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Acute Physiological, Metabolic and Perceptual Responses to different High-Intensity Interval Training formats

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SECTION 1

1. INTRODUCTION

The High-Intensity Interval Training (HIIT) represents today one of the most effective training modalities in both team and individual sports to improve physiological parameters and performance of athletes. HIIT involves repeated short-to-long bouts of rather high-intensity exercise, interspersed with recovery periods and, contrary to popular opinion, this modality of training is neither new nor revolutionary since it has been used by athletes for almost a century. For instance, in 1920s, after the studies by Hill concerning intermittent exercises, Paavo Nurmi one of the best middle- and long-distance runners in the world at that time, included some forms of HIIT in his training routines, and Hans Reindell, a Germany doctor started in 1930s to use a HIIT programs in German low-level athletes. Successively, two German trainers used the HIIT to train two important élite athletes as Rudolf Harbig and Roger Banister (Billat, 2001 a). The scientific interest and popularization of the HIIT increased in 1950s after the remarkable performances by Emil Zatopek, a highest-level athlete, who was trained by Danish researcher Per-Olof Astrand and used HIIT in his training programs. Astrand and colleagues in 1960s published some scientific research on the acute physiological responses of HIIT, creating the first scientific basis for long and short bouts of interval exercise. Coaches, sport scientist and practitioners can use different formats of high-intensity interval training to induce some specific acute physiological responses and to reach long-term physiological adaptations. These formats include long intervals exercise, long bouts of work intervals at an intensity from 95 to 105% of v/pVO_2max are used. The durations need to be longer than a minute to induce acute metabolic and neuromuscular responses. The bouts of long interval HIIT should be interspersed with short duration of passive recovery or active recovery performed at 60% of v/pVO_2max. The bouts of short interval protocols should be performed at intensity between 95–120% of v/pVO_2max for a duration between 10-60 s, alternated with a passive recovery (10-60 s), to affect the metabolic and neuromuscular responses. More recently, another HIIT form, using sprints and all-out efforts, has been applied both in laboratory and in the field. These HIIT formats include repeated-sprint training (RST; sprints lasting from 3 to 10 s, interspersed with recovery periods lasting generally between 10-60 s) or sprint interval training (SIT; 20-30 s all-out efforts interspersed with 1-4 min passive recovery periods). Some studies by Gibala and colleagues (2006; 2009; 2017) in the last years have emphasized the metabolic and neuromuscular responses of all-out efforts compared to the continuous exercise and the long bout HIIT. Another peculiar format of HIIT is represented by Game-based HIIT (GBHIIT), using the Small-Sided Games (SSGs). SSGs are used in team sport as long interval HIIT to improve the team-specific performance of players (Hammami et al., 2017). SSGs are increasingly believed to be a more suitable training than the traditional interval training for the development of physical fitness, technical skills, and decision-making ability (Arslan et al., 2017). SSGs are usually performed with modified games on reduced pitch area, using adapted rules and involving a smaller number of players then traditional games (Hammami et al., 2017). Impellizzeri (2006) has shown that SSGs are equally effective at improving aerobic fitness as common fitness training activities such as interval running at an intensity of 90–95% of HRmax. Dellal et al. (2012) also showed that some SSG formats resulted in heart rate (HR) responses comparable with short-duration intermittent running. During GBHIIT the players run for 2 to 5 minutes at a sport-specific intensity, and the recovery duration is usually passive and range from 90 to 120 seconds (Figure 1).

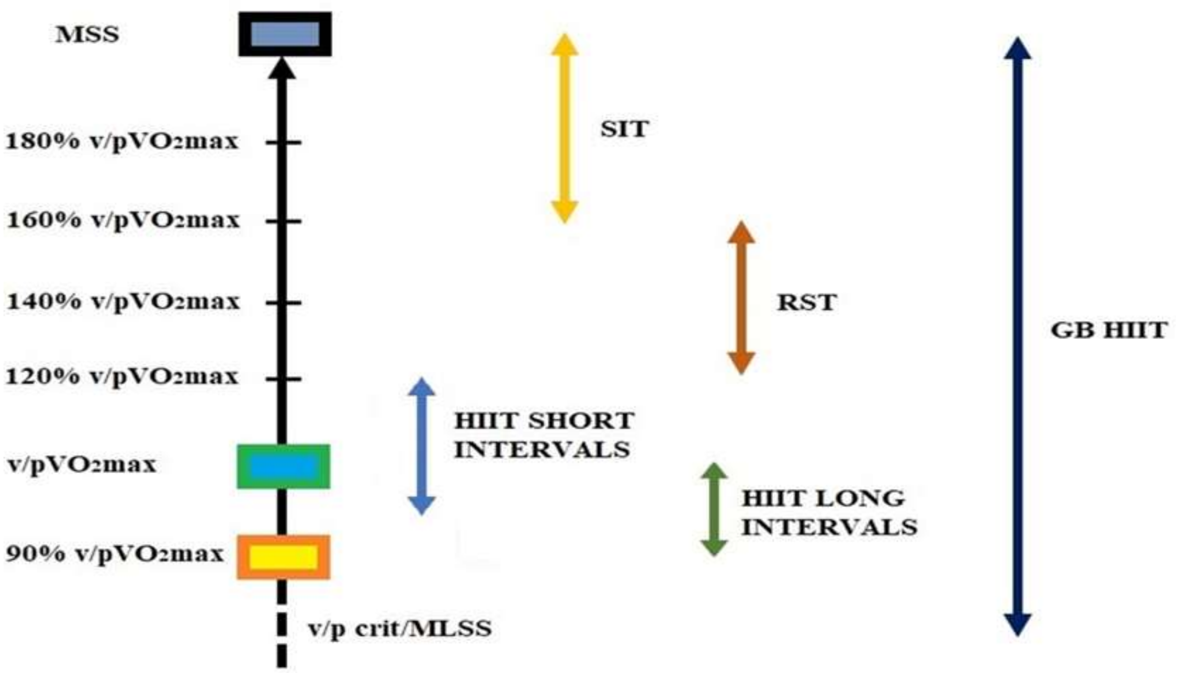

Figure 1. General guidelines for High-Intensity Interval Training protocol design. MLSS: maximal lactate steady state; v/p crit: critical velocity/power; VO_2max: maximal oxygen uptake; v/pVO_2max: minimal running speed/power required to elicit VO_2max; MSS maximal sprinting speed; RST: repeated sprint training; SIT sprint interval training. (Modified from: Laursen P and Buchheit M, 2019).

Additionally, the session volume of HIIT protocols can be defined as high (16 minutes of work) or low (4 minutes of work) (HV-HIIT or LV-HIIT, respectively). Moreover, considering the effect of training periodization, the length of HIIT intervention is classified as long-term (≥12 weeks) or short-term (≤4 weeks) duration (LT-HIIT or ST-HIIT) (Wen et al., 2019). The main parameters used to plan HIIT intensity are the speed, or the power associated with maximal oxygen uptake or VO_2max (vVO_2max or maximal aerobic speed, MAS, and pVO_2max or maximal aerobic power, MAP) (Billat and Koralsztein, 1996). It has been showed that the HIIT allows i) to increase the maximum activity of mitochondrial enzymes; ii) to reduce the use of glycogen and the blood lactate accumulation during exercise; iii) to improve performance mainly based on aerobic metabolism (Burgomaster et al., 2006); and iv) to enhance the buffering capacity of the muscle (Edge et al., 2006). The HIIT effects on physical performance depend on the intensity and duration of the working phase, intensity and duration of recovery phase and work/recovery ratio (Billat, 2001a and 2001b). Some studies demonstrated that HIIT formats allow to reach the maximal oxygen uptake (VO_2max), or at least a very high percentage of VO_2max, inducing a higher oxygen transport and a large motor unit recruitment. For this reason, the time spent at or near VO_2max during HIIT protocol is considered an optimal stimulus to improve cardiorespiratory and metabolic functions, as well as to induce peripheral adaptations (Thévenet et al., 2007). Indeed, in the last years the interest of numerous studies focused on HIIT formats that allow to maintain the highest exercise time at or more 90%VO_2max (T@VO_2max).

Nine variables can be manipulated to obtain different acute responses by HIIT sessions: intensity and duration of work and relief intervals, exercise modality, number of repetitions, number of series and between-series recovery durations and intensities. The manipulation of each variable allows to elicit different metabolic, cardiopulmonary and/or neuromuscular responses.

Additionally, today HIIT is not only used by moderately trained and competitive athletes, but also by sedentary subjects and by clinical population (Wewege et al., 2017), as described below. HIIT in sedentary older subject leads to improvements of cardiovascular health, as well as in resting haemodynamic stress, without compromising myocardial structure or strain mechanics (Grace et al., 2018). It has been also shown that HIIT can improve the low-density lipoprotein (LDL) cholesterol and peak of oxygen uptake (VO_2peak) levels in overweight and obese adults when compared to traditional moderate-intensity continuous training (MICT), despite a similar energy expenditure. However, HIIT may be associated with an increase in inflammation with short-term exercise in this population (Vella et al., 2017).

In the last 10 years, there was a growing interest for the utility and efficacy of high-intensity interval training with long intervals (1-4 minutes) at intensities ranging from 80 to 100% peak power output (PPO) on variables related to cardiometabolic health in various populations. In addition, the effects of low-volume sprint interval training (SIT), typically requiring ≤30 s "all-out" efforts at intensities greater than PPO have been examined (Gibala and McGee 2008). Some studies demonstrated the useful and effectiveness of HIIT formats in adolescent. Indeed, the HIIT formats have been shown to be more effective on the aerobic system, eliciting greater improvements in health-related factors as cardiometabolic parameters and body composition, compared to traditional training programmes as continuous training at moderate intensity, in adolescents (Zafeiridis et al., 2010, Racil et al., 2016).

In endurance sports such as running, cycling, rowing and triathlon two different training types are commonly used to improve the athlete's performance: a continuous training, performed at low intensity (70%VO_2max) for a long duration (>60 minutes), that represents the 75-90% of endurance training total time, whereas the remaining 10-25% of the total training time is occupied by alternating high-intensity intervals (≥90%VO_2max) with recovery phase (Tonnessen et al., 2014).

In this dissertation I will focus mainly on acute physiological, metabolic, and perceptual responses to different HIIT concepts, compared to those induced by Continuous Training (CT) at high or low intensity in active subjects and in élite young athletes. Overall, the results presented in this dissertation could provide new information to coaches, sport scientists and sport practitioners on the level of aerobic/anaerobic and neuromuscular engagement associated with different HIIT concepts, as well as of the different internal workloads perceived, related to the individual physiological, metabolic, and neuromuscular profiles.

2. HIIT VARIABLES

Exercise Period Intensity

The intensity of work intervals in a HIIT session is the most important factor influencing the solicitation of the maximal oxygen uptake (VO_2max) and the time spent at 90% or above VO_2max (Midgley et al., 2006; Millet et al, 2003b). The exercise period, in moderately active adults, performed at high intensity leads a higher increase in mitochondrial content than a continuous training with exercise period performed at low intensity. Furthermore, several studies showed that a low volume of exercise performed at high intensity can elicit similar skeletal muscle adaptations and reaching comparable effects on lactate threshold respect with moderate-intensity continuous exercise performed for a high volume (Abbiss et al., 2011; MacInnis et al., 2016; MacInnis and Gibala, 2017). Therefore, the exercise period intensity is an important variable to use in training planning to reach both a high level of VO_2max and a long time spent at ≥90% VO_2max during HIIT session (Laursen, 2010).

Exercise Period Duration

The intensity of HIIT bout and the proportion of muscle fibres engaged during a high-intensity interval training session, affect the duration of exercise period. The ratio between the maximum exercise period duration that can be perform and the exercise intensity, during a high-intensity interval training session is represented by an inverse function. Indeed, during an HIIT session, the higher the exercise intensity, the shorter the duration, and conversely.

Recovery period

The purpose of recovery period should be to promote the removal of blood lactate that has accumulated in the muscle during high-intensity interval training phase. The recovery can be affected by different factors such as: the duration (short or long time), modality (active or passive), types of exercise (running, cycling, and swimming) and intensities when active recovery is performed (Buchheit and Laursen, 2013 part 1). The organization of recovery period influences several aspects, as energy demand, muscle fatigue origin, individual time to exhaustion (Tlim), VO_2 and T@VO_2max (Germano et al., 2019; Zaferidis 2010). Some studies demonstrated that the active recovery leads to reach and maintain at a high level of VO_2max and maximal heart rate, so it is necessary a shorter time to return to these maximum parameters (Smilios et al., 2018). Furthermore, previous studies have showed that the active recovery allowed obtaining a significant increase in muscle and blood lactate removal, with an enhancement of maximal sprint performance (Dorado et al., 2004). Instead, it was showed that the passive recovery allows the benefits as great restoration of the intramuscular pH, increasing the exercise time (Edge et al., 2013), high resynthesis of phosphocreatine (PCr) (Buchheit et al., 2009) and consequently maintenance or improvement of performance. The cardiorespiratory and metabolic functions are influenced by the combination of time and active recovery intensities, and the profile of the subjects (élite athlete, low-level athlete, recreational) (Germano et al., 2019).

Series Duration

The duration of series is another parameter than can be used to affect the response of the HIIT training session. In the team sports, which the priorities are the tactical and technical aspects, the duration of series or the number of bouts is short and the goal of HIIT session is to improve the metabolic rate. It is used a few short series, so that for a similar total number of repetitions, get it a higher quality work volume. For example, using 2 periods of 4 min bouts with a long recovery period can be better than a single 8 min period of HIIT bouts to achieve quality or intensity during HIIT session. While the endurance athletes use a long duration of series to elicit the endurance and fatigue resistance aspects with an impact on the neuromuscular system. Generally, the series duration is between 4 minutes (quality emphasis) and 14 minutes (endurance emphasis).

Number of Interval Bout Series

The number of interval bout series is related to training workload, such as the distance run or the power produced in a single HIIT session, since the metabolic response might be balanced varying series durations and modifying the recovery type used. The greater the number of series and the duration of sessions, and the greater the workload of training. Instead, decreasing the number of interval bout series decreases the session total volume, so the workload training.

Between-Series Recovery Intensity and Duration

The duration and intensity of recovery during high-intensity interval training sessions are important variables for the HIIT sessions planification. These variables can be used to maximize the efficacity of maximal aerobic speed or power during subsequent intervals and maintaining a minimal level of VO_2 to reduce the time needed to reach VO_2max during subsequent intervals (Billat, 2001b). The intensity of recovery between the series has an important role in VO_2 response, since it affects both the actual VO_2 during the sets and exercise capacity (Dupont et al., 2004).

3. PARAMETERS OF HIIT PROGRAMMING

The main parameters used by coaches, sport scientists, and sport practitioners to prescribe and to calibrate the adequate intensity of HIIT training session are maximal aerobic speed and maximal aerobic power (MAS and MAP), heart rate (HR), and rating of perceived exertion (RPE).

Maximal Aerobic Speed and Maximal Aerobic Power

The speed and power associated with the body's maximal oxygen uptake (v/pVO_2max or maximal aerobic speed/power) (Billat and Koralsztein, 1996) has been shown to be useful reference intensity for programming HIIT (Laursen and Jenkins, 2002). The level of maximal oxygen uptake is consistently correlated with distance running and cycling performance (Buchheit and Laursen, 2013 part 1). The v/pVO_2max method represents an integrate measure of both VO_2max and the energetic cost of running or cycling/rowing in a single factor and might represent an athlete's peak locomotor ability (Billat and Koralsztein, 1996). The v/pVO_2max is the minimal speed/power needed to elicit VO_2max and it represents an ideal parameter to plan the training (Laursen and Jenkins, 2002).

The v/pVO_2max can be measured using two different methods:

1) Indirect measurement used to predict v/pVO_2max for field testing as: 5 min exhaustive run, 4 min all-out cycling and 2 km rowing time trial. The v/pVO_2max is calculated with a theoretic speed/power needed to elicit the VO_2max.
2) Direct measurement of pulmonary gas exchange during ramp running or cycling/rowing incremental tests to exhaustion are used either on a track or treadmill or by using specific ergometer (Laursen et al., 2002).

The maximal aerobic speed, or power, measured during a test can be influenced by the test method and protocol used (Midgley et al., 2007). Indeed, an estimated measurement of the maximal aerobic speed is lower (Di Prampero et al., 1986) compared to a measured vVO_2max (Harling et al., 2003). Furthermore, irrespective of the method used to measure the v/pVO_2max, the protocols including longer stage durations tend to elicit lower speed/power values (Midgley et al., 2007), while higher speed/power increments are reached with shorter tests inducing higher speed/power values, the anaerobic capacity of the individual being the confounding variable in the assessment.

Heart rate-based prescription

The heart rate (HR) measurement and monitoring of athletes is probably the most physiological parameter used to control or measure the intensity of exercise (Buchheit, 2014). The utilization of heart rate for the setting of exercise intensity is well suited for long/prolonged and submaximal exercise bouts, whereas the monitoring of work intensity during an HIIT session can be limited. Indeed, the heart rate alone cannot inform about the exercise intensity executed above v/pVO_2max, which represents an important parameter of HIIT programs (Billat, 2001b; Laursen and Jenkins, 2002). During an HIIT session with an intensity near or at v/pVO_2max the HR can reach higher values (>90-95% maximal heart rate or HRmax), but this is not always the case, during an HIIT session with very short intervals (<30 s) (Midgley et al., 2007) and medium-long intervals (1-2 min) (Seiler and Hetlelid, 2005). This is related to the HR lag at exercise onset, where the HR is much slower to respond respect with the oxygen uptake response (Cerretelli and Di Prampero, 1971). Additionally, the utilization of heart rate inertia at exercise end, called heart rate recovery

(HR recovery), can be problematic to plan the HIIT session because this can create an overestimation of the actual intensity or physiological load that occurs during recovery phase (Seiler and Hetlelid, 2005). Moreover, the temporal dissociation between heart, oxygen uptake, blood lactate concentration and work output during HIIT limits the possibility to accurately measure the intensity during HIIT sessions using heart rate alone. Indeed, it is necessary to estimate the workload during HIIT session to use the heart rate associated with another physiological and metabolic parameters as blood lactate concentration and oxygen uptake, or performance parameters as maximal aerobic speed and maximal aerobic power. Because, during a HIIT session it is more difficult for an athlete to control or adjust the exercise intensity viewing the heart rate from watch. The heart rate monitoring in endurance sports, with the athlete that know from experience as to perform the training sessions at appropriate pace or power, is used to observe the cardiac responses. Similarly, in team sports the HR responses are used to standardize the HIIT session as monitoring points throughout the season (Buchheit et al., 2015).

RPE-based prescription

The utilization of rating of perceived exertion (RPE) method (Dishman et al., 1987) to prescribe the intensity of HIIT session is most attractive and easy. Indeed, this method does not require the monitoring of physiological parameters (as heart rate or blood lactate level) or performance parameters (as maximal aerobic speed or power). Therefore, the coach could use the simple instructions with his athletes saying phrases such as, “You have run 4x4 min hard, with 2 min walk recovery intervals”. For many years, the coaches used this method to prescribe the training independently other variables such as the duration or distance of workload and relief intervals (Seiler and Sjursen, 2004). The important point is that the athletes can self-regulate the exercise intensity based on how the exercise feels, the athlete’s brain being the best sensor globally of how in balance his body is (Noakes, 2011). During an exercise in the laboratory, the target of intensity chosen is usually the maximal sustainable exercise intensity perceived as hard to extremely hard (≥6 for CR-10 Borg Scale or ≥15 on 6-20 Borg scale). The intensity selected using one the Borg’s scales is typically based on the experience of athletes in the training session, the goal of training session and the other parameters related to training periodization. Marcora (2011) demonstrated that the RPE represents “a conscious sensation of how hard, heavy and strenuous the exercise is”, moreover other studied showed that the RPE can be correlated with the physiological, biomechanical, and psychological solicitations or fatigue imposed during the training session or performance on the body. Using the RPE to calibrate the intensity of HIIT sessions it is not necessary to know the fitness level of athletes and to perform physical tests before to start the HIIT sessions (Weyand and Bundle, 2005). An interesting element of the RPE method is the possibility to use this method as a universal exercise regulator independently of the exercise modality, and variations in terrain and environmental conditions. A limitation of RPE method during HIIT session is that this method does not allow for the precise manipulation of the physiological response to a given HIIT session, which could limit the ability to reach a specific adaptation (Seiler and Sylta, 2017).

30-15 Intermittent Fitness Test (30-15 IFT)

The 30-15 IFT is a field test designed to measure the maximal oxygen uptake and the maximal heart rate in athletes of team, racket, and middle-distance running sports (Buchheit, 2008 and 2010). The protocol consists of 30 seconds shuttle runs interspersed with 15 seconds of passive recovery periods. The velocity of the first 30 second run is 8 $km \cdot h^{-1}$ and increases by 0.5 $km \cdot h^{-1}$ each 45 second stage. The subjects must run back and forth between two lines set 40 m apart at a pace governed by a pre-recorded beep at appropriate intervals that help them adjust their running speed by entering 3 m zones at each extremity and in the middle of the field while the short beep sounds (Figure 2). During the 15 second recovery period, the subjects walk in the forward direction to join the closest line (at the middle or at one end of the running area, depending on where the previous run stopped) from where they start the next run stage. Subjects are instructed to complete as many stages as possible. The example of 2 intermittent runs, during 30-15 Intermittent Fitness Test, is represented in figure 2. The test is ended when a subject could no longer maintain the imposed running speed or when he or she was unable to reach a 3 m zone around each line at the moment of the audio signal consecutively 3 times. The velocity reaches during the last completed stage is considered as the maximal aerobic speed 30-15 (MAS $30\text{-}15_{IFT}$).

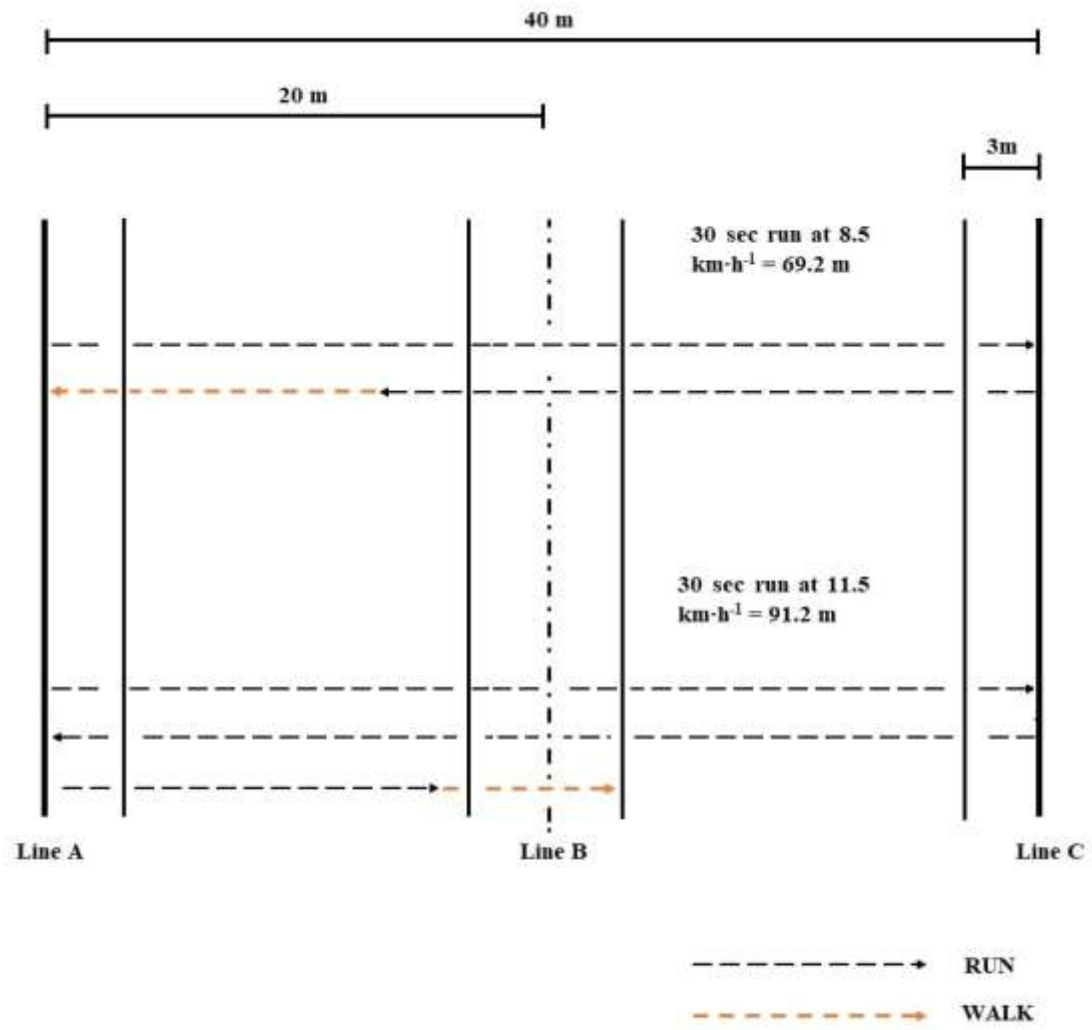

Figure 2. Schematic example of 2 intermittent runs during 30-15 IFT During a run at 8.5 km·h^{-1}, the subject starts at line A, run to line C crossing line B, and then return. After crossing line B again, they stop after 8.5 m and walk to line A during the 15-second recovery to be ready for the next stage. For a run at 11.5 km·h^{-1}, the subject starts line A and performs a complete round trip, stopping after 9.5 m when going toward line B, and then walk to line B during the 15- second of recovery for the next start. The last speed reached by the subject during the last completed step corresponds to the maximal running speed 30-15 ($MRS_{30\text{-}15IFT}$). (Modified from: Buchheit M, 2008).

Cardiopulmonary Exercise Treadmill Test (CPET)

The cardiopulmonary exercise treadmill test is a test performed in laboratory to determine the maximal cardiorespiratory responses and maximal aerobic speed in athletes, moderately, sedentary and pathological subjects. The test is carried out on a motorized treadmill, and it starts at a running velocity of 6 km·h^{-1} with a progressively increasing speed of 2 km·h^{-1} every 3 minutes and it terminates when the participants reach volitional exhaustion (Mugele et al., 2018). Verbal encouragement is provided throughout the test. During the test Heart rate (HR), oxygen uptake (VO_2), maximal aerobic speed (MAS) and respiratory exchange ratio (RER) are monitored to assess the intensity of the exercise. The exercise is considered maximal when i) anaerobic threshold, calculated with V-slope method, is exceeded, ii) peak VO_2 is 80% or higher than predicted iii) RER exceeds 1.1, and iv) rating perceived of exertion is ≥ 17 using the 6-20 Borg scale (Borg, 1982).

1500 m all-out rowing exercise test

The test is completed in laboratory using rowing ergometer (Model D, Concept 2, Morrisville, VT, USA) to assess the individual performance and physiological responses of rowers. Evaluation of rowers' performance with through a 1500 m all-out rowing exercise is the most recommendable testing for young élite rowers (Maciejewski et al., 2016).

During the test, the duration of 1500 m performance is recorded using an electronic timer included in the rowing ergometer monitor to calculate the mean power output (P1500 in W) according to the following equation: $P1500 = 2.8 \cdot (1500/T1500)^3$. The test can be performed with an ergospirometer (Sensormedics, Viasys, CA, USA) to determine cardiorespiratory parameters during the 1500 m performance.

Before the 1500 m all-out rowing exercise (1500m test), rowers perform a standardized 20 min warm-up at about 140 bpm, then they must cover the distance as fast as possible.

4. PHYSIOLOGICAL RESPONSES AND SPORT APPLICATION OF HIGH-INTENSITY INTERVAL TRAINING (HIIT)

Oxygen Uptake (VO_2) kinetics

The increase of pulmonary O_2 (VO_2p) uptake, during the transition from rest to constant-load exercise, is not instantaneously but increases exponentially to reach a new steady state. The kinetics of VO_2 consists of three phases: (I) exercise period after 15-20 seconds from the start of the exercise, it is also called the cardio-respiratory phase; (II) during this phase the VO_2 uptake increases in an exponential modality to reach a steady state after 3-4 minutes of exercise. The time constant, expressing the speed of this phase, would be related to the muscle's capacity to use O_2 locally, and to desaturate the arterial blood with O_2. It is also called the fast component of VO_2. (III) If the exercise is performed at an intensity below the lactate threshold, the VO_2 uptake remains stable, while if the exercise is performed at intensity corresponding or above the lactate threshold, VO_2 increases more slowly than in phase II. Either these increases lead to a new stationary state of VO_2 after 6 to 12 minutes of exercise, and the increase continues until exhaustion to achieve the maximal oxygen uptake (VO_2max). The maximum oxygen uptake (VO_2max) is defined as the maximum rate of oxygen uptake and used during exercise. In endurance sports, as middle- and long-distance sports, has been defined as the main performance factor indicating the individual level of cardiorespiratory fitness. Additionally, the VO_2max is considered as the best predictor of risk, at a given point in time, of getting chronic diseases as heart disease, type 2 diabetes or certain cancers, and the best predictor of chances of living a long and healthy life.

Time Spent at or Near VO_2max (T@VO_2max)

The time spent at VO_2max or near VO_2max (T@VO_2max) (Billat et al., 2000b; Dupont et al., 2003a) during a HIIT session is considered a main criterion to evaluate the solicitation of aerobic system (Thévenet et al., 2007a). During a High-Intensity Interval Training session, the T@VO_2max is influenced by the HIIT variables and formats (Buchheit et al., 2012). Therefore, for the coaches and sport scientist it is important to plan the HIIT sessions considering the HIIT formats and variables, and the strategies necessary to maximize T@VO_2max, or to define 'time-efficient' HIIT formats with respect to the T@VO_2max/exercise time ratio in relation to the total duration of the HIIT session, warm-up excluded (Buchheit and Laursen, 2013 part 1).

Anaerobic Glycolytic Energy Contribution

To optimize the athlete's training, it is important to plan HIIT sessions by monitoring the level of glycolytic anaerobic energy intake (Buchheit et al., 2012). The gold standard to measure anaerobic glycolytic energy contribution in high-intensity interval training has not been established (Buchheit and Laursen, 2013 part 2), but the two most used and preferred methods are the maximal accumulated oxygen deficit (MAOD) and the muscle lactate accumulation (Krustrup et al., 2006). The measurement of maximal accumulated oxygen deficit to assesses the anaerobic glycolytic contribution, is a method with limitations, in fact this method has been used in few studies (Noordhof et al., 2010). To evaluate the MAOD there are several methods, but best practice is currently thought to involve using 10x4 minutes submaximal exercise bouts, to establish the power output: VO_2 relationship before solving for the y-intercept value, based on the performance power output achieved using a sport-specific supramaximal exercise (Noordhof et al., 2010). The anaerobic glycolytic contribution is characterized by the blood lactate accumulation is also reported as a surrogate marker. There are some limitations regarding the use of blood lactate concentration to quantify the anaerobic glycolytic energy contribution, as prior nutritional substrate status (Buchheit et al., 2009), session timing in relation to prior exercise (Bergman et al., 1999), timing of sampling post exercise (Buchheit et al., 2012), the possible variations between different analysers and sampling sites (e.g. finger vs. ear lobe), the effect of aerobic fitness

(Burgomaster et al., 2005) and its poor association with muscle lactate (Burnley, 2008), especially following high-intensity intermittent exercise (Billat and Korlasztein, 1996). Buchheit and Laursen (2013, part 2) categorized the concentration of blood lactate concentration after a HIIT session as: very low (less than 3 $mmol \cdot L^{-1}$), low (3 to 6 $mmol \cdot L^{-1}$ mmol/L), moderate (6 to 10 $mmol \cdot L^{-1}$), high (10-14 $mmol \cdot L^{-1}$) and very high (more than 14 $mmol \cdot L^{-1}$).

Game-Based HIIT or Small-Sided Games

In the past years, there was an evolution of the traditional sport training methods regarding endurance training, to new model approach through sport-specific exercises characterized using the ball (Fernandez-Espinola et al., 2020). This approach with the use of the ball is proposed as high-intensity interval training to enhance team sport-related physical fitness. Indeed, Iaia et al., (2009) examined the effect of performing high-intensity training through soccer-specific exercises, showing that it is possible to achieve an elevated exercise intensity using the ball, as demonstrated by elevated heart rates, marked blood lactate accumulations and high rate of perceived exertion. This specific sport training method game-based conditioning or skill-based conditioning, so called Small-Sided Games (SSGs), is proposed to develop the team-specific performance of players (Hammami et al., 2017). SSGs are increasingly believed to be a more suitable training than the traditional interval training for the development of physical fitness, technical skills, and decision-making ability (Arslan et al., 2017). The acute physiological responses and load during SSGs could be manipulated modifying the games reducing pitch area, using adapted rules, and involving a smaller number of players then traditional games (Hammami et al., 2017), but the overall load cannot by default be precisely standardized. The responses, such as blood lactate and HIIT running responses, to the SSGs of the players are most variable, and the between-player variability the cardiovascular responses is higher compared to more specific run based HIIT (Hill-Haas et al., 2011). The SSGs are characterized by the repeated changes of the movement patterns and the alternating between work phase to recovery periods. Comparing the generic training run-based with games-based HIIT has been demonstrated the relationships between VO_2 vs speed (Buchheit et al., 2009) and HR vs speed (Mendez-Villanueva et al., 2013) tends to be greater during games-based HIIT than generic training run-based, that could be caused to higher muscle mass involvement. Another important aspect is that during the games based HIIT some sport-specific movements, as accelerations, decelerations, and changes of direction, are more frequently performed than during generic training run-based.

5. COMPARISON BETWEEN HIGH-INTENSITY INTERVAL TRAINING AND CONTINUOUS TRAINING

Some studies examined the physiological responses and the influence on endurance performance induced by both continuous training and HIIT formats. In most studies no significant differences were observed, while some studies have demonstrated that VO_2max, ventilatory threshold and lactate removal ability increase significantly after a period of high-intensity interval training compared to the continuous method in healthy subjects (Foster et al., 2015). The High-Intensity Interval Training exert the physiological stress through the intensity of the stimuli, while the continuous exercise programs rely primarily on the duration of the effort. The HIIT with long interval elicit a higher percentage of maximal oxygen uptake (VO_2max) and time spent at 90%VO_2max than continuous training performed at 80% of maximal aerobic speed (Zafeiridis et al., 2010). The HIIT format with 10 s working phases alternating with 20 s of passive rest and a running speed close to vVO_2max led to systemic aerobic metabolic profile like slow continuous training (Wallner et al., 2014).

VO_2max improvement in HIIT and Continuous Training

In many sports, such as long/middle distance running or cycling, the VO_2max represents a key component for success (Rønnestad and Mujika, 2013), and it is considered beneficial for sport-specific performance of young competitive players (Harrison et al., 2015b). Some studies suggested that HIIT allows to improve maximal oxygen uptake and endurance performance to a greater extent, compared to continuous training alone (Milanovic et al., 2015). Daussin et al., (2007) demonstrated that in sedentary subjects an 8 weeks HIIT programme with 3 sessions/week leads to significantly higher improvements of VO_2max than a continuous training with the same number of sessions (15% vs 9%), inducing better adaptations of central and peripheral oxygen transport and utilization. The high-intensity interval training in clinical populations allows to reach greater improvements in vascular function, insulin sensitivity, and VO_2max compared to continuous training at moderate intensity (Ramos et al., 2015; Weston et al., 2014). The 30s-30s HIIT modality performed at 100% vVO_2max significantly leads improvement of the maximal oxygen uptake compared to the continuous training at 70% vVO_2max (11% vs 7%). Both training modalities has been performed 3 times per week for 12 weeks (Gorostiaga et al., 1991).

Maximal aerobic speed improvement in HIIT and Continuous Training

A HIIT programme with working phases performed at 100% MAS allows to reach a significant increase of maximal aerobic speed compared to a training programme of continuous exercise at moderate intensity corresponding 70-75% MAS (Gharbi et al. 2008; Gonzalez-Mohino et al., 2016). In another study has been showed that in physically active men 3 sessions per week during a period of 8 weeks of HIIT performed at 90-100% of the MAS leads to reach better improvements of MAS than the continuous training carried-out an intensity corresponding to 65-75% MAS. Both training modalities had an identical external workload (% MAS × duration in minutes) (Tuimill et al., 2011).

Blood lactate concentration during HIIT and Continuous training

The blood lactate concentration at the end of exercise is frequent used to determine the metabolic response. The concentration of blood lactate is correlated with an increase of exercise intensity, and it can be useful to identify various lactate thresholds (Beneke et al., 2011). Moreover, when the intensity of exercise is variable, it has been suggested that the blood lactate recovery concentration can be considered as a good indicator for performance both for cycling and cross-country skiing (Björklund et al., 2011). Bret et al., (2003) demonstrated that different types of training seem to stimulate the lactate removal abilities as the middle-distance runners have a higher capacity respect with sprinters regarding lactate recovery in between high intensity intervals. The lactate concentration at the end of the exercise is higher for HIIT with sort intervals (30 s at VO_2max) than for continuous exercise (50% of VO_2max), for the same total workload (Edwards et al, 1973). For similar exercise intensities, Essén et al (1978b) showed that continuous exercise at VO_2max could only be sustained for a few minutes, while intermittent exercise with short HIIT intervals could be sustained for 1 hour at the same intensity. This comparison showed that blood lactate concentration ($[La]^+$) was greater after continuous versus intermittent exercise. The decrease in glycogen stock and the accumulation of $[La]^+$ were also shown to be less following intermittent exercise (15s-15s) compared to continuous exercise of the same intensity (VO_2max) (Essén, 1978 a). At the same intensity, lactate accumulation is less important during intermittent exercise compared to continuous exercise (Gharbi et al., 2008). Also, Zafeiridis et al., (2010) showed that in adolescents in post-exercise of HIIT session with long intervals, 3 minutes at 95% MAS alternating with 3 minutes at 35% of MAS, the blood lactate concentration was significantly higher compared to continuous training performed at 83% of MAS. While there was not difference of blood lactate concentration both continuous training versus HIIT with short intervals (30 seconds at 110% MAS interspersed with 30 seconds at 50% MAS), and long intervals HIIT session versus short intervals HIIT session.

Acute physiological effects: HIIT vs Continuous Training

The high-intensity interval training exercise elicit a high percentage of VO_2max for a longer duration compared to a continuous training at high intensity. Billat et al., (2000b) demonstrated that the time at VO_2max was significantly higher in 30s (100%MAS)-30s (50%MAS) performed to exhaustion than a continuous exercise performed at anaerobic threshold until exhaustion (471±398 s vs 162±189 s).

In adolescent athletes the HIIT protocol with long interval performed until exhaustion stimulates to a greater extent the aerobic system than heavy continuous protocol performed at 80% MAS. The heavy continuous exercise protocol must be for a longer time respect to HIIT with long interval, to reach the same metabolic and physiological responses (Zafeiridis et al., 2010). The utilization of HIIT protocols compared to continuous moderate training during the training session, allows to reach higher solicitation of the physiological and metabolic parameters as oxygen uptake (VO_2), heart rate (HR) and blood lactate concentration $[La]^+$ (Nicolò et al., 2014).

6. THE EFFICACY OF HIIT TO MAINTAIN OR IMPROVE HEALTH: THE IMPACT IN HEALTHY AND CLINICAL POPULATIONS

The World Health Organization recommend at least 150 minutes of physical activity of moderate-intensity (40–60% VO_2max) or 75 minutes of vigorous-intensity physical activity (60–85% VO_2max) per week for healthy adults to maintain or improve health. Despite the established therapeutic potential of physical activity, one third of the adult worldwide fails to meet the minimum physical activity guidelines, and the most frequently cited barriers to engagement in physical activity is lack of time, low motivation, and poor adherence (Godin et al., 1994; Reichert et al., 2007). One of the primary advantages of HIIT, compared to MICT, is that this training is a more time-efficient modality of physical activity, requiring less time be spent exercising, while providing similar or greater health-related benefits, compared to MICT. Several studies examined the efficacy of HIIT to maintain or improve health. Compared with MICT, HIIT has been reported to increase aerobic capacity (VO_2max) more effectively (Moholdt et al., 2009; Ciolac et al., 2010) and reduce risk factors associated with metabolic syndrome, including hypertension (Ciolac et al., 2010), and impairments in insulin action and lipogenesis (Tjonna et al., 2008), in a variety of patient populations. Recent systematic reviews and meta-analyses have reported that HIIT, compared to MICT, can significantly increase peak oxygen uptake in individuals with lifestyle-induced cardiometabolic diseases (Weston et al., 2014) and stimulate improvements in VO_2max compared to pretraining values in active non-athletic and sedentary individuals (Weston et al., 2014). HIIT is a promising method by which to reduce cardiometabolic risk factors (Kessler et al., 2012). However, reviews of HIIT, compared to MICT, to date have focused on cardiorespiratory fitness (Weston et al., 2014; Weston et al., 2014; Bacon et al., 2013) and vascular function (Ramos et al., 2015). A single meta-analysis (Hwang et al., 2011) of six studies examined the effect of HIIT on traditional cardiovascular disease (CVD) risk factors in individuals with cardiometabolic disorders and found HIIT and MICT to have similar effects on metabolic risk factors (body composition, BP, lipid profile and glucose). Recently, it has been published an extensive review of the effect of HIIT on traditional and novel markers of CVD risk factors such as inflammation. The need for a systematic review examining the efficacy of HIIT on markers of cardiometabolic health is particularly relevant, considering the growing number of individuals who develop CVD, despite the absence of traditional CVD risk factors (ie, hypertension, elevated blood glucose and high cholesterol) (Olsen, 2010). Additionally, the higher exertion and unique pattern of HIIT may induce changes in novel markers of CVD risk. For example, high-intensity exercise has been shown to reduce disease-related inflammation markers (interleukin 6 (IL-6) and tumour necrosis factor (TNF-α) in animals (Kim et al., 2014). The results of systematic review/meta-analysis suggest that HIIT is an effective intervention to improve cardiometabolic health in overweight/obese populations. Specifically, Short-Term High Intensity Interval Training (ST-HIIT) beneficially influenced waist circumference (WC), VO_2max, fasting glucose and diastolic blood pressure (DBP), whereas Long-Term HIIT (LT-HIIT) was found to beneficially influence WC, % body fat, VO_2max, resting HR, systolic blood pressure (SBP) and DBP in overweight/obese populations. Meta-analysis of ST-HIIT revealed no significant effect on body composition in normal weight populations, whereas too few studies are currently available examining the effect of LT-HIIT in normal weight populations. ST-HIIT was shown to be able to reduce WC in overweight/obese populations and LT-HIIT significantly improved WC and % body fat in overweight/obese populations. These findings suggest that HIIT is an effective stimulus for reducing body fat levels (even in the absence of weight loss) for those individuals with large fat mass. Possible mechanisms underlying HIIT-induced fat loss include generation of catecholamines that increased fat oxidation and fat release from visceral fat stores, decreased postexercise appetite and increased excess postexercise oxygen consumption resulting in an elevated fat loss state (Laforgia et al., 2006; Boutcher, 2011). A catecholamine response has been shown to be significantly elevated after HIIT (Gratas-Delamarche et al., 1994; Vincent et al., 2004). Since β 3-adrenergic receptors are located mainly in the adipose tissue (Collins and Surwit,

2001) and β -adrenergic receptor sensitivity in adipose tissue is increased following exercise (Crampes et al., 1986), these factors might explain why HIIT is effective in reducing body fat in overweight/obese individuals. One intriguing finding is the absence of weight loss despite observed decrease in body fat, this is likely to be a consequence of gain in muscle mass. HIIT is known to recruit more fast type II muscle fibres leading to greater muscle hypertrophy and muscle mass (Krustrup et al., 2004). This adaptation is likely to induce health benefits as increase in muscle mass improves insulin sensitivity (Yang, 2014). As an example, elevated muscle mass was found positively associated with reduced incidence of insulin resistance and metabolic syndrome (McPherron et al., 2013; Srikanthan and Karlamangla, 2011). Thus, if HIIT can be successfully implemented in settings outside of clinical trials, it may offer an additional strategy to induce adipose reduction in overweight/obese populations. However, more studies are required to determine whether HIIT could be a successful population-based strategy for producing health adaptations.

7. HIIT AND THE BRAIN: POTENTIAL CLINICAL BENEFITS

Increasing evidence indicates that physical activity, besides improving cardiorespiratory fitness (CRF), which associates with reduced risk of cardiovascular disease and all-cause mortality across the human ageing continuum (Garber et al. 2011), can induce neuroprotective benefits. Evidence indicates that regular physical activity and corresponding improvements in CRF can increase cerebral perfusion and vasoreactivity across the human lifespan (Ainslie et al. 2008; Bailey et al. 2013). From a clinical perspective, moderate to high levels of CRF have been shown to improve cognitive functions (Brown et al. 2010) and are associated with a markedly lower risk of stroke mortality and dementia (Prestgaard et al. 2019; Tari et al. 2019), confirming the translational neuroprotective benefits of physical activity, though the underlying mechanisms remain to be established.

Particularly, it has been attested the capacity of physical activity to improve cognitive functions in older adults, ranging from those with healthy cognition, subjective memory complaints, mild cognitive impairment, dementia, and stroke (Calverley TA, et al., J Physiol, 2020). However, as already discussed in this thesis, the optimal mode, frequency, and duration of effective physical activity remain a constant source of debate. Recently, as time demands are deemed a potential barrier to meet recommended physical activity guidelines (Guthold et al. 2018), attention has turned from moderate-intensity continuous training to an alternative paradigm, the high-intensity interval training, given its capacity to further potentiate metabolic, cardiopulmonary, and systemic vascular adaptation with the added attraction of less time spent exercising (Weston et al. 2014). Indeed, as previously stated in this dissertation, HIIT represents a more time-efficient mode of exercise, that can potentiate cardiorespiratory fitness and further enhance neuroprotection compared to more traditional moderate-intensity continuous training paradigms. While the precise mechanisms remain unclear, prolonged exposure to the mechanical forces associated with the intermittency of HIIT-induced sinusoidal hyperaemia can promote complex changes in the cerebral pressure, strain, and shear stress phenotype to induce functional/structural adaptation, that ultimately enhance neuroprotection. Establishing these mechanisms more clearly will provide new information for the prescription and future optimisation of HIIT interventions, that can have more potential to promote healthy ageing by delaying stroke, cognitive decline, and dementia. The primary mechanisms suggested to promote exercise-induced neuroprotection include, though are not exclusively confined to, accelerated neurogenesis in particular of the hippocampal dentate gyrus, that is especially vulnerable to ageing (Marlatt et al. 2012); reduction in β-amyloid (Brown et al. 2013) and neuro-oxidative-inflammatory stress (Parachikova et al. 2008); proprioceptive adaptations incurred by movements, that require sustained mental effort (Bak, 2011); and, finally, increased brain-derived neurotrophic factor (BDNF) that modulates brain plasticity by promoting neurotic outgrowth and synaptic function (Berchtold et al. 2010). Indeed, a recent study has demonstrated that physical activity can improve the synaptogenesis (i.e., formation of new neuronal synapses), and neurogenesis (i.e., generation of new neurons), via increased production of BDNF, in addition to vascular plasticity (Mekari et al., 2020). Furthermore, Stilman et al., (2016) showed that an improvement of cardiorespiratory fitness can induce some changes of cellular and molecular pathways that likely initiate changes to the macroscopic properties of the brain and behaviour, which in turn can positively affect cognitive functions in the prefrontal cortex of the brain. Finally, in a recent pilot study, it has been demonstrated that in young healthy adults the high-intensity interval training intervention, was associated with higher improvement in reaction time in the executive components of the computerized Stroop Task and in the Trail Making Test, compared to the moderate-intensity continuous training intervention (Makari et al., 2020). It is reasonable to speculate that the greater improvements in CRF (beyond those incurred through MICT for any given training volume) could simply confer additional neuroprotection through a translational 'dose–response' effect. In support of this hypothesis, it is to be noted that research has demonstrated that a better cardiorespiratory fitness in the form of elevated maximal oxygen uptake in more physically active individuals is linearly associated with improved cerebral perfusion and cerebrovascular reactivity, dissociating the brain's biological from chronological age, reducing the former by up to as much as a decade (Ainslie et al. 2008; Bailey et al. 2013). This is clinically relevant given recent evidence that cerebral hypoperfusion likely precedes dementia (Wolters et al. 2017) implying a central pathogenic role for impaired oxygen and glucose delivery during sedentary ageing. In conclusion, an increasing body of evidence shows that physical inactivity continues to be a major cause of morbidity and mortality, with overwhelming evidence supporting the musculoskeletal, cardiovascular and cerebrovascular benefits of regular exercise, which are comparable to drug interventions in a number of chronic conditions. The HIIT paradigm has emerged as a safe and more time-efficient mode of exercise, compared to moderate-intensity continuous training, that can promote further improvements in cardiorespiratory fitness, molecular and vascular function for an equivalent volume of MICT. However, to what extent HIIT can further compound cerebrovascular adaptation and potentiate neuroprotection remains to be explored.

SECTION 2

STUDY 1: Acute physiological responses to different High-Intensity Interval Training regimes and an intensive Continuous Training in active students.

STUDY 2: Acute physiological and metabolic responses to Short-Interval High-Intensity Interval Training regimes in physically active university students.

STUDY 3: High-intensity interval training in rowing: acute responses in elite adolescent males.

STUDY 4: Small-Sided Games and Official Matches: evaluation of external and internal workloads in professional junior soccer players.

STUDY 5: The effect of placebo and nocebo on running performance during high intensity interval training.

STUDY 1

ACUTE PHYSIOLOGICAL RESPONSES TO DIFFERENT HIGH-INTENSITY INTERVAL TRAINING REGIMES AND AN INTENSIVE CONTINUOUS TRAINING IN ACTIVE STUDENTS

INTRODUCTION

For improving or maintaining the public health and to reduce risk of cardiovascular diseases, it is important to perform regular physical activity with the aim to increase the cardiorespiratory fitness (CRF) (Kong et al., 2016; Shigenori, 2019). Indeed, the great level of CRF is related to cardiovascular health, and its improvement allows to decrease the risk of death (Milanovic et al., 2015). The assess of the cardiorespiratory fitness is carried out through the measurement of maximal oxygen uptake (VO_2max). Indeed, the VO_2max is currently considered the best indicator to assess cardiorespiratory fitness and it is considered one of the most important predictors of cardiovascular disease in both men and women (Arboleda Serna et al., 2016).

Some studies demonstrated that the continuous training at moderate intensity (MICT) and High-Intensity Interval Training (HIIT) allow to reach the improvements of the maximal oxygen uptake (Zaferidis et al., 2010; Naves et al., 2018; Arboleda Serna et al., 2016). These improvements can be obtained by two main parameters: training intensity and volume training. When the programmed intensity is moderate (50–80% VO_2max), the exercise is maintained without difficulty, and, therefore, it can be performed in a continuous way. On the other hand, during High-Intensity exercise (90–120% VO_2max), it is necessary to fractionate the distance (interval training) so that an enough training volume can be performed (Tuimill et al., 2011).

Several studies compared the HIIT protocols respect with continuous moderate training reporting that the physiological parameters such as oxygen uptake (VO_2), heart rate (HR) and blood lactate concentration $[La]^+$ are significantly higher during HIIT protocols than continuous training at moderate intensity (Zhang et al., 2017; Boutcher 2011). Indeed, the HIIT protocols exert their physiological stress through the intensity of the stimuli, while continuous exercise programs rely primarily on the duration of the effort. HIIT induces similar or even superior physiological effects on oxidative capacity and endurance performance compared to moderate intensity continuous training in healthy subjects (Helgerud et al., 2007). The purpose of HIIT is to repeatedly stress the physiological systems involved during a specific endurance-type exercise largely than that required during the activity. Whereas the continuous running performed at intensities above the lactate threshold, an additional slow phase increment in VO_2 is developed, but if the exercise is long and intense enough, it can elicit the VO_2 near maximal levels. The HIIT protocols allow to elicit a higher percentage of maximal oxygen uptake (VO_2max), time spent at 90%VO_2 max and anaerobic system solicitation compared to continuous training at moderate intensity (Zafeiridis et al., 2010). A lower training volume in HIIT programs allows obtaining similar cardiorespiratory adaptations compared to a higher continuous training volume program (Mc Kay et al., 2009). Furthermore, the blood lactate accumulation, at the same intensity, is lower in the HIIT compared to continuous training, because the increasing of lactate accumulation and decreasing of glycogen is higher during a continuous exercise compared to HIIT (Buccheit and Laursen, 2013).

To plan an optimal training program for stressing the aerobic system and to reach some improvements of endurance level, it is important to know how different running exercise protocols (continuous or HIIT) influence the physiological, metabolic, and psychological responses (Nicolò et al., 2014).

Panascì and co-workers (2017) in a study showed that in HIIT exercise with short interval as 30-30 an 15% increase of the maximal aerobic speed during treadmill training session is the optimal solution to reach the same physiological responses than the same exercise modality performed on track.

A recent study of Ronnestad and colleagues (2020) suggested that in HIIT protocols the approach of matching the performed work for perceived effort is closer to how athletes typical perform their HIIT training sessions. However, this method to set the workload could be influenced by the subjective nature of rating of perceived exertion, and it could not allow to reach an acceptable training volume to obtain substantial cardiorespiratory and metabolic responses. For this reason, in this study we decided to apply a traditional interval training approach, where the performed work is matched for total work.

The aim of this study was to compare physiological, metabolic, and psychological responses reached during three HIIT running protocols and a High-Intensity Continuous Training in active university students.

MATERIALS AND METHODS

Participants

Fifteen university students volunteered to participate in this study. Their mean age, body mass, height, and body mass index (BMI) were 21.0 ± 1.07 years, 64.81 ± 12.93 kg, 1.74 ± 0.09 m, 21.19 ± 2.55 kg/m^2, respectively. All participants were healthy and participated in aerobic activities and team sports trainings, such as running, cycling or soccer, 2-3 times per week. Written informed consent was obtained from all participants. The exclusion criteria included cardiac, pulmonary, or inflammatory diseases, sports inactivity, or any other medical contraindications at the time of the examinations. The local Ethics Committee approved all the procedures, and the experimental protocol was performed in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki).

Study design

All participants were tested five times in a 5-week period. The first session consisted of to perform a treadmill multistage incremental exercise test to determine VO_2max and maximal aerobic speed (MAS). Furthermore, the first session included weight and height measurements, and bioelectrical impedance analysis (Tanita BC-420 MA). Subsequently, each participant performed in four different running exercise sessions (continuous training and 10-20, 30-30 and 50-30 High Intensity Interval Training protocols) with at least 7 days of rest between each exercise session. The intensities of running exercise sessions were selected according to the standard protocols for training and rehabilitation sports in the literature. The workload was based on the result of the MAS obtained during treadmill multistage incremental exercise test. All testing sessions were performed in laboratory on a treadmill (E-motion, Runner, MO, Italy). Running exercise sessions order was randomly assigned for each participant. Participants were instructed to arrive in a rested and fully hydrated state and at least 3h after the last meal, to avoid strenuous exercise in the 24 h preceding each test session and to maintain their normal diet throughout the study. All tests were performed at the same time of the day (±1 h) to avoid influence of circadian rhythms and the participants were verbally encouraged. Participants had not experienced in interval running exercises on treadmill. Indeed, they were fully familiarized with all exercise testing procedures. All participants completed the testing sessions without complication, and it was generally well tolerated, and they did not report dizziness, light-headiness of nausea, symptoms that occasionally occur during this type of test.

Treadmill multistage incremental exercise test

The test began at a running velocity of 6 $km{\cdot}h^{-1}$ with a progressively increasing speed of 2 $km{\cdot}h^{-1}$ every 3 minutes and it terminated when the participants reached volitional exhaustion. Verbal encouragement was provided throughout the test. The participants performed the test with an ergospirometer (Sensormedics, Viasys, CA, USA) to obtain cardio-respiratory parameters all long the bouts, from warm-up to the end of the exercise. Before the measurement, the ergospirometer was calibrated following the recommendation of the manufacturer. Analysis of expired gas was sampled breath by breath. Heart rate (HR), maximal oxygen uptake (VO_2max), maximal aerobic speed (MAS) and respiratory exchange ratio (RER) were monitored to assess the intensity of the exercise. The exercise has been considered maximal when i) anaerobic threshold, calculated with V-slope method, was exceeded, ii) peak VO_2 was 80% or higher than predicted and iii) RER exceeded 1.1.

Continuous Training and High Intensity Interval Training Protocols

Following the multistage incremental exercise test, the subjects performed one continuous training and three high intensity interval training sessions, with different work-to-rest ratios (0.5 = 10-20; 1= 30-30; 1.7 = 50-30) (Figure 3), on four separate occasions. Each session was preceded by a standardized warm-up of 5 minutes running at 50% MAS and 5 minutes running at 60% MAS. The continuous training (CT) protocol was performed at a workload corresponding to 95% MAS for 8 minutes, whereas the HIIT protocols (10-20, 30-30 and 50-30) were performed for 16 minutes, alternating running phase at 95% MAS interspersed with active recovery at 40% MAS. At the end of each session the treadmill speed was reduced to 5 $km \cdot h^{-1}$ at a 0% grade for 5 minutes (cool-down phase). The choice of the running protocols used in this study was based on the facts that they have been employed by sport coaches or fitness coaches for improving the aerobic capacity, and these protocols have been studied for the effects on aerobic parameters. More specifically, the intensity of CT was selected because at this intensity continuous exercise can reach VO_2max or high level of VO_2max (Dupont et al., 2002) and the time of 8 minutes was selected because in a study of Billat and co-workers (2000), it has been reported that the duration of exhaustive run at intensity near or at 100% MAS was about 8 minutes. The work intensity for the HIIT protocols was selected based upon the notion that it has been shown that in HIIT regimes an exercise intensity set at 90-95% vVO_2max allows to significantly improve the aerobic capacity and metabolic parameters in both healthy and clinical population (Gosselin et al., 2012; Engel et al., 2018). Furthermore, recovery intensities corresponding at 25-45% VO_2max can accelerate the velocity of lactate removal allowing that the subjects will exercise at high heart rates during the HIIT sessions (Zeferidis et al., 2010). For HIIT protocols the intensity was selected using the maximal aerobic speed (MAS) since this index is simple and more practical to use for the determination of exercise training intensity and has been widely used by coaches and sports scientists when designing training programs (Billat 2001; Midgley and Mc Naughton 2006).

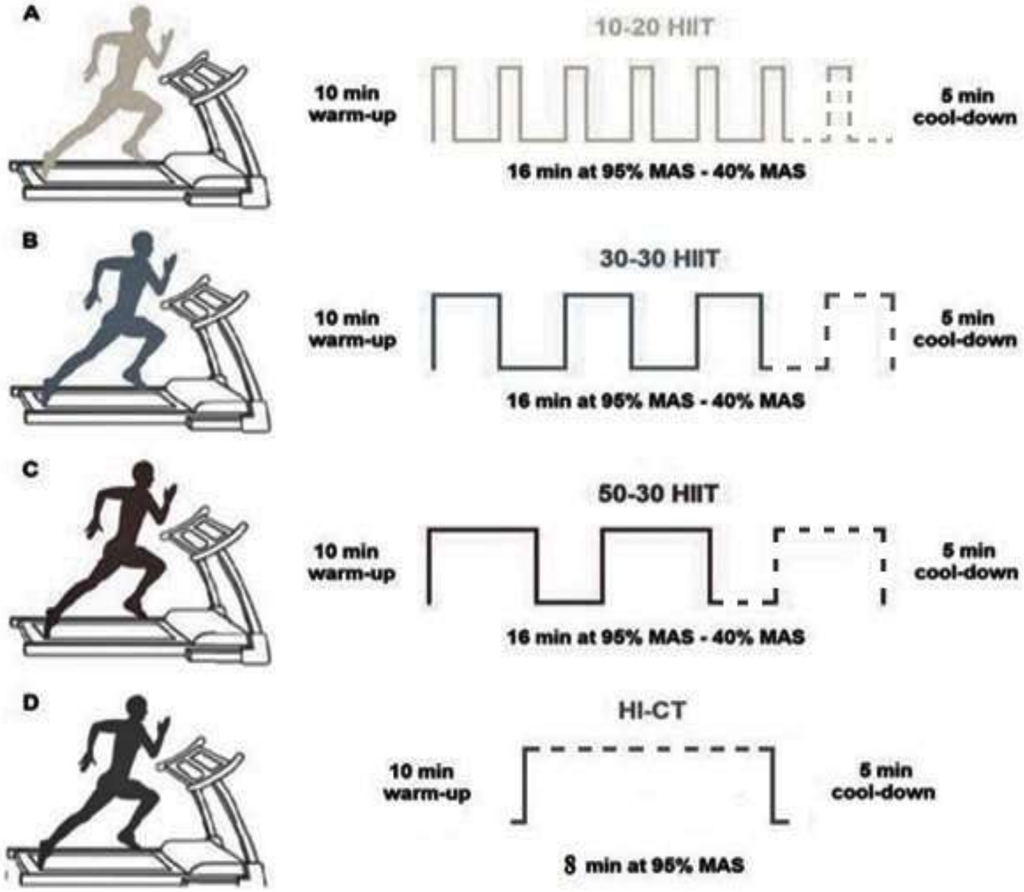

Figure 3. Experimental design: 10-20 (A), 30-30 (B), and 50-30 (C) HIIT training sessions; D: HI-CT, High-Intensity Continuous Training session.

Oxygen consumption (VO_2), volume of carbon dioxide exhaled (VCO_2), respiratory exchange ratio (RER) (Sensormedics, Viasys, CA, USA) and heart rate (HR) (Polar RS800, Kempele, Finland) were continuously monitored during each exercise session as previously described. Furthermore, we considered T@90%VO_2max as the time spent above or over 90% VO_2max. The VO_2max determined in the incremental test was compared with the peak VO_2 reached in each session. After 2 minutes each session, approximately 50 ml of blood was collected via a finger stick for the determination of blood lactate concentration using the Lactate Pro (LP, Arkray KDK, Japan). Before collection, the finger was cleansed with alcohol and allowed to air dry. At the end of each session the perceived intensity of exertion (RPE) was measured using the Borg CR 10 scale. Before the first experimental session, therefore during the familiarization phase, the Borg CR 10 scale was presented to the subjects and a verbal-anchored scale was shown for an appropriate interpretation and utilization of the scale (Borg 1998).

Statistical analysis

The normal distribution of the data was tested by means of Shapiro-Wilk test and the sphericity with Mauchly's test. We performed two types of analyses. In the first analysis, we assessed the potential differences between 10-20, 30-30 and 50-30 HIITs. The parameters considered were VO_2, VCO_2, RER, T@90%VO_2max, HR, $[La]^+$ and RPE. VO_2 and VCO_2, were normally distributed and a one-way ANOVA with SESSION (3 levels, 10-20, 30-30, 50-30) as within-subject factor, followed by Bonferroni post-hoc test, was used to perform the statistical analyses. RER, T@90%VO_2max HR, $[La]^+$ and RPE were not normally distributed and Friedman test, followed by post hoc, was applied to execute the statistical analyses. Significance level was set at $p<0.05$. The second analysis was performed to compare HIITs formats with parameters acquired from HI-CT. The parameters were VO_2, VCO_2, RER, T@90%VO_2max, HR, $[La]^+$ and RPE. Normally distributed data were analysed by means of repeated-measure ANOVAs with CONDITION as within-subjects factor (4 levels: 10-20, 30-30, 50-30 and HI-CT). Bonferroni post hoc were applied in case of significant effect. The statistical analyses were performed with SPSS (SPSS, Inc., Chicago, IL, USA). An alpha level of $p< 0.05$ was chosen.

RESULTS

Cardiorespiratory and maximal aerobic speed reached during Treadmill multistage incremental exercise test are offered in Table 1.

VO_2max (ml·kg-1·min-1)	**HRmax (bpm)**	**MAS**
48.04 ± 7.51	188.27 ± 2.27	14.05 ± 1.35 km·h^{-1}

Table 1. Cardiorespiratory and maximal aerobic speed during CPET. Mean values ± SE.

Comparison among 10-20, 30-30 and 50-30 HIIT concepts

Oxygen uptake (VO_2)

The results of the statistical analysis on VO_2 values (mL·kg^{-1}·min^{-1} and L·min^{-1}) showed a significant effect of the factor SESSION (VO_2 (mL·kg^{-1}·min^{-1}): $F(2,28)=29.05$, $p<0.0001$, $\eta^2=0.67$; VO_2 (L·min^{-1}): $F(2,28)=45.13$ $p<0.000$, $\eta^2=0.76$). Bonferroni post hoc test revealed that VO_2peak in 10-20 session (40.28±1.53 mL·kg^{-1}·min^{-1} and 2.29±0.13 L·min^{-1}) was significantly lower than VO_2peak in the other sessions (30-30: 45.09±1.94 mL·kg^{-1}·min^{-1} and 2.85±0.15 L·min^{-1}; 50-30: 46±1.23 mL·kg^{-1}·min^{-1} and 2.94±0.15 L·min^{-1}; p always<0.0001) (Figures 4 A and 4B).

Volume of carbon dioxide exhaled (VCO_2)

The one-way ANOVA on VCO_2 values showed a significant effect of factor SESSION ($F(2,28)=27.68$ $p<0.0001$, $\eta^2=0.66$). Bonferroni post hoc test revealed that VCO_2 values in 10-20 (2.09±0.14 $L \cdot min^{-1}$) were the lowest values in comparison with the other sessions (30-30: 2.76±0.15 $L \cdot min^{-1}$, $p< 0.001$; 50-30: 2.89±1.16 $L \cdot min^{-1}$, $p < 0.0001$) (Figure 4C).

Respiratory exchange ratio (RER)

The results of the statistical analysis on RER effect of the factor SESSION (RER: $F(2,28)=23.75$ $p<0.000$, $\eta^2=0.63$). Post hoc revealed that 10-20 RER (0.90±0.02) was significantly lower that 30-30 (0.96±0.01, $p<0.001$) and 50-30 0.98±0.01, $p<0.0001$) (Figure 4D).

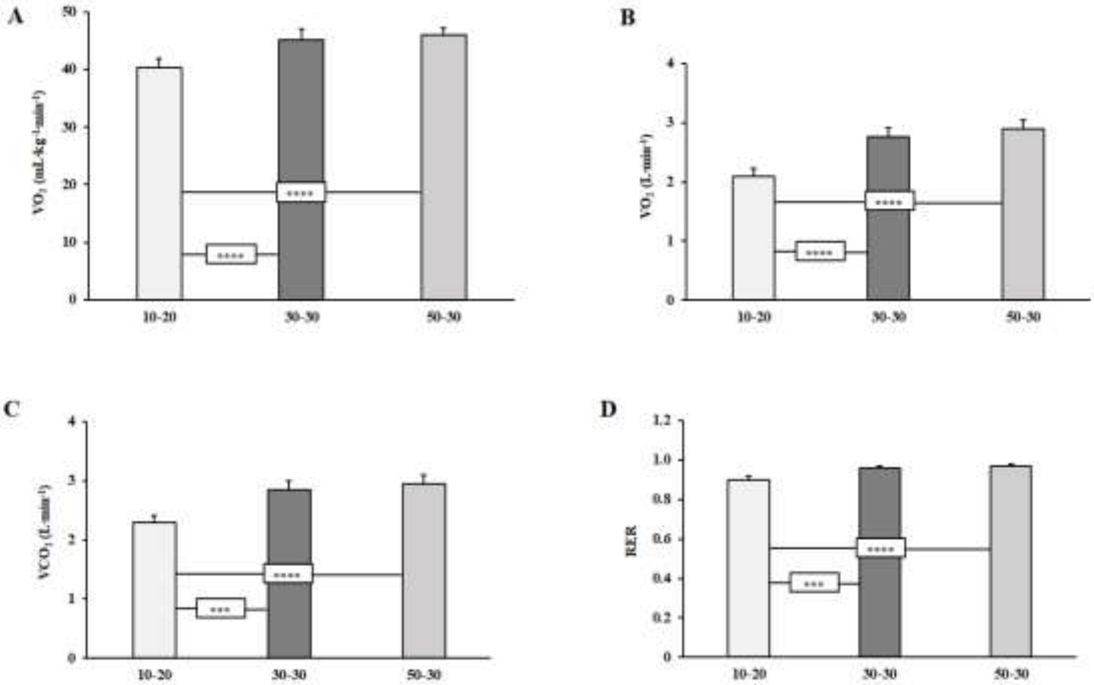

Figure 4. Oxygen uptake peak (VO_2) measured as $mL \cdot kg^{-1} \cdot min^{-1}$ (A) and as $L \cdot min^{-1}$, (B) volume of carbon dioxide exhaled (VCO_2), (C) and respiratory-exchange ratio (RER), (D) during 10-20 (white), 30-30 (dark grey) and 50-30 (light grey). Mean values ± SE. Asterisks indicate the significant differences: *** $p<0.001$, **** $p<0.0001$.

T@90%VO_2max (%)

Friedman test on percentage of T@90%VO_2max data showed a significant effect of SESSION (χ^2= (26.53), $p<0.0001$), and the following post hoc revealed that T@90%VO_2max in 30-30 (47.29±0.95%) and 50-30 (53.68±1.60%) was significantly longer than that in 10-20 (19.03±0.57%; $p< 0.001$, $p <0.0001$ respectively). Further, HI-CT T@90%VO_2max values were significantly higher than values in 30-30 ($p=0.011$). In addition, the difference of T@90%VO_2max values between 30-30 and 50-30 was not observed (Figure 5).

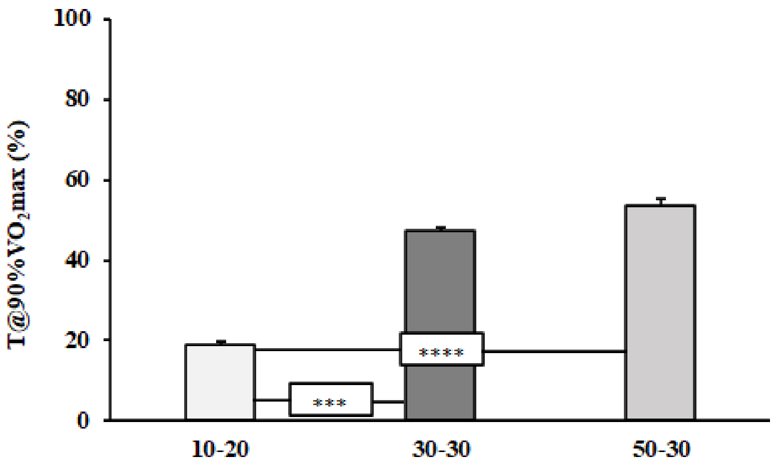

Figure 5. Percentage of time spent per session in exercise bouts at an intensity close to or above 90% VO_2max T@90% VO_2max). Mean values ± SE. Asterisks indicate the significant differences: *** p<0.001, **** p<0.0001.

Heart Rate Peak (HRpeak)

The results of the statistical analysis on heart rate peak effect of the factor SESSION (HRpeak: $F(2,28) = 98.51$ $p<0.0001$, $\eta^2=0.88$). Post hoc showed that HRpeak in 10-20 was the lowest (30-30: 182.73±1.55 bpm and 50-30: 184.27±2.09 bpm, p<0.0001 always) (Figure 6).

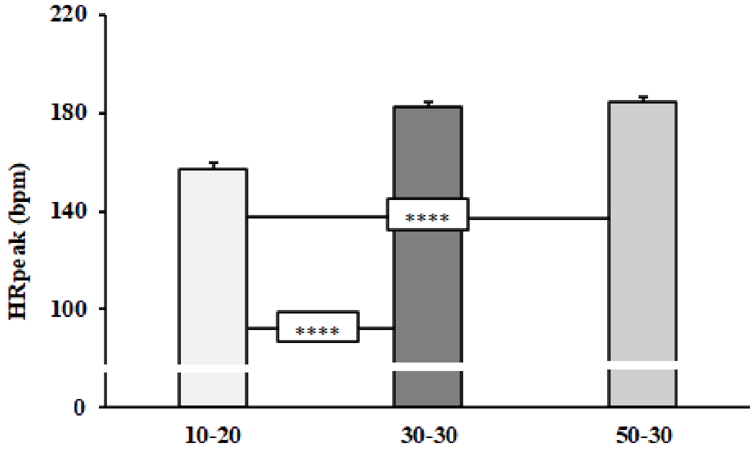

Figure 6. Heart rate peak during 10-20 (white), 30-30 (dark grey) and 50-30 (light grey). Mean values ± SE. Asterisks indicate the significant differences: **** p<0.0001.

Blood lactate ([La]$^+$)

Friedman test comparing [La]$^+$ values (Figure 7) in the different modalities showed a significant effect of SESSION (χ^2 (15,2)= 25.05, p<0.0001). Lactate values in 10-20

(1.49±0.11 mmol·L^{-1}) were significantly lower than those in 30-30 (4.08±0.18 mmol·L^{-1}, p<0.001) and 50-30 (5.73±0.8 mmol·L^{-1}, p<0.0001).

Rate of perceived exertion (RPE)

The results of the statistical analysis on RPE values showed a significant effect of SESSION (χ^2(15,2)= 26.27, p<0.0001) and post hoc revealed that RPE values in 10-20 (2.33±0.25) were significantly lower than in 30-30 (5.27±0.34) and 50-30 (6.45±0.46, p<0.0001).

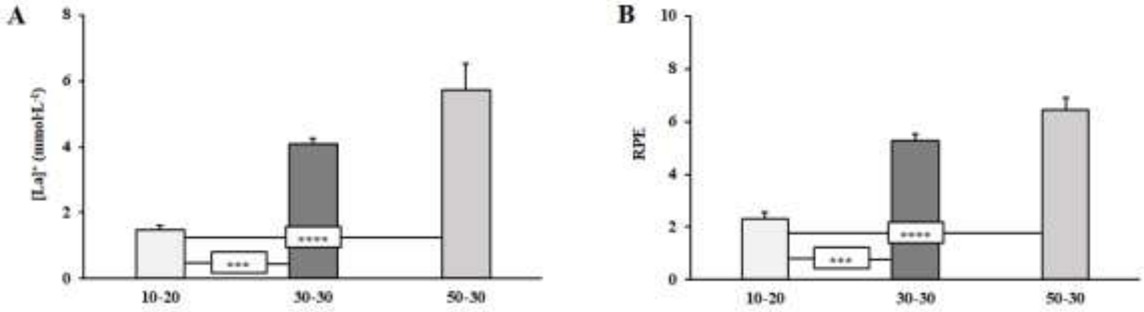

Figure 7. Blood lactate concentration (A) and Rating of Perceived Exertion (RPE) (B) during 10-20 (white), 30-30 (dark grey) and 50-30 (light grey). Mean values ± SE. Asterisks indicate the significant differences: *** p<0.001, **** p<0.000.

Comparison between HIITs and HI-CT

Table 2 presents the mean values ± SE for VO_2, VCO_2, RER, T@90%VO_2max, HR, $[La]^+$ and RPE of 10-20, 30-30, 50-30 and HI-CT. VO_2 in HI-CT were higher compared to 10-20 and 30-30 (p <0.0001 and p <0.05 respectively), but this was similar between HI-CT and 50-30. VCO_2 in 10-20, 30-30 and 50-30 was significantly lower than HI-CT (p <0.001; p <0.01; p< 0.05, respectively). RER values did not differ between HIITs and HI-CT. T@90%VO_2max, HR, $[La]^+$ and RPE were significantly higher in HI-CT than 10-20 and 30-30, but those were similar respect with 50-30.

Variables	*10-20*	*30-30*	*50-30*	*HI-CT*
VO_2 (mL·kg^{-1}·min^{-1})	40.28 ± 1.53^a	45.09 ± 1.94^b	46.01 ± 1.23	48.27 ± 1.48
VO_2 (L·min^{-1})	2.09 ± 0.14^a	2.76 ± 0.15^b	2.89 ± 0.16	3.17 ± 0.20
VCO_2 (L·min^{-1})	2.29 ± 0.13^a	2.85 ± 0.15^c	2.94 ± 0.15^d	3.49 ± 0.23
RER	0.91 ± 0.02	0.97 ± 0.01	0.98 ± 0.01	0.92 ± 0.02
T@90%VO_2max (%)	19.03 ± 0.57^a	47.29 ± 0.96^b	53.68 ± 1.60	57.50 ± 2.80
HRpeak (bpm)	157.20 ± 2.86^a	182.73 ± 1.55^c	184.27 ± 2.10	190.27 ± 1.74
$[La]^+$ (mmol·L^{-1})	1.49 ± 0.11^a	4.08 ± 0.15^c	5.73 ± 0.80	9.18 ± 0.49
RPE	2.33 ± 0.25^a	5.27 ± 0.34^c	6.45 ± 0.46	7.67 ± 0.25

Table 2 VO_2, VCO_2, RER, T@90%VO_2max, HR, $[La]^+$ and RPE measured in 10-20, 30-30, 50-30 and HI-CT. Mean values ± SE. [a] $p< 0.001$ from HI-CT-, [b] $p< 0.05$ from HI-CT; [c] $p< 0.01$ from HI-CT; [d] $p< 0.05$ from HI-CT.

DISCUSSION

To our knowledge, there few studies that compared the cardiorespiratory, metabolic, and psychological responses during continuous training at High-Intensity and three HIIT protocols with different work-to-rest ratios (10-20 = 0.5; 30-30 = 1; 50-30 = 1.7) in active university students. The physiological and psychological responses were lower during 10-20 and 30-30 HIIT protocols than during the continuous training at High-Intensity and 50-30 HIIT protocol. In addition, the physiological and psychological responses reached during continuous training at high intensity were comparable with those obtained during 50-30 HIIT protocol, but the subjects performed a lower amount of exercise.

Acute cardiorespiratory and metabolic responses

The peak of VO_2 elicited during the 10-20 HIIT protocol averaged 83.3% VO_2max, this result confirms that an exercise with 10 seconds of work phases at running speed close to vVO_2max MAS alternating with 20 s of recovery, allows reaching an aerobic metabolic profile like continuous running exercise at low intensity (Wallner et al., 2014). For the 30-30 and 50-30 protocols, the peak VO_2 averaged 93.7% and 95.8% VO_2max, respectively. These data suggest that it is sufficient an exercise alternating 30 seconds of work with 30 seconds of active recovery, to reach a high percentage of VO_2max. Whereas in continuous training exercise the peak VO_2 obtained averaged 100% VO_2max. Thus, the percentage of VO_2max reached during HIIT modalities, performed at 95% MAS, was influenced by the work to- active rest ratio. These data are in line with Gosselin et al. (2012), who observed that during different HIIT protocols with different work to-active rest ratio the VO_2 peak was lower compared to the maximal oxygen uptake obtained during the treadmill multistage incremental exercise test. Furthermore, the VO_2peak during HIIT modalities never reached the value observed during continuous training session. The oxygen consumption reflects the contribution of aerobic metabolism during exercise and depends not only on the duration or intensity of exercise, but also on the modality of exercise. Indeed, during HIIT protocols exercised at a workload corresponding to 95% MAS with recovery period at 40% MAS did not allows subjects to achieve the VO_2max. Whereas, the continuous training exercise performed at 95% MAS without recovery period allow reaching the maximal oxygen uptake.

The heart rate response in most respects mirrored the oxygen uptake response during the running exercise protocols. The heart rate was significantly lower in 10-20 HIIT protocol compared with 30-30, 50-30 HIIT's and continuous training protocols. The heart rate peak reached 157 bpm (83.5% HRmax) during 10-20, and 182 bpm (96.6% HRmax) and 184 bpm (97.8% HRmax) during both 30-30 and 50-30 protocols, respectively. During continuous training session, the HRpeak was 190 bpm (100% HRmax). Additionally, mean HR during the 30-30 and the 50-30 protocol were not significantly different than during high-intensity continuous training session. This finding indicates that 30-30 and 50-30 HIIT protocols (alternating phase at 95% MAS with recovery at 40% MAS) can stress the HR as continuous training session perform at 95% MAS.

In this study, the anaerobic glycolysis contributed significantly to energy production in continuous training than the HIIT modalities, as indicated by the blood lactate concentration after exercise. In continuous training session the high intensity of exercise, without recovery period, increased the demand for power production and was the main reason for the increasing of blood lactate concentration. The 10-20 HIIT resulted the protocol with a lower blood lactate concentration compared to HIIT modalities even though the subjects performed higher amounts of running. Relying on the rate of blood lactate accumulation (Buchheit and Laurse, 2013), we would categorize the 10-20 protocol as strongly aerobic, 30-30 as mildly anaerobic, 50-30 as anaerobic and continuous training as strongly anaerobic. As suggested by Gosselin et al., the blood lactate concentration is influenced by work-to-rest ratio. Furthermore, for the planning of HIIT sessions the monitoring of the anaerobic glycolytic energy contribution level is an important element to optimize the athlete's preparation. Indeed, the coaches can select between two different possibilities based on the session goal, to limit or enhancing the anaerobic contribution (Iaia and Bangsbo, 2009).

Effect of the exercise protocols on T@90%VO_2max

The time spent at 90-100% VO_2max, i.e., in the "red zone, during a HIIT session is considered a main criterion to evaluate the solicitation of aerobic system (Thévenet et al., 2007a). Indeed, the time spent at high percentage of VO_2max allows inducing optimal improvements of the maximal oxygen uptake (Midgley et al., 2006). The coaches of endurance sports do not prescribe HIIT sessions to exhaustion but prefer to use the series or set of HIIT sessions. This strategy is developed to maximize the T@90%VO_2max within a given period. Moreover, the T@VO_2max during High-Intensity interval training session is influenced by the HIIT variables and formats (Buchheit et al., 2012; Millet et al., 2003). In this study, the analysis of the specific effect of work interval duration between continuous training at high intensity and three HIIT protocols with different work-to-rest ratios in our group of university students, demonstrated that the HI-CT and 50-30 HIIT protocols, induced a percentage of T@90%VO_2 significantly higher than the 10-20 HIIT protocol. The time spent at 90-100% VO_2max during the 30-30 HIIT was significantly lower than HI-CT protocol. Furthermore, the T@VO_2max responses were not different during 50-30 HIIT and HI-CT protocols, allowing participants to spend a comparable time in "red zone".

Effects of the exercise protocols on the rate of perceived exertion

Rate of perceived exertion (RPE), measured with CR-10 Borg scale, showed significant lower values in 10-20 HIIT protocol respect with continuous training and 50-30 HIIT protocol. Whereas the CT induced RPE values significantly higher than 30-30 protocol. This result was probably influenced by the exercise modality (interval training vs continuous) and the work-to-rest ratio (10-20= 0.5 vs 50-30= 1.7). Furthermore, the RPE in 10-20 protocol did not differ from the 30-30 protocol. Therefore, the HIIT protocols with a work.to-rest duration of 10-20 and 30-30 can be performed by university students without undue discomfort, and both were the more feasible, pleasant, and enjoyable HIIT protocols, thus enhancing individual compliance and adherence to the prescribed training protocol.

PRACTICAL APPLICATIONS

Coaches and sport scientist may use a training modality alternating 50 second of work with 30 seconds of recovery to reach similar cardiorespiratory and metabolic acute responses compared to high intensity continues training, with a lower perceived exertion, despite a higher time of exercise. However, when using a high-intensity continues training a much shorter total exercise time is required to obtain a similar cardiorespiratory stimulus using 50-30 HIIT, making this protocol more time-efficient. If coaches and fitness practitioners aim to greater involvement of aerobic system, without a high solicitation of anaerobic contribution, the interval exercise protocol with 30 seconds work bouts interspersed with 30 second of recovery is preferable than the protocol 10-20 HIIT, because this HIIT format leads to greater both oxygen uptake and time spent at or above 90%VO_2max. When the aim of training is to recovery after competition or injury, the utilization of a protocol with 10 seconds of work interspersed with 20 seconds of recovery, allows to reach great cardiorespiratory responses with a low working load.

Therefore, in view of an evidence-based coaching, coaches could combine different types of the high-intensity training to reach several targets and to avoid the monotony of training.

CONCLUSION

The findings of this study indicate that in active university students, a HIIT with 50-s work phases at 95% MAS interspersed with 30-s of active recovery at 40% MAS and HI-CT elicited a better aerobic profile than those induced by the other HIIT formats tested. Indeed, HI-CT and 50-30 HIIT gave an optimal stimulus to elicit both maximal cardiovascular and peripheral adaptations, as, under this training condition, subjects spent the longest time in their "red zone", which means at least 90% of their maximal oxygen uptake. Furthermore, 30-30 and 50-30 HIIT protocols induced significantly higher values in VO_2 uptake compared to 10-20 protocol. In addition, the HRpeak, RER and $[La]^+$ values were significantly higher in 30-30 and 50-30, with a significantly higher internal load, compared to 10-20 protocol. The comparison between HIIT protocols and HI-CT showed that 50-30 HIIT led to similar VO_2 uptake, T@90%VO_2max, HRpeak, $[LA]^+$ and RPE values compared with HI-CT. Finally, VO_2 uptake, RER T@90%VO_2max, HRpeak, $[La]^+$ and RPE were showed to be significantly higher in HI-CT than those of 10-20 and 30-30 HIIT concepts.

STUDY 2

ACUTE PHYSIOLOGICAL AND METABOLIC RESPONSES TO SHORT-INTERVAL HIGH-INTENSITY INTERVAL TRAINING REGIMES IN PHYSICALLY ACTIVE UNIVERSITY STUDENTS

INTRODUCTION

High-intensity interval training with low-volume (LV-HIIT) is considered a practical and supportable physical activity protocol for active, healthy, and clinical populations (Farias-Junior et al., 2019). The low-volume high-intensity interval training is executed at intensity corresponding vigorous to near maximal efforts (Weston et al., 2014) specifically between 85-95% of maximal heart rate (Gibala et al., 2012). Usually, the LV-HIIT protocols involve a low amount of total work volume at 85-95% of maximal heart rate which lasted 10 minutes or less. Some studies showed that the efficacy of LV-HIIT to enhance the cardiorespiratory fitness (Currie et al., 2013) and metabolic profile in different populations (Hood et al., 2011; Little et al., 2011).

Another important factor to consider in the LV-HIIT evaluation is the affective response (feeling of pleasure or displeasure) because it is related with exercise adherence (Garber et al., 2011). To date several authors suggested that LV-HIIT leads a negative affective response (Onley et al., 2018; Thum et al., 2017), while other studies demonstrated a positive affect during LV-HIIT (Jung et al., 2014; Martinez et al., 2015). Stork et al., (2017) showed that the disagreement about the affective response during LV-HIIT session may be related to the manipulation of the HIIT variables such as intensity and duration of work and recovery intervals. Indeed, some studies reported that LV-HIIT protocols involving interval duration ≤60 seconds (short interval HIIT or S-HIIT) and carried-out at an intensity ≤85% of HRmax are more pleasant and enjoyable than HIIT with long interval or heavy continuous exercise (Martinez et al., 2015). Conversely, the protocols of LV-HIIT with duration of work interval ≥60 seconds (long interval HIIT or L-HIIT) performed at intensity higher of 85% maximal heart rate induce a negative affective response (Frazao et al., 2016; Onley et al., 2018).

It should be noted that the manipulation of each variable isolated from the others likely has a direct impact on cardiorespiratory, metabolic, psychological, and neuromuscular acute responses, and this could help to evaluate the effects induced by an HIIT protocol. In the literature, the effect of work interval duration on physiological responses during short-intervals HIIT (S-HIIT) was one of the first parameters examined (Christensen et al., 1960).

S-HIIT concept has shown to be one of the most feasible and time-efficient method for improving VO_2max, especially in healthy subjects (Wen 2019). Despite HIIT concepts represent a common approach used by coaches, athletes, and practitioners (Alves et al., 2017; Ferrari-Bravo et al., 2008; Wallner et al., 2014; Faelli et al., 2019), little data are available on HIIT formats characterized by repeated efforts lasting ≤ 20 s.

Therefore, the aim of this study was to evaluate in physically active university students the acute cardiorespiratory and metabolic responses induced by different S-HIIT protocols with low-volume (LV-HIIT) to provide coaches and athletes useful information to choose the most suitable S-HIIT, according to the expected training-induced adaptations and the individual profile. In particular, we examined the acute effects of three S-HIIT protocols (10-10, 15-15 and 20-20), characterized by different work interval durations, fixed work/rest ratio, similar work/recovery intensity (120% and 40%, respectively, of the maximal aerobic speed (MAS) and equal exercise time on systemic VO_2 responses, including time spent above 90% VO_2max, heart rate (HR), blood lactate and the rating of perceived exertion, the latter as a measure of the physiological, biomechanical and psychological stress/fatigue imposed on the subjects during the training protocols.

MATERIALS AND METHODS

Participants

A total of 20 university male students were enrolled for the study and 19 participants completed the study (age: 22.58 ± 0.61 years; height: 1.76±0.20 m; weight: 72.51±2.70 kg: BMI: 23.24±0.52 kg/m^2). The participants were recruited from the Sport Sciences Faculty of the University of Genoa. Inclusion criteria were: i) men aged from 20 to 25 years; ii) to perform ≥ 150 minutes/week of training. Exclusion criteria were: i) muscle or joint injuries, orthopaedic problems, severe visual impairment, or any other contraindication within three months before the commencement of the study; ii) BMI < 18.5 or > 25; iii) use of medications that affect cardiorespiratory function. The subjects were physically active and familiarized with aerobic activities such as running, cycling or soccer. Because subjects had not experienced in intermittent running exercises on treadmill, they were fully familiarized with all exercise testing procedures. Before entering the study, participants were fully informed about the study aims and procedures and gave their informed consent for inclusion before they participated in the study. A local ethics committee for the protection of individuals gave approval concerning the project before its initiation and conducted in accordance with current national and international laws and regulations governing the use of human subjects (Declaration of Helsinki).

Sample Size

Estimation of sample size for this investigation was performed using VO_2max as a physiological response to exercise as one of our primary outcome measures. Sample size was estimated combining the normative data and the genuine change in VO_2max determined in previous works (Barner and Kilding, 2015; Bradshaw et al., 2005). These assumptions generated a desired sample size of at least 15 subjects. However, we recruited 20 participants, to allow for drop-out during the intervention period.

Experimental Design

We completed a randomized counter-balanced order trial including three interventions. The trial compared the cardiorespiratory (Oxygen uptake, volume of carbon dioxide exhaled, respiratory exchange ratio, heart rate, metabolic (blood lactate concentration) and psychological (rating of perceived exertion) responses between three S-HIIT low-volume with different work-recovery durations (10-10, 15-15, 20-20), but matched by work recovery ratio and total work duration (1:1 and 5 minutes). Each participant underwent five different sessions: i) clinical examination where body weight and mass index (BMI) were measured using bioelectrical impedance analysis (BIA; Tanita, BC-420, MA), and height (m) was measured too; ii) After 48 hours of the clinical examination the participants were performed cardiopulmonary exercise test (CPET) on a treadmill; iii) After CPET three experimental sessions of S-HIIT (10-10, 15-15, 20-20) were carried-out with one week interval between each one. A computer-based randomization was used to determine the order of the experimental sessions. Only the participants were blinded to the order of interventions. The experimental protocol is described in Figure 8. All sessions were performed in the morning between 9:00 – 11:00 a.m. to avoid the influence of circadian rhythms.

All participants were instructed not to change their diet and physical activity practices throughout the intervention period. Subjects were also recommended not to consume alcohol, caffeine or caffeinated products, hot drinks or smoke as well as to maintain a good sleeping pattern within 24 h of each session.

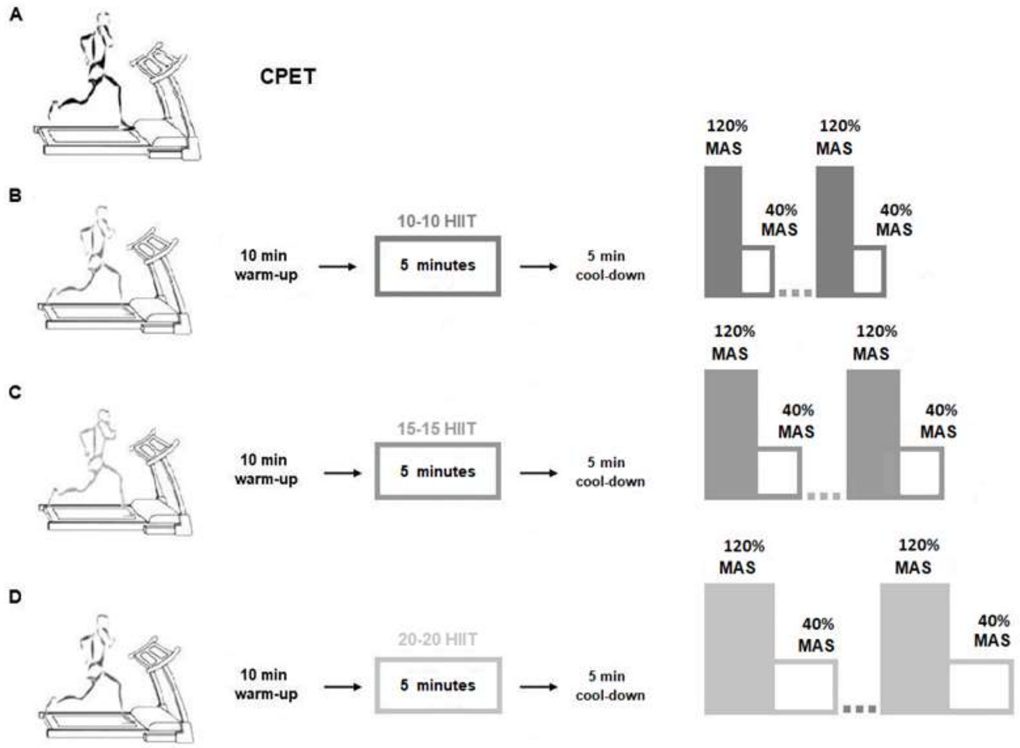

Figure 8. Experimental design. **a)** cardiopulmonary exercise test; **b)** 10-10 HIIT program; **c)** 15-15 HIIT program; **d)** 20-20 HIIT program; HIIT, High-Intensity Interval Training; MAS, maximal aerobic speed.

Cardiopulmonary exercise test

All participants performed a cardiopulmonary exercise test (CPET) to volition exhaustion on motorized treadmill (E-motion, Runner, MO, Italy) to determine the maximal aerobic speed (MAS) and the maximal oxygen uptake (VO_2max). The test started at 6 $km·h^{-1}$ speed (2% of slope) for 5 minutes as warm-up, followed by fixed increments of $km·h^{-1}$ every 3 min until volitional exhaustion (Mugele et al., 2018). Heart rate (HR) was continuously monitored and recorded using HR monitor (FORERUNNER 15, Garmin, Olathe, KS, USA). Oxygen uptake was continuously recorded using a breath-by breath with an ergospirometer (Sensormedics, Viasys, CA, USA), capable of evaluating cardiorespiratory parameters during the bouts, from warm-up to the end of the exercise. Before the measurement, the ergospirometer was calibrated following the recommendation of the manufacturer. According to Thevenet (Thevenet et al., 2007), VO_2max has been considered reached when at least 3 of the following criteria were fulfilled: i) a steady state of VO_2 (change in VO_2 at VO_2max ≤150 mL/min), ii) final RER exceeded 1.1. iii) visible exhaustion iv) a HR at the end of exercise (HRmax) within the 10 bpm of the predicted maximum [210- (0.65 x age)] and v) a lactate concentration at the end of exercise ($[La]^+$) higher than 8 $mmol·L^{-1}$

Participants' cardiorespiratory and metabolic characteristics after CPET are reported in Table 3. Maximal aerobic speed was defined as the speed reached during the last full stage added with the proportional time in the following incomplete stage before the volitional exhaustion (Midgley and Carroll, 2009).

S-HIIT sessions

The participants completed three S-HIIT (10-10, 15-15 and 20-20) sessions on a motorized treadmill (E-motion, Runner, MO, Italy). The 10-10 S-HIIT protocols consisted of 5 minutes of work intervals at 120% MAS interspersed with active recovery at 40% MAS (Dupont et al., 2004). Three sessions lasted 20 minutes, including 10 minutes of warm-up and 5 minutes of cool-down at 4 $km{\cdot}h^{-1}$. The heart rate was recorded every minute during S-HIIT sessions- The VO_2 was continuously recorded using a breath-by breath with an ergospirometer (Sensormedics, Viasys, CA, USA). The procedure was generally well tolerated, and participants completed the experimental sessions without complications, not reporting dizziness, light-headiness of nausea.

Physiological and metabolic responses

Oxygen uptake (VO_2), volume of carbon dioxide exhaled (VCO_2), respiratory exchange ratio (RER), heart rate (HR), and blood lactate ($[La]^+$) were chosen as dependent variables to describe physiological and metabolic responses. Heart rate (HR), maximal oxygen uptake (VO_2max), volume of carbon dioxide exhaled (VCO_2), respiratory exchange ratio (RER), and time spent per session in exercise bouts at an intensity close to or above 90% VO_2max (T@90%VO_2max) were measured during each S-HIIT session. Moreover, immediately after the end of each testing session, blood lactate concentration ($[La]^+$) was assessed. Blood lactate ($[La]^+$) was measured with fingertip blood samples (50 µL), using Lactate Pro (LP, Arkray KDK, Japan). Before collection, the finger was cleansed with alcohol and allowed to air dry. Measurements were performed 2 min after the end of each session.

Rating of perceived exertion

The measurement of whole-body perceived exertion was performed using Borg RPE 0-10 scale (Borg, 1998). We explained the utilization of perceived exertion scale to participants at the initial clinical examination and before each other session. Therefore, each participant was previously familiarized on the use of this scale, including anchoring procedures. The rating of perceived exertion (RPE), since it represents a non-invasive, easy, and efficient technique to be applied after training session. The low and high anchors for Borg scale 0-10 were established during the CPET. A rating of 0 (low anchor, “nothing all”) was assigned to the lowest exercise intensity, while a rating of 10 (high anchor, “maximal”) was assigned to the highest exercise intensity. RPE values were recorded during each session after 5 minutes of the end.

Statistical Analysis

Data were checked for normality with Shapiro-Wilk test. VO_2 VCO_2 and HR were normally distributed, whilst RER, $[La]^+$ and RPE were not. T@90%VO_2max in 10-10 was not normally distributed, whilst it was in 15-15 and 20-20. We compared the effects of the 3 S-HIIT regimes on VO_2, VCO_2, RER, HR, T@90%VO_2max, $[La]^+$ and RPE values evaluated at the end of the trainings. Normally distributed data were assessed by means of a one-way ANOVA with SESSION as factor (10-10, 15-15, 20-20), followed by Bonferroni post-hoc test. In case of not normal distribution, Friedman test was applied on the dataset followed by post hoc in presence of significant effect. Then, we adopted a many-to-one comparisons approach to assess the difference among CPET versus the three S-HIITs. When data were normally distributed, a one-way ANOVA with SESSION as factor was followed by Dunnett’s test comparing 10-10, 15-15, 20-20 to CPET. In case of not normal distribution, each HIIT was compared to CPET by means of Wilcoxon test. The effect size measures were presented through partial eta squared (η^2 value), with cut-off points of 0.10, 0.25, 0.40 representing small, medium, and high effect, respectively (Cohen, 1988). Significance level was set at $p<0.05$.

RESULTS

The cardiopulmonary exercise test data are offered in Table 3.

VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	*MAS ($km \cdot h^{-1}$)*	*HRmax (bpm)*	*$[La]^+$ ($mmol \cdot L^{-1}$)*
52.05±1.51	14.63±0.38	191.42±1.35	13.14±0.53

Table 3. Cardiorespiratory and metabolic parameters after CPET. Mean values ± SE.

Oxygen uptake (VO_2)

The results of the statistical analysis on VO_2 values ($ml \cdot kg^{-1} \cdot min^{-1}$ and $L \cdot min^{-1}$) of the three HIITs modalities showed a significant effect of factor SESSION (VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$): $F(2,36)=39.04$, $p<0.001$, $\eta^2=0.65$; VO_2 ($L \cdot min^{-1}$): $F(2,36)=33.14$, $p<0.001$, $\eta^2=0.65$). Bonferroni post hoc test revealed that VO_2 expressed as ml/kg/min in 10-10 session ($VO_2=44.32 \pm 0.85$ $ml \cdot kg^{-1} \cdot min^{-1}$) was significantly lower than those in 15-15 ($VO_2=48.33 \pm 1.25$ $ml \cdot kg^{-1} \cdot min^{-1}$) and 20-20 ($VO_2=50.09 \pm 1.26$ $ml \cdot kg^{-1} \cdot min^{-1}$) (p always <0.001). Concerning the analysis on VO_2 expressed as $L \cdot min^{-1}$, post hoc test showed that VO_2 in 10-10 S-HIIT ($VO_2=3.20 \pm 0.12$ $L \cdot min^{-1}$) was significantly lower than those in 15-15 ($VO_2=3.48 \pm 0.12$ $L \cdot min^{-1}$) and 20-20 ($VO_2=3.65 \pm 0.12$ $L \cdot min^{-1}$) (p always <0.001). Furthermore, VO_2 in 15-15 was significantly lower than that in 20-20 ($p<0.05$). A significant effect of SESSION was found by the one-way ANOVA comparing CPET with HIITs (VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$): $F(3,54)=22.60$, $p<0.0001$, $\eta^2=0.56$; VO_2 ($L \cdot min^{-1}$): $F(3,54)=21.14$, $p<0.0001$, $\eta^2=0.54$). Dunnett's test showed that VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) in 10-10 S-HIIT was significantly lower than in CPET ($p<0.0001$), and reached 85% VO_2max, whilst no significant differences appeared among CPET (52.05 ± 1.51 $ml \cdot kg^{-1} \cdot min^{-1}$,

3.65 ± 0.14 $L \cdot min^{-1}$) and 15-15 (93% of VO_2max) and 20-20 (96% of VO_2max) HIITs. Concerning post hoc analysis on VO_2 ($L \cdot min^{-1}$), values in CPET were significantly higher than those in 10-10 ($p<0.0001$) and 15-15 ($p<0.05$) S-HIITs (Figure 9A).

T@90%VO_2max

Paired t-test showed that 15-15 T@90%VO_2max mean value (164.21 ± 3.69 s) was significantly lower than 20-20 T@90%VO_2max (184.21 ± 4.86 s) (t (18)=3.81, $p<0.01$). Similarly, T@90%VO_2max expressed as percentage of the total training duration in 15-15 (54.74 ± 1.23) was significantly lower than those in 20-20 (61.40 ± 1.62) (t (18)=3.69, $p<0.01$). (Figure 9B).

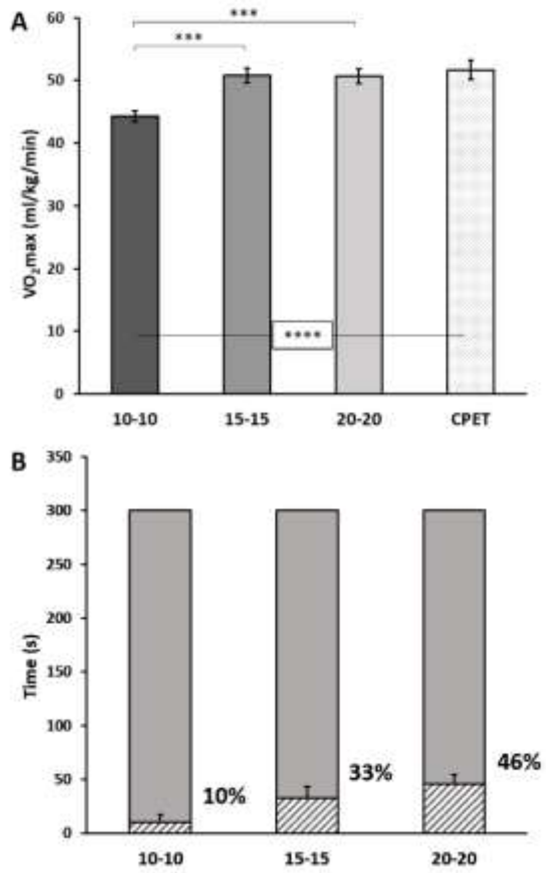

Figure 9. A) Oxygen uptake (VO_2) measured as ml/kg/min during 10-10, 15-15 S-HIITs regimes; **B)** Total time of the three HIIT protocol (s); the dashed area indicates the time spent per session in exercise bouts at an intensity close to or above 90% VO_2max (T@90%VO_2max). Mean values ± SE. Asterisk indicates the significant differences: *** p<0.001.

Volume of carbon dioxide exhaled (VCO_2)

The results of the statistical analysis on VCO_2 values of the three HIITs programs showed a significant effect of factor SESSION ($F(2,36)=22.33$, $p<0.001$, $\eta^2=0.55$). Bonferroni post hoc test revealed that VCO_2 in 10-10 session (3.12±0.13 $L \cdot min^{-1}$) was significantly lower than those in 15-15 (3.44±0.13 $L \cdot min^{-1}$, $p<0.05$) and 20-20 (3.74±0.14 $L \cdot min^{-1}$, $p<0.001$). Furthermore, VCO_2 in 15-15 was significantly lower than in 20-20 ($p<0.05$). A significant effect of SESSION was found by the one-way ANOVA comparing CPET with S-HIITs ($F(3,54)=25.93$, $p<0.0001$, $\eta^2=0.59$). Dunnett's test showed that VCO_2 in 10-10 and 15-15 HIITs were significantly lower than in CPET (3.95±0.15 $L \cdot min^{-1}$) (p always < 0.0001), whilst no significant differences appeared among CPET and 20-20 HIIT. (Figure 10A).

Respiratory-exchange ratio (RER)

The results of the Friedman test on RER did not find any significant differences among HIITs modalities. When considering CPET, a significant effect of SESSION was found ($\chi^2(19,3)=25.69$, $p<0.0001$) and the following post hoc showed that RER values in CPET (1.09±0.02) were significantly higher than in 10-10 (0.97±0.01, $p<0.0001$), 15-15 (0.99±0.02, $p<0.01$) and 20-20 (1±0.01, $p<0.01$). (Figure 10B).

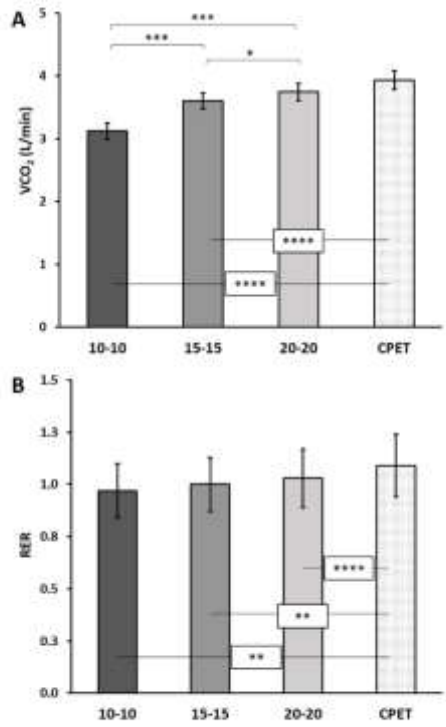

Figure 10. A) Volume of carbon dioxide exhaled (VCO_2) and **B)** respiratory-exchange ratio (RER), during 10-10, 15-15, 20-20 S-HIITs regimes and during cardiopulmonary exercise test (CPET). Mean values ± SE. Asterisk indicates the significant differences: * $p<0.05$, *** $p<0.001$.

Heart rate (HR) responses

The result of the one-way ANOVA revealed a significant effect of SESSION ($F(2,36)=47.14$, $p<0.0001$, $\eta^2=0.72$), and Bonferroni post hoc showed that HRmax values in 20-20 HIIT (181.85±1.28 bpm) were significantly higher than those in 10-10 (167.32±1.79 bpm) and in 15-15 (178.02±1.25 bpm) HIITs modalities (p always <0.001). Furthermore, HRmax values in 10-10 was significantly lower than in 15-15 ($p<0.001$). The statistical comparison among HRmax in CPET and in HIITs values showed a significant effects of SESSION ($F(3,54)=107.74$, $p<0.0001$, $\eta^2=0.86$). HRmax values in CPET (191.42±1.35 bpm) were significantly higher than in 10-10, 15-15 and 20-20 (p always <0.0001), which reached the 87%, 93%, and 95% of VO_2max, respectively. (Figure 11A).

Blood lactate ($[La]^+$)

Blood lactate ($[La]^+$) (Figure 11B). Friedman test comparing $[La]^+$ values in HIITs showed a significant effect of SESSION ($\chi^2(19,2)=31.68$, $p<0.0001$). Lactate values in 10-10 (3±0.19 mmol·L^{-1}) were significantly lower than those in 15-15 (6.71±0.71 mmol·L^{-1}, $p<0.01$) and in 20-20 (9.21±0.79 mmol·L^{-1}, $p<0.001$).

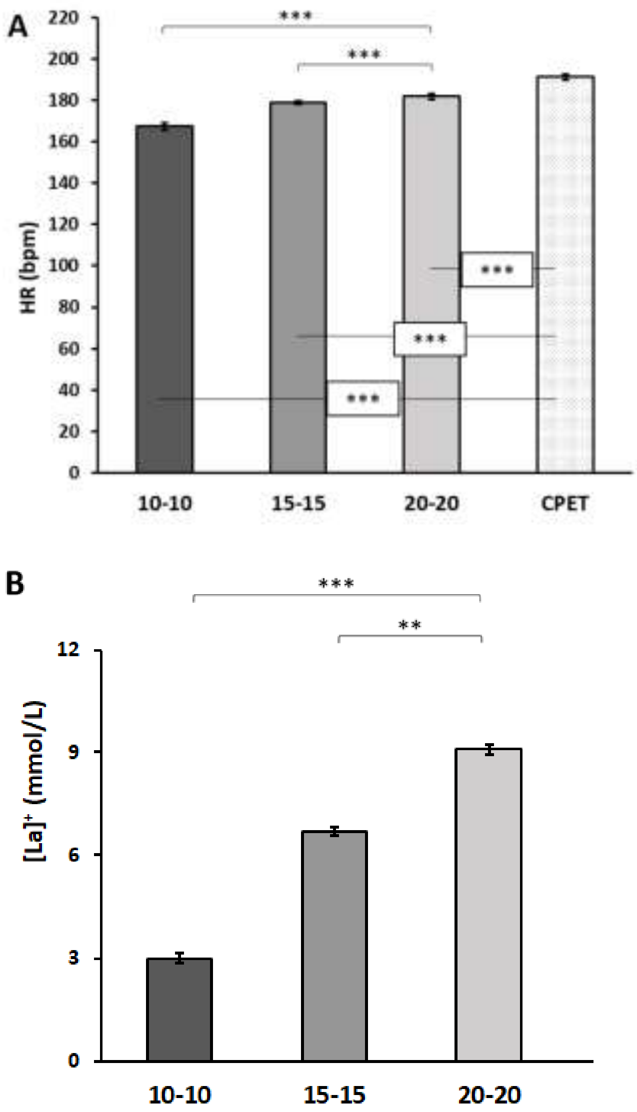

Figure 11. A) Heart rate (HR) and **B)** blood lactate ($[La]^+$) during 10-10, 15-15, 20-20 S-HIITs regimes and during cardiopulmonary exercise test (CPET). Mean values ± SE. Asterisks indicate the significant differences: ** $p<0.01$, *** $p<0.001$.

Rate of perceived exertion (RPE)

Rate of perceived exertion (RPE) (Figure 12). A significant effect of SESSION was found in RPE values ($\chi^2(19,2)= 33.79$, $p<0.0001$) and post hoc revealed that RPE values in 10-10

SI-HIIT (3.11±0.24) were significantly lower than in 15-15 (5.26±0.45, $p<0.01$) and in 20-20 (8±0.33, $p<0.001$). Furthermore, RPE in 15-15 was significantly lower than in 20-20 ($p<0.05$).

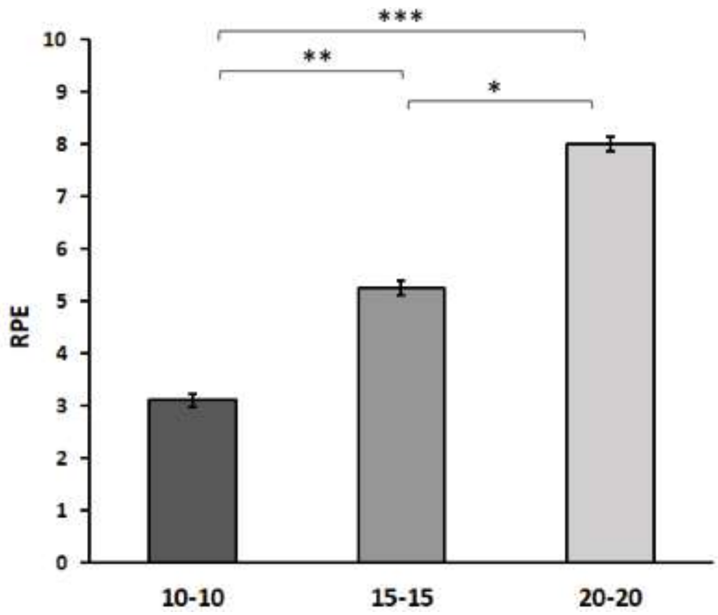

Figure 12. Rate of perceived exertion (RPE) during 10-10, 15-15, 20-20 S-HIITs regimes. Mean values ± SE. Asterisks indicate the significant differences: *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

DISCUSSION

In the present study, performed in physically active university students, we investigated the acute physiological and metabolic responses to different S-HIITs, (10-10, 15-15 and 20-20), characterized by different work interval durations, fixed work/rest ratio (=1) and fixed intensities of work and rest intervals (120% and 40% of MAS, respectively). Evaluating the acute physiological responses to the S-HIIT regimes tested, we considered the VO_2 responses as our primary variable of interest, since HIIT is, in first instance, a tool to improve cardiorespiratory fitness.

Effect of interval duration on VO_2 uptake

VO_2 uptake in 10-10 S-HIIT was significantly lower compared to the other two S-HIIT protocols, whereas no significant differences in VO_2 values were shown between 15-15 and 20-20 S-HIIT, despite of the different working interval duration between these two formats. Similarly, the comparison between VO_2 uptake and VO_2max measured through CPET, revealed that in 10-10 S-HIIT, VO_2 uptake (peak of 85% VO_2max), was significantly lower that VO_2max, whilst the VO_2 uptake in 15-15 and 20-20, (peak of 97% VO_2max for both formats), resulted to be not significantly different from VO_2max. During very short work interval (<10 s) HIIT requirements in working muscle are met predominantly by oxidative phosphorylation with more than 50% of the O_2 used derived from oxymyoglobin stores and then become available for the following work interval (Astrand et al., 1960). During the recovery period oxymyoglobin stores rapidly restore and then they become available for the following work interval. Accordingly, the cardiopulmonary responses to such efforts are relatively low. In summary, as regards VO_2 responses, under our experimental conditions, our study showed that: a) in the context of HIIT involving short-intervals, even at a work intensity of 120% MAS, work intervals >10 s are required to elicit maximal VO_2 responses; b) work intervals from 15 s to 20 s are equally effective in eliciting maximal oxygen uptake.

Effect of interval duration on T@90%VO_2max

Greater improvements in maximal oxygen uptake and cardiorespiratory function are obtained spending greater time per session in exercise bouts at an intensity close to or above the intensity corresponding to VO_2max, i.e. in the "red zone", which generally means reaching at least 90% of VO_2max (Laursen and Jenkins, 2002). According to Buchheit and Laursen (2013, part 1), it is important to develop strategies needed to maximize T@90%VO_2max within a given time, in order to define the most "time-efficient" HIIT format with respect to the T@90%VO_2max/exercise time ratio, which means the T@90%VO_2max in relation to the total duration of the HIIT session, warm-up excluded. Indeed, the study of the specific effect of work interval duration, using a fixed work/relief ratio (=1), in our group of moderately trained students, demonstrated that the 10-10 S-HIIT concept, even at high work intensity, (120% MAS), induced a T@90%VO_2 significantly lower than the other two S-HIIT concepts, whereas 20-20 S-HIIT format elicited significantly greater T@90%VO_2max and T@90%VO_2max/exercise ratio than 15-15 format. Therefore, 20-20 S-HIIT, despite causing the same effects on VO_2 uptake than 15-15 S-HIIT, enabled to spend significantly greater time at or above 90%VO_2max, with respect to 15-15. Furthermore, even considering the different training volume of 20-20 and 15-15 S-HIITs (600 s vs. 450 s), the observation of a more extensive VO_2 response elicited by 20-20 S-HIIT was reinforced by the finding that this format was demonstrated to be more time-efficient than 15-15, based upon the significantly higher T@90%VO_2max/exercise time ratio induced. Considering the importance of VO_2 kinetics for extending T@90%VO_2max, our data show that work intervals >10 s (such as 20-20 or 15-15 HIIT) are needed for individuals with slow VO_2 kinetics, to produce VO_2 responses and to significantly improve both maximal oxygen uptake and cardiorespiratory function.

HR responses to HIIT formats

The effectiveness of HR responses for controlling the intensity of a HIIT session may be limited (Buchheit, 2014), particularly for very short (<30 s) intervals, in which HR could not reach maximal values (>90-95% HRmax), when the working intensity is set at or below VO_2max, due to the well-known HR lag at exercise onset, which is much slower to respond, compared to VO_2 response (Cerretelli and Di Prampero, 1971). Indeed, under our experimental conditions, with a working intensity of S-HIIT formats fixed at 120% MAS, all the three S-HIIT regimes induced significantly lower HR responses than HRmax. Nevertheless, 15-15 and 20-20 formats reached maximal HR values, (93% and 95% HRmax, respectively). This result suggests that HR responses can provide a measure to control the intensity of HIIT session, if exercise intensity is set above VO_2max.

Anaerobic glycolytic energy contribution to HIIT formats

Anaerobic glycolytic energy contribution is the more important secondary variable to consider, when comparing the acute physiological effects of different HIIT formats. As a gold standard method to assess anaerobic glycolytic energy contribution to HIIT regimes has not been established, we have used blood lactate changes during the three different HIIT formats to estimate anaerobic energy contribution for a given exercise stimulus, focusing on post-HIIT values recorded. Indeed, in establishing the anaerobic glycolytic energy contribution, the blood lactate concentration is one of the preferred method (Buchheit and Laursen, 2013 part 2), although it has a number of limitations, including large individual responses, session timing in relation to prior exercise, timing of sampling post exercise, the possible variations between different sampling sites (e.g. finger or ear lobe) and its poor association with muscle lactate, especially following high-intensity intermittent exercise (Krustrup et al., 2006). Considering these limitations, we recorded blood lactate changes during high-intensity exercise, to estimate the anaerobic glycolytic contribution. Furthermore, accepting the fact that energy production is provided to a substantial extent by anaerobic metabolic pathways when RER value exceeds the 1.0 level (Kenney et al., 2015), we examined RER as secondary outcome to evaluate the involvement of anaerobic metabolic pathways in the three S-HIIT concepts tested. Our study showed that 10-10 S-HIIT induced significantly lower post-HIIT blood lactate values compared with the other two intermittent protocols, demonstrating that 10-10 S-HIIT moderately stressed the aerobic energy system, without significant anaerobic glycolytic energy contribution. No differences either in post S-HIIT blood lactate concentration or in RER values were found between 20-20 and 15-15 S-HIIT formats, suggesting that the two protocols, in addition to be similarly effective at stressing the aerobic energy system, they were also similarly able to induce a large anaerobic glycolytic energy contribution. Indeed, according to Bucheit and Laursen (Buchheit and Laursen, 2013 part 2), 10-10 S-HIIT session, with the exercise intensity prescribed at 120% of MAS, can be categorized between strongly aerobic/aerobic, based upon blood lactate accumulation, that was 3 $mmol \cdot L^{-1} \cdot 5\ min^{-1}$, whereas 15-15 and 20-20 S-HIITs can be classified, respectively, as anaerobic, and strongly anaerobic. Therefore, coaches and practitioners need to consider these results, to choose S-HIIT format, if the development of a high anaerobic glycolytic energy contribution is a target of their training program.

Rating of perceived exertion (RPE)

The measure of internal load, derived from RPE, showed significant lower values in 10-10 S-HIIT (intensity perceived as "moderate" on the CR-10 Borg scale, i.e. ≥ 3) compared to the other two S-HIIT concepts, whereas 20-20 induced RPE values ("very hard", i.e. ≥ 7) significantly higher than 15-15 HIIT ("hard", i.e. ≥ 5). This result, even if predictable based on the differences in training volumes between the three S-HIIT concepts, suggests that, under our experimental conditions, 10-10 S-HIIT was the more feasible, pleasant and enjoyable S-HIIT formats, thus enhancing individual compliance and adherence to the prescribed training protocol.

PRACTICAL APPLICATIONS

This study showed statistically significant differences between the cardiorespiratory and metabolic responses induced in young physically active subjects by low-volume of three different Short-Interval HIITs, 10-10, 15-15 and 20-20. Knowing the level of aerobic and anaerobic energy contribution, and rating of perceived exertion, relative to the combined physiological, biomechanical and psychological stress/fatigue imposed on the body during the exercise, associated with each HIIT format, can provide to coaches supporting sport scientists and athletes/practitioners useful information to prescribe and balance different SI-HIIT regimes based on the acute physiological and metabolic responses to HIIT concepts, and likely forthcoming adaptations.

CONCLUSIONS

In conclusion, under our experimental condition, and considering that the responses are highly athlete/subject profile-dependent, our study demonstrates that 5 minutes of the three S-HIITs tested, with similar working (120% MAS) and relief (40% MAS) intensity and a fixed work/relief ratio (=1), target the acute cardiorespiratory and metabolic responses in a significantly different manner, as concerns the relative level of aerobic and anaerobic energy contribution, and with significantly different subjective sensation of how hard each S-HIIT regime is.

STUDY 3

HIGH-INTENSITY INTERVAL TRAINING IN ROWING: ACUTE RESPONSES IN ELITE ADOLESCENT MALES

INTRODUCTION

Rowing is a sport with high demands on various components of physical fitness, such as endurance and strength, and physiological characteristics of rowers are among the highest recorded for any sport (Mäestu et al., 2005). Therefore, it is essential for a rower to simultaneously develop endurance, strength, and power capacity.

Typically, rowers perform large training volumes to reach a correct technique and high physical capacities. In fact, aerobic training is generally adopted by coaches for well-trained rowers, who perform the great majority of their training sessions at intensities below the lactate threshold (LT), despite competing at much higher intensities (Esteve-Lanao et al., 2007). However, in elite athletes, additional increases in aerobic training might not mirror improved physiological variables and endurance performance (Billat, 2001a). While endurance training relies primarily on the duration of the effort, high intensity interval training (HIIT) repeatedly stresses the physiological parameters over what is required (Zafeiridis et al., 2010). Manipulation of training intensity/duration and recovery periods during HIIT lead to differentiated training adaptations (Buchheit et al., 2013), and this is determinant for designing optimal training programs.

Several studies, performed in well-trained rowers, showed that incorporating HIIT with endurance training could enhance performance by a larger extent (Stevens et al., 2015).

Indeed, previous studies in elite rowers showed that HIIT allowed to train at, or even above, competition intensity for extended periods, tolerating continuously elevated blood lactate levels (Driller et al., 2009). More recently, HIIT was shown to induce greater improvements in VO2max and power output at LT, compared to a traditional endurance training (Ní Chéilleachair et al., 2017).

Top-level adolescent rowers have unique profiles, which must be cautiously considered when selecting training protocols. During exercise, the energy system generally hinges upon oxidative pathways more in adolescents than in older subjects (Tonson et al., 2010). Yet, other studies pinpointed at the contribution of anaerobic energy pathways in competitive adolescent rowers (Maciejewski et al., 2016). Young elite rowers carry out 12-14-h/week of training, equal to 100-km rowing. Indeed, Italian coaches generally use a polarized model to plan intensity and volume of training cycles in elite young rowers, according to the guidelines suggested by Italian Rowing Federation (FIC), very similarly to the training regimes internationally adopted. This model is characterized by ~ 80% of total training volume at an intensity below the first LT, 15-20% at an intensity between the first and the second LT, while a very small volume (~2-5%) is performed above the second LT. It is of paramount interest to evaluate the acute effects of different HIIT protocols in adolescents, providing sport scientists and coaches with new information and possibilities to personalize training schedules.

As previously reported, a recent study of Ronnestad and colleagues demonstrated that in HIIT protocols the approach of matching the performed work for perceived effort is closer to how athletes typical perform their HIIT training sessions. However, this method to set the workload could be influenced by the subjective nature of rating of perceived exertion, and it could not allow to reach an acceptable training volume to obtain substantial cardiorespiratory and metabolic responses. For this reason, in this study we applied a traditional interval training approach, where the performed work is matched for total work.

To the best of our knowledge, no crossover study has ever compared the acute physiological and metabolic responses induced by different HIIT regimes, in elite adolescent rowers. To this aim, we compared the acute physiological and metabolic responses as well as rowing performance parameters induced by two HIIT regimes (short and long), matched per training volume, chosen as paradigms of the two interventions. The assessment of internal training load through the rate of perceived exertion (RPE) at iso-time was also a focus.

MATERIALS AND METHODS

Subjects

Ten elite adolescent male rowers, with at least a) 3 years of expertise in national competition settings and b) a training volume of 6 sessions/week (90min/session), were recruited for the study. All subjects competed in the Italian Rowing National Championships the year preceding the investigation. Exclusion criteria were muscle or joint injuries, orthopaedic problems, or any other contraindication within six months before the commencement of the study. Participants' characteristics at baseline are reported in Table 4. Subjects were instructed to avoid the consumption of food in the 3 h prior to the test and not to exercise 24 h before the experimental sessions.

They were also recommended not to change their dietary habits throughout the study.

Before the experimental protocol, adolescents' parents were fully informed about the study aims and procedures, and they provided written informed consent to participate in the study. The experimental protocol was conformed to the code of Ethics of the World Medical

Association (Declaration of Helsinki) and it was approved by the Ethics Committee of University of Genoa (protocol code: 246 and date of approval: 7 July 2020).

Age (years)	*Height (cm)*	*Weight (kg)*	*BMI (kg/m^2)*	*Weekly training volume (h/wk)*	*Rowing experience (years)*
15.67±0.22	*179.78±1.67*	*68.79±2.58*	*21.22±0.51*	*11.22±0.26*	*4.11±0.42*

Table 4. Rowers' characteristics at baseline. Mean values ± SE.

Sample Size

Estimation of sample size was performed using VO_2max as a physiological response to exercise as one of our primary outcomes' measures (Lee et al., 2019). Sample size was estimated using the GPower software (3.1 software Düsseldorf, Germany), applying ANOVA repeated measures (F Test) with a significant level of 0.05, a statistical power of 80% to an effect size (ES) of 0.7 (Faul et al., 2007). This calculation generated a desired sample size of at least 10 participants.

Study Design

A randomized crossover design was carried out and performed at the same time of the day, one session/week. The experimental protocol consisted of the following three sessions: a 1500 m all-out rowing exercise test and two HIIT sessions: long (L-HIIT) and short (S-HIIT). During the first day, subjects underwent the 1500 m all-out rowing exercise test to determine their maximal oxygen uptake (VO_2max) ($mL \cdot kg^{-1} \cdot min^{-1}$) and Peak Power Output (PPO) used to quantify the intensity of the exercise during the HIIT sessions then, from the second to the third day, in a randomized order, both the L-HIIT and S-HIIT sessions were completed. The randomization was conducted in a draw form, on the assessment day (Figure 13).

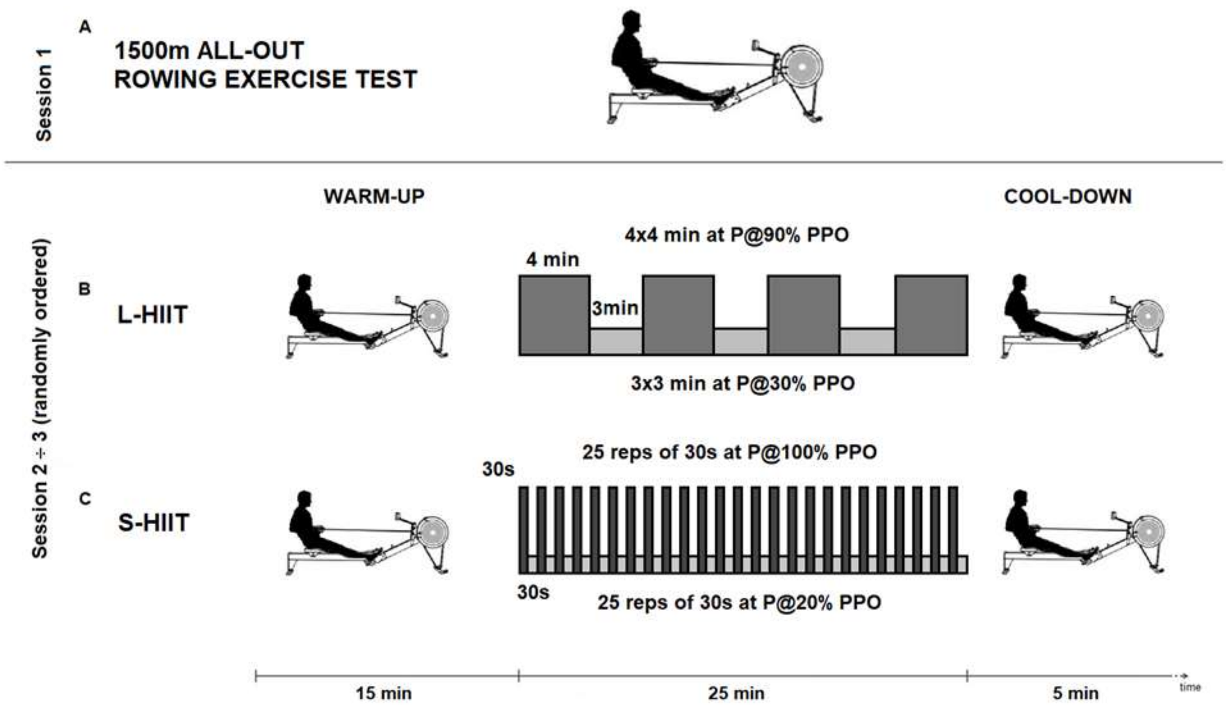

Figure 13. Experimental design.

The 1500 m all-out rowing exercise test

Rowers were tested through a 1500m all-out rowing exercise test, as this is considered the most recommendable testing distance for young elite rowers (Maciejewski et al., 2016). Initially, subjects performed a standardized 20 min warm-up at about 140 bpm, then they were asked to cover 1500 m distance as fast as possible. The test was performed on a rowing ergometer (Concept 2, Model D, Morrisville, VT, USA) using an ergospirometer (Sensormedics, Viasys, Irvine, CA, USA) and a mask (Hans Rudolph, INC., Shawnee, KS, USA) with a dead space of 30 mL. Before the measurement, the ergospirometer was calibrated following the recommendation of the manufacturer and the analysis of expired gas was sampled breath by breath. Heart rate (HR) was recorded at 5 s intervals with a Polar heart rate monitor (Polar H7, Electro, Kempele, Finland). Moreover, 2 min after the end of CPET, blood lactate concentration $[La]^+$ was measured (Assadi and Lepers, 2012).

Rowers' VO_2max was considered to be reached when at least three of the following criteria were fulfilled: i) a steady state of VO_2 (change in VO_2 $\leq$150 $mL \cdot kg^{-1} \cdot min^{-1}$ at VO_2max), ii) final respiratory exchange ratio (RER) exceeded 1.1. iii) visible exhaustion iv) an HR at the end of exercise (HRmax) within the 10 bpm of the predicted maximum [210– (0.65 x age)] and v) a lactate concentration at the end of exercise ($[La]^+$) higher than 8 ($mmol \cdot L^{-1}$) (Thevenet et al., 2007).

HIIT Sessions

HIIT sessions were performed on a rowing ergometer, in a laboratory with controlled temperature and humidity (21–24 °C and 44–56%, respectively)(Mendonca et al., 2020) and at the same time of the day (11a.m. ± 1 h) to avoid influence of the circadian rhythms. Drag factor settings of the ergometer were adjusted to 115, as recommended by the Italian Rowing Federation (FIC) for adolescent male rowers (Cattaneo, 2020). Before starting, rowers were fully familiarized with all the procedures. The experimental protocol was generally well tolerated, and subjects completed the two HIIT sessions without complication, not reporting dizziness, light-headiness, or nausea symptoms.

L-HIIT session

The L-HIIT session consisted of 15 min of warm-up (10 min at about 40%HRmax plus 5 min at the athlete's preferred pace) followed by 4x4 min at P@90%PPO (30÷32 str·min^{-1}) interspersed with 3x3 min of active recovery (P@30%PPO), and 5 min of cool-down at P@10%PPO.

S-HIIT session

During the S-HIIT session, rowers performed 15 min of warm-up (10 min at about 40%HRmax plus 5 min at the athlete's preferred pace) followed by 25 repetitions of 30 s at P@100%PPO (34÷36 str·min^{-1}) interspersed with 30 s of active recovery (P@20%PPO) and 5 min of cool-down at P@10%PPO.

Both the two HIIT sessions lasted 45 minutes.

Outcome measures

Physiological and metabolic measures.

Maximal oxygen uptake (VO_2max), volume of carbon dioxide exhaled (VCO_2), respiratory exchange ratio (RER), time spent per session in exercise bouts at an intensity close to or above 90%VO_2max (T@90%VO_2max), Total VO_2 consumed (TotVO_2), Ventilation (VE), Heart Rate (HR) and blood lactate concentration ($[La]^+$) were recorded as dependent variables to describe physiological and metabolic responses. During the whole exercise the physiological parameters were measured while, according to Assadi and Lepers (Assadi and Lepers, 2012), the metabolic response was measured 2 min after the end of each HIIT session.

The average of VO_2 value, obtained during the last 30s of the final rowing stage was considered as VO_2max. RER was averaged over the last minute of each running velocity, HRmax was identified as the highest value recorded during the test and TotVO2 was calculated by the summation of each VO_2 values ($mL \cdot kg^{-1}$) measured breath by breath during the entire time of exercise programs (Thevenet et al., 2008). Moreover, $[La]^+$ was measured with fingertip blood samples (5 μL), using Lactate Pro 2 (LP, Arkray KDK, Japan) and before each collection, according to the recommendation of the manufacturer, the finger was cleansed with alcohol and allowed to air dry (Gosselin et al., 2012).

Performance parameters

Total Distance completed (TD) and Power Peak Output (PPO) were assessed as indices of rowing performance. TD was measured as rower's total distance covered during each HIIT session while PPO was recorded as the peak value achieved during the exercise (Nevill et al., 2011).

Rate of perceived exertion

The internal training load was assessed through the RPE, using the CR-10 scale developed by Borg (Borg, 1998).

The CR-10 is a category–ratio scale that ranges from 0 (no effort at all) to 10 (maximal effort ever experienced) with a dot at the end to rate an effort that is the highest that has ever been experienced. The use of RPE has been demonstrated to be related to physiological markers, such as maximal oxygen consumption and lactate, and can be used as a surrogate for heart rate to understand the heart rate response to a specific exercise intensity (Alsamir Tibana et al., 2019).

Before the commencement of the study, each subject was informed about the RPE scale and was familiarized on the use of this scale, including anchoring procedures.

Two minutes after the end of each experimental session (Foster et al., 2001), rowers answered the question "How intense was your session?" looking at the verbal expressions and then giving the number representing their RPE. A rating of 0 (low anchor, nothing at all) was assigned to the lowest exercise intensity, while a rating of 10 (high anchor, very, very hard) was assigned to the highest exercise intensity (Alsamir Tibana et al., 2019).

Statistical Analysis

All data were normally distributed according to the Shapiro-Wilk test except T@90%VO_2max (raw values and percentage) and VE. We compared the effects of S-HIIT and L-HIIT modalities on physiological and metabolic measures (i.e., VO_2, VCO_2, T@90%VO_2max, RER, HRmax, [La]+ and TotVO_2), performance parameters (TD and PPO), and RPE. Normally distributed data (VO_2, VCO_2, RER, HRmax, $[La]^+$, TotVO_2, TD, PPO and RPE) were statistically evaluated by means of a paired t-tets. T@90%VO_2max (raw data and expressed as % of the total exercise duration) and VE values were analysed by means of Wilcoxon tests. Significance level was set at $p<0.05$. Normally distributed data are provided as means ±SE; not normally distributed data values are given as median [interquartile range].

RESULTS

Mean (median) and standard errors (interquartile range) values for both L-HIIT and S-HIIT sessions and the result of the statistical analyses are reported in Table 5.

Variables	L-HIIT	S-HIIT	Statistical analysis: L-HIIT vs. S-HIIT
Physiological and metabolic measures			
VO_2 ($mL·kg^{-1}·min^{-1}$)	58.57 (2.87)	52.50 (1.25)	t(9)=2.35, p=0.043
VO_2 ($L·min^{-1}$)	4.00 (0.12)	3.62 (0.12)	t(9)=2.52, p=0.033
TotVO_2 ($mL·kg^{-1}$)	1306.29 (70.45)	1131.65 (50.97)	t(9)=4.47, p=0.0016
VCO_2 ($L·min^{-1}$)	4.05 (0.18)	3.47 (0.15)	t(9)=2.68, p=0.025
RER	1.01 (0.03)	0.96 (0.02)	t(9)=1.49, p=0.17
VE ($L·min^{-1}$)	115.75 [105.90, 126.15]	109.95 [84.90, 116.40]	Z=-2.50, p=0.013
T@90%VO_2max (s)	790.17 [678.50, 918.00]	493.22 [301.75, 738.50]	Z=-2.80, p=0.005
HR (bpm)	196.60 (2.25)	188.80 (2.14)	t(9)=8.17, p<0.0001
$[La]^+$ ($mmol·L^{-1}$)	16.48 (1.2)	8.46 (1.05)	t(9)=8.17, p<0.0001
Performance parameters			

Total distance (TD) (m)	5470.30 (159.70)	4863.60 (137.28)	t(9)=6.50, p<0.001
PPO (W)	267.24 (9.98)	296.90 (11.03)	t(9)=26.80, p<0.0001

Internal workload

RPE (A.U.)	9.24 (0.25)	6.67 (0.21)	t(9)=9.69, p<0.0001

Table 5. Physiological and metabolic measures and performance parameters after 1500 m all-out rowing exercise test, long HIIT (L-HIIT) and short HIIT (S-HIIT). Normally distributed data are provided as means (SE); not normally distributed data values are given as median ([interquartile range]).

Physiological and metabolic measures

The results of the statistical analysis on VO_2 responses during the two testing sessions showed that VO_2 in S-HIIT was significantly lower than those in L-HIIT. A significant effect of SESSION was shown by t-test on $TotVO_2$ values revealing that $TotVO_2$ values in S-HIIT were significantly lower than L-HIIT. The results of the statistical analysis on VCO_2 values revealed that VCO_2 in S-HIIT was significantly lower than those in L-HIIT. The t-test on RER values did not find any significant difference between HIITs. The results of Wilcoxon test showed that VE values were significantly lower in S-HIIT respect to L-HIIT. Wilcoxon test on raw data showed that T@90%VO_2max in L-HIIT was significantly longer than in S-HIIT. The result of the t-test revealed that HR values in L-HIIT was significantly higher than in S-HIIT. T-test comparing $[La]^+$ values showed that in L-HIIT they were significantly higher than in S-HIIT. As a result of the statistical analysis, RPE values in L-HIIT were significantly higher than in S-HIIT.

Rowing performance parameters

The t-test on distance values showed that TD covered in L-HIIT was significantly longer than in S-HIIT. Moreover, the statistical analysis found that PPO value in S-HIIT was significantly higher than in L-HIIT.

Rate of perceived exertion

As a result of the statistical analysis, RPE values in L-HIIT were significantly higher than in S-HIIT.

DISCUSSION

The present study investigated, in elite adolescent male rowers, the acute effects induced by different HIITs on physiological and metabolic responses as well as rowing performance parameters. For this purpose, we compared two HIIT regimes: long (L-HIIT) and short (S-HIIT) characterized by different work interval durations and similar training volume.

The major finding of this study was that the L-HIIT protocol stimulated both aerobic and anaerobic systems by a greater extent, with better performance and a higher internal workload respect to the S-HIIT.

In rowing performance, the energy supply is dependent on the functional capacity of oxidative as well as anaerobic pathways, with the relative contribution of the first ones being about 80%. Therefore, rowing highlights the challenge of simultaneously developing both endurance and muscle strength. Further, as a relatively higher energy cost is attributed to the drag created by wind and water resistance, rowers must ameliorate their technique, developing a more efficient recovery phase (particularly in the timing of forces at the catch), as well as a faster stroke rate and a stronger and effective propulsive stroke. Thus, the first challenge in the rower's training, particularly for experienced athletes, including youth, is to combine endurance and strength and, at the same time, to optimize the technique.

In youth, whilst VO_2 responses during moderate-intensity exercise are poorly evident, an enhanced oxidative metabolism was shown during heavy-intensity exercise, likely associated with a more efficient oxidative phosphorylation, oxygen delivery and utilization and/or muscle fiber type recruitment patterns (Armstrong and Barker, 2009). Further, in adolescents, differently from adults, anaerobic capacity is significantly related to the time spent at a high percentage of VO_2max in severe intensity domains (Billat, 2001b).

In the present study, in order to assess the acute physiological responses of the aerobic energy system, we considered as our primary variables of interest VO_2 uptake, T@90%VO_2max and TotVO_2.

The comparison between VO_2 uptake and VO_2max measured in the 1500m all-out test, revealed that VO_2 value in L-HIIT, (peak of 97%VO_2max), was significantly higher than that measured in S-HIIT, (VO_2 uptake: 90%VO_2max) indicating L-HIIT more effective in attaining VO2max than S-HIIT.

As the optimal improvements of VO_2max may be related to the time spent at high percentages of VO_2max (T@90%VO_2max) (Buchheit and Laursen, 2013), we used this parameter to identify the most "time-efficient" protocol for stressing aerobic capacity. Our results showed that the long intermittent regime (L-HIIT) induced a T@90%VO2max value significantly longer compared to the short modality (S-HIIT).

In addition, the total oxygen consumption during exercise has been considered as a measure of the aerobic energy yield (Dorado et al., 2004). In the present study, we found that $TotVO_2$ was significantly higher in the L-HIIT than in S-HIIT.

On the whole, our study demonstrates the effectiveness of HIIT, in stressing the aerobic system of elite adolescent rowers, supporting the notion that the intermittent modality may induce significant improvements on cardiorespiratory adaptations in adolescents (Cao et al., 2019).

Moreover, comparing the two intermittent modalities, blood lactate values were significantly higher in the L-HIIT suggesting that the long work interval protocol is the most effective for stressing both the aerobic and anaerobic energy systems. According to Buchheit and Laursen(Buchheit et al., 2013), both HIIT programs can be classified as strongly anaerobic (>6 mmoL·L·5min-1), thus HIIT appears essential, when the goal is to develop the glycolytic path.

Interestingly, training above Lactate Threshold not only improves lactate response and its adaptations, but also these responses do not compromise aerobic training adaptations (Ingham et al., 2008). Furthermore, prescribing HIIT sessions could promote a delay in the accumulation of lactate, both by increasing the oxidative capacity and recruitment of muscle fibers (Poole and Gaesser, 1985) and by ameliorating lactic tolerance (Brooks, 2000). However, our results should be cautiously scrutinized as all the above cited studies were performed on endurance moderate or well-trained adult athletes.

Furthermore, our findings showed a significantly higher total distance covered with a significantly lower peak power output value during the L-HIIT session respect to the S-HIIT, demonstrating the effectiveness of the long duration intermittent modality in improving rowing performance.

Finally, as regards the internal workload, the rating of perceived exertion (RPE), measured at the end of both HIIT sessions, significantly decreased in the S-HIIT session respect to L-HIIT. Perception of effort is one of the most suitable representative indexes of fatigue, and can be defined as "the effort expended in performing a physical activity" (Marcora S, 2009), or as "a conscious manifestation of the feelings of effort produced by exercise"(Abbiss et al., 2015). Under our experimental condition, the significant lower subjective perception of effort shown after the S-HIIT session suggests the effectiveness of the short intermittent modality in making the exercise more tolerable and sustainable.

CONCLUSION

In the present work, we investigated the acute effects of HIIT regime in elite adolescent rowers, in order to provide coaches and sport scientists with new methodological intervention strategies. Our findings showed that, in this population of athletes, the HIIT protocols are an effective training modality in improving physiological and metabolic outcomes as well as performance, thus encouraging a review of the commonly applied rowing training guidelines, so to implement higher volumes in the “polarized” models. We suggest that incorporating HIIT into a traditional moderate-intensity endurance-based rowing program might lead to an increase in both aerobic and anaerobic capacity, with a significant reduction in training volume. Eventually, HIIT sessions on a rowing ergometer might be relevant in off-season training, maintaining a competition standard of quality. Follow-up studies are needed to ensure these acute responses are preserved even in the long term, following L-HIIT and S-HIIT, in elite adolescent rowers.

STUDY 4

SMALL-SIDED GAMES AND OFFICIAL MATCHES: EVALUATION OF EXTERNAL AND INTERNAL WORKLOADS IN PROFESSIONAL JUNIOR SOCCER PLAYERS

INTRODUCTION

Soccer is characterized by periods of high-intensity activity, interspersed with lower intensity actions, as well as technical and tactical components (Kunz et al., 2019). During a match, the players perform different activities such as jumping, shooting, tackling, sprinting, controlling the ball under pressure and running at different speeds, which involve high metabolic (aerobic and anaerobic energy production) and physiological requirements (Arslan et al., 2017; Hostrup et al., 2019). The soccer is a sport, characterized by a higher production of energy from aerobic metabolism, but during some phases of game the average intensity exceeds the anaerobic threshold (Borghi et al., 2021).

Several studies investigated the aspects of soccer physical performance both in adult and young soccer players. These studies showed that during an official match adult players reach on average game intensity between 80% to 90% of individual maximum heart rate (Arslan et al., 2020), and approximately 75-85% of maximal oxygen uptake (Chamari et al., 2005). Furthermore, adult soccer players cover between 8000 m and 14000 m during a match (Aguiar et al., 2012) and perform from 2 to 3 km of high intensity running (>15 $km \cdot h^{-1}$) and ~0.6 km of sprinting (>20 $km \cdot h^{-1}$) (Iaia et al., 2009). Youth soccer players reached on average > 80% of peak heart rate and approximately 75% of maximal oxygen uptake during an official match (Mendez-Villanueva et al., 2013). In a recent study (Borghi et al., 2021) was showed that the players of Under 19 professional team covered approximately 10000 m and perform between 500 and 900 m of high intensity running.

In the last decades to improve soccer physical performance, different training methods such as endurance training, high-intensity interval training and strength training have been proposed (Hammami et al., 2018). Several studies examined the effect of soccer-specific high-intensity training, showing that it is possible to achieve an elevated exercise intensity with the ball, reaching elevated heart rates, marked blood lactate accumulations and high rate of perceived exertion (Iaia et al., 2009). According to these studies, a specific sport training method, namely Small-Sided Games (SSGs), has been proposed to develop soccer-specific performance (Hammami et al., 2018). Lately, SSGs have been considered more suitable to improve the variables of performance in football, such as physical fitness, technical skills, and decision-making ability (Arslan et al., 2017). SSGs can be performed by modifying some variables such as the pitch area, number of participants, adapted games rules, presence of goalkeepers and vocal encouragement by coaches (Hammami et al., 2018). Indeed, it has been demonstrated that all these factors can influence the physiological responses, such as heart rate and blood lactate concentration with SSGs (Halouani et al., 2017; Madison et al., 2019). Previous studies showed that SSGs can be considered an appropriate training modality to improve the physical soccer performance (Hammami et al., 2018; Rabbani et al., 2019; Arslan et al., 2020). Furthermore, one of the most used SSGs formats is the Small-Sided Game with ball possession (SSG-POS), where the objective is to keep the ball for a longer time than the opposing team. This SSGs format can be considered as an efficient high-intensity interval training modality to stimulate the specific physical aspects of soccer (Gaudino et al., 2014). Although during SSGs performed as high-intensity interval training the players carry-out more acceleration and deceleration distances than in official match, the distance in high intensity running zones is less during SSGs as compared with running-based interval training and official match (Lacome et al., 2018). To our knowledge, only Koklu et al. (2020) have studied how the use of running drills can be useful to increase the distances covered in high intensity running zones during SSGs training session. For this reason, we hypothesize that the certain amount of time during a SSG bout is replaced by shuttle running, with an increase of the distance covered at high intensity running zones by the players could increase and consequently reach the demands of official match.

In this context, the main aim of this study was to assess and compare physiological responses, external load, and internal load of a SSG modality with only ball possession (SSG-POS) and a SSG regime with ball possession and shuttle (SSG-SHU) in élite junior male soccer players. Furthermore, we compared the external and internal loads between both SSGs modalities and official matches (OM).

MATERIALS AND METHODS

Experimental Approach to the Problem

SSGs were not used in training programs before the start of period of the study, for this reason one session per week for 2 weeks have been used as familiarization to 2 different SSGs formats (SSG-POS and SSG-SHU) for the subjects. During experimental period, all SSGs sessions were performed at the same time of the day (10.30 ± 1 h), on the same field (natural grass) and in the same environmental conditions (temperature, weather, and humidity) to avoid influence of circadian rhythms (Drust et al., 2003), in addition the soccer players were asked to refrain from caffeine 3h before each SSGs session. The official matches monitored during the experimental period, were performed Sunday at 11 A.M. This study was carried out during the first part of the 2019-2020 competitive season (from December 2019 to March 2020) which 10 official matches (OM) and 4 Small-Sided Games (SSGs) sessions were examined. The heart rate was monitored during all SSGs sessions and official matches because reflects the metabolic demands during an intermittent type of activity as the soccer (Aşçı, 2016). The blood lactate concentration measured at the end of exercise soccer that represents an accumulated response of the lactate production in the muscles during periods in the game with repeated intense exercise (Krustrup et al., 2006). The external load, measured as time and distance spent in selected activities, was assessed using Global Position System technology (GPS, K-sport, Montelabbate, PU, Italy) because the multifactorial nature of SSGs and official match, characterized by a great number of explosive actions and changes in velocity, implies a higher complexity in the real quantification of the external load (Varley et al., 2012). Rating of perceived exertion (RPE) is correlated both with some external load parameters such as distance at high speed (> 14.4 km·h-1) or number of accelerations (Gaudino et al., 2015), and with the heart rate (Coutts et al., 2009). For this, it was used to assess the internal stress at the end of both SSGs sessions and official matches.

Subjects

Ten élite young male soccer players (18.6 ± 1.9 years; weight 73.1 ± 5.6 kg; height 175 ± 1.5 cm, playing experience 9.5±1.5 years), were recruited from Under 19 Italian professional team. The players trained approximately 10 hours, five times/week (normally Monday, Tuesday, Wednesday, Thursday, and Friday) plus the official league-match played on Sunday. In this study only outfield players were performed the SSGs training sessions. To be included in the study, soccer players had 1) to ensure regular participation in all the training sessions; 2) to have competed regularly during the previous competitive season; and 3) to possess medical clearance for competitive sports. Before being included in the study, participants were fully informed about the study aims and procedures, and they provided written informed consent before the testing procedure. The study protocol was conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki), and it was approved by the Ethics Committee of the University of Genoa.

Procedures

After the familiarization period, the study lasted in 4 weeks, during this period the players were involved in training sessions and official matches. All subjects performed two different Small-Side Games formats, each format one time/week, in a randomized order. Both SSGs formats were performed as interval training consisting of four bouts of 4 min duration with 1 min of passive recovery between bouts.

The SSG-POS: consisted of 5 vs 5 without goalkeeper, limited ball-touches (3), 50 × 30 m field dimension, and the goal was to keep the ball possession for longer time than the opposing team; the SSG-SHU: consisted of 5 vs 5 without goalkeeper, limited ball-touches (3), 50 × 30 m field dimension with a cone 4 meters away from each side of the field. The aim was to maintain the ball possession for longer time than the opposing team, reaching the nearest cone with a shuttle running after each pass performed and returning in the SSGs pitch to continue playing (Figure 14). Each small-sided game was played with coach encouragement using standardized indications (e.g. ‘‘lose your marker’’, ‘‘find space’’, ‘‘press’’, ‘‘get back in quickly’’). Encouragement was provided by the same two individuals (coach and fitness coach). Each time the ball went out of the field, it was thrown back in by an assistant coach and stoppage time related to major injury was not counted in the total duration of the training. Each SSGs session started with a standardized warm-up, which consisted of low intensity running, striding, and stretching.

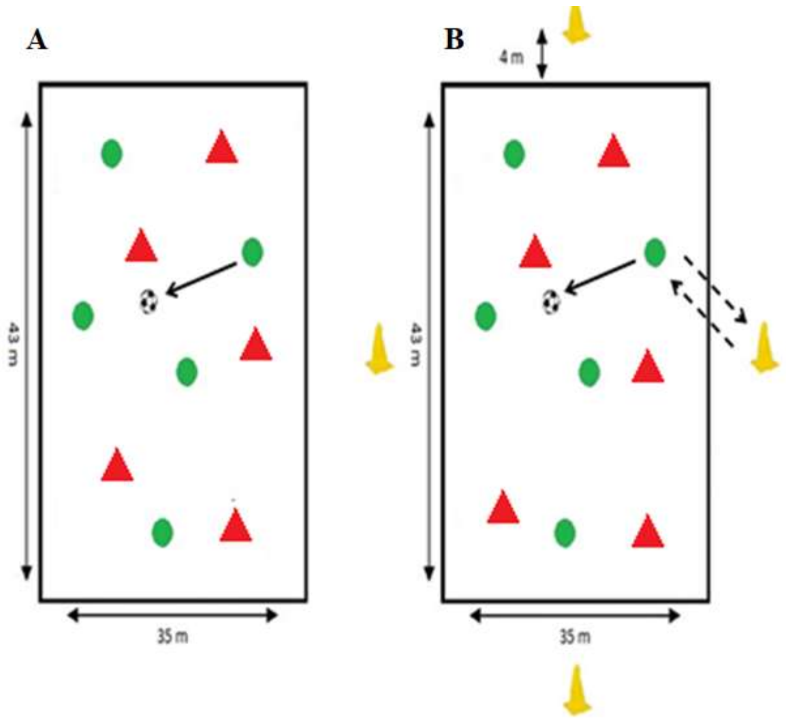

Figure 14. Schematic illustration of: (A) Small-sided games only ball possession (SSG-POS): the goal was to keep the ball possession for longer time than the opposing team; (B) Small-sided games with ball possession and shuttle (SSG-SHU): the aim was to maintain the ball possession for longer time than the opposing team,

reaching the nearest cone with a shuttle running after each pass performed and returning in the SSGs pitch to continue playing.

Equipment

During both SSGs format sessions and official matches, the physical profile was measured using a portable Global Positioning System (GPS) device, operating at a sampling frequency of 10 Hz (K-sport, Montelabbate, PU, Italy). The GPS unit was placed within a dedicated pouch between the player's shoulder blades (upper thoracic spine) in a sports vest and worn under the playing jersey. Each device was turned on at least 15-min before each session to allow for acquisition of the satellite signal. To reduce the inter-unit differences, each player wore the same unit for every training session over the whole investigation (Riboli et al., 2020). All data recorded were processed using a specific software (K-SportOnline, K-Sport, Montelabbate, Italy). The system has previously been shown to provide valid and reliable measurements of the match activity in soccer (Rampinini et al., 2015).

External load parameters

The following variables were recorded as measure of external load: Total Distance (TD, m); Relative distance (Drel; $m \cdot min^{-1}$); Distance at high speed (DHS; 14.4–19.8 $km \cdot h^{-1}$, Distance at Very High Speed (DVHS; 19.8–25.2 $km \cdot h^{-1}$) (Gaudino et al., 2014); high and very high intensity accelerations (HA; $\geq 2\ m \cdot s^{-2}$), and high and very high intensity decelerations (HD; $\leq -2\ m \cdot s^{-2}$). DHS, DVHS, HA and HD were normalized as relative distance covered in one minute ($m \cdot min^{-1}$). The average metabolic power (AMP) was calculated following previous studies (Di Prampero et al., 2015; Osgnach et al., 2010) and was normalized as watt ($W \cdot kg^{-1}$). All parameters were monitored over the entire match or over the SSG-POS or SSG-SHU training sessions.

Heart rate (HR)

Heart rate was recorded during each training session and during the official matches using the heart rate monitor (Polar H10 HR-monitor, Polar Electro Oy, Kempele, Finland), which was placed on the subjects to determine the heart rate peak during the SSGs sessions and official matches.

Blood lactate concentration

Blood lactate ($[La]^+$) was measured with fingertip blood samples (50 μL), using Lactate Pro (LP, Arkray KDK, Japan). The portable blood lactate analyser used in this study has been reported to be reliable and valid (Pyne et al., 2000). Before collection, the finger was cleansed with alcohol and allowed to air dry. Measurements were performed 1 minute after the end of the last bout in line with the paper from Rampinini et al., (2007).

Rating of Perceived Exertion (RPE)

The Borg's CR10 Scale was used to measure the internal load (Borg, 1998), and this measure was obtained 10 minutes after the end of each SSGs session and matches to prevent particularly hard or light elements following each performance from distorting the entire rating of the session (Tibana et al., 2018). Each player was asked for the score alone and without any possible interference from other players. A verbal-anchored scale was shown to the subjects, after completing each SSGs session and matches. Each subject was previously familiarized on the use of Borg CR10 scale.

Data analysis

The normal distribution of the data was checked with Shapiro-Wilk test and the sphericity with Mauchly's test. All data were normally distributed, except for RPE. We performed two kinds of analyses. In the first one, we assessed the potential differences between SSGs modalities, and between the two sessions. The outcome variables considered were TD, DHS, DVHS, AMP, HA, HD, Drel, HR, $[La]^+$ and RPE. Normally distributed data were entered in a repeated-measure ANOVA with CONDITION (2 levels, SSG-SHU and SSG-POS) and SESSION (2 levels, first and second) as within-subject factor. Bonferroni post hoc were applied in case of significant interaction. RPE values were analysed by means of Friedman test followed by post-hoc. The second analysis was performed to compare SSGs modalities with parameters acquired from the match play. Concerning SSGs, average values of the two sessions were entered in the analyses. OM data were the average of 10 match plays. The parameters were DHS, DVHS, AMP, HA, HD, and RPE. Normally distributed data were analysed by means of repeated-measure ANOVAs with CONDITION as within-subjects factor (3 levels: SSG-SHU, SSG-POS and OM). Bonferroni post hoc were applied in case of significant effect. RPE values were analysed by means of Friedman test followed by post hoc.

The statistical analyses were performed with SPSS (SPSS, Inc., Chicago, IL, USA). An alpha level of $p < 0.05$ was chosen.

RESULTS

Comparison between SSG-SHU and SSG-POS

External load parameters

The difference between conditions was not observed in the other external load parameters (DHS, DVHS, Drel, AMP, HA, HD), which were comparable between conditions and sessions (Figure 15).

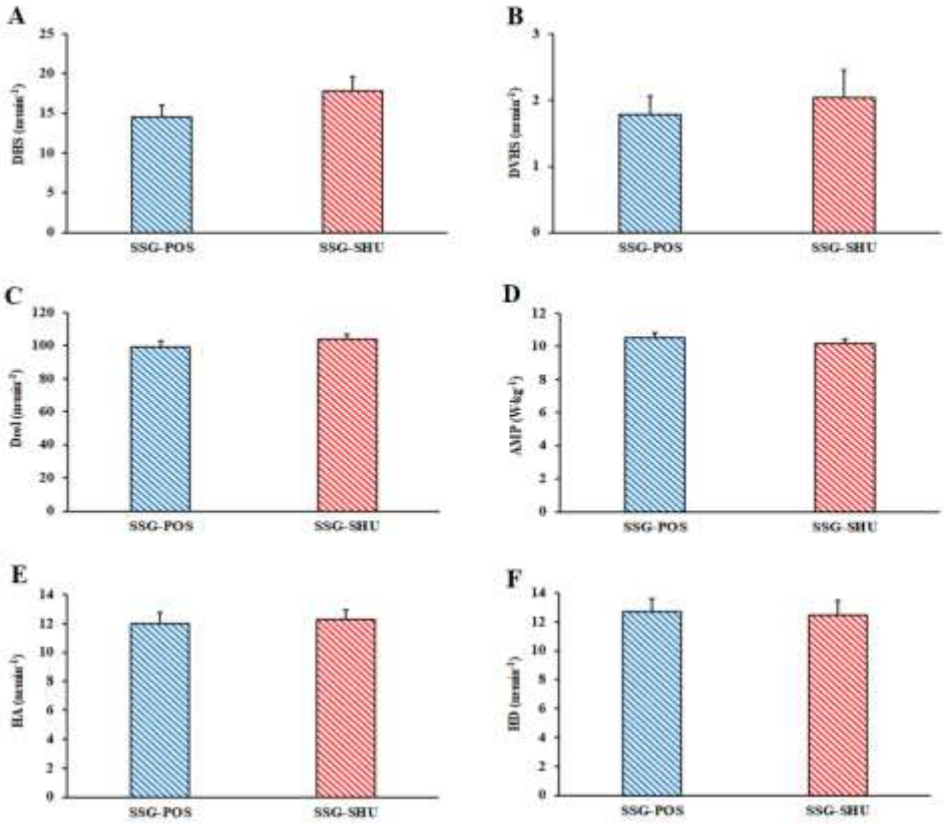

Figure 15. (A) Distance at High Speed (DHS, m·min^{-1}), (B) Distance at Very High Speed (DVHS, m·min^{-1}), (C) Relative Distance (Drel, m·min^{-1}), (D) Average Metabolic Power (AMP, W·kg^{-1}), (E) High-Intensity Accelerations (HA, m·min^{-1}) and (F) High Intensity Decelerations (HD, m·min^{-1}). Mean values ± SE. **SSG-POS** = only ball possession**; SSG-SHU** = ball possession and shuttle.

Internal load parameters

HR values (Figure 16) did not differ between conditions and sessions, and no effect of SESSION and no significant interaction were found. Friedman test on RPE values failed to find a significant difference between sessions and conditions ($\chi^2(3,10)=6.54$, p=0.09).

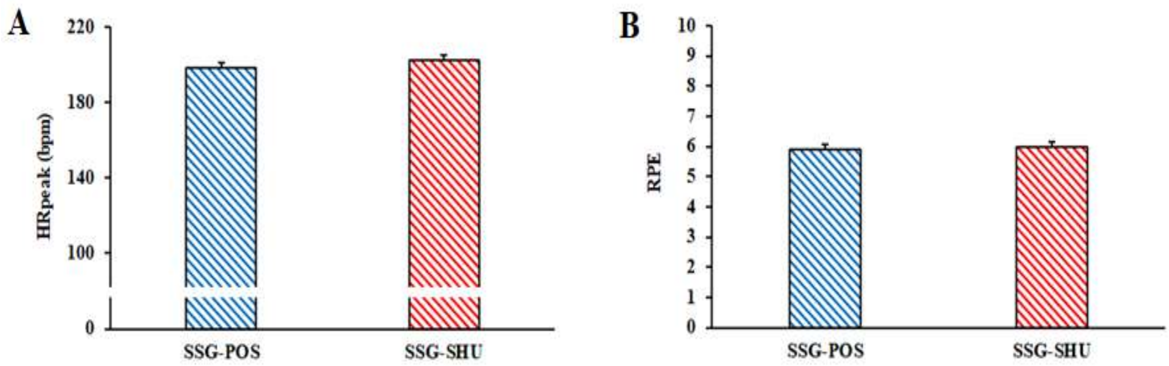

Figure 16. (A) Heart Rate Peak (HRpeak, bpm) and (B) Rating of Perceived Exertion (RPE). Mean values ± SE. **SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle.

The analysis on TD showed a significant main effect of CONDITION ($F(1,9)=25.68$, $p=0.001$, $\eta^2=0.74$), indicating that TD values were significantly higher in SSG-SHU than in SSG-POS. A significant effect of CONDITION appeared in $[La]^+$ ($F(1,9)=81.39$, $p<0.001$, $\eta^2=0.90$), showing that $[La]^+$ values in SSG-SHU were significantly higher than in SSG-POS (Figure 17).

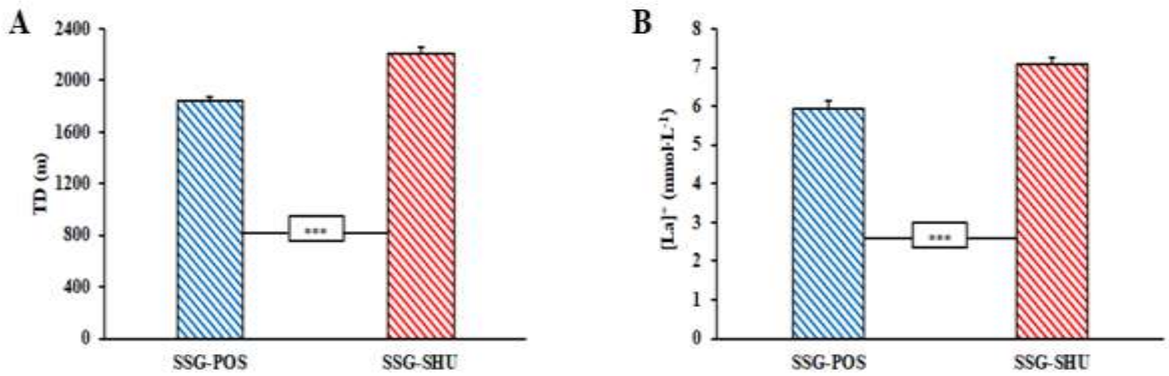

Figure 17. Total Distance (TD, m) and (B) Blood lactate concentration ($[La]^+$, $mmol{\cdot}L^{-1}$). Mean values ± SE. ***p< 0.001. **SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle.

Comparison among SSG-SHU, SSG-POS and OM

External load parameters

The one way ANOVA on DVHS showed a significant effect of CONDITION ($F(2,18)=244.29$, $p<0.001$, $\eta^2=0.96$), indicating that DVHS values in OM (mean±SE 3.39 ± 0.38 $m{\cdot}min^{-1}$) were significantly higher than both SSGs. While, DHS and Drel (CONDITION: $F(2,18)=74.14$, $p<0.001$, $\eta^2=0.89$), where values in OM (mean±SE 13.12 ± 0.57 $m{\cdot}min^{-1}$ and 100.36 ± 2.30 $m{\cdot}min^{-1}$) were significantly lower than SSG-SHU and both SSGs respectively (Figure 18).

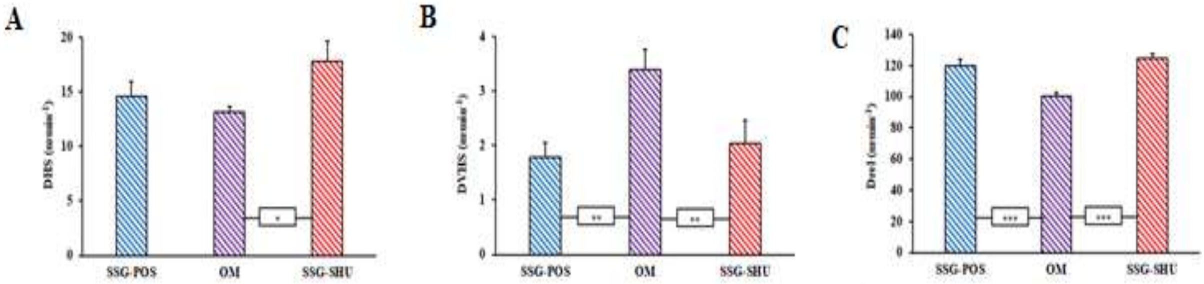

Figure 18. (A) Distance at High Speed (DHS, m·min^{-1}), (B) Distance at Very High Speed (DVHS, m·min^{-1}) and (C) Relative Distance (Drel, m·min^{-1}). Mean values ± SE. *p< 0.05; ** p< 0.01; ***p< 0.001. **SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle; **OM =** Official Matches**.**

A significant effect of CONDITION emerged by the one-way ANOVA results on AMP values ($F(2,18)=6.92$, $p=0.006$, $\eta^2=0.44$); AMP values in OM (mean±SE 9.30±0.26 W·kg^{-1}) were significantly lower than in both SSGs (Figure 19).

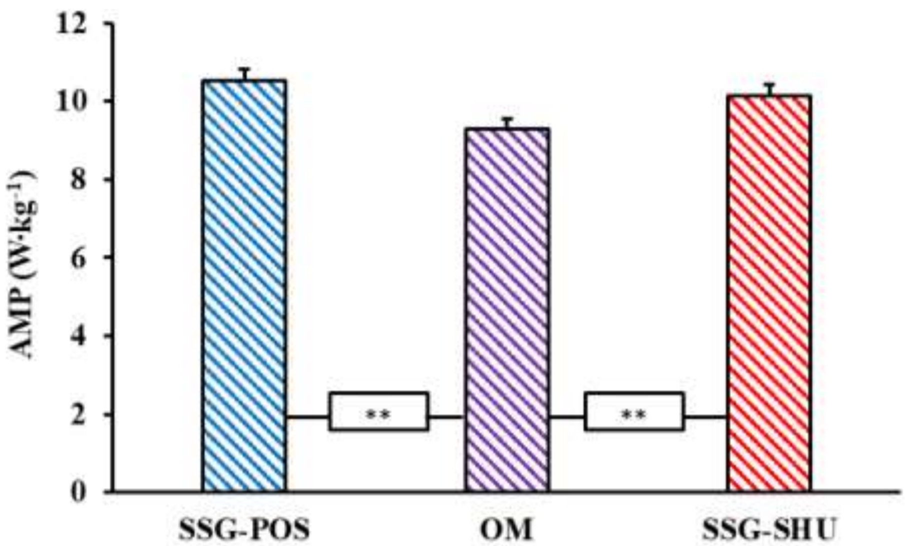

Figure 19. Average Metabolic Power (AMP, W·kg^{-1}). Mean values ± SE. **p< 0.01.**SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle; **OM =** Official Matches**.**

Both HA (CONDITION: $F(2,18)=28.04$, $p<0.001$, $\eta^2=0.76$) and HD (CONDITION: $F(2,18)=15.06$, $p<0.001$, $\eta^2=0.63$) were significantly lower in OM (mean±SE; HA 7±0.67 m·min^{-1}; HD 6.90±0.61 m·min^{-1}) than in both SSGs (Figure 20).

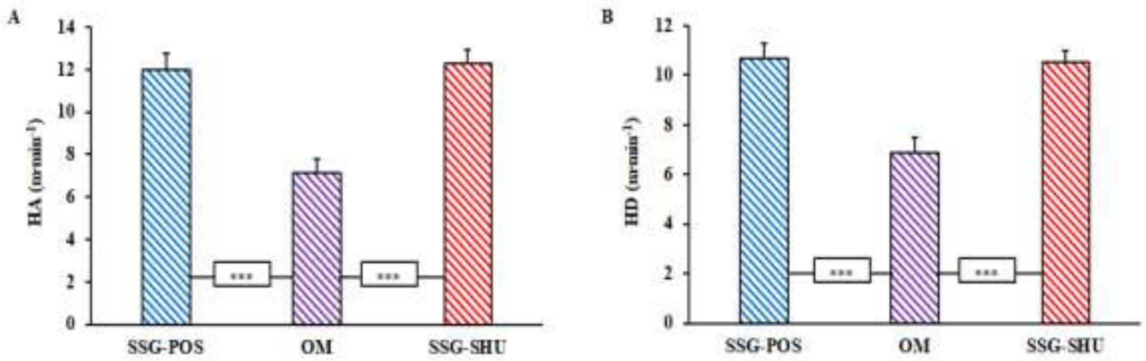

Figure 20. (A) High-Intensity Accelerations (HA, m·min^{-1}) and (B) Percentage of High Intensity Decelerations (HD, m·min^{-1}). Mean values ± SE. ***p< 0.001. **SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle; **OM =** Official Matches**.**

Internal load parameters

HRpeak values did not differ between conditions and official matches (Figure 21).

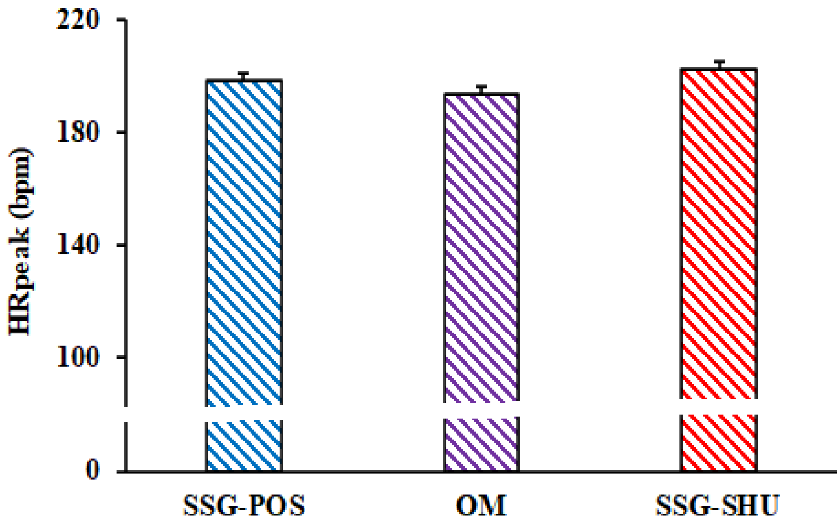

Figure 21. Heart rate peak (HRpeak, bpm). Mean values ± SE. **SSG-POS** = only ball possession**; SSG-SHU** = only ball possession and shuttle; **OM =** Official Matches**.**

DISCUSSION

This study aimed to provide soccer coaches with information about the effectiveness and characteristics of two different SSGs formats such as SSG-POS and SSG-SHU, finalized to develop specific soccer physical fitness, in particular soccer-specific endurance performance, in élite junior male players.

To this aim, the training load evolves as a function of the density of the SSGs (square meters of surface area of play for SSGs per number of players; Sangnier et al., 2019). We decided to utilize a 5vs5 format, performed in a 50x30 m pitch, which generates a density of 150 m^2. Indeed, Sangnier et al. found that players should play SSGs of 115, 150, 225 and 280 m^2 to develop strength, endurance, power, and sprint, respectively (Sangnier et al., 2019). A meticulous comparison between SSGs and official match loads may help to plan the training sessions to condition the locomotor activities typically required during the official match and to optimize performance goals (Riboli et al., 2020).

For this reason, in our study were analysed comprehensively the estimated metabolic demands (average metabolic power), the mechanical demands (distance run at high or very high speed, high-intensity acceleration and deceleration distances) and internal loads (heart rate) during two different small-sided games formats (SSG-POS and SSG-SHU) and 10 official matches in élite junior male soccer players.

The major findings of this study were that the average metabolic power, the high intensity accelerations, and decelerations ($\geq \pm 2$ $m \cdot s^{-2}$), were significantly higher during both SSGs compared to average values from 10 official matches. Furthermore, our results suggest that under our experimental conditions, both SSGs training sessions induced similar solicitations regarding the average metabolic power, the high intensity accelerations, and decelerations. The average metabolic power and the acceleration/deceleration distances recorded in our study are similar with those previously reported by other Authors (Sangnier et al., 2019). These parameters were not significantly different during SSG-POS (ball possession alone) compared to SSG-SHU (ball possession and shuttle).

Conversely, the distance covered at very high speed (19.8-25.2 $km \cdot h^{-1}$) measured in both SSGs formats were significantly lower than those in the official matches. These external load parameters were not significantly different during SSG-POS (ball possession alone) compared to SSG-SHU (ball possession and shuttle).

These results are in line with the paper from Dalen et al. (2019), who observed that élite players ran at higher intensity during official matches compared to SSGs, but the accelerations were higher in SSGs than in official matches. A possible explanation of these apparently conflicting results is that the smaller pitch size, used during training SSGs, may have limited the possibility of matching the amount of high intensity running.

Furthermore, the relative distance report significant difference between both SSGs (SSG-POS and SSG-SHU) and official matches. The total distance performed during the SSG-SHU was higher than the one measured in SSG-POS. This difference probably derives from the shuttle performed after each ball pass during SSG-SHU. This parameter was greater in official matches both for the size of the field and for the playing time than in SSGs training formats.

The internal loads during SSGs training sessions and official matches have been collected from the heart rate monitoring. These data may contribute to the evaluation of the global physical demands of the training and the official matches. Indeed, the heart rate monitoring is a very common method to quantify the intensity of physical activity, during the exercise involving high-intensity activities such as small-sided games (Casaminchana et al., 2013).

In this study, we found that the peak of heart rate during SSG-POS and SSG-SHU training sessions was similar compared to the heart rate peak achieved during the official matches. These results are in line with the paper from Asci (2016), which found that 5vs5 small-sided games format, allows to achieve a heart rate similar than values obtained during official matches. In addition, even if the players performed a shuttle after each ball pass during SSG-SHU, the heart rate peak parameters were not significantly different than the ones reached in SSG-POS. The different tactical strategies used by the players during both SSGs formats, allowed to reach similar values of peak heart rate.

Nevertheless, the heart rate monitoring is a frequently used as method to monitor exercise intensity, but it has some limitations. Indeed, the HR may overestimate the energetic cost of exercise, it may also underestimate the intensity of the very short bouts of intermittent exercise which characterize SSGs. Therefore, the exercise intensity during SSGs cannot be only established using players' heart rate (HR) responses during the game, but also by measuring the RPE and blood lactate concentration after SSG session (Koklu and Alemdaroglu, 2016).

The blood lactate concentration collected at the end of SSG-SHU training sessions was significantly higher than those recorded in SSG-POS sessions. The higher total distance covered in SSG-SHU sessions compared to SSG-POS sessions, may led to greater solicitation of the anaerobic metabolism.

Some studies in élite soccer players have used the values of blood lactate measured after the matches as the rate of anaerobic energy contribution. Bangsbo (1994) showed that lactate concentration averages 3-9 mmol·L^{-1} after the match and individual values frequently exceed 10 mmol during the match (1994). Another study reported that, the values of blood lactate concentration in soccer players were around 2-14 mmol·L^{-1} after an official match (Kurstrup et al., 2006). In the current study the mean values of RPE and blood lactate concentration measured during SSG-POS and SSG-SHU training sessions ranged between 5.90 and 6.40, 5.69 mmol·L^{-1} and 7.08 mmol·L^{-1} respectively. These results of RPE and blood lactate concentration measured for both SSGs performed in a format 5vs5, are like those reported in a study of Rampinini et al., (2007). These results demonstrate that large differences exist between players with respect to RPE and $[La]^+$ responses during SSGs sessions. These findings also reveal inter-player variability relating to using different types as a training stimulus in terms of RPE and $[La]^+$ responses, a finding also reported in previous studies (Rampinini et al., 2007; Koklu and Alemadaroglu, 2016).

In conclusion, in this study we demonstrated that in junior soccer players that two different formats of SSGs, namely SSG-POS and SSG-SHU (5vs5, 50x30m pitch dimensions) led significantly higher values of estimated metabolic and mechanical demands such as average metabolic power, high-intensity acceleration and deceleration distances than those obtained in in-season official matches in élite junior male soccer players. Nevertheless, peak heart rate measured in both SSGs formats was similar compared to official matches, demonstrating that both training modalities allow the players to reach enough time spent in high intensity zones with values like the demands of an official match. Furthermore, SSG-SHU induces higher total distance covered with higher solicitation of anaerobic contribution compared to SSG-POS, despite a similar rating of perceived exertion during two SSGs.

These results suggest that both SSG-POS and SSG-SHU an optimal training to stimulate the physiological and metabolic function, and to improve technical and tactical skills, but to train other parameters such as DVHS it is necessary to use other HIIT formats, as RST for a higher stimulus of the distance covered at very high speed.

PRACTICAL APPLICATIONS

This study demonstrated the effects of two different types of small-sided games, on young élite soccer players. Our study showed that during a 4-week intervention period, a weekly SSGs training format (ball possession only or ball possession with shuttle, 5vs5 players, 50x30m pitch dimension, with coach encouragement) could elicit significantly higher (mechanical demands) or comparable (metabolic demands) external workloads and internal workload (heart rate), than average values derived from 10 official matches, with a rate of perceived exertion (RPE) lower compared to OM. These results can assist soccer coaches in designing training programs for élite junior soccer players, suggesting that during the in-season period they could prescribe SSG training sessions in the weekly training program, to develop soccer-specific physical skills, also encompassing technical and tactical elements, with a lower subjective perception of effort, thus enhancing individual compliance and adherence to the prescribed training program.

STUDY LIMITATIONS

The main limitations of present study included a relatively small sample size of young male élite soccer players and a short training period duration (caused by COVID 19). Another limitation was the non-measurement of official matches blood lactate concentration because the portable blood lactate analysers in the field at the end of the official match is not easy and practical.

STUDY 5

THE EFFECT OF PLACEBO AND NOCEBO ON RUNNING PERFORMANCE DURING HIGH INTENSITY INTERVAL TRAINING

INTRODUCTION

In the last years, there has been a growing interest about the interaction between verbal suggestion and athletic performance during training session and sport competition. The verbal suggestion is a widely used method to stimulate this interaction and to induce the effects on expectations of performance (Colloca et al., 2010; Corsi et al., 2019). The verbal suggestion includes all words utilized to explain the effects of an inert treatment that can lead positive or negative expectations regarding the following outcome, thus influencing the real outcome. The application of inert treatment can be followed from placebo effects with physical or psychological benefits or nocebo effects with negative physical or psychological responses (Benedetti et al., 2003; Fiorio et al., 2014). It has been clearly showed that verbal suggestion and conditioning or the combination of both induce either a placebo or a nocebo response. The results of several studies supported that the combination of verbal suggestion and conditioning produces greater and longer lasting placebo and nocebo effects compared to verbal suggestion or conditioning alone (Bartels et al., 2014; Colloca et al., 2008; Jensen et al., 2012). Clark and colleagues (2000), and Beedie & Foad (2009) demonstrated that placebo effects can be applied not only in clinical situations to reduce pain or motor symptoms in neurological diseases (de la Fuente-Fernandez et al., 2001; Benedetti et al., 2004; Keitel et al., 2013), but also to balance the motor performance in healthy and physiological situations. Indeed, several other studies showed that the placebo effects in well-trained cyclists who thought to have taken caffeine, increased their output in the final test compared with baseline, even though they received a placebo (Beedie et al., 2006) and in expert runners increased their speed after drinking a glass of water that they thought to contain a special substance to increase endurance (Foster et al., 2004). It is possible to affect the motor performance of non-athletes' subjects using placebo procedure. In untrained subjects the utilization of placebo procedure led to a significantly decrease of the rating of perceived effort and improving the final motor performance (Pollo et al., 2008). Furthermore, after repeated administrations of morphine in the training phase, its replacement with a placebo on the day of competition increased pain endurance and physical performance (Benedetti et al., 2007). The nocebo effect can be defined as a negative outcome following the application of an inert treatment that the recipient believes to be effective

(Colloca and Miller, 2011). For the motor performance the application of nocebo procedure leads to a reduction of force and an increase of perceived of exertion. The factors modulating the effects of nocebo are still unclear because most of the studies were performed in clinical context (Emadi Andani et al., 2015). The different ways that induce nocebo effects have been investigated in the last years: with negative expectations (Colloca and Miller, 2011), associative learning (Colloca et al., 2010), partial conditioning (Au Yeung et al., 2014), social learning (Swider et al., 2013) and even without conscious awareness and direct learning (Ergova et al., 2015). Based our knowledge, to date no studies investigated the placebo and nocebo effects during Sprint Interval Training (SIT) session, then we hypothesize that positive and negative verbal suggestion can affect differently the motor performance in active university students during SIT performance.

In this context, the aim of this study was to test whether verbal placebo and nocebo applied during the recovery phase of Sprint Interval Training session on running performance modify participants' behaviour in term of increased resistance to fatigue and improved motor performance. Resistance to fatigue will be evaluated by means of the time to exhaustion (TTE, s) and motor performance by means of the total running distance covered during the test (TRD, m).

MATERIALS AND METHODS

Experimental Approach to the Problem

Participants were randomly assigned to the placebo group (PG), nocebo group (NG) and control group (CG) that involved 3 testing sessions over a 4-week period. This study consisted of a Cooper's 12-minute run test (CRT) followed by two experimental sessions performed on an athletic track in the following order: 1) Sprint Interval session until to exhaustion; 2) Sprint Interval session until exhaustion in different condition. Indeed, PG received placebo treatment, to the NG was given a nocebo treatment, while the CG did not receive any type of treatment. The first SIT session started with a standardized warm-up that: (a) running at low intensity for 10 minutes; (b) jogging, coordination movements, dynamic stretching for lower limbs and 4x10 m sprints. The second SIT session started with FIFA 11+ warm-up program that is a 15 to 20 minute on the field dynamic warm-up (Dvorak, 2005). All sessions were separated by 7 days and were performed at the same of the day (±1 h) to avoid influence of circadian rhythms, in similar environmental conditions (temperature of 22-23° C and relative humidity of 35-40%). The heart rate (HR), and blood lactate concentration ([La]+) were selected such as dependent variables related physiological and metabolic responses, while Time to exhaustion (TTE) and Total running distance (TRD) were assessed as indices of endurance performance. Furthermore, the rating of perceived exertion (RPE) was measured during each session to describe the perception of effort. Prior the study all participants performed a track familiarization to introduce the testing and experimental procedures. Participants were instructed to refrain from any additional strenuous physical activity during the study. Additionally, participants reported no involvement in High-Intensity Interval Training during previous 3 months of the study. Participants were also instructed to continue their normal dietary practices throughout the study but refrain from alcohol and caffeine 24 hours before each session for assessments. No additional strength, power and/or plyometric training has been performed, by the participants, during these three weeks.

Participants

Thirty-nine healthy young university students volunteered to participate in this study, and all were habitually active and completed 2 to 3 sessions of training per week, but not engaged in any sort of structured training program until least 3 months prior to the study. The sample size of 30 was determined using a priori two-sided power analysis throughout G*Power (3.1 software; Düsseldorf, Germany) and would achieve 80% power at the significance level of 0.05 (Mugele et al., 2019). To account for possible dropouts, a total of 39 participants were recruited and they were randomly separated in placebo group (PG; n=13), nocebo group (NG; n=13) and control group (CG; n=13). Prior to any participation, they were informed in detail about the nature of the experiment and possible risks and all written informed consent. The study was approved by Ethical Committee of the University of Genoa and was in accordance with the Declaration of Helsinki.

Descriptive physical and anthropometric characteristics of participants are presented in Table 6.

Group	*Placebo*	*Nocebo*	*Control*
Age (years)	21.66 ± 0.14	21.66 ± 0.14	21.92 ± 0.31
Height (m)	1.76 ± 0.03	1.76 ± 0.02	1.78 ± 0.03
Body Mass (kg)	68.72 ± 3.49	65.54 ± 3.22	66.87 ± 4.61
BMI (kg/m^2)	21.75 ± 0.50	21.06 ± 0.56	21.37 ± 0.93

Table 6. Participant’s characteristics. Mean values ± SE.

Experimental Design

Cooper test

Each participant, after 20 minutes of warm-up, ran on a 400 metres round track for a total duration of 12 minutes. They were highly motivated to run as many laps as possible. The total number of laps was counted, and the finishing point was marked. Total distance (in metres) covered in 12 minutes was calculated by multiplying the number of complete laps with 400 plus the distance covered (in metres) in the final incomplete lap (Cooper, 1968).

Sprint Interval sessions

Each sprint interval training (SIT) session consisted of repeated 30 seconds "all-out" efforts, interspersed by a period of 3 minutes of passive rest, until exhaustion. The first SIT session was started with general warm-up consisted of running at moderate-intensity and dynamic/static stretching without any interference from the experimenter. While the second SIT session started with the FIFA 11+ (Dvorak and Junge, 2005), here used as conditioning treatment. Indeed, before the warm-up begun, participants' expectancy about warm-up efficacy in not changing/improving/worsening the following SIT performance was manipulated through verbal suggestion in CONTROL/PLACEBO/NOCEBO groups, respectively. Therefore, the verbal suggestions changed in the three groups. One the warm-up was ended; participants performed the SIT. We adopted the same procedure described in Session 2, except for the information the experimenter provided to the participants during the SIT rest-periods, which changed according to the assigned groups, and acted as surreptitious manipulation. In the following, please find a detailed description of FIFA 11+, the verbal suggestion and the surreptitious manipulation.

FIFA 11+

The FIFA 11+ injury prevention program was developed in 2006 to address this matter, under the leadership of the FIFA Medical Assessment and Research Centre and in collaboration with the Oslo Sports Trauma Research Center and the Santa Monica Orthopaedic and Sports Medicine Center (Sadigursky et al., 2017). This warm-up is structured in three parts: (1) 8 minutes of running exercises (straight ahead, hip out, hip in, circling partner, shoulder contact and quick forwards & backwards); (2) 10 minutes of strength, plyometrics and balance exercise (static bench, static sideways bench, Nordic hamstrings, single leg stance, squats, vertical jumps); (3) 2 minutes of running exercises (running across the pitch, bounding, plant & cut).

Verbal suggestion

The experimenter explained to the participants of 1) the CONTROL group that "*the aim of this session was to check whether this kind of warm-up acts on the SIT performance differently from the warm-up adopted in Session 2.*"; 2) the PLACEBO group that "*the aim of this session was to check whether this kind of warm-up acted on the SIT performance differently from the warm-up adopted in Session 2. We foresee that FIFA 11+ might significantly improve the SIT performance, lowering the perceived fatigue. This is because this warm-up works on exercises crucial for SIT. For this reason, we believed you will increase both the SIT duration and the distance covered with respect to Session 2.*"; 3) the NOCEBO group that "*the aim of this session was to check whether this kind of warm-up acted on the SIT performance differently from the warm-up adopted in Session 2. We foresee that FIFA 11+ might significantly worsen the SIT performance, increasing the perceived fatigue. This is because this warm-up proposes exercises too much heavy for the following SIT. For this reason, we believed you will decrease both the SIT duration and the distance covered with respect to Session 2.*"

Surreptitious manipulation

During the SIT rest-period a surreptitious manipulation, consisting in none/positive/negative verbal feedback about the current performance, was administered to CONTROL/PLACEBO/NOCEBO group, respectively. In particular, the participants of the CONTROL group received no feedback about the SIT performance. During each rest-period, participants from the PLACEBO group were told that their performance was getting better and better with respect to Session 2, whereas participants from the NOCEBO group were told that their performance was getting worse and worse with respect to Session 2.

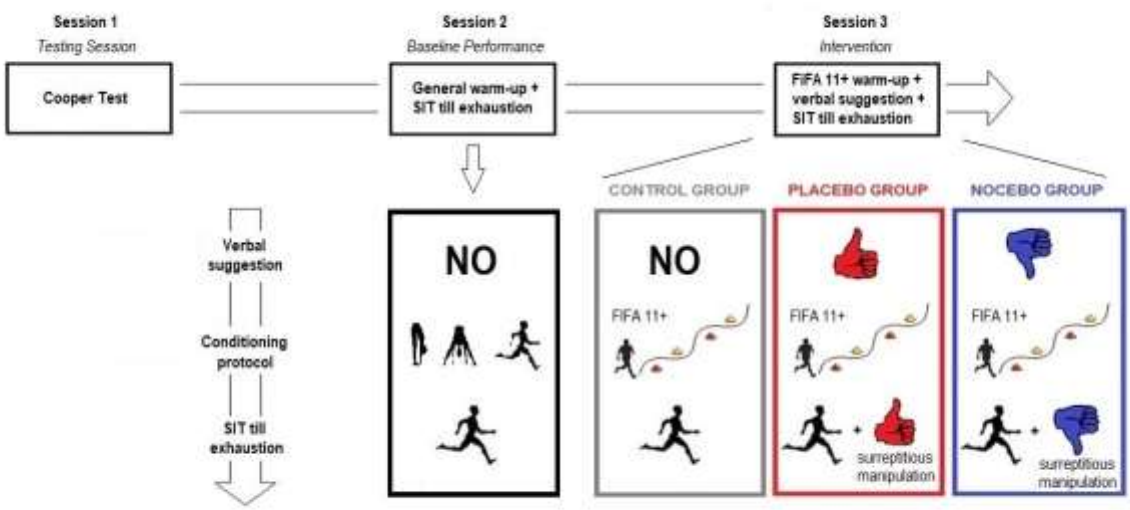

Figure 22. Experimental Design.

Heart Rate

The maximal heart rate (HRmax) during Cooper's test and the peak of heart rate (HRpeak) during Sprint Interval Training (SIT) sessions achieved both were recorded using a HR monitor (FORERUNNER Garmin 15, Olathe, KS, USA).

Blood lactate

For the measurement of blood lactate concentrations, approximately 0.3 μl of whole blood were collected form the fingertip and immediately analysed with a portable analyser (Lactate Pro 2; Arkray Factory, Inc., Japan) using an enzymatic amperometry method.

Endurance performance

Immediately after the end of the running until exhaustion, time to exhaustion (TTE) and total running distance (TRD) were measured. Time to exhaustion (TTE) and total running distance (TRD) were chosen as indices of endurance running performance. TTE was calculated as the subject's run time till exhaustion, while TRD was measured as the total distance covered during the running till exhaustion. During each running till exhaustion, the criterion to define the exhaustion was when subjects could not continue to run.

Rating of perceived exertion

The measurement of whole-body perceived exertion was performed using Borg RPE 6-20 scale (Borg, 1988). We explained the utilization of perceived exertion scale to participants at the initial clinical examination and before each other session. Therefore, each participant was previously familiarized on the use of this scale, including anchoring procedures. The rating of perceived exertion (RPE), since it represents a non-invasive, easy, and efficient technique to be applied after training session. The low and high anchors for Borg scale 6-20 were established during the familiarization session. A rating of 6 (low anchor, "very light") was assigned to the lowest exercise intensity, while a rating of 20 (high anchor, "maximal") was assigned to the highest exercise intensity. RPE values were recorded during each session after 5 minutes of the end.

Personality Questionnaires

After the whole experimental procedure, in a different day, we administered some personality questionnaires to evaluate the possible role of personality traits. The questionnaires were administered in a paper-pencil version. We considered: 1) State-Trait Anxiety Inventory (STAI) (Pedrabissi and Santinello, 1989), which is a 40-items inventory divided in two scales: STAI-I measures the level of anxiety in a precise moment and STAI-II refers to an individual's usual anxiety tendency; 2) Life-Orientation Test-Revisited (Lot-R) (Giannini et al., 2008). This is a 10-items scale developed to assess individual differences in generalized optimism versus pessimism. Four items are fillers, and the total score is obtained by summing the answers of 6 real items. We considered these tests since they were previously found to correlate with the magnitude of the nocebo effect (Corsi et al., 2016).

Statistical analysis

The distribution of the outcomes parameters was tested by means of Shapiro-Wilk test. TTE, changes in TTE %, changes in TRD % and RPE were not normally distributed, whilst TRD, HRmax, $[La]^+$, STAI-I, STAI-II and Lot-R global, Lot-R for optimism and Lot-R for pessimism were normally-distributed. To evaluate whether the three groups were homogenous at baseline (S1), two one-way ANOVAs were applied on TRD and HR_{max} values, and the Kruskal-Wallis test was applied on RPE values. Concerning the comparison between S2 and S3 among groups, TRD, HRmax, $[La]^+$ values were analysed by means of ANOVA with SESSION (2 levels, S2 and S3) as within-subject factor, and GROUP as between-subject factor. Kruskal-Wallis test, followed by post-hoc, was applied on TTE and RPE values to check for possible differences among groups in S2 and S3. Wilcoxon tests were applied to evaluate changes between S2 and S3 within each group. Changes in TTE % and changes in TRD % values were analysed by means of Kruskal-Wallis test, followed by post-hoc.

STAI-I, STAI-II and Lot-R global, Lot-R for optimism and Lot-R for pessimism were analysed by means of one-way ANOVA. Spearman correlations examined the possible relationship between these parameters and changes in TTE% and changes in TRD%. Bonferroni correction for multiple comparison was applied to set the significance level ($p=0.05/6=0.008$). Normally distributed data are described by means ± standard error values. In case of not normal distribution data are reported as median [interquartile interval]. The analyses were performed using IBM SPSS STATISTIC, version 20 for Windows. The level of significance was set at $p=0.05$.

RESULTS

Comparison among groups in Session 1

The comparison among the three groups showed that TRD, HRmax and RPE values evaluated during and after the Cooper test were not significantly different.

Data are displayed in Table 7.

	P-Group	*N-Group*	*C-Group*	*P-value*
Distance Cooper Test (m)	2207.69 ± 417.26	2243.85 ± 482.95	2180.00 ± 503.59	n.s
HRmax (bpm)	192.15 ± 2.26	192.54 ± 2.93	194.38 ± 4.42	n.s
RPE	17.92 ± 0.18	18.00 ± 0.30	18.15 ± 0.32	n.s

Table 7. Distance Cooper Test (m), Maximal Heart Rate (HRmax, bpm) and Rating of Perceived Exertion (RPE). Mean values ± SE.

Comparison between Session 2 and Session 3

Performance parameters

Time to exhaustion. Wilcoxon test comparing TTE values between S2 and S3 in the three groups showed a significant increase of TTE values in S3 (660 [450, 910] s) with respect to S2 (450 [450, 765] s) in PLACEBO group (Z=-2.46, p=0.014), a significant decrease from S2 (450 [450, 660] s) to S3 (450 [240, 540] s) in NOCEBO group (Z=-2.03, p=0.042), and no changes in CONTROL group (S2: 660 [450, 870] s; S3: 450 [240, 540] s). The results of the statistical analyses comparing TTE values in S2 and S3 among groups showed that in S2 TTE values of the three groups were homogeneous, whilst a significant effect of GROUP appeared in S3 ($\chi^2(2)=7.73$, p=0.021), when TTE values of PLACEBO group were significantly higher than those of NOCEBO group (p=0.019). No differences appeared with the CONTROL group. A graphical representation of these analyses is offered in Figure 23A.

Figure 23B showed the TTE percentage change from S2 to S3 in the three groups. As evident TTE% change in CONTROL groups did not change in S3 from S2 (0 [-3.17, 0] %), whilst increased in PLACEBO group (24.14 [0, 67.08] %) and decreased in NOCEBO group (0 [-37, 0] %). Kruskal-Wallis test showed a significant effect of GROUP ($\chi^2(2)=12.90$, p=0.002) and post hoc revealed that TTE% change of PLACEBO group was significantly higher than that of NOCEBO group (p=0.001).

Total running distance. ANOVA on TRD values showed a significant interaction between SESSION and GROUP ($F(2,36)=11.68$, p=0.0002, $\eta^2=0.39$). Bonferroni post hoc revealed that TRD values in S2 were not significantly different (PLACEBO 725.19±55.96 m, NOCEBO 711.92±55.96 m, CONTROL 734.23±55.95 m), whilst in S3 PLACEBO groups' TRD values (876.73±55.69 m) were significantly higher than those of NOCEBO group (599.62±55.69)(p=0.004). Furthermore, TRD values significantly increased from S2 to S3 in PLACEBO group (p=0.0003), and decreased in NOCEBO group (p=0.006). Results are shown in Figure 23C.

Figure 23D showed the TRD percentage change from S2 to S3 in the three groups. A significant effect of GROUP was revealed by the Kruskal-Wallis test ($\chi^2(2)=17.65$, p=0.0002). This result was motivated by the significant increase of TRD& change in PLACEBO (12.90 [0, 67.08] %) than in NOCEBO (-6.25 [-26.34, 0] %) group (p=0.0001). No changes occurred in the CONTROL group (0 [-2.02, 4.45]).

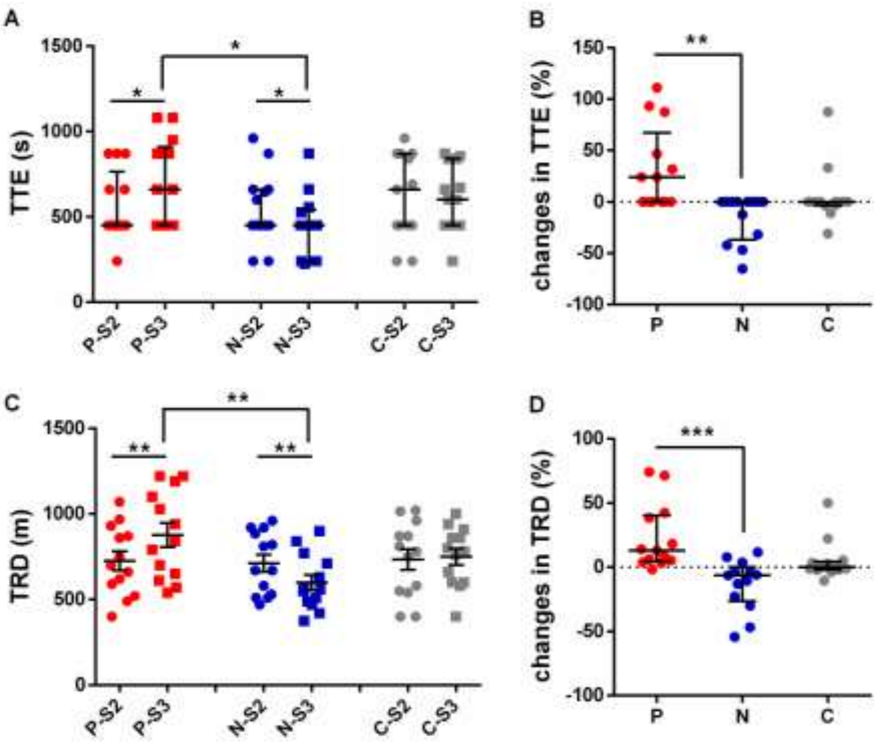

Figure 23. (A) Time to exhaustion (TTE, s), (B) Percentage of changes in TTE (%), (C) Total Running Distance (TRD; m) and (D) Percentage of changes in TRD (%). Mean values ± SE. *p<0.05; **p<0.01; ***p< 0.001. **P**= Placebo**;** **N**= Nocebo; **C** = Control**.**

Physiological and metabolic parameters

HRmax values were statistically evaluated by means of ANOVA that did not find any significant main effect of SESSION and GROUP, and no significant interaction (Figure 24A).

Similarly, no differences among groups and between Session 2 and Session 3 appeared in $[La]^+$, as shown in Figure 24B.

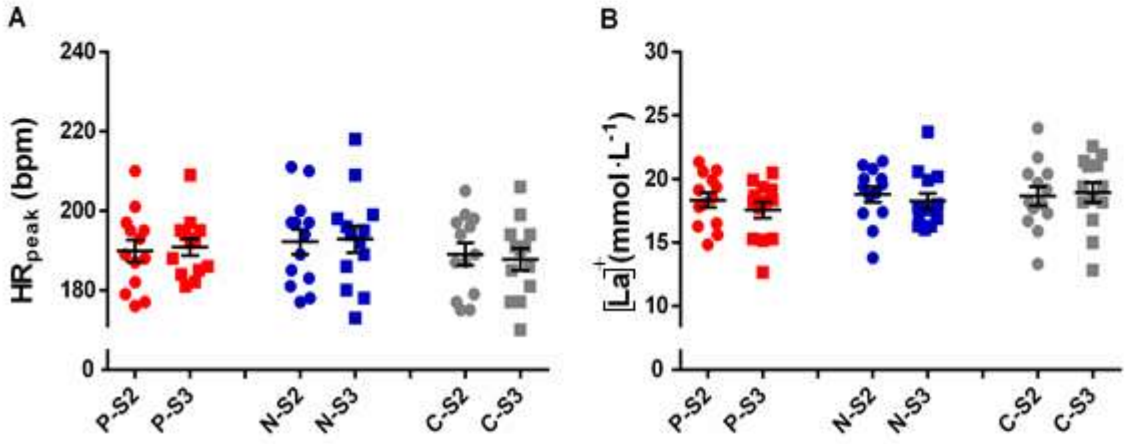

Figure 24. Mean values ± SE of (A) Heart Rate Peak (HRpeak, bpm) and (B) Blood lactate concentration ($[La]^+$, mmol·L^{-1}). Mean values ± SE. **P**= Placebo**; N**= Nocebo; **C =** Control**.**

Perception of effort

Kruskal-Wallis test comparing RPE values among the three groups in both sessions failed to find any significant effect. Furthermore, no differences appeared between RPE values in S2 and S3 in PLACEBO, NOCEBO and CONTROL groups (Figure 25).

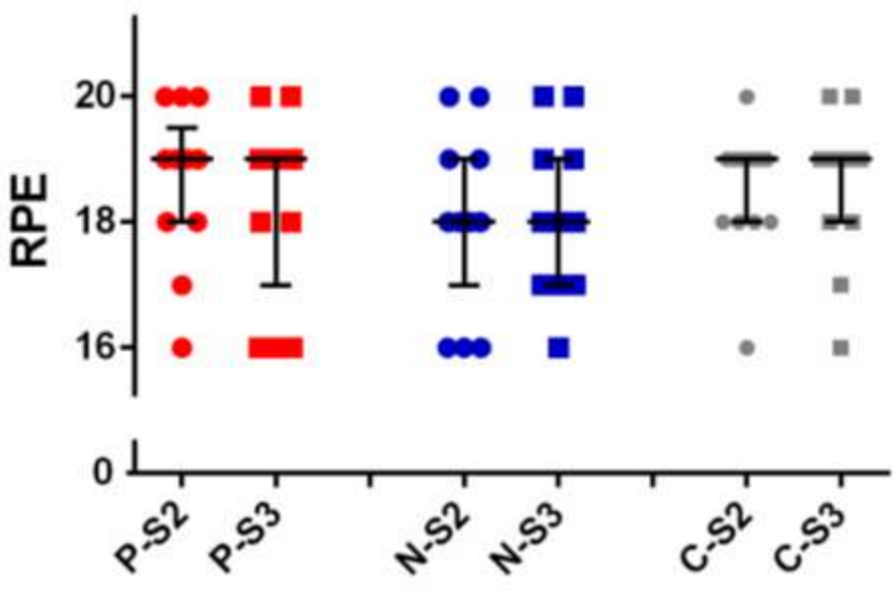

Figure 25. Mean values ± SE of Rating of Perceived Exertion (RPE). Mean values ± SE. **P**= Placebo**; N**= Nocebo; **C =** Control**.**

Personality Questionnaires

The statistical analyses revealed no difference among groups in any of the scores and no significant correlation with changes in TTE% and changes in TRD%.

DISCUSSION

This study examined the effects of a placebo or nocebo treatment consisting in conditioning manipulation and verbal suggestion on running performance parameters, such as time to exhaustion and total running distance, during a session of sprint interval training performed until exhaustion in active university students. By changing the type of conditioning (general warm-up or FIFA 11+ warm-up) following the verbal suggestion, we aimed at unveiling whether participants were more influenced by verbal suggestion or by conditioning manipulation. Corsi et al. (2019) demonstrated preponderant role in affecting the motor performance of verbal suggestion respect with conditioning.

Indeed, in our study we found that only the verbal suggestion affected the running performance outcomes, while the conditioning manipulation (general warm-up to FIFA 11+ warm-up) did not report some differences. Consistently with the results of other studies (Corsi et al., 2016; Emadi Andani et al., 2015), in this study we found a decrease in time to exhaustion and total running distance following negative verbal suggestion respect with the baseline session, while both running performance parameters increased following positive verbal suggestion.

The results of the present study show that the running performance parameters such as time to exhaustion and total running distance can be affected by positive feedback through a verbal suggestion inducing expectation and belief about the performance. We highlight, for the first time, as the use of placebo treatment through verbal suggestion, lead to enhancement of TTE and TRD during a HIIT session with sprint interval training performed until exhaustion.

Some studies demonstrated that the nocebo effect caused by pain, can be induced also by negative feedback through verbal suggestion (Colloca, Sigaudo, & Benedetti 2008; Colloca et al., 2008). These authors showed that verbal suggestion alone can induce pain perception when tactile stimulations is delivered and perception of high pain when low intensity painful stimulations is administered. Therefore, an elementary verbal suggestion could lead to the nocebo effects. The same authors demonstrated that a conditioning procedure seems to be more important in reinforcing placebo than nocebo effects (Colloca, Sigaudo, & Benedetti, 2008; Colloca et al., 2008). Particularly, the effect of placebo was stronger with verbal suggestion and conditioning than with verbal suggestion alone (Colloca, Sigaudo, & Benedetti, 2008; Colloca et al., 2008). In our study, we did not report a positive effect of conditioning to increase the time exhaustion and total running distance. Indeed, these parameters in control group did not differ, despite the use of conditioning manipulation during the intervention session.

It seems instead that verbal suggestion alone has a key role in the placebo and nocebo context, to affect the performance until exhaustion of sprint interval training in active university students.

Specifically, positive verbal suggestion induced higher time and distance of running than negative verbal suggestion in the intervention session, with statistical differences between the baseline and intervention sessions.

Finally, in line with previous studies, our findings showed no effect on either physiological, metabolic, and psychological responses following placebo, nocebo, or no verbal suggestion (control group). In agreement with Foster et al., (2004) and Porcari et al., (2006) we did not observe significant differences between baseline session and intervention session in peak of heart rate, blood lactate concentration and rating of perceived exertion (RPE).

CONCLUSION

Cognitive manipulation through placebo/nocebo intervention may significantly affect subjects' performance in trained runners. These results provide new evidence on the effects of verbal suggestion/manipulation on the outcome of performance. Athletes are often administered numerous interventions to affect various aspects relating to performance. Sport practitioners (eg, sport scientists, coaches) should be aware of the importance of words to develop their athletes' belief that interventions are effective to improve performance. These findings demonstrate that the placebo treatment can be used not only in a clinical context to reduce pain or motor symptoms in pathological subjects affected by Parkinson or multiple sclerosis, but also in healthy subjects to modulate and to affect their motor performance.

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