF norm	n.u.	LF power in normalised units LF/(Total Power–VLF) × 100	
HF	ms^2	Power in high frequency range	0·15–0·4 Hz
HF norm	n.u.	HF power in normalised units HF/(Total Power–VLF) × 100	
LF/HF		Ratio LF [ms^2]/HF [ms^2]	

2.9 Training load measure

Training load was assessed using the method of Foster et al. (2001). Approximately 30 minutes following the conclusion of each training session, the athletes had to rate their perceived level of exertion (RPE) using the category ratio (CR-10) scale developed by Borg (1987) and modified by Foster et al. (2001). A rating of 0 was to be associated with no effort (rest) and a rating of 10 was considered to be maximal effort. The scale is presented in Table 8. The product of the RPE rating (0-10) x session duration (in minutes) represents the session training load in arbitrary units (AU). This method allows an assessment of physiological and external load (training time) combined with psychological and internal load (RPE).

Table 8 Scale (CR10) modified from Foster et al. (2001) to rate the perceived level of exertion (RPE)

Rating	Descriptor
0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	-
7	Very Hard
8	-
9	-
10	Maximal

During the protocol, athletes from the guided group had to note the prescribed training load by the coach « training load » before the O2 score measures and the « adjusted training load », when the training session was adjusted. These two data allowed to calculate the load delta «Δ training load» (training load - adjusted training load). The delta provides information on the intervention of the training adjustment. When the training was adjusted, a comparative table showed in Table 9 was used to note the prescribed training intensity based on the Borg scale (CR-10.) but referring to other intensity zones prescribed by coaches.

Table 9 Guide to compare the training zone with other intensity assessment methods

Zone	Description	General ranges		
		% Critical Power	Heart rate (% HR @ CP)	RPE
1	Recovery	<56	<69	<2
2	Endurance	56-75	69-83	2-3
3	Tempo	76-90	84-94	3-4
4	Critical power	91-105	95-105	4-5
5	$\dot{V}O_{2max}$	106-120	>106%	6-7
6	Anaerobic capacity	>120	N/A	>7

2.10 Daily adjustment of training by O2 score analysis

The criteria for amending the load were arbitrarily established by the experimenter. They were not disclosed to participants in such a way that it did not influence their subjective assessment of the fatigue score.

Every morning, the training was adjusted according to the following situations:

- O2 score value more than 10 nW (= 10 Edel) above or below the rest value (reference value) and a total fatigue score (TSF) higher than 20

- O2 score value more than 30 nW above or below the rest value (reference value) despite a total fatigue score (TSF) lower than 20

- During training camps or voluntarily intensive periods, the adjustment depended on broader criteria, i.e. O2 score more than 20 nW (= 20 Edel) above or below the rest value (reference value) and a total fatigue score (TSF) higher than 25

The training adjustment was made as follows:

- Strength or endurance session at low intensity (intensity 1-2 and lower than 2 hours) → the training load was maintained

- Session longer than 2 hours at low intensity → a low volume training was prescribed

- Intensity session higher than 1-2 → a low intensity training (1-2) was prescribed

2.11 Pre- and post- test

The performance test was chosen according to the sport disciplines, in agreement with the coach. This was a maximal test with an effort duration representative of the tests performed in competition. The subjects matched at the beginning of the protocol performed the same performance test, but the tests were not always similar within the same sport discipline. In cross-country skiing, the majority of athletes completed a 3000 meters run on the track, two athletes completed a 5km uphill in roller ski and two others completed a 5min time trial test on a roller ski treadmill. In athletics, the subjects participated in a 2000 meters run on the track. In swimming, the athletes swam 400 meters freestyle in a 50m pool. Pre- and post- test were conducted under standardized and similar conditions from one time to the next with individual departures, at the same time of the day and in comparable weather conditions.

2.12 Data analysis and statistics

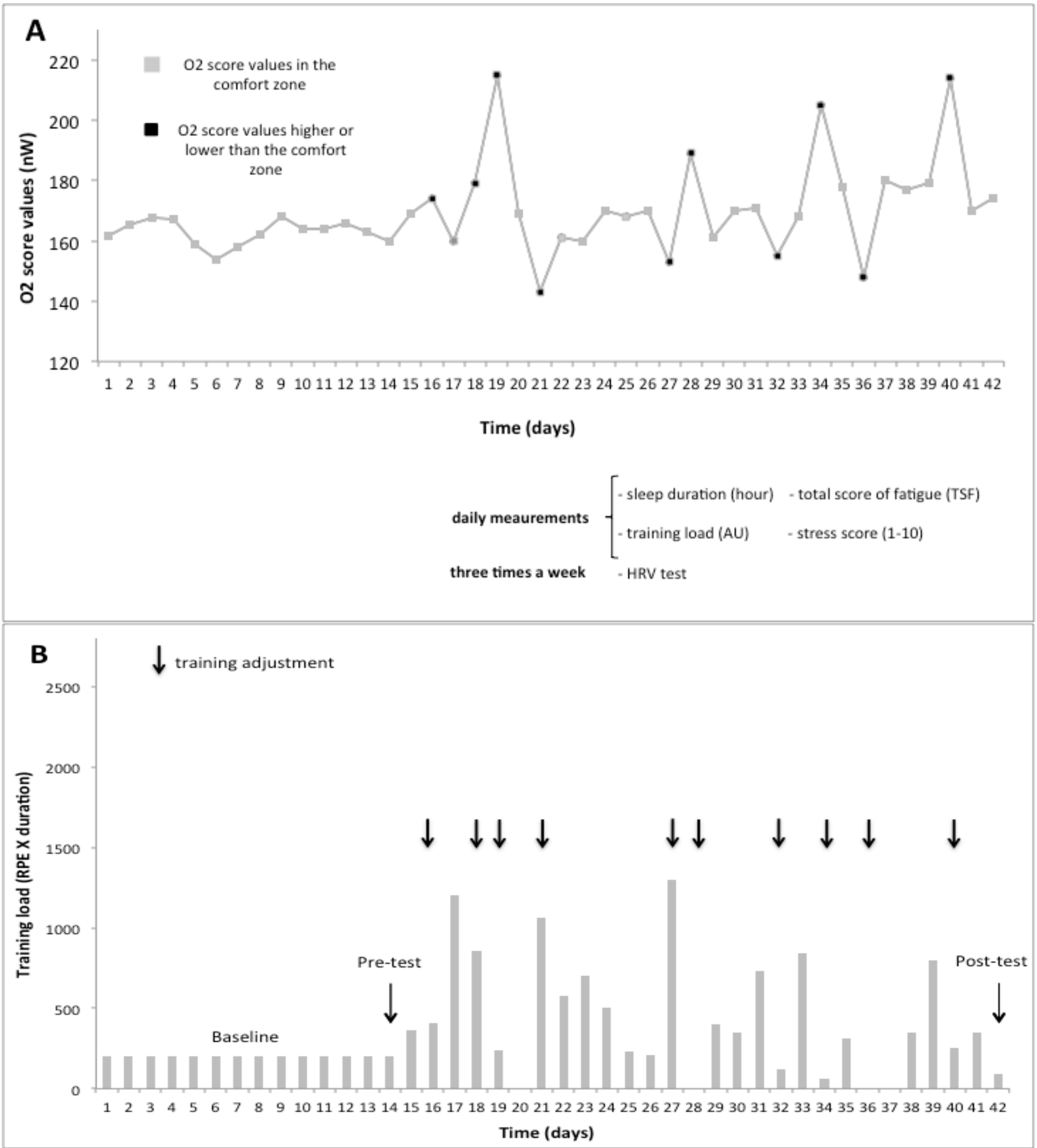
Data are reported as mean and standard deviation (SD). Data were tested for equality of variance (Fisher–Snedecor *F* test) and for normality (Shapiro–Wilk test). When both conditions were met, a two-way repeated measures ANOVA [group (guided vs control) vs. measurement (baseline, week 1 (W1), week 2 (W2), week 3 (W3) and week 4 (W4)] were performed with pairwise multiple comparison procedures (post hoc, Tukey method). T-test was used to determine if there was a significant difference between the means of two groups (guided vs control). Differences in percentage changes between the conditions were tested with a Wilcoxon signed rank sum test. When either equality of variance or normality were not satisfied, variables were analyzed for each condition using a Friedman test for repeated measures to determine time effects using pairwise multiple comparison procedures (Bonferroni test). In this case, differences between the guided and control group at baseline (pre-) were tested using a Mann–Whitney rank sum test. Each variables measured during the study were correlated using the Pearson correlation coefficient test. Null hypotheses were rejected at $p < 0.05$. All analyses were completed using SigmaStat 3.5 software (Systat Software, San Jose, CA, USA).

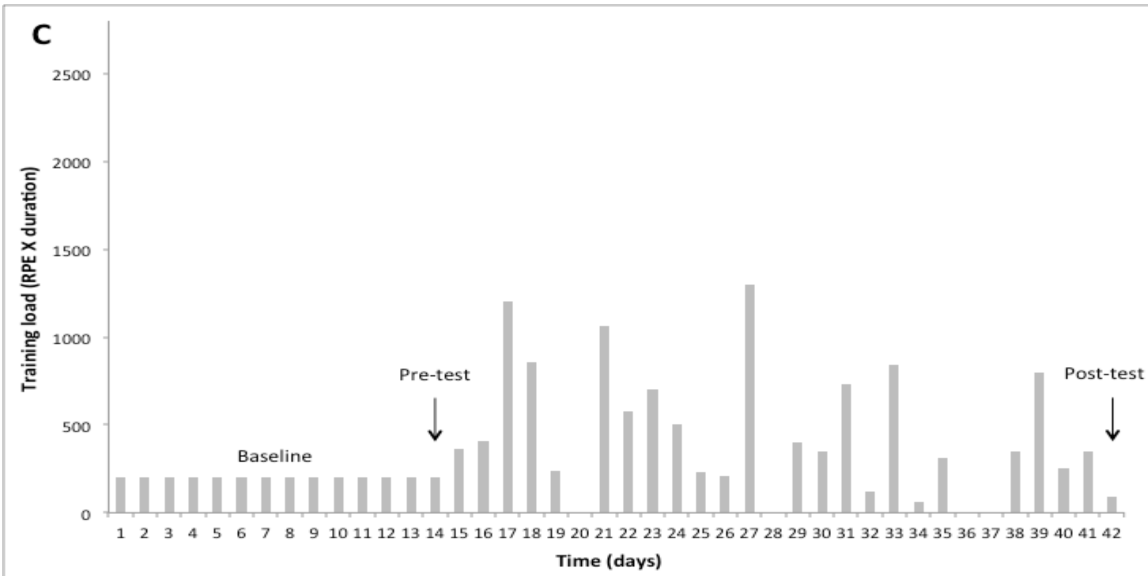
3. Results

3.1 HRV and daily measurements

A representative example of the training guided by the oxidative stress and the measurements recorded during the study are presented in figure 3.

Figure 3 Study design showing the training load adjustment in the guided group (**B**), according to the O2 score values obtained daily (**A**) while no adjustment is made in the control group (**C**). All the measurements recorded during the study are presented under the O2 score values (**A**).





The O2 score values and other parameters likely to influence the oxidative stress and measured during the study in the guided group and in the control group are reported in Table 10. The training parameters measuring the daily training load are shown in Table 11 and the HRV measurements are reported in Table 12.

Table 10 Parameters daily measured during the baseline and the protocol (week 1, 2, 3 and 4)

Parameters	Group	Baseline	week 1	week 2	week 3	week 4
sum sleep (hours)	guided		57.1 ± 4.0	57.2 ± 5.2	52.8 ± 15.8	64.0 ± 4.1
	control		58.0 ± 3.9	58.7 ± 2.7	50.3 ± 16.5	48.1 ± 20.0
mean sleep (hours)	guided	7.9 ± 0.4	8.2 ± 0.6	8.2 ± 0.7	8.4 ± 0.7	8.4 ± 1.2
	control	8.2 ± 0.6	8.3 ± 0.6	8.4 ± 0.4	8.3 ± 0.9	8.2 ± 0.3
stress (1-10)	guided	2.8 ± 0.8	2.9 ± 1.3	3.0 ± 1.2	2.2 ± 1.0	2.0 ± 0.7
	control	3.0 ± 0.8	3.4 ± 1.6	2.9 ± 1.2	2.8 ± 0.8	2.0 ± 1.0
O2score-ref (nW)	guided		171.6 ± 14.2	170.3 ± 15.7	169.9 ± 15.8	169.4 ± 14.5
	control		186.0 ± 28.6	184.9 ± 30.9	187.6 ± 30.6	197.4 ± 25.1
Δ O2score	guided		16.8 ±8.0	16.2 ± 6.6	14.3 ± 7.2	16.2 ± 13.3
	control		18.5 ± 11.5	18.6 ± 15.4	16.5 ± 6.9	13.3 ± 6.7

Data are mean ± SD. The stress indicates the subjective stress of day (rated from 1 to 10) and Δ O2 score represents the difference between the daily antioxidant (O2 score) level and the antioxidant level at its resting value (O2 score-ref) (nW).

Table 11 Training parameters daily reported during the baseline and the protocol (week 1, 2, 3 and 4)

Training parameters	Group	Baseline	week 1	week 2	week 3	week 4
volume, sum (min)	guided		669.4 ± 296.9	665.0 ± 25.4	580.1 ± 412.2	498.3 ± 416.2
	control		730.5 ± 292.7	686.5 ± 286.1	671.1 ± 449.6	775.3 ± 401.9
RPE (0-10)	guided	3.9 ± 0.7	4.3 ± 0.9	4.2 ± 0.8	4.2 ± 0.7	4.0 ± 1.0
	control	4.1 ± 0.9	4.5 ± 0.7	4.4 ± 0.9	4.0 ± 1.2	4.0 ± 1.1
training load, sum (volume X RPE)	guided		2632.6 ± 1024.5	2699.4 ± 1239.0	2463.3 ± 1990.3	1812.1 ± 1704.5
	control		3344.5 ± 1406.5	3037.7 ± 1285.2	2879.5 ± 2128.5	2845.2 ± 1856.2
training load (volume X RPE)	guided	96.9 ± 50.4	376.1 ± 146.4	385.6 ± 177.0	351.9 ± 284.3	273.5 ± 230.3
	control	121.1 ± 67.2	477.8 ± 200.9	434.0 ± 183.6	423.4 ± 296.4	449.8 ± 220.3
adjusted training load, sum (volume X RPE)	guided		2333.4 ± 994.9	2287.3 ± 1156.6	2319.2 ± 2034.5	1677.0 ± 1577.8
	control		3344.5 ± 1406.5	3037.7 ± 1285.2	2879.5 ± 2128.5	2845.2 ± 1856.2
adjusted training load (volume X RPE)	guided		333.3 ± 142.1	326.8 ± 165.2	331.3 ± 290.6	254.2 ± 212.6
	control		477.8 ± 200.9	434.0 ± 183.6	423.4 ± 296.4	449.8 ± 220.3
Δ training load, sum (volume X RPE)	guided		326.4 ± 194.7	494.5 ± 302.8	288.3 ± 171.2	315.3 ± 464.1
	control		0	0	0	0
Δ training load (volume X RPE)	guided		46.6 ± 27.8	72.9 ± 41.4	41.2 ± 24.5	45.0 ± 66.3
	control		0	0	0	0

Data are mean ± SD. Volume indicates the training session duration expressed in min and RPE represents the perceived level of exertion of the training session (rated from 0 to 10). The training load is the prescribed load before the training session and the adjusted load corresponds to the load after the intervention. Δ training load is difference between the two loads ad indicates a change in the training prescription .

Table 12 HRV parameters measured in supine (SU) and standing (ST) positions during the baseline and the protocol (week 1, 2, 3 and 4)

HRV parameters	Group	Baseline	week 1	week 2	week 3	week 4
RR_{SU} (ms)	guided	1058.4 ± 235.1	1042.6 ± 212.4	1065.8 ± 231.8	1018.6 ± 185.8	1065.1 ± 272.1
	control	1143.7 ± 140.3	1030.6 ± 217.7	1119.6 ± 173.7	1188.9 ± 204.7	1232.6 ± 94.6
HR_{SU} (bpm)	guided	59.1 ± 10.9	59.5 ± 10.4	58.4 ± 11.1	60.6 ± 9.7	59.1 ± 12.7
	control	51.7 ± 6.8	55.5 ± 11.6	53.2 ± 9.2	50.3 ± 9.2	46.9 ± 5.9
$RMSSD_{SU}$ (ms)	guided	62.4 ± 25.5	61.0 ± 25.3	64.1 ± 23.8	53.3 ± 22.1	64.1 ± 25.2
	control	89.5 ± 13.6	82.8 ± 28.1	86.3 ± 23.7	93.6 ± 43.0	76.4 ± 20.7
LF_{SU} (ms2)	guided	2172.9 ±2021.6	2264.8 ± 2561.2	2242.5 ± 1847.8	2032.2 ± 1673.9	2010.1 ± 1195.4
	control	2645.8 ± 792.9	2219.0 ± 1337.9	2875.9 ± 2400.3	2254.8 ± 1376.1	1980.6 ± 1091.0
HF_{SU} (ms2)	guided	1299 ± 794.5	1237.0 ± 899.8	1295.6 ± 835.8	986.2 ± 819.8	1612.5 ± 1054.3
	control	2744.2 ±1050.5	1895.4 ± 1427.9	2860.5 ± 2775.1	2946.1 ± 2976.6	1712.3 ± 1416.9
LF_{SU} (n.u.)	guided	55.9 ± 16.2	56.8 ± 18.7	55.2 ± 22.1	61.1 ± 18.2	54.9 ± 13.6
	control	51.0 ± 14.0	50.0 ± 17.1	48.3 ± 13.5	52.0 ± 9.2	52.0 ± 9.4
HF_{SU} (n.u.)	guided	44.1 ± 16.2	42.5 ± 18.2	44.8 ± 22.1	38.9 ± 18.2	45.0 ± 13.5
	control	49.0 ± 14.0	50.0 ± 17.1	51.7 ± 13.5	47.9 ± 9.2	47.9 ± 9.4
LF + HF_{SU} (ms2)	guided	3471.9 ± 2338.8	3501.8 ± 2822.9	3536.9 ± 2141.6	3018.4 ± 2304.4	3622.6 ± 1941.8
	control	5390.1 ± 1250.8	4114.4 ± 2578.0	5736.5 ± 5077.5	5200.8 ± 4219.4	3692.9 ± 2176.5
LF/HF_{SU}	guided	2.5 ± 3.0	2.3 ± 2.4	2.3 ± 2.2	2.2 ± 1.4	1.8 ± 1.6
	control	1.2 ± 0.6	1.6 ± 1.3	1.2 ± 0.5	1.3 ± 0.7	1.8 ± 1.5
RR_{ST} (ms)	guided	752.0 ± 124.2	731.5 ± 163.0	811.4 ± 148.5	738.2 ± 172.9	821.9 ± 204.4
	control	600.7 ± 299.9	721.0 ± 39.2	699.6 ± 82.3	700.5 ± 40.4	764.8
HR_{ST} (bpm)	guided	81.5 ± 10.6	84.9 ± 15.8	72.6 ± 13.5	85.0 ± 18.2	75.3 ± 18.7
	control	77.4 ± 11.7	76.7 ± 15.4	78.0 ± 21.7	77.1 ± 20.0	59.2 ± 27.2
$RMSSD_{ST}$ (ms)	guided	56.3 ± 38.1	31.1 ± 19.1	37.6 ± 22.1	33.3 ± 20.8	29.8 ± 21.0
	control	50.6 ± 34.4	28.8 ± 8.6	38.4 ± 30.5	37.9 ± 32.7	67.7 ± 73.9
LF_{ST} (ms2)	guided	6159.4 ± 9103.0	2470.4 ± 2272.4	3106.6 ± 2161.6	2407.2 ± 2304.0	2505.9 ± 2665.3
	control	3453.1 ± 1732.3	2319.7 ± 1506.3	3215.8 ± 2625.9	2507.0 ± 1228.4	2812.2 ± 1205.5
HF_{ST} (ms2)	guided	1370.5 ± 1834.7	316.5 ± 242.8	429.2 ± 451.9	435.6 ± 471.2	174.9 ± 133.0
	control	1586.0 ± 1831.3	305.6 ± 177.5	994.9± 1747.1	592.9 ± 899.8	
LF_{ST} (n.u.)	guided	82.1 ± 10.7	87.8 ± 1.9	86.9 ± 8.6	83.9 ± 14.0	90.6 ± 5.5
	control	83.7 ± 13.0	87.7 ± 12.1	87.8 ± 5.7	93.6 ± 3.1	96.9
HF_{ST} (n.u.)	guided	17.9 ± 10.6	12.2 ± 1.9	13.1 ± 8.5	16.0 ± 14.0	9.4 ± 5.5
	control	16.3 ± 13.0	12.3 ± 12.0	12.1 ± 5.7	6.3 ± 3.1	3.1
LF + HF_{ST} (ms2)	guided	7529.8 ± 10780.6	2786.9 ± 2511.1	3535.8 ± 2466.3	2842.8 ± 2518.0	2680.7 ± 2778.3
	control	5039.1 ± 3331.2	2625.3 ± 1567.5	4210.7 ± 4075.5	3099.9 ± 1702.2	5687.8 ± 5182.4
LF/HF_{ST}	guided	7.7 ± 5.2	7.7 ± 2.0	12.7 ± 12.5	10.3 ± 7.2	11.9 ± 7.6
	control	8.6 ± 7.6	11.7 ± 8.7	10.1 ± 9.0	16.3 ± 14.2	15.8 ± 21.4

Data are mean ± SD. SU = supine position; ST = standing position; HR = heart rate (beat per minute); RMSSD = root-mean-square differences of successive R-R intervals (ms); LF = low frequencies in the spectral analysis of HRV (ms^2); HF = high frequencies in the spectral analysis of HRV (ms^2); LF + HF = total spectral power (ms^2); LF/HF = ratio LF (ms^2)/HF (ms^2); nu = normalized units.

3.2 Performance tests (pre and post)

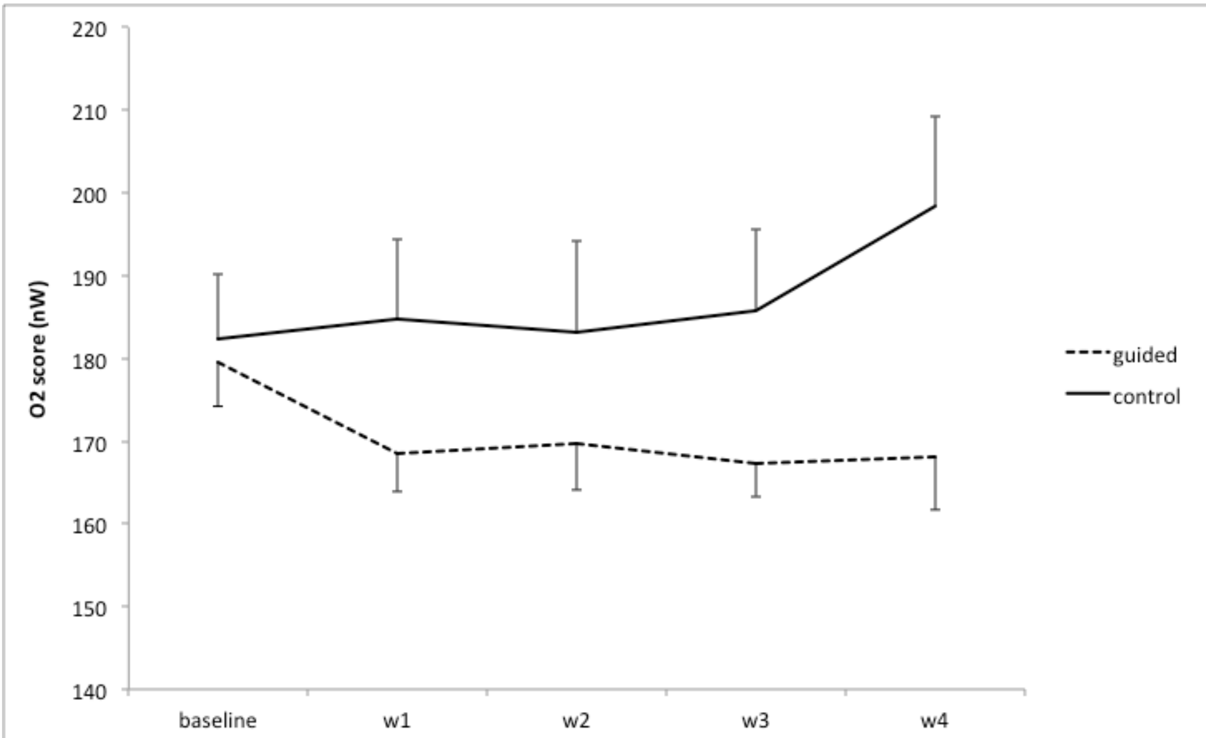
No change in performance was observed in the guided and control group (pre– to post-change 0.4 vs. -0.2%).

3.3 Comparison between groups

Figure 4 to figure 6 compare the 2 groups according to a parameter and over the different periods of the study (baseline, week 1, 2, 3 and 4). These figures show the impact of the training intervention on different parameters.

Figure 4 shows the evolution of O2 score in both groups. There is a group-time interaction (F = 2.45 and P = 0.02) indicating a different change over time and between groups.

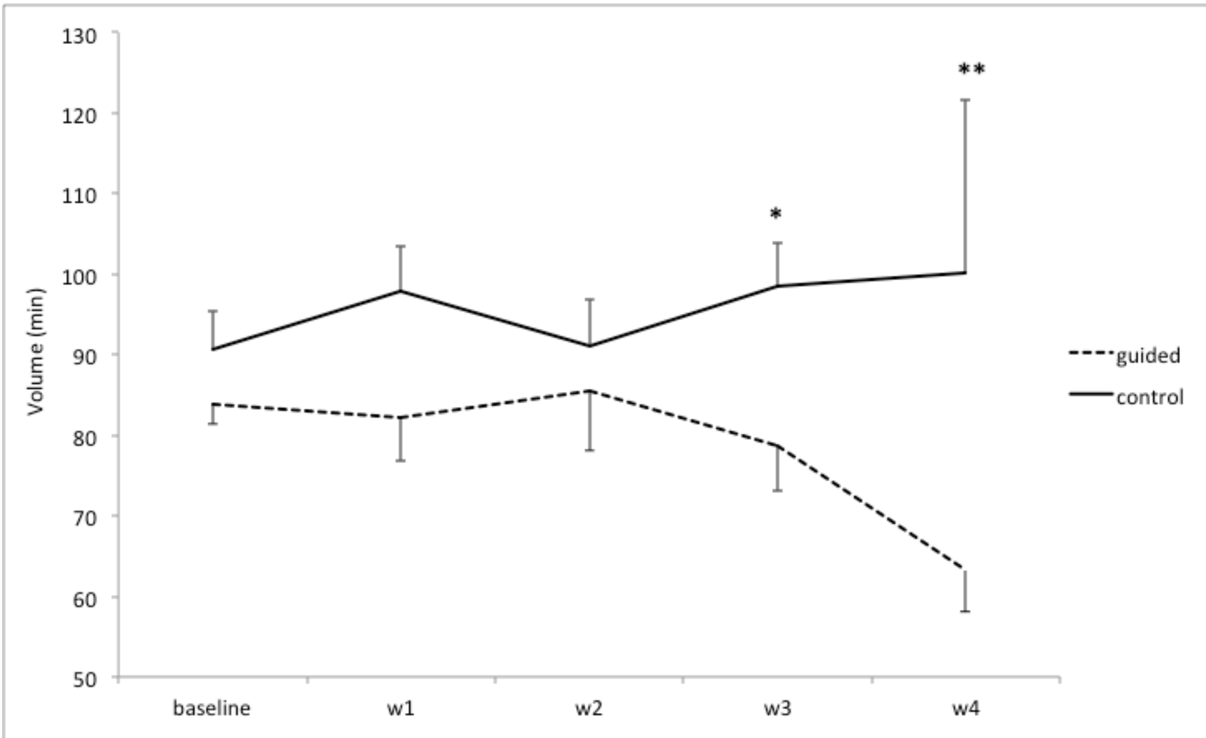
Figure 4 Time course of O2 score values (nW) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol



Data are mean ± SE.

The evolution of the volume for both groups is shown in Figure 5. No significant change in volume was observed over the time of the study. However, significant differences were found in the volume between the guided and the control group.

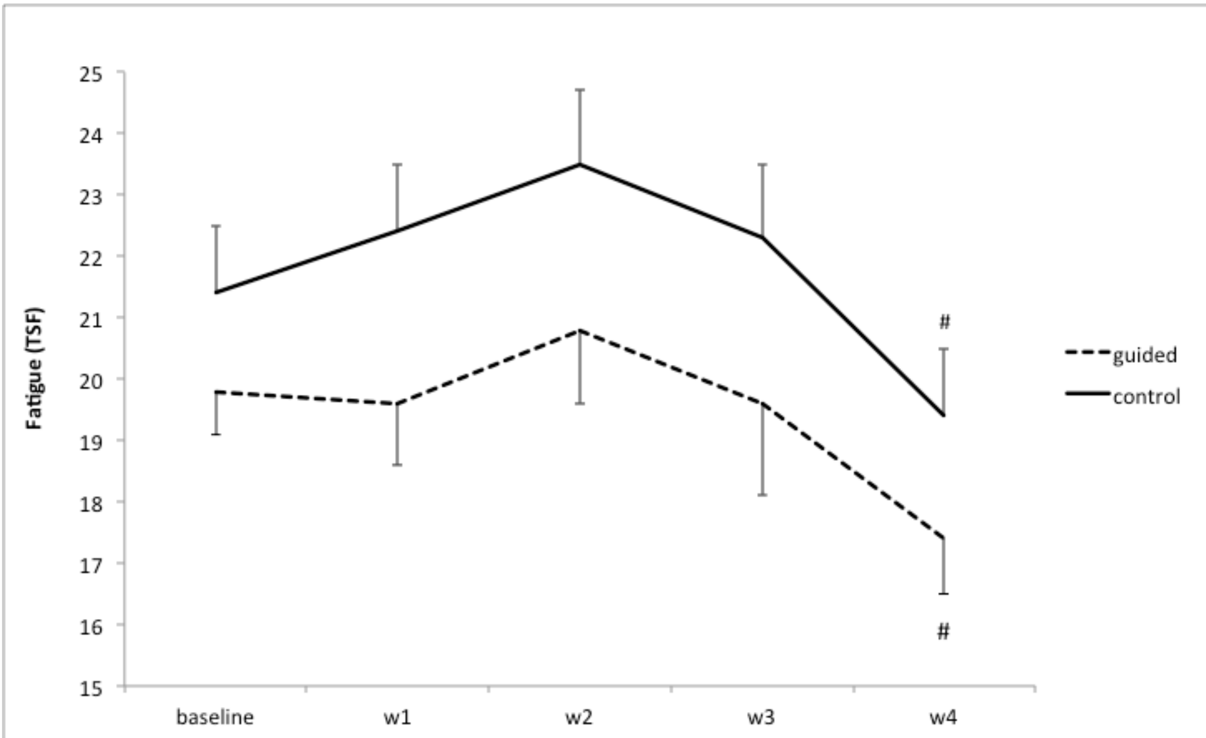
Figure 5 Time course of the volume (min) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol.



Data are mean ± SE. Volume indicates the training session duration expressed in min. * P < 0.05, ** P < 0.001 for differences with guided group.

Figure 6 shows the evolution of the fatigue in both groups.

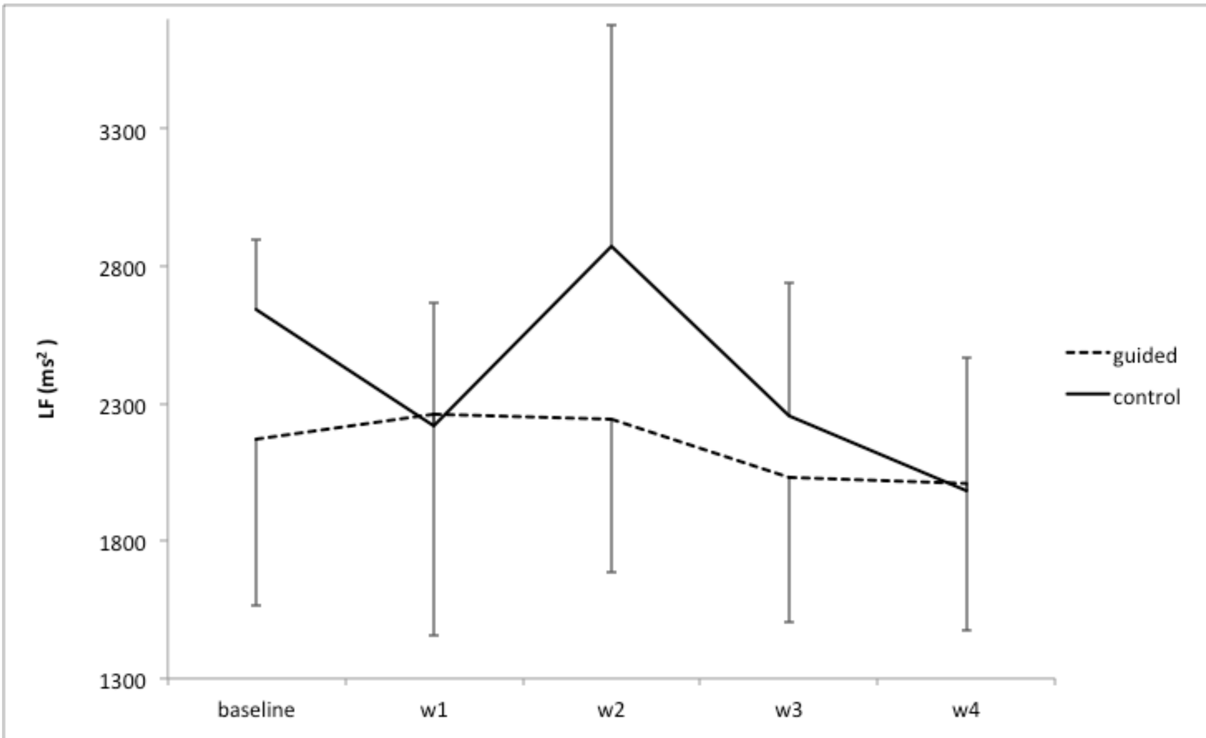
Figure 6 Time course of the fatigue (TSF) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol.



Data are mean ± SE. Fatigue represents the total score of the fatigue questionnaire (8 items rated from 1 to 7). # $P < 0.05$ for differences with w2

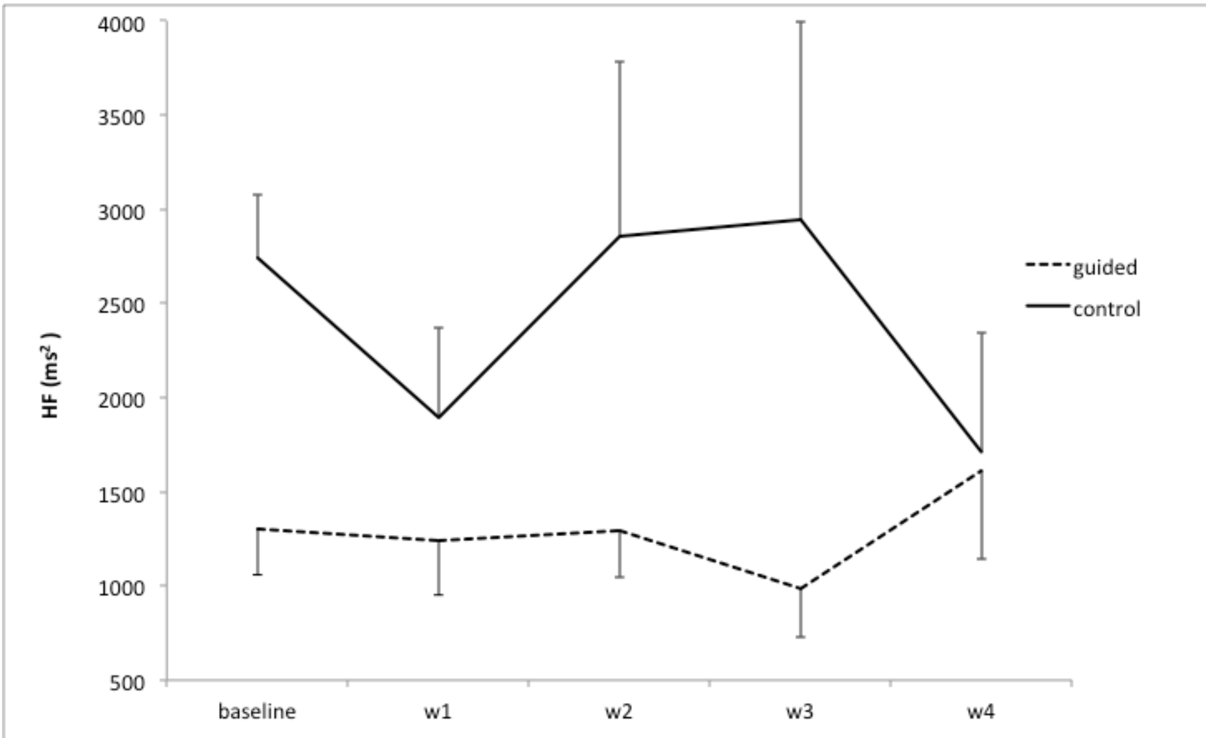
The figure 7 and 8 show the evolution of LF_{SU} (ms^2) and HF_{SU} (ms^2) for both groups. No significant differences were observed between groups and over the time.

Figure 7 Time course of LF (ms²) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol.



Data are mean ± SE. LF is the low frequencies in the spectral analysis of HRV calculated in supine position (SU).

Figure 8 Time course of HF (ms^2) over the baseline, week 1 (w1), week 2 (w2), week 3 (w3) and week 4 (w4) of the protocol.

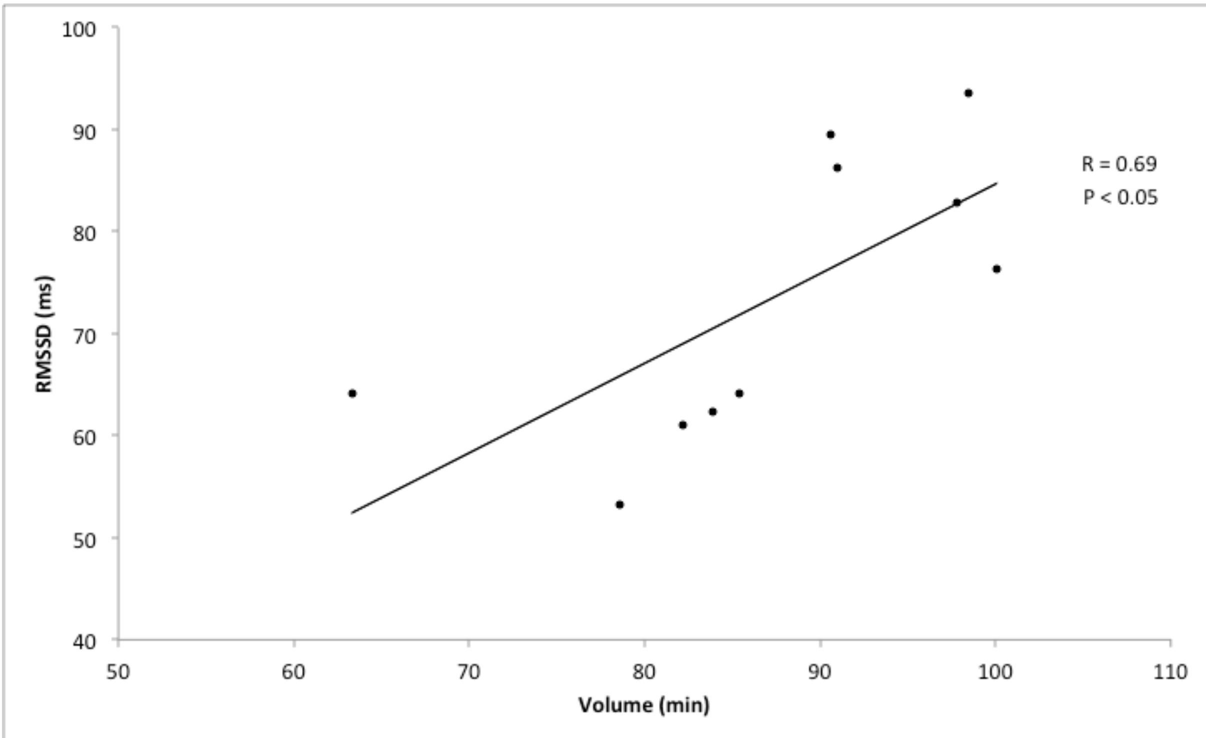


Data are mean ± SE. HF is the high frequencies in the spectral analysis of HRV calculated in supine position (SU).

3.4 Relationships with the training volume

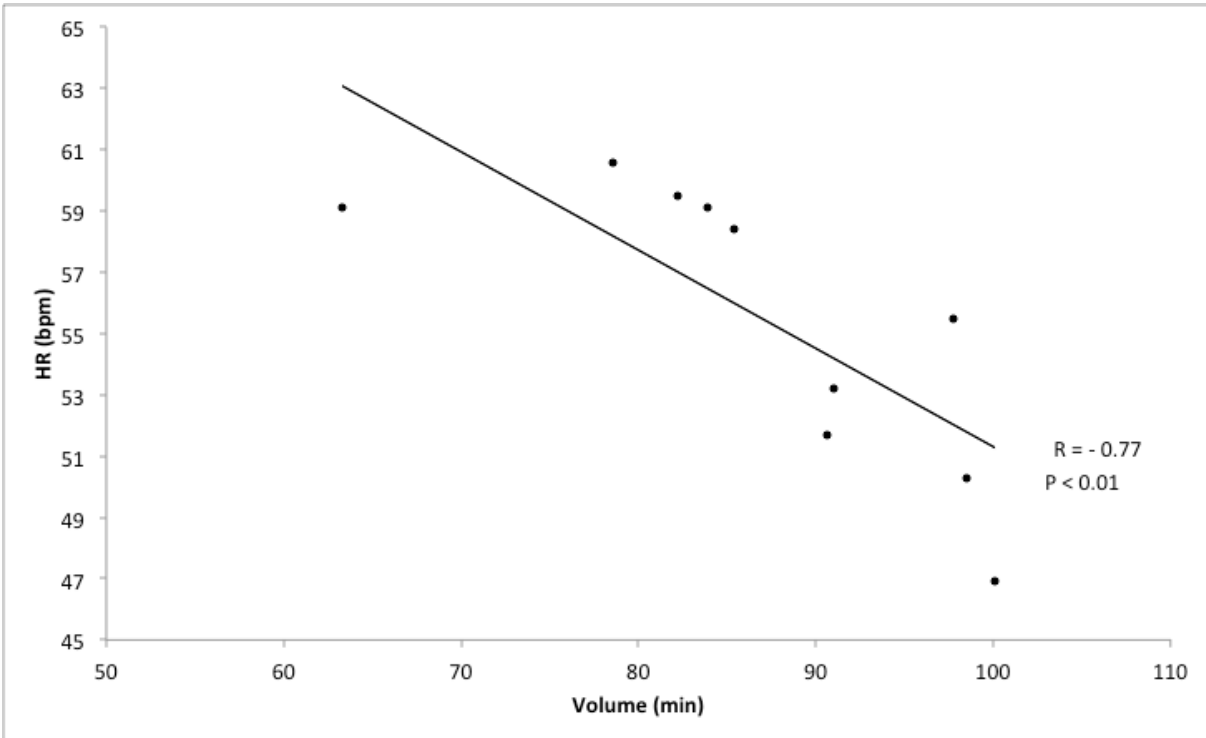
Figures 9 to 11 show the relationships between the training volume and $RMSSD_{SU}$, HR_{SU} or fatigue.

Figure 9 Relationship between the volume (min) and $RMSSD_{SU}$ (ms)



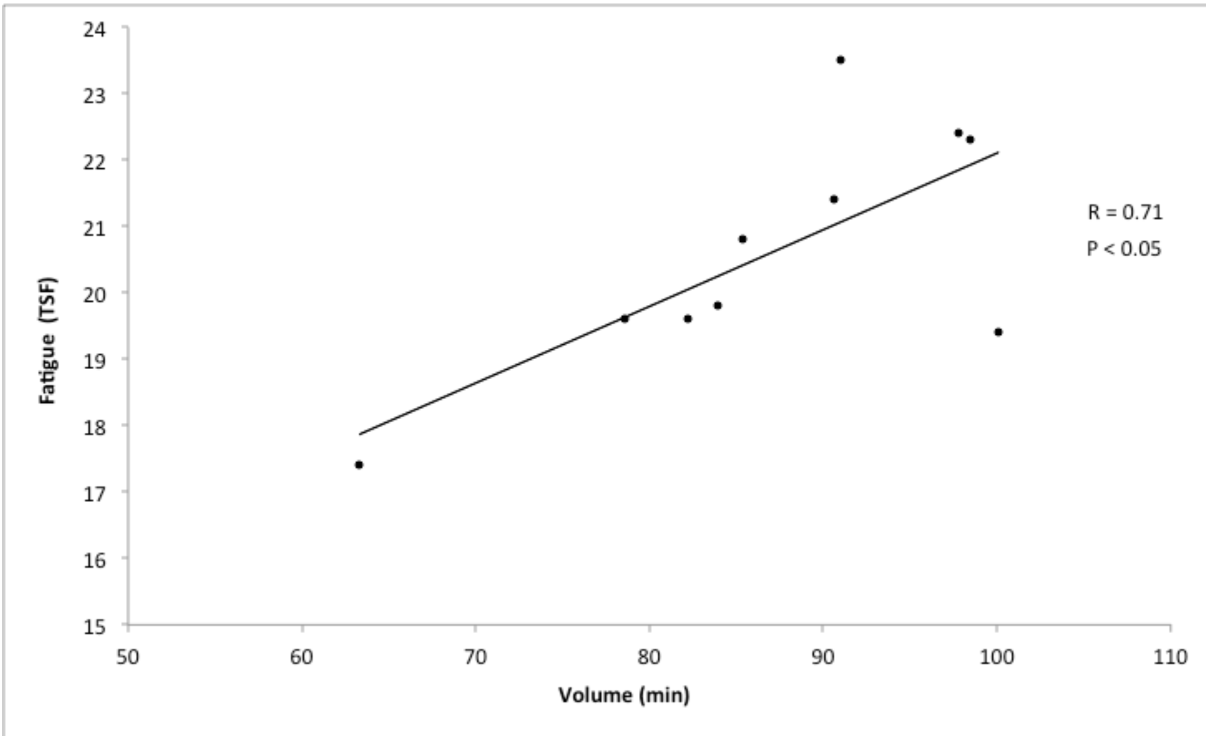
Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. RMSSD is the root-mean-square differences of successive R-R intervals (ms) calculated in supine position (SU). The volume indicates the training session duration expressed in min.

Figure 10 Relationship between the volume (min) and HR_{SU} (bpm)



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. HR is the heart rate (beat per minute) calculated in supine position (SU). The volume indicates the training session duration expressed in min.

Figure 11 Relationship between the volume (min) and the fatigue (TSF)



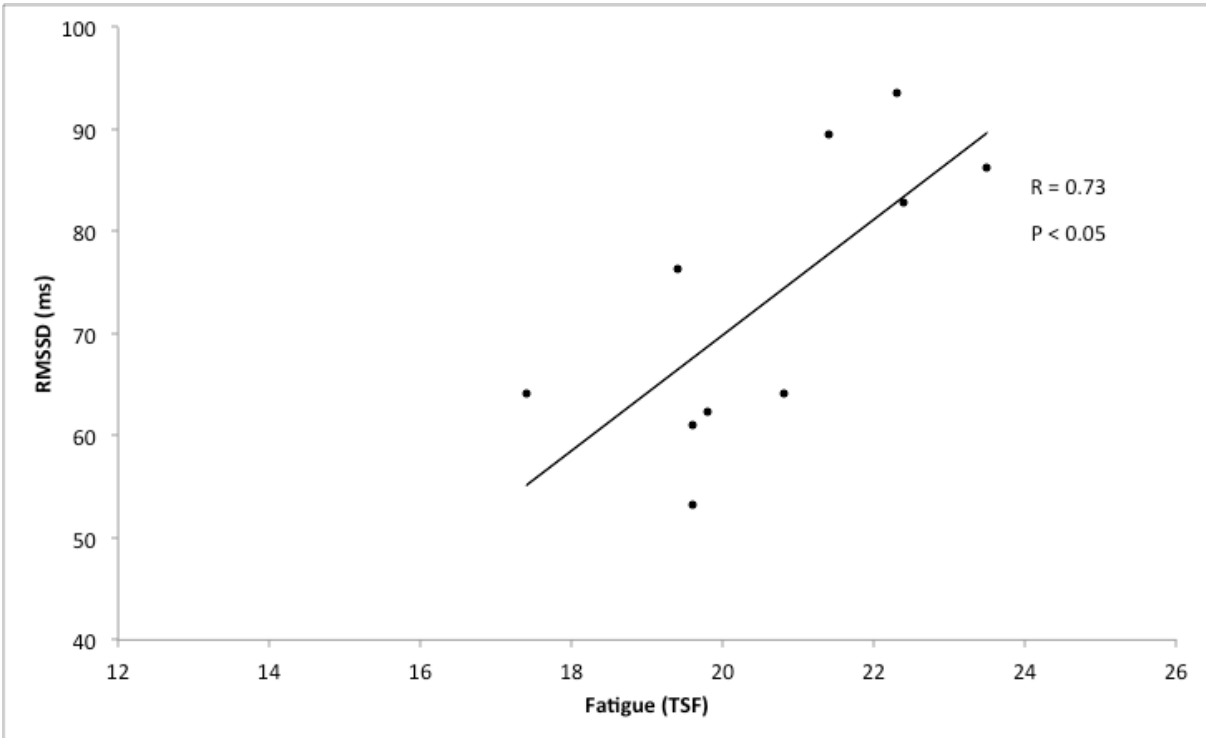
Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. Fatigue represents the total score of the fatigue questionnaire (8 items rated from 1 to 7) and the volume indicates the training session duration expressed in min.

3.5 Relationships with the fatigue

Figure 12 to figure 14 show the relationships between fatigue and $RMSSD_{SU}$, HF_{SU} (ms^2) or LF_{SU} (ms^2).

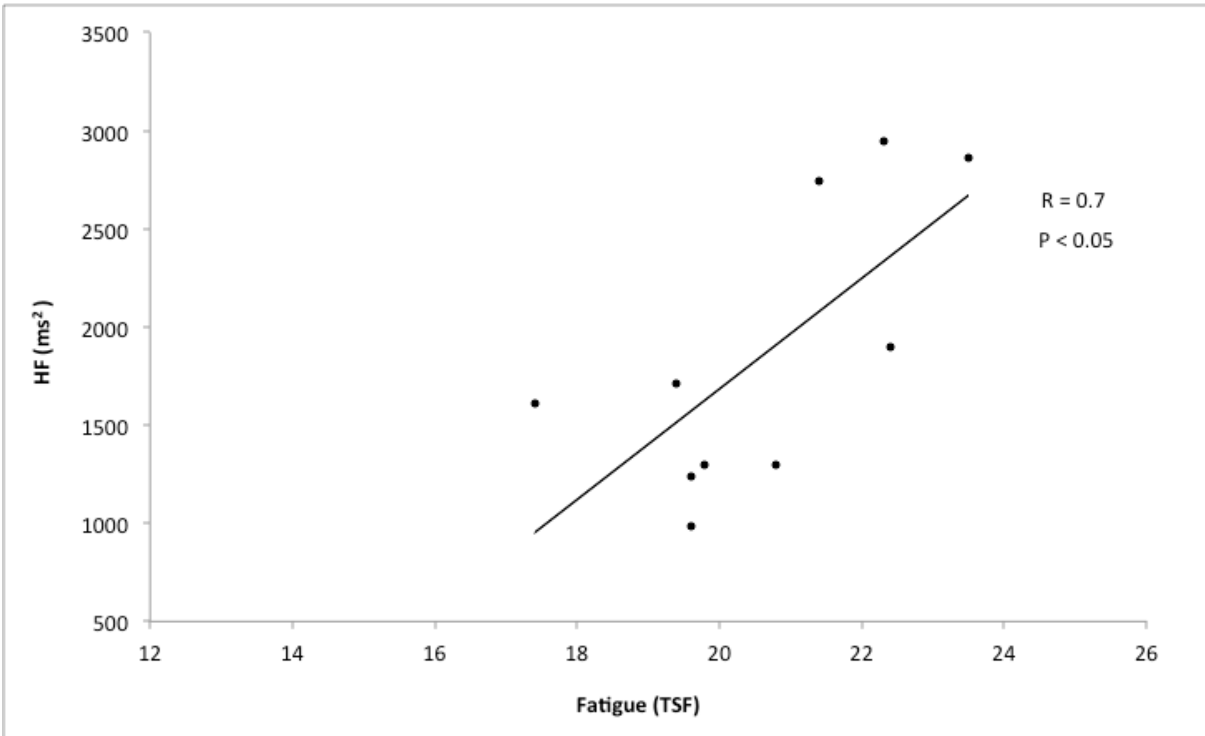
When expressed in normalized units, HF_{SU} (nu) and LF_{SU} (nu) showed a significant correlation with the subjective fatigue. HF (nu) was positively correlated with the fatigue ($R = 0.67$, $P < 0.05$) while LF (nu) was inversely correlated ($R = -0.67$, $P < 0.05$).

Figure 12 Relationship between the fatigue (TSF) and $RMSSD_{SU}$ (ms)



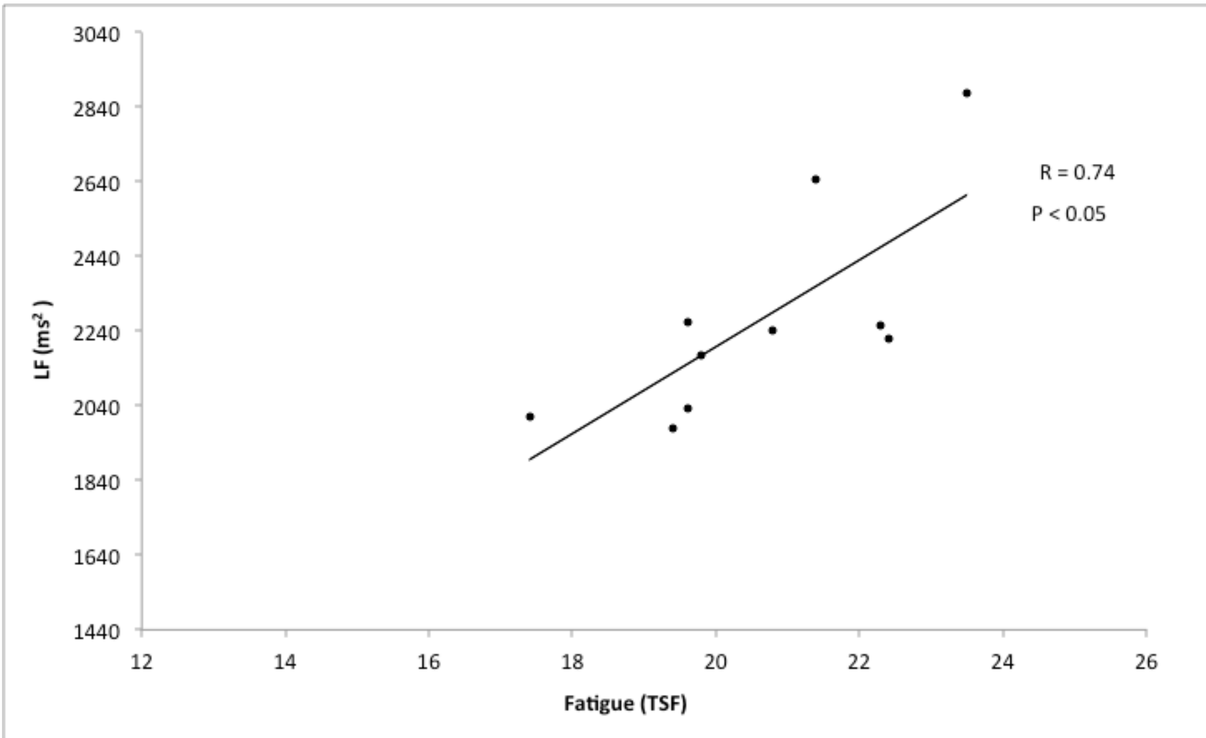
Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. RMSSD is the root-mean-square differences of successive R-R intervals calculated in supine position (SU). The fatigue represents the total score of the fatigue questionnaire (8 items rated from 1 to 7).

Figure 13 Relationship between the fatigue (TSF) and HF_{SU} (ms^2)



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. HF is the high frequencies in the spectral analysis of HRV calculated in supine position (SU). The fatigue represents the total score of the fatigue questionnaire (8 items rated from 1 to 7).

Figure 14 Relationship between the fatigue (TSF) and LF_{SU} (ms^2)



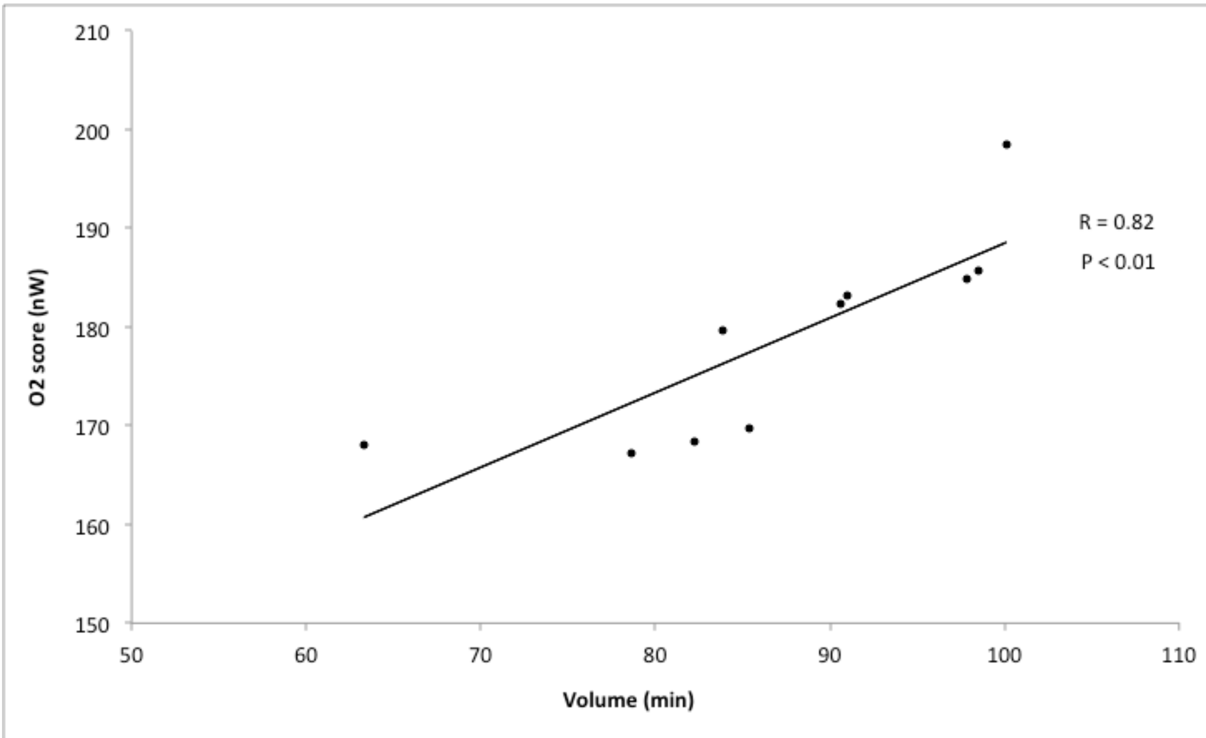
Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. LF is the low frequencies in the spectral analysis of HRV calculated in supine position (SU). The fatigue represents the total score of the fatigue questionnaire (8 items rated from 1 to 7)

3.6 Relationship with O2 score values

Figure 15 to figure 17 show the interaction of O2 score with the volume, $RMSSD_{SU}$ and HR_{SU}.

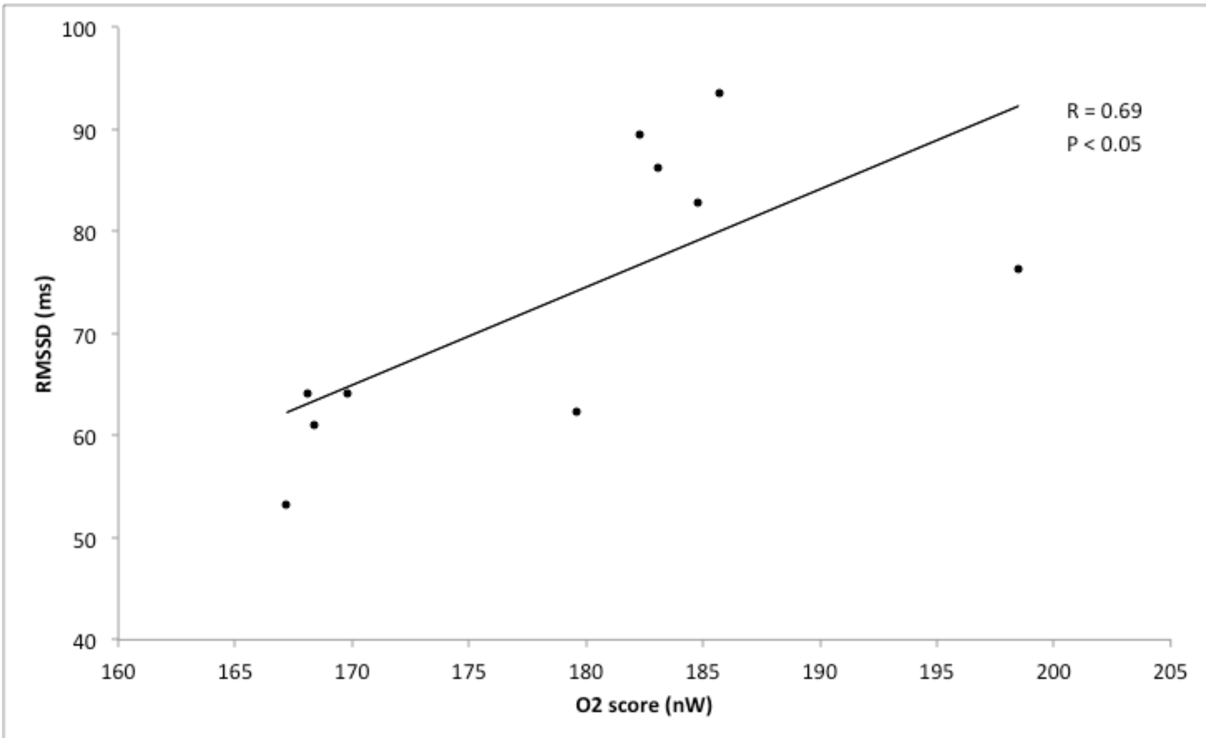
"O2 score-ref" (reference value) demonstrated the same interactions as O2 score. A positive relationship was found with the volume ($R = 0.85$, $P < 0.01$) and $RMSSD_{SU}$ ($R = 0.77$, $P < 0.05$) and a negative relationship was reported with HR_{SU} ($R = -0.96$, $P < 0.001$). When expressed in normalized units, HF_{SU} (nu) and LF_{SU} (nu) showed a significant correlation with O2 score as well as "O2 score-ref". HF (nu) was positively correlated with both O2 score ($R = 0.70$, $P < 0.05$) and O2 score-ref ($R = 0.71$, $P < 0.05$) while LF (nu) was negatively correlated with O2 score ($R = -0.69$, $P < 0.05$) and O2 score-ref ($R = -0.71$, $P < 0.05$).

Figure 15 Relationship between the volume (min) and O2 score (nW)



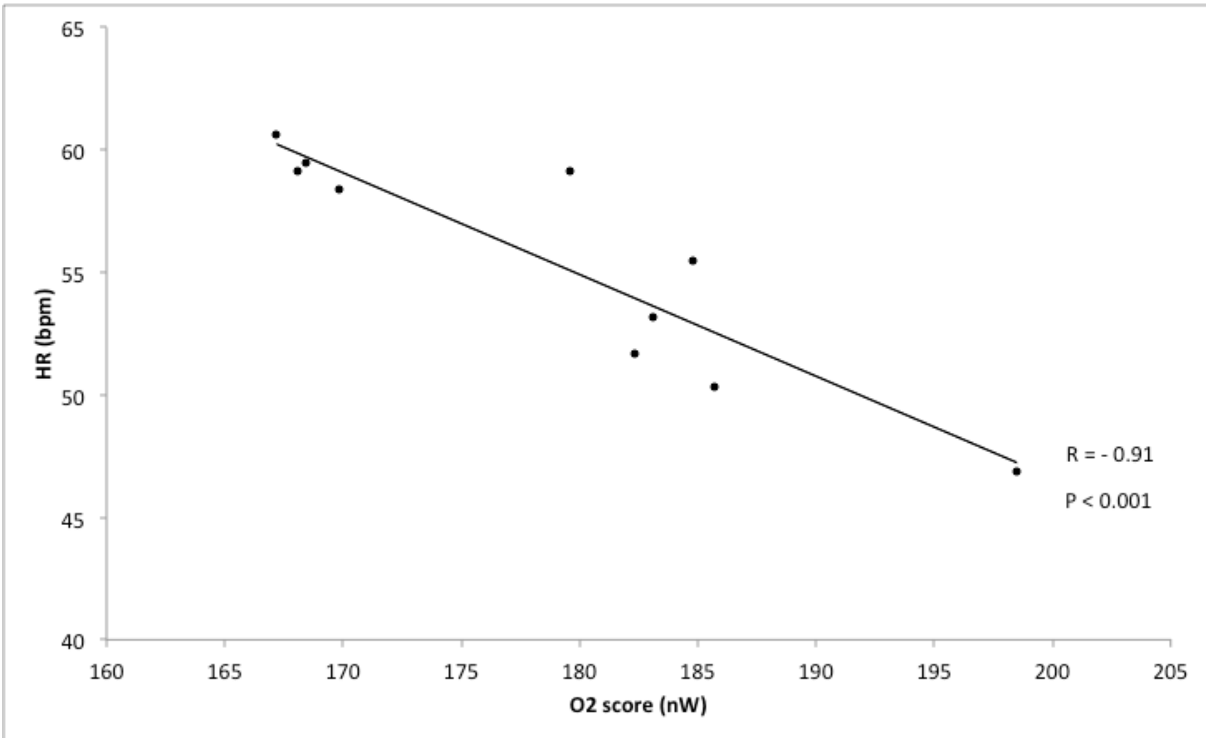
Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. O2 score represents the antioxidant capacity (nW) and the volume indicates the training session duration expressed in min.

Figure 16 Relationship between O2 score (nW) and $RMSSD_{SU}$ (ms)



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. RMSSD is the root-mean-square differences of successive R-R intervals calculated in supine position (SU). O2 score represents the antioxidant capacity (nW).

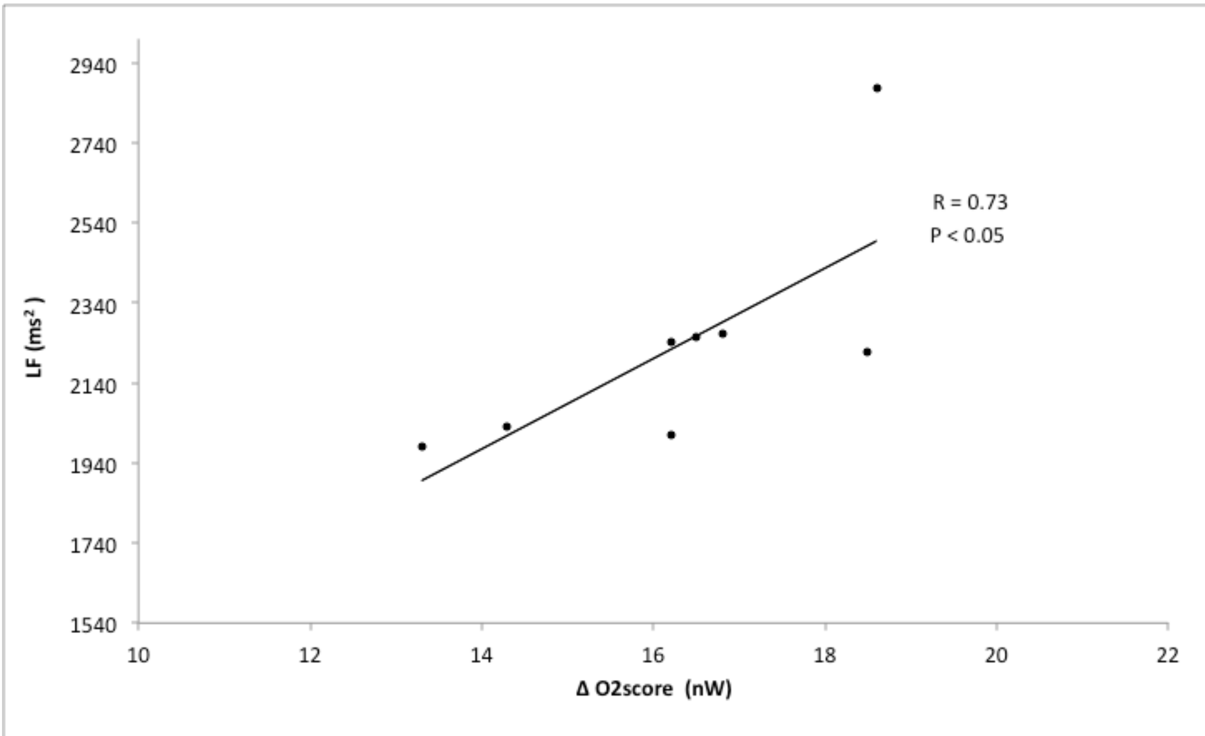
Figure 17 Relationship between O2 score (nW) and HR_{SU} (bpm).



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. HR is the heart rate (beat per minute) calculated in supine position (SU). O2 score represents the antioxidant capacity (nW).

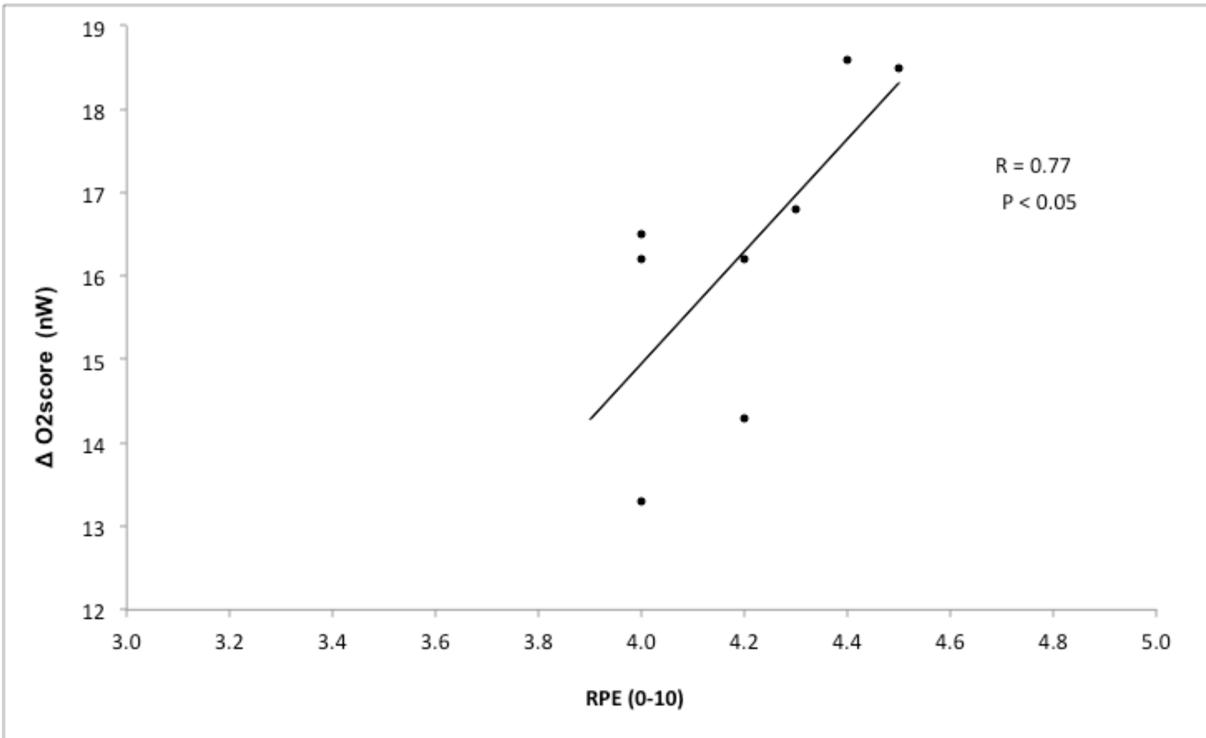
Figure 18 and figure 19 show the interaction of Δ O2 score with LF_{SU} (ms^2) and RPE.

Figure 18 Relationship between Δ O2 score (nW) and LF_{SU} (ms2).



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. LF is the low frequencies in the spectral analysis of HRV calculated in supine position (SU). Δ O2 score represents the difference between the daily antioxidant level (O2 score) and the antioxidant level at its resting value (O2 score reference).

Figure 19 Relationship between RPE (0-10) and Δ O2 score (nW).



Data are mean of baseline, week 1, week 2, week 3 and week 4 for both groups. Δ O2 score represents the difference between the daily antioxidant (O2 score) level and the antioxidant level at its resting value (O2 score reference) and RPE indicates the perceived level of exertion of the training session (rated from 0 to 10).

4. Discussion

4.1 Effectiveness of training guided by oxidative stress

This study demonstrated that the daily individualized adjustment of the training loads based on the oxidative stress analysis stabilized the O2 score values in the guided group while it continued to increase in the control group. The rationale was to adjust the daily training stimulus when the antioxidant capacity was altered (i.e., not in the reference range or comfort zone). It indicates indirectly an increased oxidative stress production that may reflect insufficient recovery from the previous training, (Tacchini et al. 2013). The impact of this oxidative stress guided prescription was significant since the average training volume was lower in the guided group in comparison to the control group (w3: P = 0.029, w4 P= 0.008). It stabilized the values measured with O2 score device (antioxidant power) and limited the changes in some sympathetic and parasympathetic HRV parameters. These findings confirmed the hypothesis that endurance training guided by oxidative stress would regulate the daily values.

The second hypothesis suggested that monitoring oxidative stress would allow better respecting the individual recovery states and thus induce an improved endurance performance. This was not observed since performance remained similar in both groups. The study took part at the beginning of the general preparation. Many authors have recently showed with elite cross-country skiers that a large part of the annual training volume is performed during this period (Tonnessen et al. 2014; Sandbakk et al. 2016; Solli and al. 2017). The objective of this period is to create physiological adaptations following these training loads and improve the athlete’s work capacity (Turner, A. 2011).

The lack of significant difference between the two performance tests could be explained by the timing chosen to perform the pre- and post-test. The pre-test was performed at the end of the baseline with a deliberately light average training load without an intensive training period prior to this test. The post-test was performed at the end of the protocol, i.e. after 4 weeks for most athletes. The results showed a significantly difference in training load between protocol weeks compared to the baseline week. The load being higher in the weeks following the baseline, the expected physiological responses wouldn't have had enough time to take place, maybe due to a lack of recovery time.

4.2 Correlations with O2 score values

Another finding of the present study is the correlation between O2 score values, the training volume and the fatigue. The results demonstrated a positive correlation between the volume and the O2 score value as well as the reference value (O2 score-ref). They also showed a positive correlation between the volume and the fatigue. Moreover the increase of these three parameters was positively correlated with the temporal marker $RMSSD_{SU}$ (ms). There is therefore an O2 score - fatigue relationship that is found through indirect markers of fatigue (such as HRV). However, the volume and O2 score value showed more direct links and correlations since they are related in the same way with HRV parameters such as HR_{SU} (bpm), $RMSSD_{SU}$ (ms), LF_{SU} (nu) and HF_{SU} (nu).
This O2 score redox sensor should be a practical tool to monitor the endurance training since the results showed positive correlations between the O2 score values and objective measurements like training volume or HRV parameters.

4.3 The training volume

Taking the results in a global way, the volume of training prescribed by the coaches during the protocol seems to have been adapted to general preparation but also to the athletes of the different disciplines. The results showed a significant correlation between the increase in training volume and some vagal-related indices of HRV.
Indeed, some time-domaine measures such as HR (bpm) and RMSSD (ms) decreased and increased respectively in supine position, when the volume was higher. This was also the case with the HF power (nu) which took over from LF power (nu) when the volume increased.
The observation of higher HF (nu) and RMSSD (ms) at supine rest supports the idea that a balanced autonomic control shifted towards parasympathetic predominance when the volume increased. Plews et al. (2013) reported that in endurance sports, positive adaptations to training would be marked by a decrease in resting HR and an increase in vagal-derived indices of HRV. Pichot, V. (2000) confirmed that heavy training shifted the cardiac autonomic balance toward a predominance of the sympathetic over the

parasympathetic drive with middle-distance runner. Iellamo (2002) also observed with high-performance athletes that strenuous endurance training enhanced sympathetic activation while submaximal training increased vagal and tended to decrease sympathetic cardiac modulation. In the present study, the relation between the training volume and the HRV modulation supports the idea that the training dose prescribed at this specific period induced positive autonomic nervous system adaptations.

4.4 The fatigue

Subjective fatigue was positively correlated with an increase in training volume. However, when the total score of fatigue was greater, RMSSD (ms), HF and LF (ms^2) increased in supine position. Therefore this acute fatigue does not appear to have been negative in the short term since it tended to enhance some HRV parameters. Meeusen et al. (2013) would describe it as acute fatigue, since it is not accompanied by decreased performance. In which case, it would be an overreaching state. The questionnaire used in the present study has been validated by (Atlaoui, Duclos 2004; Chatard, Atlaoui 2003). It makes it possible to highlight a state of fatigue but does not allow to distinguish sub-categories of fatigue such as an acute and functional or a chronic and non-functional fatigue state. Many authors including Meeusen et al. (2013) reported the importance of an adequate recovery following an intense training period. If the balance between appropriate training stress and adequate recovery is respected, an acute fatigue should be followed by positive adaptations. The present study showed that this acute and subjective fatigue perceived by the athletes could have been due to the training volume but did not generate negative physiologic responses such as overreaching.

4.5 O2 score values

The training volume resulted in increased O2 score values which were also accompanied by an increase in the O2 score reference values. These results support the findings of Finaud et al. (2006) that summarized the effect of aerobic training on antioxidant enzymes. He reported an increase in antioxidant enzyme activity in plasma and other tissues following a controlled protocol of endurance training.

Moreover, Venditti et al. (1997) reported that chronic endurance in rats increased antioxidant defenses and thus limiting tissue damage caused by oxidative stress. In the present case, the increase in the O2 score and reference values following an increased training volume supports the idea of an adaptive antioxidant defense mechanism. The antioxidant power at rest (reference value) was positively correlated with the volume, which would indicate a stronger defense system following a higher training volume. This adaptative mechanism seems to be a positive indicator since when these two values increased, time-domain measures in supine position such as HR (bpm) and RMSSD (ms) decreased and increased respectively. In addition, the results showed that when the O2 score value and the reference value increased, there was an increase in HF_{SU} (nu).

Finally, the results showed in both groups that when the subjective stress and the RPE of the training sessions were higher, the O2 score deviated from the reference value, resulting in an increase in the delta O2 score. Mrakic-Sposta et al. (2015) reported in a recent study the acute adaptative antioxidant defense mechanisms after high-intensity discountinous training in master swimmers. The antioxidant capacity was increased while it significantly decreased the oxidative stress production. Finaud et al. (2006) reported that oxidative stress is especially important after intensive or traumatic exercise. O2 score value being an indirect indicator of the oxidative stress presence, this would explain its evolution when the RPE of the training was higher.

When O2 score deviated from the reference value, it was correlated with a higher frequency marker LF_{SU} (ms^2). Some authors such as Pichot et al. (2000) or Iellamo et al. (2002) also observed a sympathetic predominance in cardiovascular autonomic modulation by the marked increase in LF (ms^2) during intensive trainings.

5. Strengths and limits

As most field studies conducted on athletes doing competitions, the present study encountered a number of limits; **i)** the timing chosen for the pre- and post-test was not always respected due to training camps, illnesses or injuries during the protocol. The duration of the protocol was slightly different between athletes. This impacted the pre- and post- performance test, which not always took place under standard conditions, not allowing for an ideal pre- and post-comparison. This first limitation could explain the insignificant results obtained on the evolution of the two performances. **ii)** The watches recording R-R intervals experienced technical problems and some athletes could not measure or missed HRV measurements. **iii)** Comparative analyses of a variable between both groups (guided vs. control) were limited by the lack of homogeneity of the athletes. Indeed, all athletes of a group practiced various sports (cross-country skiing, athletics and swimming) at different levels (regional, national and international), whose training loads were sometimes very heterogeneous. In order to obtain a sufficient amount of data for each variable measured over the entire protocol, the statistical analysis was based on individual data normalized over a week. **iv)** O2 score values can be influenced by many factors other than training such as sleep, diet or stress. Although these factors were measured to control the values obtained, it is difficult to know from which training amount the values obtained are due to the stress load or other factors.

Despite all the limitations mentioned, this study also has strengths for various reasons; **i)** The study was conducted on real elite athletes with qualitative and ecological results. **ii)** The quantity of variables daily measured and recorded is significant and increases the richness of the analysis. **iii)** From a practical point of view, this study establishes a first step in the interest of daily oxidative stress analysis to monitor the training endurance athletes.

6. Perspectives

The issue of the study was to test the effectiveness of the endurance training adjustment by the oxidative stress analysis, through the O2 score redox sensor. This monitoring method wasn't widely used in endurance athletes and its effectiveness has not yet been demonstrated in the literature. The findings of the study showed encouraging and supportive interactions in favor of this method that could be practical in the daily adjustment of training. The delta O2 score nevertheless seems more sensitive than the absolute O2 score value and more likely to prevent an overreaching state.

In order to obtain a further analysis of oxidative stress, it would be preferable to have a long-term longitudinal follow-up to test the relevance of the O2 score values during more intensive training periods.

Finally, it would also be interesting to compare this recent measurement tool in the monitoring field with daily HRV measurements on a daily basis. These two daily values would allow a comparative analysis day to day and not over an average period of one week.

7. Conclusion

Daily individualized adjustment of the training loads based on the oxidative stress analysis stabilized the O2 score values while it continued to increase in the control group following a predefined training. The impact of the adjustment was significant since the average training volume was lower in the guided group in comparison to the control group. However, no significant improvement in aerobic performance was observed in both groups.

The second important result highlighted the positive correlation between the O2 score values and the training volume. These two parameters are related in the same way with some HRV parameters such as $RMSSD_{SU}$ or HR_{SU} (bpm). This O2 score redox sensor should be a practical tool to monitor the endurance training, since it is linked to other objective and subjective methods known to be effective in the monitoring field.

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9. Annexes

9.1 Consent document

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UNIL | Université de Lausanne
Institut des sciences du sport
de l'Université de Lausanne

Formulaire de consentement

Pour le suivi des athlètes afin de mieux gérer leur récupération

Information

Le système de défense antioxydant permet de contrôler le stress oxydatif qui correspond à un déséquilibre entre les antioxydants et les radicaux libres, en faveur des radicaux libres. Son suivi permet de prévenir le stress oxydatif et créer les conditions favorables pour mieux récupérer et améliorer les performances.

Les mesures sont utilisées à des fins expérimentales et de façon anonyme. Les résultats permettront de mieux analyser la relation entre le système de défense antioxydant, sa gestion et les performances.

J'ai compris que l'on recourt à des volontaires pour effectuer cette étude préliminaire de 6 semaines, dans le cadre d'un travail de master. La réalisation de ce travail dépend de mon engagement et de ma rigueur quant aux prises de mesure et aux données à tenir à jour.

Objet

Chaque participant mesurera quotidiennement, avec l'outil O2score la valeur de son système de défense antioxydant à l'aide d'une microgoutte de sang.

Prendra sa variabilité cardiaque trois fois par semaine et remplira son carnet d'entrainement quotidiennement.

Participera à un test de performance standardisé et reproductible un mois plus tard.
Il est tenu de maintenir à jour tous les résultats obtenus à fournir.

Amélie Parolini, étudiante en Master entraînement et performance
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Tél : 077 437 12 92

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Institut des sciences du sport de l'Université de Lausanne

Consentement

Le représentant légal autorise l'athlète à prendre part à l'étude décrite ci-dessus

Nom, Prénom du représentant légal : **Date :**

Signature :

L'athlète donne son accord pour participer à cette étude de 6 semaines, conformément aux informations fournies ci-dessus.

Je suis en possession d'un appareil O2 score pour la durée de l'étude. J'ai la responsabilité de le garder intègre pour la durée du protocole.

A la fin de l'étude, si l'appareil s'est avéré utile et pertinent pour l'entraînement, il est possible de le garder pour un prix spécial de 100.- au lieu de 290.-

Nom, Prénom de l'athlète : **Date :**

Signature :

9.2 *O2 score* questionnaire

Questionnaire EDEL

Consignes : Lorsque des réponses sont proposées, merci de **cocher la case** de la réponse correspondante. Ce questionnaire devrait vous prendre environs 5 minutes.
Les données recueillies sont traitées de manière confidentielle et rendues anonymes.

Informations générales :

-Sexe:

- ☐ Homme
- ☐ Femme

-Taille =_____ m

-Poids =_____ kg

-Age = _____ ans

Alimentation :

Si vous ne consommez pas un des types d'aliments proposés au moins une fois par semaine, merci d'indiquer « 0 ».

En général, combien de fois par semaine consommez-vous des:	0	1-3	4-7	Plus de 7
Aliments à base de céréales non raffinées (pain complet, riz complet, pâtes complètes…) ?				
Légumineuses (Lentilles, haricots, pois chiches…) ?				
Légumes (carottes, poireaux, courgettes, pommes de terres…) ?				
Boissons sucrées/sodas ?				
Poissons ou fruits de mer ?				
Viandes rouges et produits dérivés (bœuf, agneau, porc, charcuterie, farce à la viande, etc.) ?				
Viandes blanches (Poulet, pintade, autre volaille, lapin…) ?				
Sucreries, biscuits ou pâtisseries ?				

-En général, combien de fois par jour mangez-vous des fruits ?

- ☐ 0
- ☐ 1 - 2
- ☐ 3 et plus

-A quelle fréquence utilisez-vous de l'huile d'olive dans la préparation des plats ?

- ☐ Jamais
- ☐ Parfois

- ☐ Souvent
- ☐ Tout le temps

Consommation d'alcool :

En cas de question sur les quantités, référez-vous à l'image ci-contre pour plus de précision

En général, combien de fois par semaine buvez-vous de verres de	0	1-3	4-7	Plus de 7
Vin				
Autre alcool (bières, alcopop, alcools forts…)				

Alicaments/médicaments :

-Prenez-vous au moins une fois par semaine un (des) complément(s) alimentaire(s)/nutritionnel(s) ?

- ☐ Oui
- ☐ Non

-Si oui, le(s)quel(s) ?

- ☐ Vitamine A
- ☐ Vitamine C
- ☐ Vitamine E
- ☐ Oligo-éléments (iode, fer, cuivre, zinc, sélénium, manganèse, silicium, etc.)

Si autre, indiquez : ____

-Prenez-vous au moins une fois par semaine un anti-inflammatoire non stéroïdien (Ibuprofen, Irfen, aspirine, etc.):

- ☐ Oui
- ☐ Non

-Si vous êtes une femme, prenez-vous un contraceptif oral (la pilule) ?

- ☐ Oui
- ☐ Non

Conditions de vie :

En général, quelle est votre fréquence d'exposition	Jamais	Parfois	Souvent	Tout le temps
À la Fumée passive ?				
À la Pollution urbaine ?				
Au soleil ?				

-En général, vous vous estimez :

- ☐ Pas stressé
- ☐ Faiblement stressé
- ☐ Plutôt stressé

- ☐ Très stressé

-En général, combien d'heures <u>par nuit</u> dormez-vous?

_____ heures.

-En général, combien d'heures <u>par semaine</u> de sport faites-vous?

_____ heures de sport.

Ce questionnaire est maintenant terminé, nous vous remercions pour votre participation.

9.3 Training diary: daily measurements, fatigue questionnaire and HRV recorded

Semaine		Sommeil (heure)	Fatigue	O2 Score (EDEL)	Activité	Descritpion	Volume (minute)	Intensité (RPE)	Charge	Charge/jour	Charge/semaine
	Lundi								0		
									0		
									0	0	
	Mardi								0		
									0		
									0	0	
	Mercredi								0		
									0		
									0	0	
	Jeudi								0		
1									0		
									0	0	
	Vendredi								0		
									0		
									0	0	
	Samedi								0		
									0		
									0	0	
	Dimanche								0		
									0		
									0	0	0

Changement d'intensité de l'activité	Charge (min)	Intensité (RPE)	Charge	Charge/jour	Charge/semaine	Modification de la charge	Stress(1-10)	Remarques (shake, gros repas, complément)
			0					
			0					
			0	0		0		
			0					
			0					
			0	0				
			0					
			0					
			0	0				
			0					
			0					
			0	0				
			0					
			0					
			0	0				
			0					
			0					
			0	0				
			0					
			0					
			0	0	0			

Date	Supine									Standing								
	RR	HR	RMSSD	LF	HF	LF(nu)	HF(nu)	TP	LF/HF	RR	HR	RMSSD	LF	HF	LF(nu)	HF(nu)	TP	LF/HF
j1																		
j2																		
j3																		
j4	1127.2	53.228	105.37	2917.8	3102.5	48.418	51.482	6020.3	0.94048									
j5	1216.8	49.309	89.219	3518.5	1224.5	74.161	25.81	4743	2.8733									
j6	1065.5	56.31	93.65	3576.6	1713.5	67.61	32.39	5290.1	2.0874	660.24	90.876	26.59	1748.4	680.46	71.978	28.013	2428.86	2.5694
j7																		
j8																		
j9	1419.7	42.262	108.31	2484.8	2641.1	48.473	51.522	5125.9	0.94082	838.7	71.539	44.692	5542.6	579.88	90.518	9.4703	6122.48	9.5581
j10																		
j11																		
j12																		
j13	1075.1	55.809	80.799	2691.5	2277.7	54.153	45.827	4969.2	1.1817									
j14																		

		Description	Score
		Pas du tout	1
			2
			3
		Normal	4
			5
			6
		Beaucoup	7

Semaine	Jour	Questions	score
	Lundi	J'ai trouvé l'entraînement plus difficile	
		J'ai plus dormi	
		Mes jambes étaient plus lourdes	
		J'ai attrapé froid ou eu une infection	
		Ma concentration était plus difficile	
		J'ai travaillé mois efficacement	
		Je me suis senti plus irritable	
		J'ai été plus stressé à la maison ou é l'école	
		Score total	0
	Mardi	J'ai trouvé l'entraînement plus difficile	
		J'ai plus dormi	
		Mes jambes étaient plus lourdes	
		J'ai attrapé froid ou eu une infection	
		Ma concentration était plus difficile	
		J'ai travaillé mois efficacement	
		Je me suis senti plus irritable	
		J'ai été plus stressé à la maison ou é l'école	
		Score total	0
	Mercredi	J'ai trouvé l'entraînement plus difficile	
		J'ai plus dormi	
		Mes jambes étaient plus lourdes	
		J'ai attrapé froid ou eu une infection	
		Ma concentration était plus difficile	
		J'ai travaillé mois efficacement	
		Je me suis senti plus irritable	
		J'ai été plus stressé à la maison ou é l'école	
		Score total	0

9.4 O2 score device