HEART RATE VARIABILITY TRAINING AND CONTROL OF EMOTIONS

CHARALAMPOS FOUNTOULAKIS

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To my son Stelios

who was the main reason why I did not give up, as I have always wanted to teach him that we have to try hard to achieve our goals.

ABSTRACT

This thesis outlines an investigation into the effects of heart rate variability training (HRVT) on stress and performance, and participants' experiences of undertaking HRVT. Data were collected from male Middle-Eastern adolescent student-athletes. Study 1 examined the acute effects of a single 20-minute HRVT session on performance under pressure. Thirty-six participants completed a reactive stress tolerance test after both HRVT and a control condition (group discussion about pre-competition routines). Completing HRVT did not improve performance. Study 2 built on Study 1 by exploring the effects of a 5-week HRVT protocol consisting of five lab-based and five home-based 20-minute HRVT sessions on biomarkers of the stress response. Fifty-seven participants were randomly assigned to an experimental (n=30) and a control (n=27) group, comprising five educational sessions. There were acute effects of HRVT on a-amylase levels within each session, with a-amylase levels decreasing over the course of the session. Both cortisol and a-amylase levels reduced over the course of the 5-week HRVT protocol. There were significantly lower cortisol levels and skin conductance levels in the experimental compared to the control group at the end of the training programme. Study 3 focused on the reflections of 22 participants who took part in the HRVT programme in Study 2. Participants proposed that apart from the identification of the individualized resonant breathing frequency, the customization of the inhalation-exhalation ratio is highly related to the participants' experience. That change to the HRVT programme may further enhance effectiveness, and, that effectiveness can be increased by practicing it regularly and including it as part of a pre-competition routine.

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PREFACE

Research reported in this thesis was presented in the annual conference of the Division of Sport and Exercise Psychology (DSEP) of the British Psychological Society, on the 2nd December 2019 at Solihull/ UK.

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CHAPTER 1: LITERATURE REVIEW

'The key to winning is poise under stress.'

Paul Brown (American football coach)

1.1 Introduction

High-performance and competitive sport is a context that can create an emotional experience for athletes which may vary from negative to positive under different circumstances. An emotional state may also affect an athlete during a training session or an important competition. As such, during their sport career, athletes will typically develop an arsenal of strategies that can help them overcome such issues and deal with negative emotions.

The present thesis focuses on one potential part of this arsenal, a relatively new psychophysiological strategy that can help an athlete reach a better level of emotional control. This psychophysiological strategy is heart rate variability training (HRVT). Its aim is to increase heart rate variability (HRV), and is a strategy based on slow breathing, and has been researched within the last two decades in domains, and associated with various physiological but also psychological benefits (e.g. Lehrer, Vaschillo & Vaschillo, 2000; Lehrer & Gevirtz, 2014). This thesis will discuss how HRVT can be used to strengthen the mental side of performance by regulating emotions more effectively.

Understanding emotion is not a simple process given its complexity and the different states it may describe (Jones, 2003). Theorists suggest that an emotion can be

a reaction to an incoming stimulus, whereas this stimulus may be real or imaginary (Deci, 1980), or, as described within the individual zones of optimal functioning (IZOF) framework, an emotion is a component of the psychobiosocial state of an individual at a given time and acts as a multimodal manifestation of human functioning (e.g. Hanin, 1997).

In everyday life, we often use "emotions" as a term that can be used interchangeably with "feelings". The term "emotions" may often refer to either positive or negative feelings that one can experience in a particular situation. The truth, however, is that emotions can mean more than that, as they include patterns of physiological responses and species-typical behaviours (Carlson, 1999), which are accompanied by feelings, in the case of humans. These physiological responses and species-typical behaviours along with hormonal responses constitute what we call emotional responses.

Emotions are manifested through facial expressions and relate to respiratory sinus arrhythmia (RSA), which is the phenomenon of heart rate acceleration during inhalation and heart rate deceleration during exhalation. RSA is considered as a component of heart rate variability (HRV) has been also related to emotional control. As the aim of HRVT is to contribute to the athletes' better preparation for a competition, HRVT should have positive effects on the athletes' stress levels to a reasonable extent, so that the athletes have a good reason to include it in their pre-competition routine but also, it should be able to create long-lasting effects that will help athletes reach a better level of self-regulation in the long run. To understand why

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HRVT may be potentially helpful for an athlete in regulating stress levels it is important

to understand the nature of the stress response, and that is the focus of the next

section.

1.2 The Role of the Autonomous Nervous System in Emotional Responses

Physiological responses to emotion are often responses from the autonomous

nervous system (ANS), the "autonomic" responses, which consist of sympathetic and

parasympathetic responses as well as visceral afferents. While the magnitude of the

sympathetic and parasympathetic responses is regulated by the nature of the situation

and is strengthened by hormonal responses, visceral afferents communicate

information to the brain which, in turn, leads to the creation of a psychological

experience of emotions (Porges, 2011). For instance, in a context where one feels fear,

a secretion of epinephrine takes place, or a stressful situation may lead to

gastrointestinal complications. Within a sporting context, anxiety is often present due

to trait and/ or state and/ or mood and is also a result of strong social desirability; this

may result in a heightened sympathetic tone, which is a consequence of the stress

response induced by those factors (Tanguy, Sagui, Fabien, Martin-Krumm, Canini, &

Trousselard, 2018).

Once the sympathetic nervous system (SNS) is activated, fight-or-flight

behaviours are also triggered which, along with adrenal activity, are responsible for

mobilization (e.g. increased skeletal activity of the major limbs); the SNS has been

considered by most researchers, following the suggestion of Cannon (1928), one of the

main determinants of emotion, as it prepares the body for an emergency by accelerating the heart rate, lubricating the skin with sweat and inhibiting the gastrointestinal tract (Porges, 2011). However, this does not take into consideration the substantial role that the parasympathetic system plays in emotion, as, neurophysiologically, it is highly connected to facial structures, pupil dilation, salivation, hearing, eye movements, breathing and swallowing (Porges, 2011), which are responses and functions linked to emotions. For instance, a situation causing anxiety may lead to elevated salivation or a relaxed state may lead to a constriction of the eye pupil. So, while, the term "parasympathetic" -derived from Greek- describes a nervous system "which guards against feelings", "sympathetic" is a term that describes a nervous system which is associated with "feelings".

Incoming stimuli trigger responses mainly regulated by the two branches of the ANS; these responses may have positive or negative effects on performance, based on the individual's subjective interpretation and idiosyncrasy. This means that, in the context of sport, the electrobiochemical responses that follow emotions may ameliorate or deteriorate sport performance, depending on the responses' valence and intensity (Lazarus, 2000; Russell & Barrett, 1999). This is the reason why athletes participating in competitive sport try to learn how to perform, while managing their stress response and experiencing negative emotions (e. g. while feeling anxious).

1.3 Emotional Regulation in Competitive Sport

When psychological skills training is regularly included in the mental

preparation of an athlete, they train themselves on how they can regulate their

emotions more efficiently, whereas emotional regulation is actually the process by

means of which "…individuals influence which emotions they have when they have

them…" (Gross, 1998, p. 275) and, also, refers to how they express and experience

these emotions. Emotional regulation has two different aspects: the intrapersonal and

the interpersonal aspect, with the former referring to coping strategies individuals use

to deal with stressors, as well as the appraisal of every situation (Thatcher & Day, 2008;

Martinent, Campo, & Ferrand, 2012; Gaudreau, Nicholls, & Levy, 2010; Mellalieu,

Hanton, & Shearer, 2008), and the latter referring to the interaction of individuals

within a context in which they can influence each other to improve or worsen

performance outcomes (Tamminen, Gaudreau, McEwen, & Crocker, 2016). Indeed,

theorists have claimed that emotion can have a multi-faceted impact on sport

performance (Jones, 2003). It can have an effect on motivational aspects, as it can lead

an athlete towards an action or an object, or can lead them away from those (i.e. the

action or object). For instance, an athlete who scores an own goal in football may avoid

getting possession of the ball because he feels embarrassed, or in the case of anger, a

footballer may demand the ball from her team-mates to try to make amends (Jones,

2003; Vallerand & Blanchard, 2000). Another aspect of performance affected by

emotions is cognitive functioning; researchers have shown that acute stress may impair

cognitive flexibility due to elevated sympathetic arousal (e.g. Marko & Riečanský,

2018), whereas if increased arousal is caused due to somatic anxiety, it may create

positive effects on perceptuo-motor speed (Jones & Cale, 1989; Parfitt & Pates, 1999) or may lead to a narrower attentional focus which, in turn, may lead to positive or negative results depending on the extent to which attention is narrowed as well as on the context the athlete is in (Jones, 2003). Last but not least, elevated arousal has always been associated with some emotions although the latter do not necessarily cause the former, since an arousal increase may also exist due to different reasons (e.g. as a result of exercise). Whereas elite athletes report that heightened arousal has helped them in important competitions (Gould Eklund & Jackson, 1992), researchers maintain that it can have a positive effect on anaerobic power (e.g. Hardy, Jones & Gould, 1996) and a negative effect on fine motor tasks (e.g. Parfitt et al., 1990).

In terms of strategies used to control emotions, researchers have investigated two different broad categories, namely, cognitive strategies and strategies for arousal control (Jones, 2003). While under the cognitive strategies umbrella, there are such popular strategies as imagery, self-statement modification, reframing and Socratic dialogue, the extent to which arousal control strategies are important has been pointed out by researchers (Parfitt et al., 1990; Jones, 2003), as regulating physiological arousal can help in emotional regulation which, in turn, can enhance performance. However, researchers claim that applied practitioners should use arousal control strategies with caution, as potentially it may result in lowering positive emotions as well those that benefit performance (e.g. Maynard, Hemmings, & Warwick- Evans, 1995).

1.4 Biofeedback and Emotional Control

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An effective way in which an athlete can regulate his/her level of emotions is by means of biofeedback/ neurofeedback (Edmonds & Tenenbaum, 2012). Biofeedback is the process of increasing the awareness of a client towards a physiological function with instrumentation attached to various sites of the human body, such as muscles, fingers, scalp, around the waist, (etc.), while neurofeedback is the application of biofeedback to monitor brain waves by attaching electrodes to the scalp. Biofeedback first appeared in late 1960s and was initially used for controlling musculature, states of consciousness and visceral physiology (Miller, 1969; Basmajian, 1967). The concept of a supermind was also introduced by one of the first initiators of biofeedback research, Barbara Brown, to describe the new mind that can be built by means of biofeedback practice, which has a more advanced level of consciousness and has also unlimited potential. Initially, it was expected that electroencephalogram (i.e. EEG biofeedback, a.k.a. neurofeedback) would help humans increase their creativity and make significant advancements in their level of consciousness (Brown, 1980). However, this proposition was not supported by evidence, and practitioners started using biofeedback and neurofeedback together to help individuals improve awareness of their physiological responses and develop control over those responses with a view to optimizing self-regulation, which turned out to be a more realistic expectation.

Apart from the very basic forms of biofeedback in everyday life (e.g. weighing scale, heart rate on a treadmill, etc.), other, more advanced, uses of biofeedback have been developed. For example, biofeedback applications to enhance learning in an academic environment, clinical applications of biofeedback, its applications to enhance

creativity of artists, and the development of biofeedback strategies to optimize performance at work but also in sport. Within the context of performance enhancement, the field of optimal performance psychophysiology emerged, which is widely known as sport psychophysiology when it deals with athletes, or performing arts psychophysiology when it deals with artists, with its primary objective being better stress management, since stress management programmes benefit from the addition of a psychophysiological component (De Witte, Buyck & Van Daele, 2019), and the use of biofeedback and neurofeedback to optimize performance.

Within the sporting domain, applications of psychophysiology to performance enhancement preceded the modern era of biofeedback. Indeed, Coleman Griffith (as cited in Edmonds & Tenenbaum, 2012) first discussed the investigation of a psychophysiological approach to performance in 1918, whereas Jacobson (1938) and Basmajian (1967) contributed to the development of the biofeedback movement by examining the neurophysiology of musculature. Later on, correlates between heart rate fluctuation and performance were analyzed by Porges (1972) and Lacey and Lacey (1974). However, it was not until 1982 that the importance of a field of applied psychophysiology was made clear when Zaichowsky and Sime (1982) linked psychophysiology with stress management strategies in a pioneering journal article in which it was claimed that biofeedback, if coupled with other self-regulatory strategies, such as imagery, progressive muscle relaxation or autogenic training, can assist athletes in their preparation for an important competition.

1.4.1 Biofeedback modalities. Different biofeedback modalities have been researched for performance enhancement; these include surface electromyography (sEMG, or even EMG sometimes), electrodermal activity (EDA, often referred to as skin conductance [SC]), peripheral skin temperature (TEMP), respiration rate and pattern (RESP), electroencephalography (EEG), heart rate (HR) and heart rate variability (HRV). The use of biofeedback in applied sport psychology has enabled practitioners to quantify interventions (Zaichowsky, 2009) and has been used as a proof for the positive outcome of their intervention, as the nature of their work is such that they (i.e. applied sport psychologists) are not often in position to provide evidence on the effectiveness of their work. Nevertheless, due to lack of knowledge by practitioners, high cost, and delay in the development of portable and easy-to-use devices, biofeedback has not been as widely used as might be expected (Perry, Shaw, & Zaichowsky, 2011).

In this section, each of the different ways in which biofeedback has been used is outlined.

1.4.1.1 Surface electromyography (sEMG). Since the beginning of biofeedback practice, surface electromyography (sEMG, often referred to as simply EMG) was used to monitor muscle tension patterns and is often used nowadays to minimize harmful muscle habits that lead to chronic (or even acute) pain which, in turn, may hinder the improvement of performance. For instance, an athlete may have the habit of continuously raising his/her left shoulder, whenever they feel stressed, and, as a result of it, tensing his/her left trapezius muscle without realizing it, which may result in chronic pain of the left shoulder; in this case, an EMG session may help the athlete gain

awareness of this bad habit. In a relaxed (non-stressed) state, a practitioner should see

a low -in terms of μV- smooth signal.

The initiators of this biofeedback application used sEMG to minimize muscle

tension in the hamstrings of gymnasts, with a view to increasing their flexibility as well

as to improve muscle flexibility in sprinters (Wilson & Bird, 1981; Cummings, Wilson, &

Bird, 1984). In the study with male gymnasts, the biofeedback group showed a faster

improvement across trials (Wilson & Bird, 1981), whereas, in the case of sprinters,

superior flexibility gains in the retention period were shown in the groups who took

part in the biofeedback and the relaxation treatment methods (Cummings et al., 1984).

1.4.1.2 Electrodermal activity (EDA). This is also known as skin conductance

(SC) or even galvanic skin responses (GSR) in honor of Luigi Galvani who was the first

scientist in the 18th century to claim that muscles produce electricity. This modality is

actually based on an infinitesimal amount of electrical current that the sensor imposes

on the skin; it travels through the skin and measures how conductance fluctuates with

the secretion of sweat caused by stress. As sweat consists of salt water, it can conduct

electrical signals, whereas the more sweat indicates that more electricity flows through

the skin (Peper**,** Tylova**,** Gibney, Harvey**,** & Combatalade, 2008). Monitoring EDA helps

practitioners develop strategies for cognitive self-quieting and emotional regulation

(Edmonds & Tenenbaum, 2012). In a relaxed (non-stressed) state, a practitioner should

see a low signal, in terms of micro-Siemens (μS), which is the scale used to measure

electrodermal activity. Besides, EDA has been found to be a good index for arousal-

activation in shooting and can discriminate between good and poor performers

(Guillot, Collet, Dittmar, Delhomme, Delemer, &Vernet-Maury, 2003).

However, EDA has not received wide acceptance from the scientific community

yet, due to lack of faith in the consistency of EDA data (Posada-Quintero, Dimitrov,

Moutran, Park & Chon, 2019). There is still room for further researching this

biofeedback modality; the next step would be to analyze long-term EDA data and

develop advanced algorithms for detecting and removing artifacts.

1.4.1.3 Peripheral skin temperature (TEMP). Peripheral skin temperature or

skin temperature is a modality often used in applied psychophysiology to teach

performers how to self-monitor skin temperature and use hand-warming strategies

and imagery for relaxation purposes. As the thermistor (i.e. the sensor by means of

which skin temperature is monitored) is placed on the individual's hand to measure

temperature, it actually reflects the extent to which hand vessels are constricted

which, according to what is assumed physiologically, results from higher levels of

stress. Indeed, vasoconstriction is caused by a lower blood flow in the extremities

which, in turn, causes a decrease in the skin temperature, whereas vasodilation is a

result of greater blood flow in the extremities which, in turn, results in a temperature

increase. Therefore, skin temperature is reversely related to vasoconstriction in the

client's hands as the more the constriction, the colder the hands of the client will be

(Pepper et al., 2008).

__1.4.1.4 Respiration rate and pattern (RESP).__ In stress management, breathing control is crucial, as poor stress tolerance may result in an imbalance of the chemicals involved in the respiration process (i.e. mainly oxygen and carbon dioxide) which in turn, may result in a lack of acid-base balance within the individual's body. Respiration as a function occurs involuntarily, although we can often intervene and change our breathing pattern (Peper et al., 2008). In fact, the autonomous nervous system (ANS) is responsible for our respiration rate and pattern as this is a - mostly - unconscious function of the human body; an individual becomes more aware of their breathing rate and pattern once they face difficulty in oxygen intake, or in any other aspect of the respiration process (e.g. they cannot take a deep breath, or they have difficulty while speaking). Then, breathing becomes a more conscious process and can be changed through self-observation. Using biofeedback for observing one's breathing rate and pattern can be beneficial, as training effortless diaphragmatic breathing may lead to better homeostatic and regenerative processes.

In applied psychophysiology used for optimizing performance, respiration is often monitored with strain gauges. In performance enhancement, respiration is monitored with thoracic and/ or abdominal strain gauges, which are used to indicate the extent to which the chest and abdomen – respectively - of an individual expand/ are compressed while breathing (Peper et al., 2008). In a relaxed (non-stressed) state, a practitioner should see a normal sine-type wave (i.e. normal periodic oscillation), whereas the average respiration of an adult should be around 12 to 18 breaths per minute.

__1.4.1.5 Electroencephalography (EEG).__ Electroencephalography, often referred to as neurofeedback, is a broad area which deals with monitoring the current, produced by the electrical activity of neurons, that reaches the human scalp and the voltage differences of such scalp phenomena (Thompson, Steffert, Ross, Leach & Gruzelier, 2008). In other words, EEG is a strategy that teaches an individual how they can change some aspect of their cortical activity, as the whole approach is based on different aspects of human cortical activity (Vernon, 2005). This may include changing of one's amplitude or frequency or coherence of signals received from one's brain with EEG. In brief, by means of EEG biofeedback, one can learn how a specific state of brain feels like, learn what they need to do to reach this state, and rehearse this as many times, as it is needed in order for them to be able to re-activate this specific state voluntarily, when this is necessary. For instance, a practitioner can help a client quiet his mind while, at the same time, the client can watch his beta activity declining. After a while, the client will be in position to repeat this, since he will remember what reducing one's beta activity feels like. It is worth mentioning that, in optimal performance psychophysiology, EEG training is conducted on the grounds that the client learns to activate optimal states of cognitive functioning which lead to performance enhancement. This is based on the identification of associations between specific patterns of cortical activity and states of optimal functioning. This is possible up to a certain extent, as research (e.g. Vernon, Frick, & Gruzelier, 2004) has shown that one cannot always re-activate an EEG amplitude pattern, which they have rehearsed during training, while being at rest. However, as the field of EEG biofeedback

is rapidly expanding, many new areas of application emerge both in a clinical setting but also in performance enhancement (e.g. Cheron, Petit, Cheron, Leroy, Cebolla, Cevallos, Petieau, et al., 2016; Peper et al., 2008).

1.4.1.6 Heart rate (HR). Heart rate monitors have been used extensively by physical coaches and physiologists to monitor the heart rate of athletes; slower resting heart rates have been associated with aerobically fit athletes (e.g. Lopes & White, 2006) whereas the way to monitor HR during exercise is as simple as by using a wristwatch-type fitness tracker. In a lab-based environment, heart rate can be monitored either through electrocardiography (ECG) or by using the blood volume pulse (BVP) finger sensor, which is based on the technology of photoplethysmography (Schwartz & Andrasik, 2003). The readings of this sensor are based on the changes of the light which occur due to the changes of blood volume in the arteries and capillaries which, in turn, reflect changes in heart rate. It is also worth adding that the BVP sensor has been also used for information on the elasticity of the vascular bed as well as blood pressure and cardiovascular health (Peper, Harvey, Lin, Tylova, & Moss, 2007). In a relaxed (non-stressed) state, a practitioner should see an average heart rate which is approximately from 60 beats per minute to 90s, depending on the cardiovascular fitness of the individual (i.e. the more the cardiovascular fitness, the lower the average heart rate) (e.g. Tulppo, Hautala, Makikallio, Laukkanen, Nissila, Hughson Huikuri, 2003).

1.4.1.7 Heart rate variability (HRV). Another important cardiovascular aspect, which has been researched in and is the epicentre of this project, is heart rate

variability. The training of this physiological parameter results in a better control of the autonomic responses which occur when one experiences stress. Additionally, practicing the training of this parameter is really easy and practical, as it can be rehearsed at any time and place that an individual can rest for a few minutes.

Heart rate variability (HRV) is the variation of both instantaneous heart rate and R-R intervals (Papaioannou, 2007). The variation in the R-R intervals actually reflect responses of the cardiovascular control system to physiological perturbations. Changes in the blood pressure (arterial and venous blood pressure) occur; these changes result from cyclic variation on intrathoracic pressure which, in turn, is a result of respiration movements. Another factor responsible for this is the blood flow autoregulation which causes fluctuations in the peripheral vascular resistance (Papaioannou, 2007). The SNS and the PNS work, at the same time, towards achieving a homeostatic balance when the above-mentioned perturbations are detected by chemoreceptors and baroreceptors.

Slow breathing has been found to affect HRV which, in turn, has been found to affect the ANS positively, thus leading to a state of better self-regulation. Indeed, the training of HRV has been discussed extensively due to its psychophysiological benefits as well as other practical advantages, like the ease to learn how to practice it, its non-invasiveness and that there is no expensive equipment involved in it (Jimenez-Morgan & Molina-Mora, 2017).

History of HRV. The variations of heartbeat have been discussed since the early days of medicine, since humans could easily understand the difference in heartbeat during physical exercise or sexual arousal (Billman, 2011). The first scientist who described human pulse in his scripts was Herophilus, which is the Latinized name of the ancient Greek physician Ηρόφιλος, who lived approximately between 335 to 280 BC (Billman, 2011). Among other scientists who researched the pulse after Herophilus, the most influential physician was Cladius Galenus, which is the Latinized version of Γαληνός, an ancient Greek scientist who lived in 131-200 AD, and managed to write eighteen books on human pulse; the greatest advancement in Galenus' work was that, in his scripts, he included eight treatises which discussed the predictive value of pulse towards the prognosis of disease (Boylan, 2007). Galenus maintained that one should feel the human pulse in health to be able to understand its deviation when it comes to sick people. He also pointed out the variations of pulse that exist due to different age or gender, different country, pregnant/ non-pregnant, after exercise, alcohol use or sleep deprivation (Bedford, 1951).

Later on, in the beginning of the 18th century, John Floyer, an English physician invented what he called "the physician pulse watch" by means of which he could create tabulations between pulse and respiration for different conditions. The physician watch was actually a portable clock with a second hand where Floyer (1707) could use a push-piece that could stop the watch to make the appropriate measurements. As time went by, timepieces became more and more accurate which allowed scientists to start investigating periodic fluctuations in the arterial pulse. Rev.

Stephen Hales was the first ever to discuss the fluctuation of the beat-to-beat interval as well as the blood pressure during the respiratory cycle (Hales, as cited in Billman, 2011).

In an experiment he conducted with a dog and a new invention of his, a smoked drum kymograph (i.e. a device used to measure mechanical activity), Ludwig (as cited in Billman, 2011) managed to discuss what was later known as respiratory sinus arrhythmia (RSA), since he observed that a respiratory cycle caused periodic oscillations in the amplitude and timing of the arterial blood pressure waves, when he recorded that the pulse of the dog accelerated during inhalation, whereas it decelerated during exhalation. Moreover, it was not until the end of the 19th century that Einthoven also managed to record electrical activity on human heart with his experiment with galvanometers.

The rapid development of technology helped the realization of such recordings in a more sophisticated way; ambulatory ECGs were first recorded in the early 1960s, when Holter (1961) developed a small portable device for long-term ECG recording (i.e. sometimes more than 24 hours) which, in turn, resulted in associations between disease, heart interval and beat-to-beat variations. A power spectral analysis came later in the 1970s when researchers (e.g. Hyndman, Kitney, & Sayers, 1971; Sayers, 1973; Chess, Tam, & Calaresu, 1975) were able to investigate the breakdown of individual frequencies that constitute the periodic variations in heart rate, physiologically.

1.4.1.8 Applications of HRV. The turning point in HRV cardiological research, however, occurred when scientists (Wolf, Varigos, Hunt, & Sloman, 1978), observed an association between the standard deviation of normal beat-to-beat intervals (SDNN) and acute myocardial infarction (AcMyoI). This happened when Wolf et al. (1978) found out that there was a correlation between sinus arrhythmia and survival after AcMyoI.

HRV as an index of estimating risk in health. Kleiger, Miller, Bigger and Moss (1987) as well as other researchers (La Rovere, Specchia, Mortara, & Schartz, 1988; Forslund, Bjorkander, Ericson, Held, Kahan, Rehnqvist, & Hjemdahl, 2002; Balanescu, Corlan, Dorobantu, & Gherasim, 2004; Stein, Domitrovich, Huikuri, & Kleiger, 2005) examined correlates between cardiological parameters and HRV; their results prompted the founding of the joint task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Ernst, 2017). This task force standardized certain processes within the field of HRV and proposed further clinical research with a view to shedding light on more HRV-related correlates within the clinical context.

After all the technological developments in applied biofeedback and the inclusion of HRV in more than 14000 articles and more than 2000 clinical trials as well as the invention of electronic devices, mobile applications and other gadgets, it has been concluded that the HRV's most important clinical application is risk stratification. Indeed, there have been many studies that have shown that HRV is reversely related to sudden cardiac death (i.e. there is a correlation between sudden cardiac death and low

HRV (e.g. Huikuri, Makikallio, Peng, Goldberger, Hintze, & Moller, 2000; La Rovere,

Pina, Maestri, Mortara. Capomolla, Febo, Ferrari, Franchini, Gnemmi, Opasich, Riccardi,

Traversi, & Cobelli, 2003; Maheshwari, Norby, Soliman, Adabag, Whitsel, Alonso, &

Chen, 2016) whereas the use of HRV has been widely recognized as an index of cardiac

health (Maheshwari et al. , 2016; Adabag, Luepker, Roger, & Gersh, 2010; Adabag,

Smith, Anand, Berger, & Luepker, 2012). Additionally, HRV has been used for the

identification of cardiac autonomic neuropathy (Schonauer, Thomas, Morbach,

Niebauer, Schonauer, & Thiele, 2008; Cygankiewicz & Zareba, 2013) caused by diabetes

and is also used for the prediction of systemic infections in critical care medicine (Seely

& Macklem, 2004; Mazzeo, La Monaca, Di Leo, Vita, & Santamaria, 2011).

HRV and performance. Research has also focused on developing correlates

between heart rate variability and performance; specifically, cardiac autonomic

function has been found to be strongly interdependent with aerobic running

performance, with higher HRV resulting in better aerobic running performance, while

post-exercise HRV measurements have been shown to have predictive value towards

changes in aerobic endurance capacity (Buchheit, Chivot, Parouty, Mercier, Al Haddad,

Laursen, & Ahmaidi, 2010). Moreover, in psychological research, higher levels of HRV

have been shown to correlate with enhanced cognitive performance (Johnsen, Thayer,

Laberg, Wormnes, Raadal, Skaret, Kvale, & Berg, 2003), resting levels of vagally-

mediated HRV have been found to correlate positively with cognitive performance

(Thayer, Hansen, Saus-Rose, & Johnsen, 2009), whereas resting respiratory sinus

arrhythmia, a component of HRV, has been associated with higher attentional capacity (Hansen, Johnsen, & Thayer, 2003; Porges, 1992).

HRV and stress. As mentioned above, heart rate variability has been associated with psychological benefits as well; research in this field (e.g. Holzman & Bridgett, 2017) has also shown that individuals may have a better response to environmental demands when they have higher levels of HRV, which are also associated with more parasympathetic activity. This has been researched in clinical contexts for the better treatment of anxiety disorders. Researchers (Appelhans & Luecken, 2006b) pointed out that anxiety disorders occur mainly in parallel with a breakdown of the central autonomic network, which is in line with the neurovisceral integration model, according to which, a functional and structural network is formed from the integration of autonomic, attentional and affective networks, which is responsible for emotional regulation and dysregulation (Thayer & Lane, 2000). This breakdown is characterized by a malfunction of any inhibitory processes of the central autonomic network which results in a decreased activity of the parasympathetic branch of the ANS and a state of continuous, excessive worry of the individual. Different correlates with HRV have also been researched; the most important of them are correlates with general anxiety (Thayer, Friedman, & Borkovec, 1996), panic disorder (Friedman & Thayer, 1998), social anxiety (Mezzacappa, Tremblay, Kindlon, Saul, Arseneault, Seguin, Pihl, & Earls, 1997), trait anxiety (Fuller, 1992) and stress-associated anxiety (Sgoifo, Braglia, Costoli, Musso, Meerlo, Ceresini, & Troisi, 2003). In all of these studies, HRV parameters negatively correlated with the development of the above-mentioned conditions/

disorders, which is an indication of the fact that higher HRV is linked with better

psychological health, since, as mentioned above, higher HRV results in elevated

parasympathetic activity.

1.4.1.9 HRV measurement. The metrics used to describe heart rate variability

depend on the nature of the HRV measurement that needs to be analyzed. An HRV

signal could be either very long (i.e. more than 24 hours) or ultra-short (i.e. shorter

than even 5 minutes); researchers (e.g. Shaffer & Ginsberg, 2017) have suggested that

short HRV signals (e.g. signals recorded for approximately 5 minutes) have different

properties from long-term HRV signals (e.g. signals recorded for, say, 24 hours). As

such, the measurement method used, for example, for a 24-hour signal should be

different from the method used for a brief 5-minute signal, as different indices have

been developed to analyze HRV signals of different nature and duration. In other

words, HRV can be calculated using signals of different duration (i.e. short as opposed

to long signals), which have different properties and cannot be used interchangeably,

as this would lead to erroneous conclusions. For this reason, such different signals are

usually measured by means of different metrics, which is explained, in detail, below:

Time-domain methods. This appears to be the simplest method for analyzing

HRV; it, actually, provides the practitioner with information on the heart rate at any

point in time, but also on the intervals between successive normal complexes (Task

Force of the European Society of Cardiology [ESC] and the North American Society of

Pacing and Electrophysiology [NASPE], 1996). If a practitioner uses

electrocardiography, then the detection of every single QRS complex is possible, which

gives the possibility for the analyses of normal-to-normal intervals (NN); these are the

intervals resulting from consecutive QRS complexes which, in turn, result from sinus

node depolarizations.

Figure 1.1 depicts two subsequent heartbeats along with the name of their

components. The RR interval is the time between two consecutive R-peaks, which,

when they are free from artifact, are referred to as NN intervals (NB Artifacts may

occur due to arrhythmic events as well as faulty sensors which may result in erroneous

figures and analyses. For this reason, only normal R-peaks are selected for analysis

[Citi, Brown & Barbieri, 2012]).
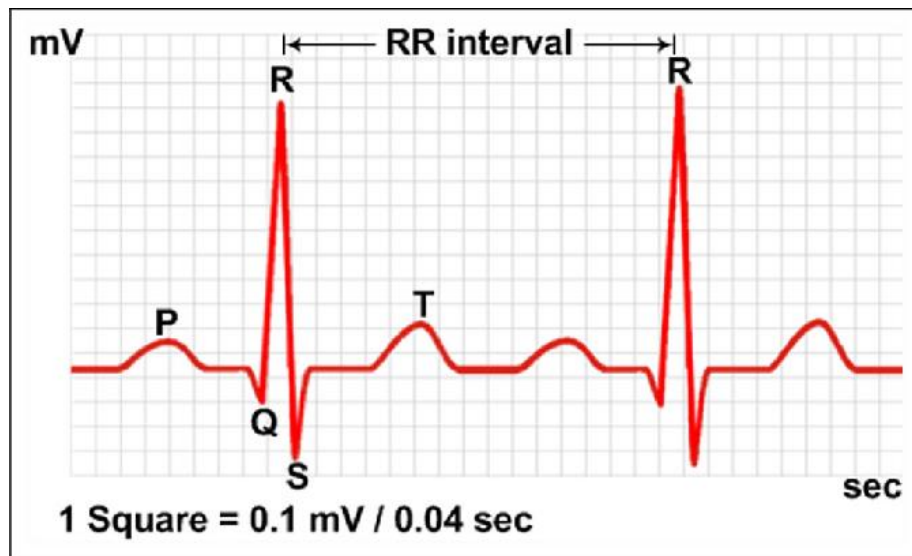
Figure 1.1 Illustration of QRS complexes[1] and RR interval of ECG signals (Lin, Yang, Zhou

& Wu, 2018, p. 5).

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[1] P symbolizes atrial depolarization, QRS complex is ventricular depolarization, and T symbolizes papillary
muscle repolarization (Medicine-on-line.com, 2019).

Moreover, statistical indices to describe HRV have been created by means of analyzing the NN intervals. The SDNN, which is the standard deviation of the NN intervals, is, actually, the square root of the variance and reflects all the cyclic components, which determine variability in the period of recording (Task Force of the ESC and the NASPE, 1996). The SDNN is more accurate when obtained from signals recorded for over 24 hours than during short periods resulting from usual biofeedback sessions (Shaffer & Ginsberg, 2017).

Other popular time-domain methods are the standard deviation of the average NN intervals for each of the 5-minute segments recorded over a period of 24 hours (SDANN). SDANN and SDNN cannot be used interchangeably as they are different ways of analyzing the HRV signal (NB While SDANN analyses 5-minute segments over a long period, SDNN is an index for an entire 24-hour time series [Kuusela, 2013]). The mean number of times per hour in which the change in consecutive normal sinus (NN) intervals exceeds 50 milliseconds is another index known as pNN50. This index is closely associated with parasympathetic activity and correlates well with such indices as RMSSD (i.e. root mean square of successive differences) and HF (i.e. high frequency) power, which will be discussed later on.

The RMSSD is derived by calculating every single successive difference between heartbeats in milliseconds, then, square these differences and calculate the average of those; then, the square root of the total is computed to give the RMSSD. This index is also used for assessing vagally-mediated changes in heart rate variability (Shaffer & Ginsberg, 2017). Interestingly, researchers have suggested that RMSSD can be even

used for ultra-short-term recordings, such as 10 seconds (Salahuddin, Cho, Jeong & Kim, 2007), 30 seconds (Baek, Cho, Cho & Woo, 2015) and 60 seconds (Esco & Flatt, 2014). The parasympathetic nervous system appears to influence the RMSSD more than the SDNN, whereas the RMSSD does not seem to be affected by respiration as much as the RSA (Hill & Siebenbrock, 2009).

Furthermore, the index of HR Max-HR Min is often used by researchers (e.g. Shaffer & Ginsberg, 2017) and refers to the average difference between the maximum and minimum recorded heart rate in each respiratory cycle; however, this index is particularly sensitive to breathing rate and should not be considered as reliable for recordings shorter than 2 minutes. As slower breathing paces can result in higher RSA's without having affected significantly the vagal tone, this index is mainly indexing RSA amplitude and not vagal activity.

Geometrical methods. Furthermore, N-to-N intervals, can be also used in a different way to geometrically give information about the HRV under investigation. Such characteristics as the sample density distribution of the NN intervals, or the sample density distribution of differences between adjacent NN intervals can provide a practitioner with useful information (Task Force of the ESC and the NASPE, 1997). The most popular geometrical methods for analyzing HRV are the HRV triangular index (HTI) and the triangular interpolation of the NN interval histogram (TINN), with the former calculating the density of the RR interval histogram divided by its height, and the latter being the baseline width of the distribution measured as a base of a triangle

(Task Force of the ESC and the NASPE, 1996). Both of these methods are based on 24-hour recordings.

Frequency-domain methods. In addition to the time-domain methods for measuring HRV, different spectral methods have been discussed for the same reason since the 1960s (Kay & Marple, 1981). Power spectral density (PSD) shows at which frequency variations are strong and which frequency variations are weak at (Task Force of the ESC and the NASPE, 1996). Mathematical algorithms (e.g. Fast Fourier Transform (FFT)) can lead to a more detailed spectral analysis, by distinctly separating different frequency bands which depict different functionalities of the autonomous nervous system (Task Force of the ESC and the NASPE, 1996).

HRV frequency bandwidths: In terms of frequency bandwidths, HRV can be separated into ultralow frequency (ULF), very low frequency (VLF), low frequency (LF) and high frequency (HF) (Shaffer & Ginsberg, 2017).

The ultralow frequency band, which refers to frequencies lower than 0.003 Hz, can be useful only if it has been derived from long recordings (i.e. at least 24-hour recordings) and is highly correlated with SDANN (Bigger, Fleiss, Steinman, Rolnitzky, Kleiger, & Rottman, 1992). Although circadian rhythms appear to be the primary driving force of this frequency band, the extent to which the two different branches of the ANS contribute to this frequency has not been clear yet (Shaffer, McCraty & Zerr, 2014). As ULF is derived from 24-hour recordings, it may give information about certain

psychiatric disorders which are associated with specific circadian HR patterns
(Stampfer, 2013; Stampfer & Dimmitt, 2013).

The very low frequency band ([VLF] 0.0033-0.04 Hz) is the band that can be
useful even if it is derived from short recordings (i.e. 5 minutes), though, it can be more
precise when it is obtained from 24-hour recordings. VLF has also been connected
powerfully with all-cause mortality (Tsuji**,** Venditti**,** Manders**,** Evans**,** Larson**,** Feldman, &
Levy, 1994; Tsuji, Larson, Venditti, Manders, Evans, Feldman, & Levy, 1996; Hadase,
Azuma, Zen, Asada, Kawasaki, Kamitani, Kawasaki, Sugihara, & Matsubara, 2004;
Schmidt, Muller-Werdan, Hoffmann, Francis, Piepoli, Rauchhaus, Prondzinsky,
Loppnow, Buerke, Hoyer, & Werdan, 2005). Very low frequency has also been
associated with arrhythmic death (Bigger et al.,1992), post-traumatic stress disorder
(Shah, Lampert, Goldberg, Veledar, Bremner, & Vaccarino, 2013), whereas low value in
this frequency band is linked with high inflammation in various studies (Carney,
Freedland, Stein, Miller, Steinmeyer, Rich, & Duntley, 2007; Lampert, Bremner, Su,
Miller, Lee, Cheema, Goldberg & Vaccarino, 2008) and low levels of testosterone
(Theorell, Liljeholm-Johansson, Bjork, & Ericson, 2007). While the SNS has been found
to affect the amplitude and frequency of the VLF oscillations (Shaffer et al. , 2014), and
the heart's intrinsic nervous system contributes to the VLF rhythm, there is uncertainty
as to the physiological mechanisms determining activity in this frequency band (Kleiger,
Stein, & Bigger, 2005).VLF may also be triggered by thermoregulatory, renin-
angiotensin, and endothelial influences on the heart (Akselrod, Gordon, Ubel, Shannon,
Barger, & Cohen, 1981; Claydon & Krassioukov, 2008), whereas it has been found that

PNS activity contributes to VLF since inhibition of PNS minimizes VLF (Taylor, Carr,

Myers & Eckberg, 1998), in contrast with the SNS activity in which a blockade may not

affect VLF (Task Force of the ESC and the NASPE, 1996; Berntson, Bigger, Eckberg,

Grossman, Kaufmann, Malik, Nagaraja, Porges, Saul, Stone, & van der Molen, 1997).

However, as the physiological explanation of VLF is not crystal-clear to researchers

(Task Force of the ESC and the NASPE, 1996), interpreting VLF in short-term recordings

is usually avoided because it may lead to erroneous conclusions.

The low frequency band ([LF] 0.04-0.15 Hz) gives meaningful information once

it is derived from a recording of – at least- 2 minutes (Shaffer et al., 2014) and has been

strongly associated with baroreceptor activity at rest; hence, researchers often refer to

it as baroreceptor range (McCraty & Shaffer, 2015). The low frequency range is thought

to be affected both by sympathetic and parasympathetic activity; researchers also

claim that it may also be affected by blood pressure through baroreceptors (Task Force

of the ESC and the NASPE, 1996; Akselrod et al., 1981; Berntson, Cacioppo, &

Grossman, 2007; Lehrer, 2007), or by the PNS alone (Reyes del Paso, Langewitz,

Mulder, Van Roon, & Duschek, 2013), or even by baroreflex activity alone (Goldstein,

Bentho, Park, & Sharabi, 2011). It is worth pointing out that vagally-mediated

influences, which result from respiration, may be present in the lower frequency band

if the breathing rate is below 8.5 breaths per minute (Brown, Beightol, Koh, & Eckberg,

1985); this is attributed to the fact that when an individual breathes at a slow rate,

heart rhythm oscillations, caused by vagal activity, may enter the LF band (Ahmed,

Harness & Mearns, 1982; Lehrer, Vaschillo, Vaschillo, Eckberg, Edelberg, Shih, Lin,

Kuusela, Tahvanainen, & Hamer, 2003; Tiller et al., 1996). Last but not least, Shaffer et

al. (2014) also claimed that, at rest, low frequency does not reflect cardiac sympathetic

innervation; rather, it reflects baroreflex activity.

The high frequency band ([HF 0.15-0.40 Hz], or increased for children and

infants who breathe faster, [Finley & Nugent, 1995]) depicts mainly parasympathetic

activity and is influenced by the respiration rate (e.g. Frenneaux, 2004). It is often

called respiratory band because it follows the fluctuations of the HR that occur during

inhalation and exhalation (as it happens with the RSA). In fact, the RSA phenomenon

refers to the acceleration of the heart rate which occurs during inhalation and results

from an inhibition of the vagal outflow, whereas this outflow is restored as an

individual breathes out and slows down HR by releasing acetylcholine (Eckberg &

Eckberg, 1982). It is worth pointing out that low high frequency has been linked with

increased stress, anxiety, worry or even panic; HF has also been observed to be

augmented during the night and decrease during the day (McCraty & Shaffer, 2015).

Moreover, proper vagal functioning leads to a better state of autonomic regulation

which is needed to keep cardiovascular health at a good level (Thayer, Yamamoto, &

Brosschot, 2010).

Composite frequency-domain indices. Other indices often used by experts in this

field are (a) the ratio of low frequency to high frequency (LF/HF), which reflects

balance between the sympathetic and parasympathetic branches of the ANS and

usually ranges from 1 to 2, and (b) the natural logarithm of the HF (lnHF), which under

controlled conditions, is thought to estimate vagal tone (Egizio, Eddy, Robinson, & Jennings, 2011).

Additional HRV metrics. Other methods of measuring HRV include rhythm pattern analysis (Schechtman, Kluge, & Harper, 1988; Courmel, Hermida, Wennerblom, Leenhardt, Maison-Blanche, & Cauchemez, 1991) which refers to such analyses as the interval spectrum and spectrum of counts; in fact, these are methods used to link HRV with the variability of other physiological measurements. Additionally, non-linear measurements have also been used to explain aspects of HRV that cannot be described by linear methods, as the mechanisms regulating HRV appear to be complicated and are sometimes unpredictable (e.g. Poincare plot, approximate entropy, sample entropy, detrended fluctuation analysis etc. [Stein & Reddy, 2012]).

Therefore, it appears that HRV can be measured in different ways by researchers, with each way examining HRV from a different perspective and answering different types of research questions. The software used in this research project uses time-domain and frequency-domain methods to analyze HRV; consequently, these indices will be further used in the resulting statistical analyses.

1.5 Heart Rate Variability Training

As discussed above, heart rate variability has been associated with anxiety, with higher levels of HRV resulting in lowered anxiety. It appears that a strategy leading to higher levels of HRV could result in reduced anxiety and stress and, as a result of this,

in enhanced performance. This chapter discusses how this can be achieved by means of stimulating the baroreflex system**.**

1.5.1 The effects of rhythmical stimulation on the baroreflex system.

Ringwood and Malpas (2001) suggested that the baroreflex system can be considered as a "closed-loop" system, in the sense that it is a control system with feedback. The baroreflex system's main function is to protect the human body from acute blood pressure changes; therefore, increases or decreases in blood pressure (BP) lead to decreases or increases in heart rate (HR) respectively. As the human cardiovascular system has characteristics of a natural vibration frequency or resonance (Sakakibara, Kaneda & Oikawa, 2019), research has expanded on the oscillatory nature of the baroreflex system describing the three different cardiovascular functions connected via the baroreflex branches; the baroreflex system is linked to HR via the HR baroreflex branch and with vascular tone (VT) via the VT baroreflex branch (Christou, Jones, & Seals, 2003; Jones, Christou, Jordan, & Seals, 2003; Jordan, Tank, Shannon, Diedrich, Lipp, Schroder, Arnold, Sharma, Biaggioni, Robertson, & Luft, 2002; Just, Wittmann, Nafz, Wagner, Ehmke, Kirchheim, & Persson, 1994). The HR and VT baroreflex branches are both considered as closed-loop systems with negative feedback which respond to blood pressure fluctuations (Vaschillo, Vaschillo, Pandina, & Bates, 2011); in other words, HR and VT slow down when the blood pressure increases. However, due to the delay in the closed-loop baroreflex system, any shift in the blood pressure is not compensated immediately. A change in blood pressure triggers the baroreflex system which, after the inherent delay, eliminates the BP shift. This elimination of the BP shift

elicits a new baroreflex response, which, again after the delay, results in another BP

shift of lower magnitude this time and so forth, until the magnitude of the new BP shift

equals to zero. As Vaschillo et al. (2011, p. 928) outlines:

> " *Thus, any BP shift will evoke a faded BP oscillation at a resonance frequency*
>
> *that depends on the time of the delay."*

According to Hammer and Saul (2005), the delay in this closed-loop control

system, giving negative feedback, results in the baroreflex system becoming a

resonance system with oscillations whose frequency equals the magnitude of the delay

multiplied by 2; thus, in the case of humans, this resonance frequency of the 10-second

oscillation period (i. e. 5-second delay multiplied by two) equals to 0.1Hz (i. e. six

oscillations per minute) (Deboer et al., 1987; Saul et al., 1991; Vaschillo et al., 2002).

These oscillations can be elicited with any type of rhythmical stimulation of the

cardiovascular system (Vaschillo, Vaschillo & Lehrer, 2006); however, an easy way to

do this is by regulating the respiratory pattern of an individual, since inhalation induces

an acceleration of the heart rate whereas exhalation causes a deceleration of it, a

phenomenon also discussed earlier in this chapter and is known as respiratory sinus

arrhythmia (RSA). The acceleration and deceleration of the heart rate is caused by the

tenth cranial nerve, the vagus nerve (Frazier, Strauss & Steinhauer, 2004). In brief,

during inhalation vagal activity is reduced and the heart rate accelerates, while during

exhalation the activity of the vagus nerve is reinstated which results in the heart rate

deceleration.

Therefore, HRV can be trained and can produce beneficial effects in different

contexts, as discussed below.

1.5.2 HRV biofeedback and management of medical conditions. Low HRV has

been correlated with different physical and mental health problems in the last years.

Various conditions/disorders have been proved to benefit from HRV biofeedback,

probably because of the stimulation of the vagus nerve which also has effects on the

central nervous system, and/or the autonomic homeostasis caused by HRV training

and/ or because there is an increased stimulation of the cholinergic anti-inflammatory

pathway (Gevirtz, 2013); such conditions are asthma (Lehrer, Carr, Smetankine,

Vaschillo, Peper, Porges, Edelberg, Hammer, & Hochron, 1997; Lehrer, Vaschillo,

Vaschillo, Lu, Scardella, Siddique, & Habib, 2004; Lehrer et al. , 2006), coronary artery

disease (Cowan, Pike & Budzynski, 2001; Del Pozo, Gevirtz, Scher, & Guarneri, 2004),

coronary heart disease (Nolan, Kamath, Floras, Stanley, Pang, Picton, & Young, 2005),

chronic obstructive pulmonary disease (Giardino, Chan, & Borson, 2004), fibromyalgia

(Hassett, Radvanski, Vaschillo, Vaschillo, Sigal, Karavidas, Buyske, & Lehrer, 2007),

heart failure (Swanson, Gevitz, Brown, Spira, Guarneri, & Stoletniy, 2009), major

depressive disorder (Karavidas, Lehrer, Vaschillo, Vaschillo, Marin, Buyske, Malinovsky,

Radvanski, & Hassett, 2007), posttraumatic stress disorder (Zucker, Samuelson,

Muench, Greenberg & Gevirtz, 2009), hypertension/ normotension (Overhaus, Ruddel,

Curio, Mussgay, & Scholz, 2003), whereas a study with ADHD patients, which combined

neurofeedback with HRV biofeedback as an intervention, showed that adults and

children improved in ADHD- related parameters (Groeneveld, Mennenga, Heidelberg,

Martin, Tittle, Meeuwsen, Walker & White, 2019). As effectiveness of HRV biofeedback has emerged in all of the above studies, further investigation of the effects of HRV biofeedback is needed to provide more robust evidence on its effects on such medical conditions/disorders (Wheat &Larkin, 2010). Besides, HRV biofeedback has been shown to be beneficial in the case of diseased people, though, its effects on healthy individuals have not been thoroughly researched (Deschodt-Arsac, Lalanne, Spiluttini, Bertin & Arsac, 2018).

1.5.3 Respiratory sinus arrhythmia (RSA) and emotions. Research (Chernigovskaya, Vaschillo, Petrash, & Rusanovskii, 1991) has shown that the amplitude of respiratory sinus arrhythmia (RSA), which is a component of HRV, can be increased with slow-paced breathing; this is, actually, a method used for treating stress-related problems and autonomic dysfunction. In addition, it has been claimed that our facial displays of emotion also relate to RSA; studies discussing structural evidence (Porges, 1995; Porges, Doussard-Roosevelt & Maita, 1994) associated RSA with emotions; on the one hand, the larynx, which is an important means of vocalizing emotional state is innervated by efferent fibres from the nucleus ambiguus, and, on the other, the nucleus ambiguus is thought to be connected with the facial and trigeminal nerves, by means of afferent fibres (NB Facial expressions and vocalization are emotion behaviours which are facilitated by the facial and trigeminal nerves). In essence, a covariation of RSA and increased vagal influence on the heart is observed when spontaneous social behaviours are exhibited, as well as an inter-relation of a

vagal withdrawal, increased sympathetic influences and the mobilization of flight-and-

fight behaviours (Porges, 2008).

What Chernigovskaya et al. (1991) claimed was also supported by research

conducted, later on, by Lehrer, Smetankin and Potapova (2000) and Lehrer, Vaschillo

and Vaschillo (2000). Specifically, it was supported that the amplitude of RSA was

found to be maximized at the frequency of six breaths per minute (i.e. 0.1 Hz) and be

minimized at the frequency of three breaths per minute (i.e. 0.05 Hz) (Vaschillo, Lehrer,

Rishe, & Konstantinov , 2002). In the above study of Vaschillo et al. (2002), participants

used biofeedback to regulate their respiration rate and achieve maximal (or minimal)

amplitudes of RSA. As described earlier in this chapter, the frequency band from 0.05

to 0.15 Hz has been denoted as "low frequency" (LF) HRV, and is influenced by both

the parasympathetic and sympathetic systems (Berntson et al., 1997), while it appears

to be more closely linked to the baroreflex (BR) activity than HRV in other frequency

bands (Bernardi, Leuzzi, Radaelli, Passino, Johnston, & Sleight, 1994). Things are much

more complicated in the case of very low frequency (VLF), however, due to the various

factors determining activity within this specific frequency band; this makes it difficult

to conduct analyses and reach concrete conclusions based on VLF, whereas high

frequency (HF) depicts vagal or parasympathetic modulation of heart rate and cardiac

parasympathetic influence due to RSA. As such, since resonant breathing occurs within

the LF frequency band (Vaschillo et al., 2002) and low-frequency periodicities are

produced by baroreflex feedback (Papaioannou, 2007), research focusing on HRV

training has been focusing on LF periodicities with a view to achieving better flexibility of autonomic functioning.

1.5.4 Heart rate variability training and emotions. The positive effects of biofeedback (Kessler, Soukup, Davis, Foster, Wilkey, Van Rompay, & Eisenberg, 2001) and, especially, heart rate variability training (e.g. Lehrer & Gevirtz, 2014) on stress and anxiety were discussed and further investigated by researchers. As already discussed, low HRV has been associated with many different clinical conditions (e.g. Hofmann, Moscovitch, Litz, Kim, Davis, & Pizzagalli, 2005), while high resting HRV has been linked to good self-regulation (e.g. Khodik, 2013). Therefore, it appears that HRV training could be used as an index of self-regulation (Segerstrom & Nes, 2007), whereas it may also be used as an effective way for helping individuals regulate their physiological functioning by means of breathing training (Khazan, 2013). HRV biofeedback has also appeared in a few qualitative studies as a strategy for reducing stress and anxiety (Futterman & Shapiro, 1986; Gevirtz, 2013; Tabachnick, 2015), whereas conditions like generalized anxiety disorder and post-traumatic stress disorder have also been thought to be effectively treated by means of HRV biofeedback (e.g. Kemp, Quintana, Felmingham, Matthews, & Jelinek, 2012; Zucker et al. , 2009), since it was also shown that HRV training could be used for improving self-regulatory capacities (Lehrer & Gevirtz, 2014). In addition, a meta-analytic study (Goesslt, Curtisst & Hofmann, 2017) focusing on 24 articles relevant to HRVT and stress, showed that HRV training is a very promising intervention for reducing anxiety and stress and is becoming more and more attractive especially with the advancements of technology in this field. A study

conducted with twelve sports students showed that even short-lasting heart rate variability training sessions have positive effects on young athletes' stress levels in view of university examinations (Deschodt-Arsac et al., 2018). However, HRV training or any other biofeedback-based intervention should not be considered as a standalone intervention for anxiety in children and adolescents when there are long-term physical conditions; further research using different modalities or together with other psychotherapeutic or pharmacological therapies designed with the appropriate methodology may provide more robust evidence on the use of biofeedback in such cases (Thabrew, Ruppeldt, & Sollers III, 2018).

Furthermore, as maximization of RSA has been related to activity within the low-frequency (LF) frequency band of HRV, and low-frequency periodicities have been associated with better flexibility of autonomic functioning, researchers claim that better control of sympathetic and parasympathetic activities can be achieved within the LF bandwidth (Lehrer et al. , 2000; Vaschillo et al. , 2002). In other words, by regulating one's breathing pattern, individuals can achieve a better state of emotional control. This better flexibility of the ANS, which results in better emotional control, can be accomplished in approximately 10 half-hour sessions of HRVT, as previous literature suggests (Lehrer et al., 2000), whereas the approximate breathing frequency for this training is around 6 breaths per minute, which is a frequency within the LF bandwidth.

1.5.5 HRV training in sport. Apart from the various applications in a clinical context for the treatment and/or alleviation of various conditions, HRV training has

been used and researched in performance as well as sport, for performance-enhancement-related purposes.

The benefits of biofeedback were investigated in a study with ballroom and Latin dancers (Raymond, Sajid, Parkinson & Gruzelier, 2005). Different types of biofeedback were used in this study, as the participants were divided into three groups, with one group receiving EEG biofeedback (i.e. neurofeedback) on the levels of their alpha and theta activity; the second group practiced HRV training following Lehrer et al.'s (2000) protocol, whereas the third group acted as a control group receiving no intervention. That was a study with a relatively small sample size (i.e. a few of the participants dropped out and the study ended up with 18 participants), and, as a result of this, any conclusion should be discussed with caution. The results showed the beneficial effects of the two different types of biofeedback on the dancers, with each of the different types of biofeedback having a different influence on the participants; as such, the alpha/ theta EEG training appeared to have positively affected the "timing" dimension of the dance assessment, whereas the HRV biofeedback training had a positive effect on "technicality". The dance performers were assessed by means of a customized scale consisting of the following dimensions: technicality, musicality, timing, partnering skill, performing flair and overall execution. Based on these results, and given that HRV has been associated with smart social gesture and expression (Porges, 2003), the authors of this study (Raymond et al., 2005) claimed that high HRV may result in improvement in dance performance, given that it (i.e. HRV) has been associated with emotional reactivity and lowered aggression.

The effects of HRV biofeedback training on a golfer's physiology, mood and sport performance were also investigated in a case study with a 14-year-old male golfer (Lagos, Vaschillo, Vaschillo, Lehrer, Bates, & Pandina, 2008). In this study, the standard protocol of Lehrer et al. (2000) was used with the golfer; so, there were 10 weekly lab-based sessions, whereas the golfer was asked to practice HRV training at home, twice daily for 20 minutes each time. The results of this study showed that there were large acute and chronic effects on indices of autonomic function (e.g. increase in low frequency, increase in total HRV) which followed HRV biofeedback training. So, Lagos et al. (2008) concluded that HRV biofeedback may have helped the golfer improve his ability to cope with stress and, also, helped him increase the probability that he performs at an optimal level during a competition. Of course, further investigation is needed in order to be able to generalize these findings. Another case study was conducted with another golfer using again the original protocol of Lehrer et al. (2000) (Lagos, Vaschillo, Vaschillo, Lehrer, Bates, & Pandina, 2011); this study was conducted in a virtual reality golf center where the golfer was asked to practice resonant breathing frequency during golf performance. Psychometric and physiological measures were taken before and after the HRV biofeedback training, while golf performance was recorded in the virtual reality environment. The measures taken in this study detected changes in the cardiovascular indices after the fourth session of HRV biofeedback; however, it was not until after the 10th lab-based session that great psychological, physiological and performance-related changes were recorded. Lagos et al. (2011) concluded that HRV training skills can be acquired as early as after the fourth

HRV biofeedback session but desirable psychological and performance-related effects can be achieved after a longer engagement in regular practice of HRV training. However, as this is a case study, such conclusions cannot be generalized, and further research is needed on that.

Furthermore, in a study conducted with university basketball players, the athletes were divided into control, placebo and experimental groups (Paul, Garg & Sandhu, 2012). The experimental group went through ten 20-minute HRV biofeedback training sessions, for 10 consecutive days (NB. a modified version of Lehrer et al.'s (2000) protocol). The placebo group watched 10-minute motivational clips for 10 consecutive days and the control group received no intervention. The participants were assessed at Session 1, Session 10 and one month after the completion of the protocol (1month follow-up) in such parameters as HRV, response time (i.e. reaction time plus movement time), respiration rate, concentration and basketball shooting performance. The results of this study showed that the experimental group demonstrated an improved response time (i.e. reaction and movement time), and heightened concentration levels which could be attributed to the balance achieved between the two branches of the ANS (Paul et al., 2012). It was maintained in this study, that due to this balance, the participants reached a state of mental readiness that helped them gain control over their ability to focus on the task at hand which, in turn, results in maximal performance, as the interaction between an efficient level of psychomotor skills (i.e. in this case, response time) and a good level of psychological qualities (concentration) can lead to an athlete reaching an optimal state of composure

and mental readiness. In another research paper referring to the same cohort of basketball players, the assessed psychological variable was anxiety; additionally, HRV and RR intervals were also measured physiologically, whereas the players' dribbling ability, pass accuracy and perimeter throws for 3 minutes were also assessed (Paul & Garg, 2012). In this research paper, it was concluded that basketball players managed to find their 'zone of excellence' by practicing HRV biofeedback training. Their level of self-efficacy was elevated after the HRV biofeedback training, which was also the case in the follow-up measurements after 1 month. Paul and Garg (2012) then suggested that HRV training may have a profound influence on psychosocial processes which, in turn, may serve as an index of sport performance and, that, this psychophysiological strategy may be used for emotional and cognitive restructuring.

Heart rate variability training has been used with volleyball players as well (Tanis, 2012). Fourteen female collegiate volleyball players participated in a study where the HRV training protocol suggested that they attend a weekly HRV training session for 6 consecutive weeks, while they keep a diary on their affective control and progress in the practice of HRV training. The practitioner in this study (Tanis, 2012) adopted Culbert, Martin and McCraty's (2004) suggestion on psychophysiological coherence and self-regulation skills as well as Blumenstein, Bar-Eli and Tenenbaum's (1997) five-step approach to mental training incorporating biofeedback, and conducted a quasi-experimental study the results of which showed that the participants were able to self-regulate when they wanted to. However, the quantitative analyses did not yield statistically significant results; in order words, this means that the idea that athletic

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performance may be improved with HRV training was not supported by this study. Tanis (2012) attributed that to the fact that the volleyball players' performance was above average before and after the intervention and, as a result of this, small improvements in performance, which might be very important in sport, were not significant statistically.

Finally, a study with track/ long-distance athletes was conducted using the original protocol of Lehrer et al. (2000) (Choudhary, Trivedi & Choudhary, 2016). Participants were divided into two groups, namely, an experimental group that went through the protocol and a control group that did not do anything additional apart from their regular training. Skin conductance, HRV variables (e.g. LF/HF), VO$_2$Max and sport performance were assessed before and after the 10-week protocol for both the experimental and the control groups. The results of this study indicated that after the 10-week intervention, there was significant improvement in the skin conductance levels but also VO$_2$Max of the participants in the experimental group. Additionally, LF HRV was increased within the HRV training session which is an indication of positive oscillation effects in the cardiovascular system of the participants. It was concluded that HRV training may have helped track athletes to cope with stress effectively and also helped them to perform at an optimal level during the study (Choudhary et al., 2016).

1.6 The Focus of this Research Project

There has been limited research into HRV training in sport and the present programme of research aims to add to this sparse literature. In particular, there has also been limited research exploring whether HRV training can lead to a better performance while being under pressure, or a better level of stress-related indices such as EMG, heart rate, electrodermal activity, skin temperature, and salivary cortisol and a- amylase.

The present programme of research initially aims to address previous research limitations to more robustly assess the notion that HRV training is positively related to emotional control, which, from an applied practitioner's standpoint, is mainly related to stress and anger management. In this thesis, it is possible to examine the relationships between HRV training and emotional control using HRV training protocols discussed and validated in previous studies (e.g. Lehrer et al., 2000; Lehrer, Vaschillo, Zucker, Graves, Katsamanis, Aviles, & Wamboldt, 2013).

Previous literature in this field suggests that HRVT results in better stress management, but this assertion has not been tested within a context of male adolescent athletes in the Middle East. If, as predicted, HRVT is related to better emotional control, ways in which HRVT should be included in the athletes' weekly routine should be promoted. Therefore, secondly this thesis will assess the practical aspects of a newly-introduced 5-week protocol proposed with a view to teaching an athlete a skill they can use as and when they want before a psychologically important competition.

This thesis extends previous research (e.g. Lehrer et al. 2000; Lehrer et al., 2013) by introducing HRVT to athletes in a sport academy in the Middle East and examines the effectiveness of this protocol. Gaining an understanding of how individuals respond psycho(physio)logically after a single session of HRVT, an HRVT training programme, and what aspects of HRVT could be improved, will be valuable for athletes and sport psychologists. By formulating strategies (via HRVT) to help an individual control their emotions more efficiently, it may be possible to help an athlete approach an important event with more probabilities to perform well and, therefore, succeed.

1.6.1 Aims. This thesis builds on previous research examining the effects of HRVT on emotional control, and how HRVT can be used for the better preparation of an athlete for competition. It also adds to previous literature exploring how HRVT affects different stress-related psycho(physio)logical and biochemical indices. The main aims of this thesis are:

1. Investigate the acute effects of a single session of HRVT on the participants' performance under pressure.

2. Explore the acute effects of a single session of HRVT on the participants' psychophysiological responses and biochemical markers of stress.

3. Explore the long-lasting effects of an HRV training programme on participants' psychophysiological responses and on biochemical markers of stress.

4. Investigate the participants' reflections on their experience of an HRVT protocol and make recommendations for the improvement of the programme.

5. Make recommendations on whether HRVT can be used to help athletes prepare

   for competition by reducing levels of stress and how this can be achieved.

CHAPTER 2- HEART RATE VARIABILITY TRAINING AND EMOTIONAL CONTROL:

ACUTE EFFECTS

2.1 INTRODUCTION

This study addresses aim 1 of the thesis; acute effects of a single HRVT session

on performance under pressure will be investigated by means of a reactive stress

tolerance test. The construct of reactive stress tolerance has appeared with different

descriptions in the literature, as, for example, in a study conducted within the field of

traffic psychology to investigate criterion validity of test batteries, it is referred to as

"…resilience of attention and reaction speed under conditions of sensory stress."

(Risser, Chaloupka, Grundler, Sommer, Hausler, & Kaufmann, 2008, p. 152), or in

another study, in the same field, it was described as complex choice reaction (Sommer,

Heidinger, Arendasy, Schauer, Schmitz-Gielsdorf, & Hausler, 2010), or researchers have

even referred to it as stress tolerance and reaction speed in the presence of rapidly

incoming stimuli, in a study dealing with personnel selection (Vorster, Pires & Taylor,

2011).

HRV biofeedback has been found to have positive effects on various medical

conditions, as explained in the literature review section, but also on stress and anxiety.

This is because HRV biofeedback contributes to the increase of the amplitude of the

RSA and, eventually, of the HRV. As low HRV has been linked with physical and

psychological disorders, such a strategy aiming to enhance HRV would also result in the

alleviation of such conditions (Wheat & Larkin, 2010). In psychophysiology and

behavioral medicine, results of HRV biofeedback are promising, indicating that there might be acute improvements in HRV and baroreflex gain observed during the training sessions (Wheat & Latkin, 2010). It is exploring the potential effects of HRVT on performance that is the focus of the present chapter.

2.1.1 Immediate Effects of HRV Training

Breathing at one's resonant breathing frequency, thus, stimulating HRV, had been already proved to result in the amplification of vagal response, which was presented (Lehrer et al., 2003) as a way to efficiently train the baroreflex system.

In the same study investigating HRVT as a method for improving pulmonary function and vagal baroreflex gain (Lehrer et al., 2003), acute increases in low frequency and total spectrum HRV were observed along with strong long-term effects on resting baroreflex gain and pulmonary function whereas it was also observed that the participants' baroreflex system became more responsive, as they went on repeating HRV training over time, irrespective of breathing rate and pattern which led the researchers, Lehrer et al. (2003), to conclude that the human baroreflex system acquires some type of plasticity by means of repetition, which makes it intrinsically more responsive as the training continues over time.

Immediate effects of HRV training on HRV and baroreflex gain were also detected in a study conducted with asthmatic patients; the researchers in this study (Lehrer et al., 2004) claimed that alleviation of the asthma symptoms could not be attributed to long-term effects caused by HRV training, and suggested that such

improvements may have resulted from long-lasting bronchodilation effects. In another study with asthmatic patients, it was pointed out that HRV training should be used as a complementary and not alternative treatment for asthma, as it cannot substitute inhaled steroid medication (Lehrer et al., 2018).

Hassett et al. (2007) pointed out that, while effects of a single session of HRV biofeedback were detected immediately on HRV parameters in their study, a delay was observed in such results as better blood pressure, baroreflex gain or any other therapeutic effects. Additionally, Hassett et al. (2007) detected a carry-over effect of the HRV intervention they tested; resting baroreflex gain was observed to be higher after the HRV biofeedback session compared to resting baroreflex gain before the session. Karavidas et al. (2007) also discussed acute HRV changes detected during their protocol to treat major depressive disorder by means of a 10-week HRV biofeedback protocol. They suggested that, while their data showed that there was a vagus nerve stimulation between the weekly sessions, such a stimulation was also detected within the HRV biofeedback sessions, thus, suggesting acute effects. Additionally, during the HRV training sessions, any increase in the LF amplitude was attributed to resonance effects reflecting both respiratory sinus arrhythmia but also baroreflex gain, which, in turn, suggests, that HRV training may result in beneficial effects on the vagus nerve activity, on the one hand, and on major depression, on the other; therefore, it appears that HRV training may be – potentially - an effective intervention used for major depression with lower risk and cost than the usual interventions.

In a study conducted with male participants employed in senior managerial positions, EEG changes were recorded after a single session of HRV training, which were associated with increased internal attention and relaxation during the HRV training session as well as after that (Prinsloo, Rauch, Karpul & Derman, 2013a). Not only was that one of the first studies which measured the effects of a single HRV training session on humans and, specifically, on the power recorded in the different EEG bandwidths, but also, Prinsloo et al. (2013a) reported that they recorded changes in EEG during and, also, immediately after an HRV training session which suggested that HRVT resulted in increased attention, increased relaxation and reduced anxiety both during and after the HRVT. It is worth mentioning that findings related to a better regulation of stress had also been found earlier (Sherlin, Muench, & Wyckoff, 2010); however, this study used a 15-minute intervention as opposed to the 20-minute HRV session used by Prinsloo et al. (2013a), whereas similar changes have been also reported in studies which used meditation as their intervention strategy (Chan, Han, & Cheung, 2008).

Moreover, an interesting differentiation between alert relaxation and sleepy relaxation was made to describe a relaxation state in which an individual experiences a diminished level of anxiety paired with maximized cognitive functioning (Prinsloo, Rauch, Lambert, Muench, Noakes, & Derman, 2011). Indeed, during their research, Prinsloo et al. (2011) found out that a 10-minute HRV training session resulted in better cognitive performance during a Stroop task, in better reaction times but also in a less-sleepy relaxation state. This is an important differentiation as it allows athletes to

include HRVT in their pre-competition routine, as a sleepy relaxation state would not be a desirable outcome just before a competition. This differentiation of relaxation states was also, later, adopted by Prinsloo et al. (2013a), who concluded that both augmented attention and increased relaxation were recorded during as well as after the session and, therefore, their participants had entered a state of less-sleepy alert relaxation as well. Last but not least, another study, conducted again with males holding senior managerial positions, showed that a 10-minute biofeedback-induced deep-breathing session using HRV biofeedback resulted in short-term carry-over effects modifying the participants' physiological responses within the 5 minutes of rest, making them, at the same time, reach a less-sleepy/more-alert relaxation state, and also, that those effects were detected, later, in lab-induced stress following the 5-min rest period as well (Prinsloo, Derman, Lambert, & Rauch, 2013b); Therefore, it appears that even a 10-minute HRVT session is enough to cause this state of alert relaxation as described by Prinsloo et al. (2011), Prinsloo et al. (2013a) and Prinsloo et al. (2013b).

2.1.2 HRV Training and Reactive Stress Tolerance

The possibility that one HRV training session may create acute positive effects is something that has been investigated by researchers; however, it is worth investigating further, as research findings, so far, have not created robust foundation based on which one can confidently use a single session and expect immediate effects. Psychological stress is a major issue in many different domains of life and has been the epicenter of discussion and research for many decades now. This study focuses on

exploring the acute effects of HRVT on performance in a reactive stress tolerance test

and addresses Aim 1 of the thesis.

The extent to which one can handle stress or not can be attributed to stress

tolerance (Bland, Melton, Welle, & Bigham, 2012). In the field of performance where

individuals have to deal with multiple incoming stimuli and respond correctly to all of

them, the concept of reactive stress tolerance has also been introduced:

> '…reactive stress tolerance is defined as the ability of an
> individual to react quickly and accurately in a situation where
> he or she is overstretched.'
>
> (Neuwirth & Benesch, as cited in Ong, 2017)

In the fitness industry, a study investigating the effects of a karate and fitness

training program on older adults used reactive stress tolerance as one of the constructs

measured along with other tests measuring cognitive functioning (Witte, Kropf, Darius,

Emmermacher, & Bockelmann, 2015). In this study, the karate and fitness training

program created significant improvements in different aspects of cognitive functioning

as well as in reactive stress tolerance and attention, which is a construct measured by

the reactive stress tolerance test, whereas the improvement of reactive stress

tolerance was attributed to that karate training may counteract the deterioration of

reactivity under stress for older adults (Witte et al, 2015).

Furthermore, in a study conducted within the sport domain, researchers tried

to identify differences between fencers and non-fencers in reactive stress tolerance,

which they defined as an individual's ability to give quick serial answers to continuously

changing stimuli, but also, they tried to measure differences as far as the participants'

personality characteristics were concerned (Patocs, Melia, Kovacs, Fozer-Selmeci,

Revesz, & Toth, 2016). The results of this study showed that the decision-making ability

of fencers was determined by two main personality factors, namely, determination and

self-congruence, which were found to be also associated with reactive stress tolerance

and the associated ability to react; these personality factors were also found to be

significantly linked with the decision-making ability of the fencers, which, in turn, can

be used to distinguish between elite and non-elite fencing athletes. Reactive stress

tolerance was found to be significantly higher in females than males, but also, in

athletes practicing open-skill sports, as well as athletes of higher competitive level

(Ong, 2017). While the different level of reactive stress tolerance in females compared

to males may be attributed to various factors (e.g. types of sports female athletes

practice in Singapore, culture, etc.), the fact that athletes of open-skill sports appear to

have better reactive stress tolerance is because they have been used to competing in

unpredictable environments, thus, having strengthened their ability to face new –

sometimes - highly demanding - pressure wise - situations. Additionally, athletes, who

are at a higher level, have developed a better level of mental toughness which is

related to stress tolerance and, as an extent of that, reactive stress tolerance (Ong,

2017).

2.1.3 The objectives of the Present Study

Aim 1 of the thesis refers to the exploration of acute effects of HRVT, as

practically meaningful findings in this area would directly inform practice. This would

extend the work of Prinsloo et al. (2011; 2013a; 2013b) as well, who showed the existence of some immediate effects after a single session of HRV but also it would contribute to the studies of Lehrer et al. (2003) and Karavidas et al. (2007) who showed that there were immediate effects on HRV within the sessions of their protocols; in the present study the investigation of the existence of such effects will be expanded in the sport domain and, specifically, in adolescent athletes who live in the Middle East.

In Study 1, the effect of a single session of HRV training on reactive stress tolerance is explored. This is important, as any psychophysiological strategy must have acute effects on the athletes' stress tolerance levels to a reasonable extent, so that they have a good reason to include it in their routine. In other words, this will give information on the extent to which there is an immediate impact on the participants' reactive stress tolerance after a 20-minute HRVT session. It is expected that the reactive stress tolerance in the post-intervention testing session of the experimental group will be significantly better (i.e. higher ratio of correct responses and/ or lower ratio of wrong responses) compared to the control group at the .05 level of significance.

2.2 METHOD

2.2.1 Participants

Forty-two participants were initially recruited for the study (ethnicity: 81% Middle-Eastern and 19% Caucasian). Participants were recruited in ASPIRE academy, and in Clement Fencing Academy; both academies are based in Doha, Qatar. They were eligible for inclusion if they (a) were student-athletes of ASPIRE academy or athletes of Clement Fencing Academy, (b) were healthy individuals without any physical or mental disability (i.e. were free from conditions that restrict physical activity), (c) had no history of cardiovascular or breathing (i.e. asthma) problems, (d) were aged between 11 and 18 years. Four participants were not able to complete all the steps of the study due to other commitments, and two athletes could not finish the taught HRV training protocol successfully. The mean age of the 36 participants who completed the study was 14.40 years (SD= 1.63). Fifteen of them (41.66%) were athletes of Clement Fencing Academy, whereas 21 (58.33%) were student-athletes of ASPIRE academy. All of the participants were male adolescents (100%) who practiced a sport regularly; 5 of the participants were athletes of table tennis (13.80%), 27 of them were athletes of fencing (75%), and 4 athletes were athletes of shooting (11.11%).

2.2.2 Design

The study was a crossover experimental design. Participants completed both experimental and control conditions. Initially, the recruited participants were randomly assigned to an experimental condition (E) or a control condition (C), and after a 2-week

break (i.e. wash-out period) participants completed the remaining condition. So, participants who had completed the experimental condition completed the control condition and participants who had completed the control condition completed the experimental procedure. The independent variable of the study (IV) was the different condition, whereas the dependent variable (DV) of the study was reactive stress tolerance.

2.2.3 Instruments

2.2.3.1 Reactive stress tolerance test. Reactive stress tolerance is measured by means of the Determination Test, which is a Vienna Test System-based test, a computerized platform of psychodiagnostics manufactured by Schuhfried GmbH (Schuhfried GmbH, 1996). The test requires that the respondent use their cognitive ability to distinguish different sounds, colours, and memorize relevant characteristics of stimulus configurations; the difficulty in this test is that the client is asked to respond to rapidly changing visual and acoustic stimuli in the way instructed during the practice phase of the test. The client responds to the relevant incoming stimuli by pressing one of the five round coloured buttons, with each of them corresponding to a visual stimulus displayed on the screen, or one of two rectangular coloured buttons, with each of them corresponding to a different pitch of an acoustic stimulus, or one of two foot pedals (i.e. right or left), with each of them corresponding to a white rectangular visually distinct field in the bottom left - and right- corners of the screen respectively. This specific test form is the adaptive mode, which allows the system to always follow the speed of the respondent. As a result of this, a different total number of stimuli may

be generated within the 4 minutes of the test, for each respondent. To control for the different number of stimuli generated in different test administrations, the total number of correct responses and the total number of wrong responses (i.e. incorrect plus omitted responses) have been divided by the total number of stimuli generated in each administration, which results in the two ratios used as reactive stress tolerance indices in the statistical analyses.

The total duration of the instruction/practice phase and the test itself is approximately 6 minutes (i.e. approximately 2 minutes for the instruction/practice phase and 4 minutes for the test).

2.2.3.2 Mindroom testing and training. The 'mindroom' is an ASPIRE laboratory used for integrated psychophysiological testing and training within a standardized environment. The ASPIRE mindroom is a four-station mindroom, with each of the stations consisting of an armchair, an audio headset, a laptop with biofeedback software installed in it, a biofeedback/neurofeedback encoder and sensors. The mindroom has a client area, where all the stations are placed, and a control room, divided from the client area by means of a glass partition; in the control room, practitioners can view the graphs and statistics of each session but, also, they can have full control of the clients' laptops. The audio headsets installed at each mindroom station allow practitioners to communicate with any of the clients using a microphone installed in the control room.

2.2.3.2.1 Biofeedback equipment.

The manufacturer of the biofeedback hardware and software used in this study is Thought Technology Ltd, which is based in Montreal/ Canada. The specifications of the hardware are explained below:

ProComp Infiniti encoder. This is an 8-channel, multi-modality encoder which is used for real-time computerized biofeedback and neurofeedback as well as data acquisition. The encoder is connected to a fiber optic cable which, in turn, is connected to the client's laptop by means of a USB module (ProComp Infiniti Manual, 2017).

Blood volume pulse (BVP) sensor. This is the sensor used for measuring/ monitoring heart rate in this study. Measurement with this sensor is based on photoplethysmography; the sensor, actually, monitors the change of the reflected light caused by blood flow. Each heartbeat results in more red light being reflected, which is recorded by the sensor, whereas less red light is reflected between pulses (Thought Technology Ltd., 2003). During this study, the BVP sensor was placed on the palmar surface of the non-dominant index fingertip of the clients. The type of BVP sensor used was BVP-Flex/ Pro. In this project, the BVP sensor was placed on the clients' non-dominant index finger.

Respiration sensor (RESP). A respiration sensor was used for monitoring the pace and pattern of abdominal respiration of the clients. This sensor has a velcro strap which is stretched around the client's abdomen and, actually, transforms the expansion or contraction of the abdominal area of the client to a rise or fall of the wave being displayed on the screen. The respiration sensor is placed slightly above the naval

area of the client. The type of respiration sensor used in this study was RESP- Flex/Pro (Physiology suite manual, v. 5.1).

Skin conductance sensor (SC). This is a sensor used to monitor conductance across the skin and has two fingerband electrodes which, in this project, were attached to the middle and small finger of the participants. Skin conductance is, actually, the ability of the skin to conduct electricity; real-time variations in conductance reflects activity of the sympathetic nervous system and are related to levels of stress. A relaxed state usually gives a conductance of approximately 2 μS (i.e. micro-Siemens), however, this varies according to the skin type of the client as well as environmental factors. The sensor used was the Skin Conductance Sensor- SA9309M, by Thought Technology Ltd. (Thought Technology's sensors and accessories manual, SA7511 Rev.4).

Skin temperature sensor (TEMP). This is, actually, a thermistor measuring surface temperature on the skin and, in this project, was placed on the non-dominant ring finger of the participants. The role of this sensor is to identify changes in the temperature of the client's hand as this reflects increased or decreased blood flow to the extremities which result from vasodilation or vasoconstriction respectively. The skin temperature sensor used was the Temperature Sensor-SA9310, manufactured by Thought Technology Ltd. (Thought Technology's sensors and accessories manual, SA7511 Rev.4).

EMG (electromyography) sensors. Surface electromyography (SEMG) was used, during this study, to monitor electrical activity on both of the clients' trapezii (i.e. left

and right trapezius) which results from muscle contraction when an individual is stressed. For this project, MyoScan-Pro (SA9401M) EMG sensors, manufactured by Thought Technology Ltd., were used. (Thought Technology's basics of surface electromyography, 2008).

Furthermore, all of these sensors were connected to different channels of the above-mentioned encoder.

Biograph Infiniti version 6.0.4. This is the main psychophysiology software on which the resonant breathing frequency assessment as well as the HRV training sessions were based. Biograph Infiniti has been designed and manufactured by Thought Technology Ltd. The first version of it was available in the market in 2003 whereas the version used for the present study was designed and distributed in 2014. Furthermore, the HRV training sessions were conducted using Thought Technology's Physiology Suite, which acts as a plug-in software within Biograph Infiniti, and, specifically, with its pre-designed screen labelled 'HRV-% power, pacer & animation'. Resonant breathing frequency was assessed by means of another software which acts as a plug-in to the Biograph Infiniti as well and has been created by Inna Khazan (2013) in collaboration with the Biofeedback Federation of Europe (BFE).

2.2.4 Procedure

Following the institutional ethical approval and the approval of the IRB committee of the ADLQ (i.e. Anti-Doping Lab Qatar), participants were recruited from both within ASPIRE academy from the sports of fencing, shooting and table tennis, and,

also, from Clement Fencing Academy. Information sheets describing the nature and purpose of the study were distributed to the coaches and the student-athletes. The athletes from the Clement Fencing Academy were not covered by the blanket parental consent form that parents sign in ASPIRE academy, at the beginning of each school year, and, as such, consent forms were distributed to the parents of the 15 athletes from that academy and assent forms along with information sheets were distributed to the athletes themselves.

Participants were assigned to either an experimental condition or a control condition. The procedure for both groups is shown in Appendix A.1 and a detailed description of each stage is discussed below:

2.2.4.1 Experimental condition (E). The participants who were initially assigned to the E condition had three main sessions with the main researcher.

2.2.4.1.1 Session 1E. In this session, participants took the reactive stress tolerance test. Each participant sat in in front of the screen and went through the whole test (i.e. instructions/practice phase and testing phase).

Then the participants followed the researcher to the mindroom where their knowledge about diaphragmatic/abdominal breathing was refreshed. The researcher asked them to practice abdominal breathing for a while so that he could make sure that everyone was breathing using their diaphragm. The researcher instructed the participants that during the assessment, they had to breathe in from the nose and breathe out through pursed lips.

The assessment took place in groups of four to five participants. After making

sure that all of the participants of a group could practice diaphragmatic breathing, the

researcher attached a respiration sensor and a blood volume pulse sensor to all of

them. Then, the resonance frequency assessment software was started and

participants read the instructions. During this assessment, the participants were asked

to follow a paced breathing exercise where they had to progressively slow down their

breathing from 7.0 breaths/minute to 4.5 breaths/minute, in order for the researcher

to identify the breathing rate that maximized their heart rate variability (Lehrer,

Vaschillo, & Vaschillo, 2000). From a starting point of 7.0 breaths/minute participants

slowed their breathing down by half a breath per minute in every subsequent step,

with each of these steps lasting for 90 seconds (i.e. in total, the assessment consisted

of six steps). After each step, a break was given to the participants who were told to

continue only if they felt they were ready for the next step. During this assessment, the

participants had to follow the breathing pacer displayed on their laptop's screen; when

the ball moved upwards, they had to breathe in, when it moved downwards they had

to breathe out, whereas they had to pause their breathing when the ball moved

horizontally.

The researcher watched the progress of the assessment from the control room

within the mindroom and could communicate with each of the participants

individually, or collectively, when this was necessary. At the end of this assessment, a

report was produced for each of the participants which provided the researcher with

four major HRV parameters for each of the breathing steps, and also information on

the extent to which the clients could follow the breathing pacer accurately or not. The

researcher reinforced participants to follow the pacer more accurately, had the latter

deviated from the instructed pace, which could be easily detected by the former.

The HRV parameters (i.e. SDNN, Pnn50, Heart rate [Max-Min] and LF percent of

total power) indicated which breathing pace helps each participant maximize their

HRV. This pace was identified as the resonant breathing frequency of each of the

participants.

2.2.4.1.2 Session 2E. Two weeks later, participants returned for their next

session. The first part of this session took place in the mindroom where participants,

being wired with the same sensors as in session 1E, were asked to go through a 20-

minute computerized exercise of paced breathing (i.e. HRV training session); this 20-

minute session took place at a mindroom station whose breathing pacer had been

previously configured by the researcher to run at the specific participant's resonant

breathing frequency. The researcher instructed the participants to breathe effortlessly

without taking any deep breaths; he also strongly advised them to let him know

immediately in case they felt dizzy or they experienced any difficulty in breathing.

Again, during this session the researcher had continuous visual contact with the

participants and could also monitor in real time the indices and graphical

representations of their respiration rate and pace as well as their heart rate. Using the

audiovisual equipment, he was in position to reinforce them to continue breathing at

their resonant breathing frequency, should they deviate - even slightly - from the pre-

set breathing pace. At the end of the 20-minute session, the researcher removed the

respiration and blood volume pulse sensors from the participants and asked them to

follow him to the area where the reactive stress tolerance tests were located,

approximately a 6-minute walk. Upon reaching these laboratories the researcher

allocated each of the participants to the same lab and testing station they had initially

completed the reactive stress tolerance test in Session 1E. After them completing the

reactive stress tolerance test, the participants were thanked, and an appointment was

arranged 2 weeks later for the next session.

2.2.4.1.3 Session 3E. In this session, the participants who had already gone

through sessions 1E and 2E, took part in a control condition, since the experimental

part of the study had been completed and a crossover had taken place after a washout

period of 2 weeks. At the beginning of this session, the researcher developed a group

discussion about pre-competition routines; he displayed a PowerPoint presentation

with the help of a wall projector which triggered interested topics of discussion with

the participants on pre-competition routines. The discussion lasted for 15 to 20

minutes and then, the participants were taken immediately to the labs where reactive

stress tolerance could be tested. Again, they were allocated to the same lab and testing

station that they had been administered the test throughout the E condition.

At the end of this session, the researcher thanked the participants for their

participation in the study.

2.2.4.2 Control condition (C). Likewise, the participants who were initially

assigned to the C condition, had three main sessions with the main researcher.

2.2.4.2.1 Session 1C. In this session, the participants were administered the

reactive stress tolerance test. After finishing this, the researcher thanked them and

asked them to come back after 2 weeks.

2.2.4.2.2 Session 2C. At the beginning of this session, the participants took part

in a group discussion about pre-competition routines which, again lasted for 15 to 20

minutes; after that, they were asked to follow the researcher to the labs, where

reactive stress tolerance could be completed. Again, participants were allocated to the

same labs and stations as the ones they had used in Session 1C. Participants then

completed the reactive stress tolerance test. After that, the participants went to the

mindroom where they were connected to the RESP and BVP sensors and were taken

through the resonance breathing frequency assessment (as was the case for the

participants in Session 1E). At the end of this assessment, the participants were

thanked, and an appointment was made in two weeks for the final session.

2.2.4.2.3 Session 3C. This was the last session of the C condition. In this session,

the participants who had already gone through sessions 1C and 2C, took part in the

experimental condition, since the control part of the study had been completed and a

crossover had taken place after a washout period of two weeks. As such, they all went

through a 20-minute HRV training session in exactly the same way as the participants

of the E condition in Session 2E. At the end of that, they were all taken to the labs,

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where the reactive stress tolerance test could be administered, were allocated to the

same lab and testing station as the ones they used throughout the C condition and

were administered the reactive stress tolerance test. After that, the researcher

thanked them for their participation in the study.

Appendix A.1 displays, concisely, the procedure followed in this study.

2.2.5 Data Analysis

The Statistical Package for the Social Sciences (SPSS) version 21.0 was used as a

tool for data analysis. Statistical analyses included descriptive statistics such as

percentages and means and standard deviations and repeated-measures analyses of

covariance (ANCOVA). The level of significance for all statistical analyses was set at the

.05 alpha level. Repeated-measures ANCOVAs were conducted to investigate whether

there were statistically significant differences in the main variables of reactive stress

tolerance of participants recorded after the control condition (C) compared to the main

variables of reactive stress tolerance recorded after the experimental condition (E),

controlling for the main variables of reactive stress tolerance of the participants

recorded in the first session (i.e. Session 1E and Session 1C).

Therefore, the following tests were conducted:

- ✓ A repeated-measures analysis of covariance (ANCOVA) was conducted to
  investigate:
  - ➢ whether there were statistically significant differences in the ratio of
    correct responses recorded in the experimental condition compared to the

ratio of correct responses recorded in the control condition controlling for the ratio of correct responses recorded during the participants' baseline testing session.

➢ whether there were statistically significant differences in the ratio of wrong responses recorded in the experimental condition compared to the ratio of wrong responses recorded in the control condition controlling for the ratio of wrong responses recorded during the participants' baseline testing session.

➢ whether there were statistically significant differences in the number of correct responses recorded in the experimental condition compared to the number of correct responses recorded in the control condition controlling for the number of correct responses recorded during the participants' baseline testing session.

➢ whether there were statistically significant differences in the number of incorrect responses recorded in the experimental condition compared to the number of incorrect responses recorded in the control condition controlling for the number of incorrect responses recorded during the participants' baseline testing session.

➢ whether there were statistically significant differences in the number of omitted responses recorded in the experimental condition compared to the number of omitted responses recorded in the control condition controlling

for the number of omitted responses recorded during the participants'

baseline testing session.

➢ whether there were statistically significant differences in the median

reaction time recorded in the experimental condition compared to the

median reaction time recorded in the control condition controlling for the

median reaction time recorded during the participants' baseline testing

session.

➢ whether there were statistically significant differences in the number of

generated stimuli recorded in the experimental condition compared to the

number of generated stimuli recorded in the control condition controlling

for the number of generated stimuli recorded during the participants'

baseline testing session.

2.3 RESULTS

2.3.1 Measures of Central Tendency and Dispersion

The descriptive performance data are outlined in Table 2.1. The mean ratio of correct responses that the participants achieved in the reactive stress tolerance test was lower in the measurement taken immediately after the control condition (M = .89, SD = .03) than the measurement taken after an HRV training session (M = .91, SD = .03) and much lower in the baseline measurement (M = .87, SD = .05); likewise, the standard deviations of the ratio of correct responses did not vary significantly across the three conditions, with the standard deviation of the baseline measurement being slightly higher than the ones of the other two conditions, as shown in Table 2. 1.

Table 2. 1

Means and Standard Deviations of the Recorded Variables Across all Three Conditions

	Baseline		HRV training		Control	
Variables	M	SD	M	SD	M	SD
Ratio of Correct Responses	0.87	0.05	0.91	0.03	0.89	0.03
Ratio of Wrong Responses	0.22	0.09	0.14	0.05	0.19	0.07
Correct Responses	258.42	49.19	268.31	51.69	270.53	46.16
Incorrect Responses	42.94	18.41	27.11	15.47	37.67	18.12
Omitted Responses	20.81	8.69	15.67	6.62	18.89	6.52
Median Reaction Time	0.66	0.11	0.72	0.13	0.67	0.11
Number of Stimuli	297.06	49.68	294.28	54.274	304.08	49.18

Furthermore, the ratios of wrong responses (i.e. incorrect plus omitted responses) appear to vary considerably across the three conditions. As such, the participants' mean ratio of wrong responses was reduced to .14 after one session of HRV training from .22 recorded at the beginning of the study, whereas it was .19 after the control condition. Similarly, the standard deviations ranged from .09 for the measurements taken at the beginning of the study, down to .05 after the HRV training condition, whereas it was .07 after the control condition.

Table 2.1 also displays the actual values of the correct, incorrect and omitted responses as well as the median reaction time of the participants, the mean number of stimuli generated within the 4 minutes of the test and the mean total number of reactions across the three conditions. The mean values in Table 2.1 indicate that there was an improvement compared to the baseline measurement in the two conditions, with the mean value recorded in the experimental condition being the best and the value in the control the second best recorded, as far as the variables "ratio of correct responses", "ratio of wrong responses", "incorrect responses" are concerned. Correct responses were slightly worse in the experimental condition (M = 268.31) compared to the control condition (M = 270.53), whereas, interestingly, median reaction time appeared to be slightly slower in the control condition (M = 0.67) than in the baseline measurements (M = 0.66) and much slower in the experimental condition (M = 0.72). On the other hand, the mean total number of stimuli generated within the 4 minutes of the test appeared to be higher in the control condition (M = 304.08) than in the

baseline (M = 297.06), whereas it appeared to be lower in the experimental condition
(M = 294.28).

2.3.2 Investigation of Statistically Significant Differences

The following ANCOVAs investigate the extent to which the above variables are significantly different across the two conditions when controlling for the baseline measurement:

2.3.2.1 Analysis of covariance of the ratio of correct responses (RCR) recorded in the experimental and control conditions. The ANCOVA conducted on the ratio of correct responses (M = 0.91, SD = 0.03 in the experimental condition and M = 0.89, SD = 0.03 in the control condition) showed that there was no significant difference in the ratio of correct responses recorded in the experimental and control conditions $F_{(1, 34)}$ = 0.35, $p > .05$. Table 2.2 (Appendix A.2) displays the results of this ANCOVA.

Therefore, we fail to reject the first null hypothesis which means that the ratio of correct responses in the reactive stress tolerance test is not significantly higher after an HRV-training condition than after a control condition when controlling for the baseline ratio of correct responses.

2.3.2.2 Analysis of covariance of the ratio of wrong responses (RWR) recorded in the experimental and control conditions. The ANCOVA conducted on the ratio of wrong (i.e. incorrect plus omitted) responses (M = 0.14, SD = 0.05 in the experimental condition and M = 0.19, SD = 0.07 in the control condition) showed that there was no significant difference in the ratio of wrong responses recorded in the experimental and

control conditions F (1, 34) = .39, p > .05. Table 2.3 (Appendix A.2) displays the results
of the ANCOVA.

Therefore, we fail to reject the second null hypothesis which means that the
ratio of wrong responses in the reactive stress tolerance test is not significantly higher
after an HRV-training condition than after a control condition when controlling for the
baseline ratio of wrong responses.

2.3.2.3 Analysis of covariance of the correct responses (ZV) recorded in the
experimental and control conditions. The ANCOVA conducted on the correct
responses (M = 268.31, SD = 51.69 in the experimental condition and M = 270.53, SD =
46.16 in the control condition) showed that there was no significant difference in the
number of correct responses recorded in the experimental and control conditions F (1,
34) = 0.16, p > .05. Table 2.4 (Appendix A.2) displays the results of this ANCOVA.

Therefore, it appears that additional analyses on the actual number of correct
responses does not yield any statistically significant results, which means that the
number of correct responses is not significantly different between the experimental
and control conditions when controlling for the baseline measurement of correct
responses.

2.3.2.4 Analysis of covariance of the incorrect responses (F) recorded in the
experimental and control conditions. The ANCOVA conducted on the incorrect
responses (M = 27.11, SD = 15.47 in the experimental condition and M = 37.67, SD =
18.12 in the control condition) showed that there was no significant difference in the

number of incorrect responses recorded in the experimental and control conditions F (1, 34) = 0.85, p > .05. Table 2.5 (Appendix A.2) displays the results of the ANCOVA.

Therefore, it appears that additional analyses on the actual number of incorrect responses does not yield any statistically significant results, which means that the number of incorrect responses is not significantly different between the experimental and control conditions when controlling for the baseline measurement of incorrect responses.

2.3.2.5 Analysis of covariance of the omitted responses (A) recorded in the experimental and control conditions. The ANCOVA conducted on the omitted responses (M = 15.67, SD = 6.62 in the experimental condition and M = 18.89, SD = 6.52 in the control condition) showed that there was no significant difference in the number of omitted responses recorded in the experimental and control conditions F (1, 34) = 0.12, p > .05. Table 2.6 (Appendix A.2) displays the results of the ANCOVA.

Therefore, it appears that additional analyses on the actual number of omitted responses does not yield any statistically significant results, which means that the number of omitted responses is not significantly different between the experimental and control conditions when controlling for the baseline measurement of omitted responses.

2.3.2.6 Analysis of covariance of the median reaction time (MDRT) recorded in the experimental and control conditions. The ANCOVA conducted on the median reaction time recorded (M = 0.72, SD = 0.13 in the experimental condition and M =

0.67, SD = 0.11 in the control condition) showed that there was no significant

difference in the median reaction time recorded in the experimental and control

conditions $F_{(1, 34)} = 0.73$, $p > .05$. Table 2.7 (Appendix A.2) displays the results of the

ANCOVA.

Therefore, it appears that additional analyses on the median reaction time does

not yield any statistically significant results, which means that the median reaction

time is not significantly different between the experimental and control conditions

when controlling for the baseline measurement of MDRT.

2.3.2.7 Analysis of covariance of the number of generated stimuli (S) recorded

in the experimental and control conditions. The ANCOVA conducted on the number of

generated stimuli (M = 294.28, SD = 54.27 in the experimental condition and M =

304.08, SD = 49.18 in the control condition) showed that there was no significant

difference in the number of generated stimuli recorded in the experimental and

control conditions $F_{(1, 34)} = 0.07$, $p > .05$. Table 2.8 (Appendix A.2) displays the results

of this ANCOVA.

Therefore, it appears that additional analyses on the number of generated

stimuli does not yield any statistically significant results, which means that the number

of generated stimuli is not significantly different between the experimental and control

conditions when controlling for the baseline measurement of the number of generated

stimuli.

To sum up the findings of this section, although the measures of central tendency and dispersion show that both ratios of correct and wrong responses were better immediately after an HRV training session compared to after a control session (i.e. discussion about pre-competition routines), the investigation of significant differences by means of a one-way analysis of covariance for each of the main variables (i. e. ratio of correct responses and ratio of wrong responses) controlling for the measures of RCR and RWR, respectively, taken at the baseline, showed that there are no significant differences both in the RCR and the RWR between the experimental and the control conditions. Additional analyses on the actual number of correct, incorrect, omitted responses but also analyses on the median reaction time and the number of generated stimuli within the 4 minutes of the reactive stress tolerance test showed no signs of statistical significance as well. Consequently, we failed to reject both null hypotheses of this study.

2.4 DISCUSSION

The purpose of this study was to investigate the effect of a single 20-minute session of HRV training on reactive stress tolerance, which is an individual's ability to give quick serial answers to continuously changing stimuli (Patocs et al., 2016) or, in other words, stress tolerance and reaction speed in the presence of rapidly incoming stimuli (Vorster et al., 2011). The ultimate objective of that would be to build scientific evidence by means of which, athletes and coaches would support the idea of including such a strategy in the athletes' pre-competition routine. It was expected that reactive stress tolerance, in the post-intervention testing session of the experimental group, would be significantly better compared to the control group at the .05 level of significance.

However, the results of the statistical analyses did not support this as there was no difference in performance on the reactive stress tolerance between the two conditions. Considering that reactive stress tolerance is also the ability of an individual to react quickly and accurately in a situation they are overstretched (Neuwirth & Benesch, as cited in Ong, 2017), the fact that the ANCOVA on the ratio of wrong responses (i.e. incorrect plus omitted responses) and the ratio of correct responses showed that there was no statistically significant difference immediately after a single 20-minute HRV training, indicates that the findings of the present study are not in accordance with Prinsloo et al.'s (2011) results which showed better reaction times, diminished anxiety and cognitive performance after a 10-minute HRV training exercise. Furthermore, the results of this study are not consistent with the beneficial effects that

Prinsloo et al (2013a) found in their study where a single HRVT session resulted in increased internal attention, increased relaxation and reduced anxiety. Nor are they consistent with the results of Prinsloo et al (2013b) which demonstrated that a single deep-breathing exercise based on HRV training may result in maintained levels of HF power and RMSSD after induced stress, thus better self-regulation.

Of course, the present study measured the performance of participants in a task where they had to use assigned rules to respond correctly to rapidly incoming stimuli, a task that one can complete efficiently only if they are able to tolerate stress and perform under pressure; therefore, the measured constructs are not exactly the same as those measured by Prinsloo et al. (2011), Prinsloo et al. (2013a) or Prinsloo et al. (2013b), though one would expect that there should be a linear relationship between such as a better level of self-regulation (as, for example, discussed by Prinsloo et al., 2013b) and responding correctly to rapid stimuli, which was not reflected by the discussed findings.

Nevertheless, the measures of central tendency showed that most of the indices were even slightly better in the experimental condition compared to the control condition. Indeed, the ratio of correct responses and ratio of wrong responses, which are both highly indicative of the level of reactive stress tolerance of the athletes, were even slightly better immediately after a 20-minute HRV training session (i.e. experimental condition) compared to immediately after the discussion on pre-competition routines (i.e. control condition). As the reactive stress tolerance was a test where the athletes had to use different assignment rules and respond correctly to

different rapidly incoming visual and acoustic stimuli, it is the performance of the athlete in this task that is actually measured; in the context of sport, when it comes to performance, even a small improvement is worth considering, as this small difference may lead to a completely different result, depending, of course, on the nature of the sport. In the present results, clearly, a small improvement is detected in the ratio of wrong responses indicating that the proportion of the sum of incorrect and omitted responses in the total number of generated stimuli within the 4 minutes of the test, got slightly lower (i.e. better). Likewise, the proportion of correct responses in the total number of generated stimuli within the 4 minutes of the test got slightly higher (i.e. better).

Controlling for the baseline measurement, the number of correct responses did not differ significantly between the control and experimental conditions. This means that the 20-minute HRV training session did not help the participants to respond correctly to significantly more incoming stimuli. Likewise, controlling for the baseline measurement, the number of incorrect responses did not differ significantly between the control and experimental conditions. In other words, after a single 20-minute HRV training session, participants did not give less incorrect responses to incoming stimuli. The same was true (i.e. there was no statistically significant improvement) for the following variables: omitted responses, median reaction time and number of generated stimuli within the 4 minutes of the test.

Moreover, that fact that this study failed to show statistically significant improvements might have been because the reactive stress tolerance test was not

sport specific or because the conducted HRVT session was not enough in order for the participants to familiarize themselves with the slow-breathing strategy and practice HRVT efficiently.

Although the findings of this study do not appear to be so promising, the study actually provided evidence that a 20-minute HRVT session was feasible for adolescent athletes; one of the initial concerns, before commencing the data collection in this study, had been whether athletes of this age (i.e. adolescent athletes) would be capable of finishing the 20-minute session of slow breathing. It proved that this was feasible without any serious problems, though one single session was not proved to be enough in order to cause statistically significant performance effects.

2.4.1 Limitations of the Study

The present study aimed to investigate whether there were immediate effects on reactive stress tolerance after a single 20-minute HRV training session. This presupposes that reactive stress tolerance be measured immediately after the completion of the 20 minutes of HRV training. Although ASPIRE academy in Doha/ Qatar, is an environment with the most sophisticated equipment that can be used for sport psychological testing and training, the set-up of the labs did not help much in this respect. In other words, participants had to walk for - at least - 6 to 7 minutes after completing the 20 minutes of biofeedback session before sitting down in front of the testing station and go through the reactive stress tolerance test. As such, on the one hand, the participants had to do physical activity (i.e. brisk walking from the one lab to

the other) immediately after the HRV training session and before the post-intervention testing and, on the other, at least 6 to 7 minutes had already elapsed from the completion of the HRV training session.

There was also a shortcoming in the design of the sessions of this study that the participants had to go though: Whereas the design of the present study was randomized controlled trials with a crossover and the sessions planned for the participants were based on this rationale, there was no pre-intervention testing session planned for both of the groups of participants after the crossover. It was initially thought that the existing baseline measurement, at the beginning of the study, was enough; however, there is a school of thought, according to which, post-crossover pre-intervention measurement could be slightly different from the initial baseline measurement which should have been reflected, somehow, in the results.

Moreover, it was assumed that the resonant breathing frequency of each of the participants would be identified within one session, which is not always realistic. Sometimes, it takes more than one, even three sessions to identify the resonant breathing frequency of a client, as the 9-minute software assessment is not always enough and – sometimes a lot more - additional qualitative analysis is needed in order to decide what the client's resonant breathing frequency is. Therefore, it might have been that, due to the rush to proceed to the next step of the study, in certain cases, the identified frequencies were not those frequencies that resulted in the best phase angle (i.e. optimal cardiorespiratory synchrony), or maximal RSA.

2.4.2 Conclusions

A single 20-minute heart rate variability training session did not have significant effects on reactive stress tolerance. Of course, a psychobiological system, as the human being, does not function in a linear way and cannot be reducible to one variable only; this means that whereas an HRV training session seem to have non-significant –statistically - effects on one's reactive stress tolerance, it might be that acute effects do exist in some other form which may, in turn, create beneficial effects on performance. The next study will expand the investigation of acute effects to other aspects of the human psychobiological system with a view to aligning such effects with the findings of Prinsloo et al (2011), Prinsloo et al. (2013a) and Prinsloo et al. (2013b) and understand the potential mechanisms and changes in stress biomarkers that may occur as a result of HRVT.

CHAPTER 3: HEART RATE VARIABILITY TRAINING AND EMOTIONAL CONTROL: LONG-LASTING EFFECTS AND FURTHER INVESTIGATION ON ACUTE EFFECTS

3.1 INTRODUCTION

In Study 1, acute effects on a reactive stress tolerance test, used to measure performance under pressure, were investigated. HRVT did not improve performance. Specifically, performance under pressure caused by rapidly incoming visual and acoustic stimuli was not linked with acute beneficial effects caused by a single 20-minute HRVT session. The present study (Study 2) addresses Aim 2 and Aim 3 of the thesis and builds on the investigation of the effects of an HRV training programme on emotional control. It considers the extent to which such effects can be long-lasting and, also, sets the framework for a comprehensive investigation of HRV training on biochemical and psychophysiological parameters that are related to sport performance. The present study is the epicentre of the thesis and describes the effects of a 5-week protocol on biochemical and psychophysiological variables. The collection of salivary samples needed for the biochemical variables also allows for further examination of acute effects, on biomarkers of stress, to build on the exploration of performance effects in Study 1. Therefore, this study builds on the existing literature by investigating acute, and long-lasting effects on psychophysiological, biochemical and HRV-related variables while, at the same time, investigating acute effects on the measured biochemical markers as well.

Heart rate variability has been extensively discussed as an index of adaptability of the autonomous nervous system to varying psychological and psychophysiological demands (Bertch, Hagemann, Naumann, Schachinger & Schulz, 2012), whereas an increase in HRV would mean higher adaptability to new situational demands. The autonomous nervous system (ANS) consists of two branches, namely, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The role of the SNS and the PNS and, consequently, of the ANS, is the regulation of homeostatic balance, as they are related to a neural network responsible for the regulation of various target organs such as the lungs, the adrenal, sweat, lacrimal and salivary glands, the eyes, kidneys, pancreas, trachea, blood vessels, bronchi, stomach, intestine, bladder, external genitalia, larynx and the heart (Porges, 2011).While the SNS is associated with dealing with challenges outside the body by providing increased metabolic output, the PNS is linked with a growth and restorative system. The two branches of the ANS act as antagonists in most cases. Activation of the PNS results, for example, in the constriction of the pupil, deceleration of the heart rate, peristaltic movement, as well as the relaxation of the rectal and vesical sphincters, whereas, when the SNS is stimulated, the heart rate gets faster, the blood vessels constrict, the pupils dilate, sphincter contracts, peristaltic movement is minimized and blood pressure may increase.

3.1.1 Stress and Psychophysiological Variables

Each individual reacts in a different way when they find themselves in a stressful situation, whereas the amplitude of the physiological response varies as well

(Peper et al., 2008). As a result of this, practitioners have been using various

biofeedback sensors to monitor and quantify such psychophysiological responses as

muscle tension, which is monitored and quantified by means of EMG (e.g. Peper et al. ,

2008; De Witt, 1980; Schleifer, Spalding, Kerick, Cram, Ley, & Hatfield, 2008; Lundberg,

Forsman, Zachau, Eklof, Palmerud, Melin, & Kadefors, 2002), skin conductance (e.g.

Peper et al. , 2008; Binboga, Guven, Cattikas, Bayazit, & Tok, 2012; Marko, 2016), skin

temperature (e.g. Peper et al. , 2008; Baker & Taylor, 1954; Vinkers, Pennings,

Hellhammer, Verster, Klaessens, Olivier, & Kalkman, 2013), respiration rate and pattern

(e.g. Peper et al. , 2008; Suess, Alexander, Smith, Sweeney, & Marion, 1980; Conrad,

Muller, Doberenz, Kim, Meuret, Wollburg, & Roth, 2007), and heart rate/ HRV which is

monitored either by means of electrocardiograph (ECG) (e.g. Lundberg et al. , 2002;

Laborde, Mosley, & Thayer, 2017) or a sensor measuring blood volume pulse (BVP)

(Peper et al. , 2008).

Psychophysiological indices of stress can also provide useful information on

acute stress without interfering with the stressful task (Pakarinen, Korpela, &

Torniainen, 2016). In a study quantifying acute stress with HRV and EDA in real-world

conditions (Pakarinen et al. , 2016), electrodermal activity was found to correlate

significantly with self-reported stress, while the same was true for LF/HF ratio, which is

an index of sympathovagal balance or it reflects sympathetic modulation (Task Force of

the ESC and the NASPE, 1996). Besides, HRV and EDA have been thought to be the two

non-invasive methods to assess central and peripheral dynamics of the SNS (Posada-

Quintero et al., 2019), whereas the latter has been discussed as an index of emotional

arousal as well (Sabatinelli, Bradley & Lang, 2001). Surface electromyography

responses on the vastus lateralis, on the other hand, have been proved to correlate

with cardiac autonomic modulation in a study with male professional cyclists (Saraiva,

Silva, Simões, Garcia, Menegon, Sakabe, Ortolan, Martins, Oliveira, & Catai, 2016).

In practice, the SNS is responsible for responding to external challenges

whereas the PNS deals with any visceral demands of the human organism, thus,

ensuring homeostasis (Porges, 2011). Therefore, it seems that the PNS tone of the

human organism, before the appearance of a challenge, indicates stress or physiologic

vulnerability; likewise, the withdrawal of PNS activity, as a result of the appearance of a

challenge, defines stress. In ANS-related terms, homeostasis is defined as the

physiological state that smoothly covers the visceral needs when there is no external

challenge, while stress is the disruption of homeostasis, which can also be

characterized by a withdrawal of PNS tone.

3.1.1.1 Biochemical markers of stress. From a biochemical standpoint, salivary

a-amylase can be considered as an index of the stress response of the ANS in young

people (e.g. Fortunato, Dribin, Buss, & Granger, 2008; Davis & Granger, 2009; Keller &

El-Sheikh, 2009; Yim, Granger, & Quas, 2010) but also in older adults (e.g. Bosch,

Veerman, de Geus, & Proctor, 2011) Salivary a-amylase has attracted the interest of

researchers, as it is a non-invasive way for measuring the ANS response to stress (e.g.

Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011), is also a non-invasive

biomarker of SNS (Nater & Rohleder, 2009), and is a consequence of the secretion of

catecholamines resulting from the activation of the SNS (e.g. Nater & Rochleder, 2009),

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which is one of the two biological systems involved in the stress response, along with

the hypothalamic-pituitary-adrenal (HPA) axis activity, measured by means of salivary

cortisol (e.g. Chroussos & Gold, 1992). Figure 3.0 depicts the concentration levels of a-

amylase and cortisol, as recorded under normal circumstances during a day due to

circadian rhythms (Adam, Hoyt, & Grager, 2011).
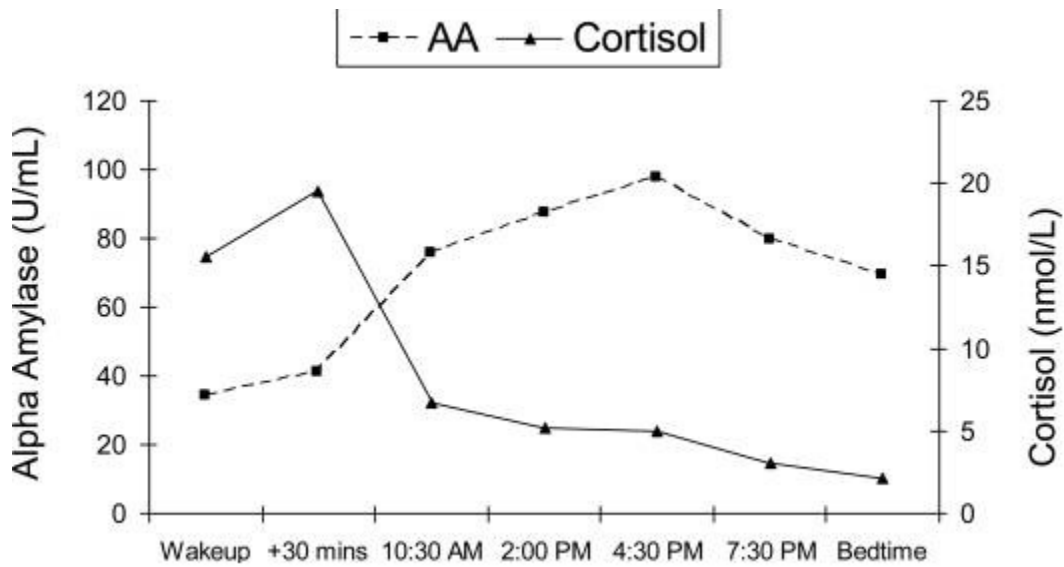
Figure 3.0. Average diurnal patterns of salivary alpha amylase and salivary cortisol

across the day in a sample of late adolescents (Adam et al., 2011, p.7).

Salivary a-amylase (sAA) has also been found to fluctuate in parallel with HR

and LF and correlate significantly with LF/HF, which is thought to be an index of

sympathovagal balance, during stress, whereas an increase in sAA was followed by a

decrease in HF within the same conditions (Nater, La Marca, Florin, Moses, Langhans,

Koller, & Ehlert, 2006). Salivary a-amylase has been also found to be inversely

correlated with heart rate variability, which may indicate reciprocity between

sympathetic and parasympathetic reactivity (De Vries-Bouw, Jansen, Vermeiren,

Doreleijers, van de Ven, & Popma, 2012). A-amylase was first reported to correlate

with elevated stress in the late 1970s (Gilman, Fischer, Biersner, Thornton, & Miller,

1979), whereas cortisol, on the other hand, has been used as an index of stress in

various studies as well (e.g. Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011;

Haneishi, Fry, Moore, Schilling, Li, & Fry, 2007; Daughters, Gorka, Matusiewicz, &

Anderson, 2013), and is also called glucocorticoid because it is associated with glucose

metabolism. The secretion of cortisol and generally glucocorticoids help a human to

cope with the detrimental effects of stress, by increasing sugar (glucose) in the

bloodstream; to illustrate how important cortisol is, those who have had their adrenal

glands removed and, therefore, do not have a normal secretion of cortisol, need

supplements to cope with stress.   (Carlson, 1999). As collection of saliva is a minimally

invasive method, it is preferred for the investigation of cortisol levels in a given

situation, especially in a context with minors. Additionally, the method of detecting

cortisol within saliva minimizes anticipatory stress which may be caused during the

collection of blood and may also be reflected in the cortisol levels. Salivary cortisol has

also been found to correlate with heart rate variability (Michels, Sioen, Clays, De

Buyzere, Ahrens, Huybrechts, Vanaelst, & De Henauw, 2013); a natural stressor

occurring during students' examinations has been shown to increase cortisol and

decrease HRV (Sgoifo et al, 2003), whereas a higher cortisol awakening response was

shown to be related with lower LF and HF levels (Stalder, Evans, Hucklebridge & Clow,

2011), though this relationship was not proved to be mediated by emotional

dysregulation and stress (Stalder et al. .2011).

It is important to note, that circadian rhythms play a significant role in the way

biochemical markers fluctuate during the day. Figure 3.0 shows how both biochemical

markers develop during the day in late adolescents, whereas in the case of adults,

salivary a-amylase appears to decrease within the first 30 minutes after awakening and

then it has an increasing trend until the evening (Nater, Rohleder, Schlotz, Ehlert, &

Kirschbaum, 2007). On the other hand, salivary cortisol reaches its peak approximately

30 minutes after awakening time and declines gradually as time goes by, which seems

to be the same pattern in both groups.

3.1.2 HRV Training Protocols

Research has shown that regulation of ANS-related undesirable responses as

well as alleviation of various medical conditions can be achieved by means of HRV

training (Goesslt, Curtisst, & Hofmann, 2017; Lehrer & Gevirtz, 2014; Wheat & Larkin,

2010). The first HRV training protocol was introduced by Lehrer, Vaschillo and Vaschillo

(2000); the ultimate objective of that protocol was to teach an individual the process of

slowing down their breath to a breathing pace at which there is a resonance caused by

respiration-induced oscillations and oscillations produced by baroreflex activity; that

protocol suggested that the individual should practice slow breathing at that frequency

of resonance in order to achieve optimal result. The duration of this protocol is 10

weeks with one controlled (i.e. lab-based) session every week and two 20-minute daily

sessions given as homework to the client. Whether this protocol has been designed to bring about a relatively permanent change in the individual or it actually teaches the client a skill they can use as and when they want before a psychologically important competition has not been clear (P. Lehrer, personal communication, April 15, 2014). Nevertheless, this 10-week protocol has a large learning component for the clients participating in it, as it teaches them step-by-step how they can optimize the effects of resonant breathing frequency using the practitioner's instructions efficiently (e.g. by using diaphragmatic breathing, by using pursed lips, and other instructions given during this protocol). It is the permanent change that needs to be researched further, as outlined by Lehrer (personal communication, April 15, 2014).

More recently, a modified version of the above-mentioned protocol was suggested; that was, actually, a shorter version of the already existing protocol which had resulted from the authors' research experience in HRV biofeedback (Lehrer, Vaschillo, Zucker, Graves, Katsamanis, Aviles, & Wamboldt, 2013). In other words, after having used the longer, 10-session, protocol many times, Lehrer et al. (2013) decided that HRV training could be taught in only five visits. This had been already tested successfully for the treatment of such medical conditions as asthma (Lehrer et al., 2004), depression (Karavidas et al., 2007), fibromyalgia (Hassett et al., 2007) but also other physical conditions (Katsamanis, Lehrer, Escobar, Gara, Kotay, & Liu, 2011). Again, the objectives of this protocol were to first identify the resonant breathing frequency of the client, teach them how to breathe from the abdominal area (i.e. use of diaphragmatic breathing), teach them how to exhale using pursed lips and, beyond

all, make sure that the RSA phenomenon is maximized with the instructed resonant breathing frequency (RBF) but also that the client is comfortable with this RBF.

Another 5-week protocol consisting of 5-minute HRVT exercises twice daily (for 5 weeks) was also introduced in a context of athletes before their university examinations (Deschodt-Arsac et al., 2018). Despite the short duration of the HRVT exercises (i.e. 5 minutes), this study showed that subjects demonstrated better cardiac autonomic control and better anxiety levels, which persisted even after 12 weeks from the cessation of the protocol. A more abbreviated version of a 5-week protocol was introduced by Gross, Shearer, Bringer, Hall, Cook, and Kilduff (2016) in which elite sport support and management staff were subjected to psychological and physiological measures, RBF identification and 5-minute paced breathing exercises as well as 3-minute, 4-minute and 5-minute RBF accuracy tests in Sessions 3, 4, and 5 respectively; in the accuracy tests, participants had to rehearse their RBF without the help of a breathing pacer. The results provided evidence that HRV biofeedback helped participants to enhance emotional regulation and is an effective strategy for elite sport support and management staff to use on demand.

Although, the above-mentioned protocols have been the most prevalent protocols introduced for HRV biofeedback, variations of those protocols have also been used mainly in the sport domain. For instance, the 10-session protocol discussed by Lehrer et al. (2000) was again used, as already discussed earlier in this project, but within a period of 4 weeks and without any homework given to the participants in a study with dance performers (Raymond et al. , 2005), or in another study with

basketball players, the original protocol was modified to ten 20-minute daily sessions for 10 consecutive days (Paul & Garg, 2012; Paul et al., 2012). Another approach was also introduced to female volleyball players, where the athletes attended one weekly session for 6 consecutive weeks of HRV biofeedback (Tanis, 2012), as per Culbert et al.'s (2004) suggestion on psychophysiological coherence and self-regulation skills, as well as Blumenstein et al. 's (1997) five-step approach to mental training incorporating biofeedback.

3.1.3 The Present Study

To date, there has been no robust scientific evidence on the long-lasting as well as acute effects of HRV training on the biochemical markers of cortisol and a-amylase. Moreover, there has been no study investigating the long-lasting effects of a pre-defined HRV- training protocol on various psychophysiological variables in Middle-Eastern male adolescent student-athletes.

The objective of the present study is to build the scientific foundation of the effectiveness of HRV training in adolescent student-athletes. This study is the first to investigate the effects of a 5-week protocol of five controlled (i.e. lab-based) and five uncontrolled (i.e. home-based) HRVT sessions on such stress-related psychophysiological responses as electrical activity on trapezius muscles, electrodermal activity, skin temperature and HRV, in addition to the above-mentioned biochemical markers of stress, that is, salivary cortisol and a-amylase.

Second, the study is the first to explore the viability of such a 5-week training programme in adolescent Middle-Eastern athletes. This approach was taken given the

challenges of incorporating the longer 10-week programme (Lehrer et al., 2000) but also the shorter version of it (Lehrer et al., 2013) into athletic populations who often travel to competitions and training camps and, as a result of this, they would not be able to commit to such a long protocol, nor would they be able to commit to the required homework of two 20-minute HRVT exercises per day, given their long days at the academy. Furthermore, it was decided that this study will focus, for the first time, on stress-related variables of psychophysiological and biochemical nature since those are related with arousal, which is highly related to performance (e.g. Landers, 2007), whereas these variables were primarily monitored in an environment of integrated psychophysiological testing and training (the "Mindroom").

It is hypothesized that psychophysiological and biochemical variables will have reduced significantly after the 2-week wash-out period following the suggested 5-week protocol (Peper et al., 2008; Nater & Rohleder, 2009). It is also hypothesized that there will be significant acute reductions on the participants' salivary a-amylase after each single lab-based weekly 20-minute HRV training session (e.g. Nater & Rohleder, 2009) (NB As discussed cortisol has a delay in response [Maruyama et al., 2012] and is not ideal for measuring acute effects. In the present thesis, acute biochemical effects refer to immediate responses coming from the SNS and are reflected in the concentration of a-amylase).

Overall, Study 2 addresses Aim 2 and Aim 3 of the thesis which focus on the acute effects of HRVT on biochemical markers of stress but also its long-lasting effects on psychobiophysiological variables.

3.2 METHOD

3.2.1 Participants

Fifty-seven participants were initially recruited for the study. Participants were recruited from the Doha-based ASPIRE academy. They were eligible for inclusion if they were (a) student-athletes of ASPIRE academy, (b) healthy individuals (i.e. were free from conditions that restrict physical activity and had no history of cardiovascular or breathing conditions such as asthma), and (c) aged between 11 and 18 years. The mean age of the 57 participants who completed the study was 15.79 years (SD= 0.98). From the 57 recruited athletes, 30 of them (52.63%) were randomly assigned to an experimental group, whereas the rest 27 (47.37%) were assigned to a control group. All of the participants were male adolescents (100%) who practiced a sport regularly; five of the participants were fencing athletes (8.8%), seven of them were table tennis athletes (12.3%), three were squash athletes (5.3%), twelve were track and field athletes (21.1%), eleven were football players (19.3%), five were football referees (8.8%), four were gymnastics athletes (7.0%), eight of the participants were shooting athletes (14.0%) and two were golf athletes (3.5%).

3.2.2 Design

This study was a pre-test post-test experimental design. Participants were randomly assigned (by tossing a coin) to an experimental and a control condition. The independent variable of the study (IV) is the different condition in which athletes participate, whereas the dependent variables (DVs) of the study are biochemical markers (i.e. cortisol levels and a-amylase levels) and psychophysiological variables

(i.e. total electrical activity on the clients' trapezii, skin temperature, skin conductance, heart rate and heart rate variability).

3.2.3 Instruments

3.2.3.1 Mindroom testing and training. The mindroom is an ASPIRE laboratory used for integrated psychophysiological testing and training. The ASPIRE mindroom is a four-station mindroom, with each of the stations consisting of an armchair, an audio headset, a laptop with biofeedback software installed in it, a biofeedback/ neurofeedback encoder and sensors. The mindroom has a client area, where all the stations are placed, and a control room, divided from the client area by means of a glass partition; in the control room, practitioners can view the graphs and statistics of each session in real time but, also, they can have full control of the clients' laptops. The audio headsets installed at each mindroom station allow practitioners to communicate with any of the clients using a microphone installed in the control room.

3.2.3.1.1 Biofeedback equipment.

The manufacturer of the biofeedback hardware and software used in this study is Thought Technology Ltd, which is based in Montreal/ Canada. The specifications of the hardware are explained below:

ProComp Infiniti encoder. This is an 8-channel, multi-modality encoder which is used for real-time computerized biofeedback and neurofeedback as well as data acquisition. The encoder is connected to a fiber optic cable which, in turn, is connected to the client's laptop by means of a USB module (ProComp Infiniti Manual, 2017).

Blood volume pulse (BVP) sensor. This is the sensor used for measuring/ monitoring heart rate in this study. Measurement with this sensor is based on photoplethysmography; the sensor, actually, monitors the change of the reflected light caused by blood flow. Each heartbeat results in more red light being reflected, which is recorded by the sensor, whereas less red light is reflected between pulses (Thought Technology Ltd., 2003). During this study, the BVP sensor was placed on the palmar surface of the non-dominant index fingertip of the clients. The type of BVP sensor used was BVP-Flex/ Pro. In this project, the BVP sensor was placed on the clients' non-dominant index finger.

Respiration sensor (RESP). A respiration sensor was used for monitoring the pace and pattern of abdominal respiration of the clients. This sensor has a velcro strap which is stretched around the client's abdomen and, actually, transforms the expansion or contraction of the abdominal area of the client to a rise or fall of the wave being displayed on the screen. The respiration sensor is placed slightly above the naval area of the client. The type of respiration sensor used in this study was RESP- Flex/Pro (Physiology suite manual, v. 5.1).

Skin conductance sensor (SC). This is a sensor used to monitor conductance across the skin and has two fingerband electrodes which, in this project, were attached to the middle and small finger of the participants. Skin conductance is, actually, the ability of the skin to conduct electricity; real-time variations in conductance reflects activity of the sympathetic nervous system and are related to levels of stress. A relaxed state usually gives a conductance of approximately 2 µS (i.e. micro-Siemens), however,

this varies according to the skin type of the client as well as environmental factors. The

sensor used was the Skin Conductance Sensor- SA9309M, by Thought Technology Ltd.

(Thought Technology's sensors and accessories manual, SA7511 Rev.4).

Skin temperature sensor (TEMP). This is, actually, a thermistor measuring

surface temperature on the skin and, in this project, was placed on the non-dominant

ring finger of the participants. The role of this sensor is to identify changes in the

temperature of the client's hand as this reflects increased or decreased blood flow to

the extremities which result from vasodilation or vasoconstriction respectively. The

skin temperature sensor used was the Temperature Sensor-SA9310, manufactured by

Thought Technology Ltd. (Thought Technology's sensors and accessories manual,

SA7511 Rev.4).

EMG (electromyography) sensors. Surface electromyography (SEMG) was used,

during this study, to monitor electrical activity on both of the clients' trapezii (i.e. left

and right trapezius) which results from muscle contraction when an individual is

stressed. For this project, MyoScan-Pro (SA9401M) EMG sensors, manufactured by

Thought Technology Ltd., were used (Thought Technology's basics of surface

electromyography, 2008).

Furthermore, all of these sensors were connected to different channels of the

above-mentioned encoder.

Biograph Infiniti version 6.0.4. This is the main psychophysiology software on

which, the psychophysiological stress profile, the resonant breathing frequency

assessment as well as the HRV training sessions were based. Biograph Infiniti has been

designed and manufactured by Thought Technology Ltd. (Thought Technology, 2016).

The first version of it was available in the market in 2003 whereas the version used for

the present study was designed and distributed in 2014. Furthermore, the HRV training

sessions were conducted using Thought Technology's Physiology Suite, which acts as a

plug-in software within Biograph Infiniti, and, specifically, with its pre-designed screen

labelled 'HRV-% power, pacer & animation' (Thought Technology's Physiology Suite, v.

5.1). Resonant breathing frequency was assessed by means of another software which

acts as a plug-in to the Biograph Infiniti as well and has been created by Inna Khazan in

collaboration with the Biofeedback Federation of Europe (BFE) (BFE's resonance

frequency assessment, 2014).

The "Optimizing Performance and Health Suite" by Vietta Sue Wilson was used

for the psychophysiological stress profile used in the pre-intervention and post-

intervention phases of this study (BFE, 2017). For the purposes of the current study, a

shortened version of the original protocol was used. In sum, the psychophysiological

stress profile will consist of the following activities:

- ✓ Eyes closed (baseline)

- ✓ Eyes open (baseline)

- ✓ Stroop Test (stressor 1)

- ✓ Recovery 1

- ✓ Math Task (stressor 2)

- ✓ Recovery 2

The total duration of this shortened version of stress profile is 9 minutes and 5 seconds.

3.2.3.2 Saliva Collection. SalivaBio oral swabs (SOS) and swab storage tubes were used for the collection of saliva from the participants of this study. Both the swabs and the tubes were manufactured by Salimetrics LLC (USA). Furthermore, ELISA (i.e. enzyme-linked immunosorbent assay) assay kits were used for the analysis of cortisol in the participants' saliva, by Salimetrics LLC. The analysis of a-amylase in saliva was conducted with alpha-amylase kinetic enzyme assay kits, also by Salimetrics LLC. (USA).

3.2.4 Procedure

Following institutional ethical approval and the approval of the IRB committee of the ADLQ (i.e. Anti-Doping Lab Qatar), participants were recruited within the ASPIRE academy's school. Table 3.1 presents the procedure followed in Study 2.

3.2.4.1 Experimental condition (Exp). The participants who were assigned to the Exp condition had eight main sessions with the main researcher.

3.2.4.1.1 Pre-intervention testing session (Pre-Int$_{exp}$)- Session 1. Participants attended this session in groups of four. They had been instructed to refrain from eating anything for at least 1 hour before the session and rinse their mouth with water at least 10 minutes before the session, as saliva collection had been scheduled at the beginning of the session. Upon entering the mindroom, the participants sat down at one of the four stations (i.e. an armchair equipped with a laptop and biofeedback

hardware and software), where they were connected with the sensors, namely, the BVP, SC, TEMP, RESP and two EMG sensors.

Then, they put a SalivaBio oral swab in their mouth and kept it under their tongue for 3 minutes; during this time participants were instructed not to speak, nor chew the swab. The swab was then placed in the pre-labelled swab storage tubes provided. All four participants put on the mindroom headphones to communicate with the researcher and, also, isolate themselves from anything happening in the surrounding area.

After putting the swab in the storage tube, the researcher administered the shortened version of the psychophysiological stress profile to the participants. At the end of this protocol, the researcher collected saliva from the participants again, whereas they were all given two pre-labelled storage tubes and oral swabs in order to collect saliva in the first (i.e. after 45 minutes) and second (i.e. after 90 minutes) school breaks. The participants were instructed not to eat anything until the collection of the last sample, nor drink water within less than ten minutes before the collection of a sample.

3.2.4.1.2 Resonant breathing frequency assessment (RBFtest)- Session 2. In the second session, the participants came to the mindroom where diaphragmatic/ abdominal breathing was discussed and their knowledge was refreshed. The second session was scheduled either a week after the Pre-Int$_{exp}$, or within the week following the Pre-Int$_{exp,}$ as it did not really matter whether the RBFtest would take place a few

days or exactly a week after the first session. The researcher asked the participants to practise abdominal breathing for a while so that he could make sure that everyone was breathing using their diaphragm. The researcher instructed the participants that during the assessment they should breathe in from the nose and breathe out through pursed lips. A demonstration of that also took place.

The assessment took place in groups of five participants (i.e. four stations within the mindroom and a fifth portable station that could be used for this type of assessment). After making sure that all of the participants of the group could practice diaphragmatic breathing, the researcher attached a respiration sensor (RESP) and a blood volume pulse sensor (BVP) to each participant. Then, the resonance frequency assessment software was started on their computers and the participants were asked to read the instructions carefully (The protocol used for the RBFtest was exactly the same as the protocol used in Study 1). The assessment started with 7.0 breaths/minute and slowed down by half a breath per minute in every subsequent step, with each of these steps lasting for 90 seconds (i.e. in total, the assessment consisted of six steps); after each step, a break was given to the participant who were told to continue only if they felt they were ready for the next step. During this assessment, the participants had to follow the breathing pacer displayed on their laptop's screen;

Table 3.1

Procedure Followed in Study 2

when the ball moved upwards, they had to breathe in, when it moved downwards, they had to breathe out, whereas they had to pause their breathing when the ball moved horizontally.

The researcher watched the progress of the assessment from the control room within the mindroom and could communicate with each of the participants individually, or collectively, when this was necessary. If the participants deviated from the instructed pace the researcher could detect this and reinforced the participants to follow the pacer more accurately. At the end of this assessment, a report was produced for each of the participants with four major HRV parameters for each of the breathing steps (i.e. heart rate (Max-Min), pNN50, SDNN, LF percent of total power), and the extent to which the clients could follow the breathing pacer accurately or not.

The above-mentioned HRV parameters indicated which breathing pace helps each participant maximize their HRV (i.e. in all four variables, a higher value was considered as a more desirable value). This pace was identified as the resonant breathing frequency of each of the participants. In case, however, that the report did not yield clear results as to what the client's resonant breathing frequency is, the researcher made a note so that more than one frequency (i.e. usually two and, less frequently, three frequencies) would be tested with that specific participant at the beginning of the next session (i.e. before proceeding to the actual HRVT session).

3.2.4.1.3 Heart rate variability training (HRVT1, HRVT2, HRVT3, HRVT4, HRVT5)- Sessions 3 to 7. The third session with the participants of the experimental

group, was planned either one week exactly after Session 1 or two weeks after that, depending on when Session 2 had been planned (i.e. if the participants were available for the RBFtest sooner than one week after the Pre-Int$_{exp}$, then both Pre-Int$_{exp}$ and RBFtest took place within the first week). Again, participants had been instructed to refrain from eating anything for at least one hour before the session and rinse their mouth with water at least 10 minutes before coming to the mindroom, as saliva collection had been scheduled at the beginning of the HRVT session. At the beginning of the session, the participants were connected to the BVP and RESP sensor, whereas their station had been configured to the resonant breathing frequency that had been assessed in the previous session. Before starting the HRVT session, they were asked to put a SalivaBio oral swab in their mouth and keep it under their tongue for 3 minutes. The HRVT session commenced immediately after the participants had put the oral swab in the pre-labelled storage tube.

In order to make sure that the calculated breathing frequency was indeed the clients' resonant breathing frequency, the participants were asked to go through a 3-minute slow breathing test, following the pacer on their screens; at the end of this test, they gave feedback to the researcher as to whether they felt comfortable with that pace. The researcher had already collected information on whether LF power had been increasing during those 3 minutes, there was cardiorespiratory synchrony, and HR peak-to-valley fluctuation was maximal (NB This quick test took place only in Session 3). When each client agreed with the researcher that, indeed, that was their resonant breathing frequency, they proceeded to the actual session which included a 20-minute

computerized exercise of paced breathing (i.e. HRV training session); the researcher instructed the participants to breathe effortlessly without taking any deep breaths.

Again, during this session the researcher had continuous visual contact with the participants and could also monitor in real time the indices and graphical representations of their respiration rate and pace as well as their heart rate. Using the audiovisual equipment, he was in position to reinforce them to continue breathing at their resonant breathing frequency, should they deviate - even slightly - from the pre-set breathing pace.

As in session Pre-Int$_{exp,}$ saliva was collected from the participants again, at the end of the HRVT session, whereas they were all given two pre-labelled storage tubes and oral swabs in order to collect saliva in the first (i.e. after 45 minutes) and second (i.e. after 90 minutes) school breaks. Again, the participants agreed that they would not eat anything till the collection of the last sample, nor would they drink water within less than 10 minutes before the collection of a sample.

Before leaving the mindroom, the participants were instructed to download specific free breathing apps on their smartphones, which were simple breath pacers and were configured to each SA's resonant breathing frequency by the researcher. The participants were instructed to go through a 20-minute exercise with their mobile app at home, three days after session 3 (NB these were uncontrolled practice sessions, since it was not possible to monitor the participants). Additionally, weekly

appointments with the participants were arranged on the same day and same time of each week until the participants finished the pre-agreed protocol.

In Session 4, Session 5, Session 6 and Session 7, the participants again went through a 20-minute HRVT session which was designed in the same way as in Session 3 as well as collection of saliva in exactly the same way as in HRVT1 (i.e. before the HRVT session, immediately after, 45 minutes after and 90 minutes after the HRVT session). Again, they were instructed to do their homework, which was a 20-minute home-based exercise of slow breathing with their mobile app, configured at their resonant breathing frequency, three days after each lab-based session.

In total, all the participants participated in five controlled (i.e. lab-based) and five uncontrolled (i.e. home-based) HRVT sessions/ exercises.

3.2.4.1.4 Post-intervention testing session (Post-Int$_{exp}$)- Session 8. As this study mainly investigates long-lasting effects, it was decided that the Post-Int$_{exp}$ session would take place two weeks after the last HRVT session, in order for any short-term HRVT effects to be washed out. This is common practice in non-pharmacological interventions (e.g. Oliveira, Radanovic, Mello, Buchain, Vizzotto, Celestino, Stella, Piersol, & Forlenza, 2015). Therefore, the Post-Int$_{exp}$ session was planned two weeks after the participants' last home HRVT exercise which followed HRVT5.

Before this session, participants had again been instructed to refrain from eating anything for at least 1 hour before the session and rinse their mouth with water at least 10 minutes before the session, as saliva collection was scheduled at the

beginning of this session. When they came to the mindroom, they were connected to the sensors BVP, SC, TEMP, RESP and two EMG sensors.

As in Pre-Int$_{exp,}$ they were asked to put a SalivaBio oral swab in their mouth and keep it under their tongue for 3 minutes; after that, they had to put the swab (without touching it) in the pre-labelled swab storage tubes they had been provided with. Again, all four participants were asked to put on the mindroom headphones so that communication would be better with the researcher and, also, they would isolate themselves from anything happening in the surrounding area.

After putting the swab in the storage tube, the researcher administered the psychophysiological stress profile to the participants; the latter were asked to pay attention to the laptop screen and follow the instructions written on it. At the end of this protocol, the researcher collected saliva from the participants anew, whereas they were all given two pre-labelled storage tubes and oral swabs in order to collect saliva in the first (i.e. after 45 minutes) and second (i.e. after 90 minutes) school breaks. As in the previous sessions, they all agreed that they would not eat anything until the collection of the last sample, nor would they drink water within less than 10 minutes before the collection of a sample.

3.2.4.2 Control condition (Ctrl). The participants who were assigned to the Ctrl condition had seven main sessions with the researcher (NB The participants in the control condition were offered the possibility of familiarizing themselves with the researched HRVT protocol, after the completion of the study).

3.2.4.2.1 Pre-intervention testing session (Pre-Int$_{Ctrl}$)- Session 1. The

participants followed exactly the same procedure as in Pre-Int$_{exp.}$ The only difference

with Pre-Int$_{exp}$ is that only two salivary samples were collected from the participants in

Pre-Int$_{Ctrl}$, that is, before and immediately after (i.e. the participants were not told to

collect saliva 45 minutes and 90 minutes after the end of the session as was the case in

Pre-Int$_{exp}$).

3.2.4.2.2 Educational sessions (EducS1, EducS2, EducS3, EducS4, EducS5)- Sessions 2 to

6. These were 20-minute educational group sessions organized with the participants of

the control group. The participants of the control group attended five weekly sessions

on sport science that were educational in nature and did not comprise practice of

specific strategies designed to enhance performance (NB In the 1st week, EducS1 took

place, in the 2nd week EducS2 and so on).

Each of these educational sessions were scheduled at 12:30 p.m. every Tuesday

or Thursday. On the day that EducS3 had been arranged, the control group participants

were asked to come and meet the researcher in the morning at 8:30 a.m. for saliva

collection. One salivary sample was taken at 8:30 a.m. and a second one was taken at

8:50 a.m. (NB The collection should take place always at the same time of the day in

order to avoid circadian-rhythm-related fluctuations of salivary cortisol and a-amylase

(e.g. Ghiciuc, Cozma-Dima, Pasquali, Renzi, Simeoni, Lupusoru, & Patacchioli, 2011;

Wilcox, Granger, Szanton, & Clark, 2014).

3.2.4.2.3. Post-intervention testing session (Post-Int$_{Ctrl}$)- Session 7. As

mentioned above, this study mainly investigates long-lasting effects; therefore, it was

decided that the Post- Int$_{Ctrl}$ session would take place 2 weeks after the last

educational session, in order for any short-term effects to be washed out. Therefore,

the Post-Int$_{Ctrl}$ session was planned 2 weeks after EducS5 (e.g. Oliveira et al. , 2015)

Again, before this session, participants had been instructed to refrain from

eating anything for at least 1 hour before the session and rinse their mouth with water

at least 10 minutes before the session, as saliva collection was scheduled at the

beginning of this session. When they came to the mindroom, they were connected to

the sensors BVP, SC, TEMP, RESP and two EMG sensors. Saliva[1] was collected before

the commencement of the psychophysiological stress profile and immediately after it

(NB the procedure was exactly the same as in Pre-Int$_{Ctrl}$).

3.2.5 Data Analysis

The Statistical Package for the Social Sciences (SPSS) version 21.0 was used as a

tool for data analysis. Statistical analyses included descriptive statistics such as

percentages and means and standard deviations.

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[1] It is worth adding that, as the initial purpose of the study was to investigate long-lasting

effects, the collection of saliva during the five weeks of the protocol (in this case educational sessions)

was not considered as necessary. The idea of including four instances of saliva collection in every HRVT

was created to be able to monitor how biochemical markers develop before and after each session of

HRVT, whether any effects of HRVT last for some time and compare those with what was expected

(Adam, Hoyt & Granger, 2011). In this way, the cost of the consumables needed for the analyses of the

samples was considerably restricted as well, which made the realization of the study more feasible.

Other statistical analyses were repeated- measures analyses of variance

(ANOVA), paired-samples t-tests used for post-hoc comparisons, and repeated-

measures (mixed model) analyses of covariance (ANCOVA). The level of significance for

all statistical analyses was set at the .05 alpha level. The variables measured during this

study are summarized in Appendix B.1. First, we explore the impact of HRVT on key

aspects of HRV, such as RMSSD and LF, with the former being considered to be the

primary time-domain metric to reflect vagally-mediated changes in HRV and a more

reliable index than short-term SDNN and pNN50 (Shaffer & Ginsberg, 2017), whereas

the latter is affected by both SNS and PNS and maximization of it would be desirable.

Other key aspects of HRV are investigated as described below, as well as the impact of

HRVT on biochemical and psychophysiological aspects of stress. Therefore, the

following tests were conducted:

✓ A repeated-measures analysis of covariance – mixed model - (ANCOVA) was
   conducted to investigate:

> whether there were statistically significant differences in the root mean
   square of the successive differences (RMSSD) recorded for the participants
   of the experimental group during their post-intervention testing session (i.e.
   Post-Int$_{exp}$) compared to the RMSSD recorded for the participants of the
   control group during their post-intervention testing session of (i.e. Post-
   Int$_{Ctrl}$), controlling for the RMSSD recorded during the participants' pre-
   intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the power of the low frequency range (LF) recorded for the participants of the experimental group during their post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the LF recorded for the participants of the control group during their post-intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the LF recorded during the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the standard deviation of normal-to-normal R-R intervals (SDNN) recorded for the participants of the experimental group during their post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the standard deviation of normal-to-normal R-R intervals (SDNN) recorded for the participants of the control group during their post-intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the standard deviation of normal-to-normal R-R intervals (SDNN) recorded during the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the proportion of the number of pairs of successive NN (R-R) intervals that differ by more than 50ms divided by the total number of NN (R-R) intervals (i.e. pNN50) recorded for the participants of the experimental group during their post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the pNN50 recorded for the participants of the control group during their post-

intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the pNN50

recorded during the participants' pre-intervention testing session (i.e. Pre-

Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the mean ratio low

frequency to high frequency (LF/HF) recorded for the participants of the

experimental group in the post-intervention testing session (i.e. Post-Int$_{exp}$)

compared to the LF/HF recorded in the post-intervention testing session of

the participants in the control group (i.e. Post-Int$_{Ctrl}$), controlling for the

LF/HF recorded in the participants' pre-intervention testing session (i.e. Pre-

Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the cortisol levels

detected in the participants of the experimental group before the post-

intervention testing session (i.e. Post-Int$_{exp}$) compared to the cortisol levels

detected in the participants of the control group before their post-

intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the cortisol

levels detected before the participants' pre-intervention testing session (i.e.

Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the cortisol levels

detected in the participants of the experimental group immediately after

the post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the

cortisol levels detected in the participants of the control group immediately

after their post-intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling

for the cortisol levels detected immediately after the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

> whether there were statistically significant differences in the a-amylase levels detected in the participants of the experimental group before their post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the a-amylase levels detected in the participants of the control group before their post-intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the a-amylase levels detected before the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

> whether there were statistically significant differences in the a-amylase levels detected in the participants of the experimental group immediately after their post-intervention testing session (i.e. Post-Int$_{exp}$) compared to the a-amylase levels detected in the participants of the control group immediately after their post-intervention testing session of (i.e. Post-Int$_{Ctrl}$), controlling for the a-amylase levels detected immediately after the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

✓ Furthermore, a repeated-measures analysis of variance (ANOVA) was conducted to investigate:

> whether there were statistically significant differences in the a-amylase levels of the experimental group participants collected before the HRVT compared to the other three instances of collection, that is, immediately after, 45 minutes later and 90 minutes after the end of the HRVT.

➢ whether there were statistically significant differences in the cortisol levels of the participants of the experimental group collected at the same time from Session 1 to Session 8 (i.e. seven measurements X four different times of collection).

➢ whether there were statistically significant differences in the a-amylase levels of the participants of the experimental group collected at the same time from Session 1 to Session 8 (i.e. seven measurements X four different times of collection).

✓ Additionally, a repeated-measures analysis of covariance – mixed model - (ANCOVA) was conducted to investigate:

➢ whether there were statistically significant differences in the total electrical activity recorded in the trapezius muscles (EMG) in the post-intervention testing session of the participants in the experimental group (i.e. Post-Int$_{exp}$) compared to the EMG recorded in the post-intervention testing session of the participants in the control group (i.e. Post-Int$_{Ctrl}$), controlling for the electrical activity recorded in the trapezii of the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the skin conductance levels (SC) recorded in the post-intervention testing session of the participants in the experimental group (i.e. Post-Int$_{exp}$) compared to the SC recorded in the post-intervention testing session of the participants in

the control group (i.e. Post-Int$_{Ctrl}$), controlling for the SC recorded in the

participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$ and Pre-Int$_{Ctrl}$).

➢ whether there were statistically significant differences in the skin

temperature levels (TEMP) recorded in the post-intervention testing session

of the participants in the experimental group (i.e. Post-Int$_{exp}$) compared to

the TEMP recorded in the post-intervention testing session of the

participants in the control group (i.e. Post-Int$_{Ctrl}$), controlling for the TEMP

recorded in the participants' pre-intervention testing session (i.e. Pre-Int$_{exp}$

and Pre-Int$_{Ctrl}$).

Appendix B.1 outlines the different parameters measured during the different sessions

of the study.

In sum, better understanding of psychophysiological/biochemical responses to

the newly-introduced 5-week slow-breathing strategy is important. First, as stress

management and, as an extent of that, emotional control, in general, is one of the key

issues an athlete faces prior to competition, strategies for better mental preparation

are needed. Second, if HRV training is proposed as an intervention, then understanding

whether a single 20-minute session has acute effects and/or within a 5-week period

have increasing or gradually attenuated as well as long-lasting benefits is important. In

the current study, the following three research questions were addressed: (A) Does a

single 20-minute HRV training session create acute biochemical effects? (B) With the

passage of time within the 5-week period of the suggested protocol, are there any

increasing or attenuated effects? (C) Are there any long-lasting effects recorded two

weeks after the completion of the 5-week protocol? Answering these questions will

result in a clearer understanding on the benefits of HRV training on emotional control.

3.3 RESULTS

3.3.1 Demographic Data

The demographic information of the participants is displayed in Table 3.2 (Appendix B.2). All of the athletes were males (100%); the majority of the participants who took part were student-athletes of athletics (21.1%); there were also student-athletes of football (19.3%), shooting (14.0%), table tennis (12.3%), fencing (8.8%), artistic gymnastics (7.0%), squash (5.3%), golf (3.5%), whereas there were also student-athletes who study in Aspire academy to be football referees (8.8%). The distribution of their age is also shown in Table 3.2; the majority of the student-athletes participating in this study were 16 years old (53.4%).

3.3.2 Measures of Central Tendency and Dispersion

The mean and standard deviations of the measured variables are shown in Table 3.3. In that table, it is shown that the mean electrical activity on the trapezius muscles (i.e. EMG) of the participants in the experimental group during the baseline (M = 7.43, SD = 13.37) was much higher than the EMG recorded after the intervention (M = 3.85, SD = 3.86), whereas it appeared to be slightly higher in the case of the control group (M = 6.04, SD = 7.28 compared to M = 7.59, SD = 8.77) in the post-intervention measurement. Likewise, skin conductance appeared to be lower in the post-intervention measurement for the experimental group (M = 2.93, SD = 1.84, compared to M = 1.73, SD = 1.46); for the control group it was lower before the intervention (M = 2.71, SD = 2.62) compared to (M = 3.21, SD = 3.27) after the intervention. On the other

Table 3.3

Means and Standard Deviations of the Recorded Variables Across the Two Groups

Groups	Variables	PRE		POST	
		M	SD	M	SD
	EMG[1]	7.43	13.37	3.85	3.86
	Skin Conductance (SC)	2.93	1.84	1.73	1.46
	Skin temperature (Temp)	30.29	3.94	32.30	3.26
	HRV-low frequency (LF)	428.25	397.80	786.32	683.62
	HRV-high frequency (HF)	617.76	548.32	768.82	710.41
	Heart rate (HR)	68.65	11.58	65.61	15.75
	Respiration rate	15.44	2.44	15.41	2.81
	Standard deviation of NN (SDNN)	60.81	21.60	77.26	24.59
	pNN50	0.19	0.10	0.20	0.09
Experimental	RMSSD	61.71	23.45	78.05	29.74
	Cortisol 1	0.26	0.18	0.29	0.21
	Cortisol 2	0.19	0.11	0.13	0.10
	Cortisol 3	0.15	0.08	0.14	0.09
	Cortisol 4	0.15	0.10	0.13	0.07
	A-amylase 1	61.94	42.64	75.65	64.17
	A-amylase 2	61.02	42.89	68.16	53.86
	A-amylase 3	100.46	57.34	102.27	57.74
	A-amylase 4	97.41	51.43	108.24	70.73
	EMG[1]	6.04	7.28	7.59	8.77

	Skin Conductance (SC)	2.71	2.62	3.21	3.27
	Skin temperature (Temp)	29.22	4.03	30.36	3.84
	HRV-low frequency (LF)	484.89	331.26	486.10	433.74
	HRV-high frequency (HF)	566.07	549.68	624.57	556.74
	Heart rate (HR)	71.83	13.11	72.97	12.16
	Respiration rate	16.91	1.55	16.03	2.40
	Standard deviation of NN (SDNN)	64.41	18.79	71.62	24.93
	pNN50	0.18	0.09	0.18	0.09
Control	RMSSD	63.67	25.32	64.40	26.26
	Cortisol 1	0.42	0.31	0.37	0.25
	Cortisol 2	0.31	0.19	0.29	0.19
	Cortisol 3	N/A	N/A	N/A	N/A
	Cortisol 4	N/A	N/A	N/A	N/A
	A-amylase 1	104.54	89.35	89.06	77.89
	A-amylase 2	124.77	99.11	78.52	49.25
	A-amylase 3	N/A	N/A	N/A	N/A
	A-amylase 4	N/A	N/A	N/A	N/A

[1] Total electrical activity recorded in the trapezius muscles.

hand, skin temperature was found to be higher in the case of the experimental group

after the intervention (M = 32.30, SD = 3.26) compared to the measurement conducted

before the intervention (M = 30.29, SD = 3.94), which, in the case of this

psychophysiological variable, is a positive development; even for the control group,

there was a small increase in the skin temperature in the post-intervention

measurement (M = 30.36, SD = 3.84), compared to the baseline measurement (M = 29.22, SD = 4.03).

Moreover, the root mean square of the successive differences for the experimental group was higher when measured after the intervention (M = 78.05, SD = 29.74) than before the intervention (M = 61.71, SD = 23.45), whereas for the control group there was just a slight increase in the post-intervention measurement (M= 63.67, SD = 25.32 compared to M = 64.40, SD = 26.26 after the intervention). A similar trend was observed in the mean total power in low frequency range (LF) recorded for the experimental group (M = 428.25, SD = 397.80 before the intervention and M = 786.32, SD = 683.62 after the intervention) and the control group (M = 484.89, SD = 331.26 before and M = 486.10, SD = 433.74 after). The standard deviation of all NN intervals (SDNN) was found to be elevated after the intervention both for the experimental (M = 77.26, SD = 24.59 compared to baseline measurements M = 60.81, SD = 21.60) and the control group (M = 71.62, SD = 24.93 compared to baseline measurements M = 64.41, SD = 18.79).

A comparison of the biochemical markers between the two instances of measurement across the two groups showed that all four measurements for a-amylase in the case of the experimental group were higher in the post-intervention measurement (e.g. $M_{a\text{-amylasepost-int1}}$ = 75.65, SD = 64.17 compared to the pre-intervention $M_{a\text{-amylasepre-int1}}$ = 61.94 and SD = 42.64), for a-amylase 2 ($M_{a\text{-amylasepost-int2}}$ = 68.16, SD = 53.86 compared to $M_{a\text{-amylasepre-int2}}$ = 61.02 and SD = 42.89), and similar trends for a-amylase 3 and 4 as shown in Table 3.3, whereas for the control group

there was a declining trend in the post-intervention measurement ($M_{a\text{-}amylasepost\text{-}int1}$ = 89.06, SD = 77.89 compared to the pre-intervention $M_{a\text{-}amylasepre\text{-}int1}$ = 104.54 and SD = 89.35) and for a-amylase (2 $M_{a\text{-}amylasepost\text{-}int2}$ =78.52, SD = 49.25) compared to the pre-intervention measurement ($M_{a\text{-}amylasepre\text{-}int2}$ = 124.77 and SD = 99.11). For salivary cortisol, there was a declining trend in the post-intervention measurements for all the measurements of both groups, as show in Table 3.3, apart from the first measurement in the experimental group ($M_{cortisolpre\text{-}int1}$ = 0.26, SD = 0.18) compared to the post-intervention measurement ($M_{cortisolpost\text{-}int1}$ = 0.29, SD = 0.21).

3.3.3 Investigation of Statistical Significance

3.3.3.1 Heart rate variability. The following analyses of covariance (ANCOVAs) were conducted to investigate significant difference in HRV-related parameters:

3.3.3.1.1 Standard deviation of NN intervals (SDNN). An ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the SDNN levels measured in post-intervention session, controlling for the SDNN levels measured in the pre-intervention session ($M_{exp\text{-}post}$ = 77.26, $SD_{exp\text{-}post}$ = 24.59 and $M_{ctrl\text{-}post}$ = 71.62, $SD_{ctrl\text{-}post}$ = 24.93 compared to $M_{exp\text{-}pre}$ = 60.81, $SD_{exp\text{-}pre}$ = 21.60 and $M_{ctrl\text{-}pre}$ = 64.41, $SD_{ctrl\text{-}pre}$ = 18.79)[2].

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[2] $M_{exp\text{-}post}$ = mean value in the post-intervention session for the experimental group, $SD_{exp\text{-}post}$ = SD in the ost-intervention session for the experimental group, $M_{ctrl\text{-}post}$ = mean value in the post-intervention session for the control group, $SD_{ctrl\text{-}post}$ = SD in the post-intervention session for the control group, $M_{exp\text{-}pre}$ = mean value in the pre-intervention session for the experimental group, $SD_{exp\text{-}pre}$ = SD in the pre-intervention session for the experimental group, $M_{ctrl\text{-}pre}$ = mean value in the pre-intervention session for the control group, $SD_{ctrl\text{-}pre}$ = SD in the pre-intervention session for the control group.

Table 3.4 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the SDNN levels, showed no statistically significant differences, $F_{(1, 52)} = 1.65$, $p > .05$. Therefore, SDNN levels measured in the post-intervention session of the experimental group were not found to be significantly different from those measured in the control group, when controlling for SDNN levels measured in the pre-intervention session.

3.3.3.1.2 Proportion of the number of pairs of successive NNs that differ by more than 50ms, divided by the total number of NNs (pNN50).

An ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the pNN50 levels measured in post-intervention session, controlling for the pNN50 levels measured in the pre-intervention session ($M_{exp-post} = 0.20$, $SD_{exp-post} = 0.09$ and $M_{ctrl-post} = 0.18$, $SD_{ctrl-post} = 0.09$ compared to $M_{exp-pre} = 0.19$, $SD_{exp-pre} = 0.10$ and $M_{ctrl-pre} = 0.18$, $SD_{ctrl-pre} = 0.09$). Table 3.5 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the pNN50 levels, showed no statistically significant differences, $F_{(1, 52)} = 0.37$, $p > .05$. Therefore, pNN50 levels measured in the post-intervention session of the experimental group were not found to be significantly different from those measured in the control group, when controlling for pNN50 levels measured in the pre-intervention session.

3.3.3.1.3 Root mean square of successive differences between adjacent NNs (RMSSD).

An ANCOVA was conducted to determine if there was a statistically significant

difference between the experimental condition and the control condition on the RMSSD levels measured in post-intervention session, controlling for the RMSSD levels measured in the pre-intervention session. ($M_{exp-post}$ = 78.05, $SD_{exp-post}$ = 29.74 and $M_{ctrl-post}$ = 64.40, $SD_{ctrl-post}$ = 26.26 compared to $M_{exp-pre}$ = 61.71, $SD_{exp-pre}$ = 23.45 and $M_{ctrl-pre}$ = 63.67, $SD_{ctrl-pre}$ = 25.32). Table 3.6 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the RMSSD levels, was found to be statistically significant, $F (1, 52)$ = 4.96, p = .03, η_p^2 = .09.

Therefore, RMSSD levels measured in the post-intervention session of the experimental group were found to be significantly different from those measured in the control group, when controlling for RMSSD levels measured in the pre-intervention session.

3.3.3.1.4 Low frequency (LF). An ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the LF levels measured in post-intervention session, controlling for the LF levels measured in the pre-intervention session. ($M_{exp-post}$ = 786.32, $SD_{exp-post}$ = 683.62 and $M_{ctrl-post}$ = 486.10, $SD_{ctrl-post}$ = 433.74 compared to $M_{exp-pre}$ = 428.25, $SD_{exp-pre}$ = 379.80 and $M_{ctrl-pre}$ = 484.89, $SD_{ctrl-pre}$ = 331.26). Table 3.7 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the LF levels, was found to be statistically significant, $F (1, 51)$ = 4.05, p = .05, η_p^2 = .07.
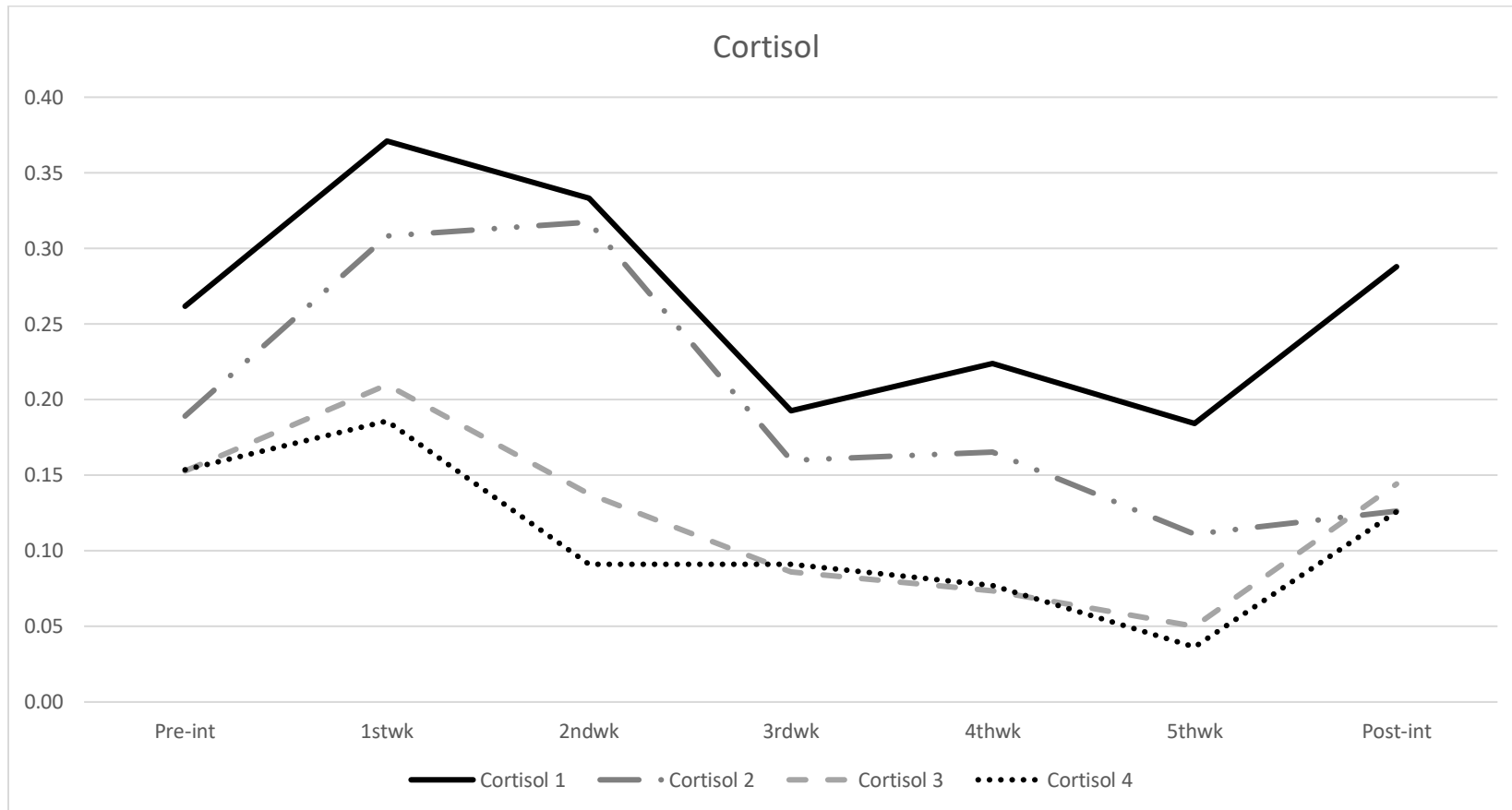
Figure 3.1. Development of salivary cortisol at the same time of collection over the 7 weeks of the protocol (i.e. pre-intervention,

weeks 1 to 5 of the intervention, post-intervention), (where Cortisol 1= before the session, Cortisol 2= immediately after the session,

Cortisol 3 = 45 minutes after the session, Cortisol 4 = 90 minutes after the session), for the experimental group.
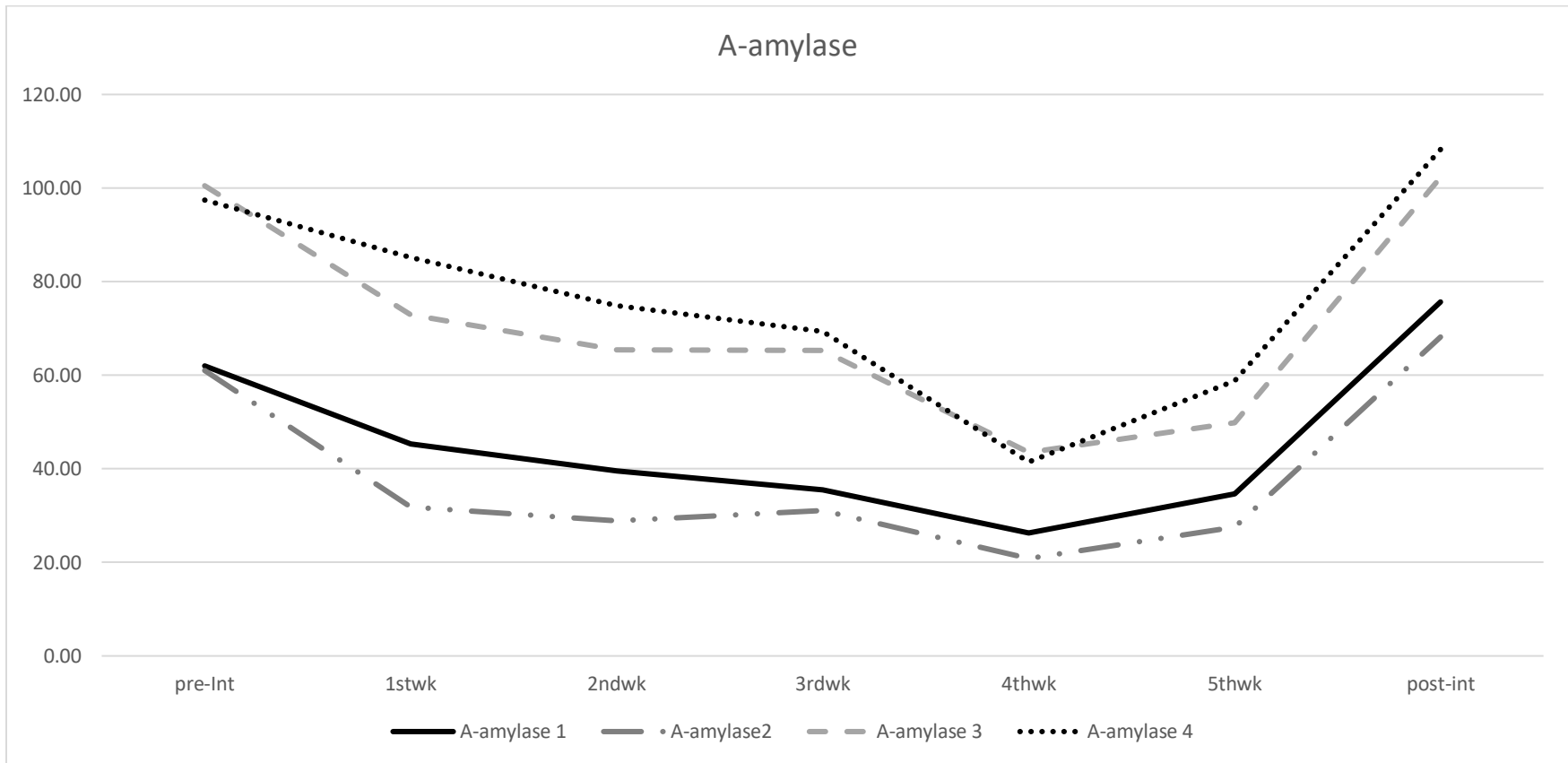
Figure 3.2. Development of salivary a-amylase at the same time of collection over the 7 weeks of the protocol (i.e. pre-intervention, weeks 1 to 5 of the intervention, post-intervention), (where A-amylase 1= before the session, A-amylase 2= immediately after the session, A-amylase 3 = 45 minutes after the session, A-amylase 4 = 90 minutes after the session), for the experimental group.

Therefore, LF levels measured in the post-intervention session of the experimental group were found to be significantly different from those measured in the control group, when controlling for LF levels measured in the pre-intervention session.

3.3.3.1.5 Ratio of low frequency to high frequency (LF/HF). An ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the LF/HF ratio levels measured in the post-intervention session, controlling for the LF/HF ratio levels measured in the pre-intervention session ($M_{exp-post}$ = 1.02, $SD_{exp-post}$ = 0.96 and $M_{ctrl-post}$ = 0.78, $SD_{ctrl-post}$ = 0.78 compared to $M_{exp-pre}$ = 0.69, $SD_{exp-pre}$ = 0.72 and $M_{ctrl-pre}$ = 0.86, $SD_{ctrl-pre}$ = 0.60). Table 3.8 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the LF/HF ratio levels, was not found to be statistically significant, $F (1, 51) = 2.81, p > .05$.

Therefore, LF/HF ratio levels measured in the post-intervention session of the experimental group were not found to be significantly different from those measured in the control group, when controlling for LF/HF ratio levels measured in the pre-intervention session.

Last but not least, statistical significance was found in the indices RMSSD and LF; the former is considered to be the primary time-domain metric to reflect vagally-mediated changes in HRV and a more reliable index than short-term SDNN and pNN50

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(Shaffer & Ginsberg, 2017), whereas the latter is affected by both SNS and PNS and maximization of it would be desirable. Consequently, the results showed that the proposed 5-week HRVT protocol did result in statistically significant changes in RMSSD and LF, though the effect sizes were very small.

3.3.3.2 Other long-lasting effects. The two biochemical markers (i.e. cortisol and a-amylase) were analyzed in comparison with the salivary samples collected from the control group, as explained below:

3.3.3.2.1 A-amylase. A one-way analysis of covariance (ANCOVA) was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the a-amylase levels detected in the first salivary sample collected in the post-intervention session (i.e. a-amylase$_{post-int1}$), controlling for the a-amylase levels detected in the first salivary sample collected in the pre-intervention session (i.e. a-amylase$_{pre-int1}$) ([for the experimental group]: $M_{a-amylasepost-int1}$ = 75.65, SD = 64.17 compared to the pre-intervention $M_{a-amylasepre-int1}$ = 61.94 and SD = 42.64; [for the control group]: $M_{a-amylasepost-int1}$ = 89.06, SD = 77.89 compared to the pre-intervention $M_{a-amylasepre-int1}$ = 104.54 and SD = 89.35). Table 3.9 (Appendix B.2) summarizes the results of this ANCOVA this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on the a-amylase 1 levels, was found to have no statistically significant effects, $F(1, 50) = 0.99$, $p > .05$. Therefore, a-amylase levels detected in the first sample collected in the post-intervention session (a-amylase$_{post-int1}$) of the experimental group were not found to be significantly different from those detected in the control group, when

controlling for a-amylase levels detected in the first salivary sample collected in the pre-intervention session (a-amylase$_{pre-int1}$).

Furthermore, a one-way ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the a-amylase levels detected in the second salivary sample collected in the post-intervention session (i.e. a-amylase$_{post-int2}$), controlling for the a-amylase levels detected in the second salivary sample collected in the pre-intervention session (i.e. a-amylase$_{pre-int2}$). ([for the experimental group]: M$_{a-amylasepost-int2}$ = 68.16, SD = 53.86 compared to the pre-intervention M$_{a-amylasepre-int2}$ = 61.02 and SD = 42.89; [for the control group]: M$_{a-amylasepost-int2}$ = 78.52, SD = 49.25 compared to the pre-intervention M$_{a-amylasepre-int2}$ = 124.77 and SD = 99.11). Table 3.10 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on a-amylase 2 levels, was found to have no statistically significant effects, $F (1, 50) = 0.61$, $p > .05$. Therefore, a-amylase levels detected in the second sample collected in the post-intervention session (a-amylase$_{post-int2}$) of the experimental group were not found to be significantly different from those detected in the control group, when controlling for a-amylase levels detected in the second salivary sample collected in the pre-intervention session (a-amylase$_{pre-int2}$).

3.3.3.2.2 Cortisol. Likewise, a one-way analysis of covariance (ANCOVA) was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the cortisol levels detected in the

first salivary sample collected in the post-intervention session (i.e. cortisol$_{post-int1}$), controlling for the cortisol levels detected in the first salivary sample collected in the pre-intervention session (i.e. cortisol$_{pre-int1}$) ([for the experimental group]: M$_{cortisolpost-int1}$ = 0.29, SD = 0.21 compared to the pre-intervention M$_{cortisolpre-int1}$ = 0.26 and SD = 0.18; [for the control group]: M$_{cortisolpost-int1}$ = 0.37, SD = 0.25 compared to the pre-intervention M$_{cortisolpre-int1}$ = 0.42 and SD = 0.31). Table 3.11 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on cortisol 1 levels, was found to have no statistically significant effects, $F (1, 50) = 0.00003$, $p > .05$. Therefore, cortisol levels detected in the first sample collected in the post-intervention session (cortisol$_{post-int1}$) of the experimental group were not found to be significantly different from those detected in the control group, when controlling for cortisol levels detected in the first salivary sample collected in the pre-intervention session (cortisol$_{pre-int1}$).

Moreover, a one-way ANCOVA was conducted to determine if there was a statistically significant difference between the experimental condition and the control condition on the cortisol levels detected in the second salivary sample collected in the post-intervention session (i.e. cortisol$_{post-int2}$), controlling for the cortisol levels detected in the second salivary sample collected in the pre-intervention session (i.e. cortisol$_{pre-int2}$) ([for the experimental group]: M$_{cortisolpost-int2}$ = 0.13, SD = 0.10 compared to the pre-intervention M$_{cortisolpre-int2}$ = 0.19 and SD = 0.11; [for the control group]: M$_{cortisolpost-int2}$ = 0.29, SD = 0.19 compared to the pre-intervention M$_{cortisolpre-int2}$ = 0.31 and SD = 0.19). Table 3.12 (Appendix B.2) summarizes the results of this ANCOVA; this analysis of

covariance, conducted for the two different groups of participants (i.e. experimental versus control group) on cortisol 2 levels, was found to be statistically significant, $F_{(1, 49)} = 7.57$, $p = .008$, $\eta_p^2 = .13$. Therefore, cortisol levels detected in the second sample collected in the post-intervention session (cortisol$_{post-int2}$) of the experimental group were found to be significantly different from those detected in the control group, when controlling for cortisol levels detected in the second salivary sample collected in the pre-intervention session (cortisol$_{pre-int2}$). From the descriptive statistics of Table 3.3, it is also concluded that cortisol$_{post-int2}$ was significantly lower in the case of the experimental group than in the control group.

3.3.3.3 Acute effects. Acute effects were investigated in a-amylase as well. As such, a repeated-measures analysis of variance was conducted (ANOVA) to examine the extent to which the average a-amylase detected in the salivary sample collected before each of the five HRV training sessions run during the 5-week training period of the experimental group (a-amylase 1) was significantly different from the average of the a-amylase detected immediately after the HRV-training sessions run during the 5-week period of training (a-amylase 2), the a-amylase detected 45 minutes after the HRV training sessions (a-amylase 3) and the a-amylase detected 90 minutes after the HRV training sessions (a-amylase 4). The graphical representation of the development of the mean sAA across the four different times of collection is shown in the figure below (Figure 3.3):
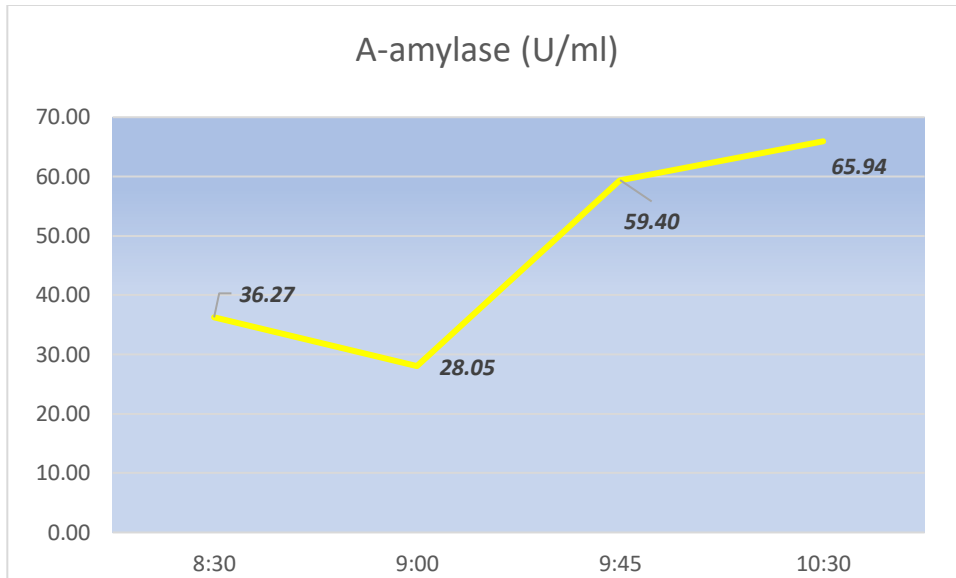
Figure 3.3. Mean levels of sAA collected at different times across the five weeks of

HRVT (a-amylase 1= 8:30, a-amylase 2= 9:00, a-amylase 3= 9:45, a-amylase 4= 10:30).

As shown in Table 3.13a (Appendix B.2), the repeated-measures ANOVA

conducted for the averaged a-amylase detected over the five different weeks of HRV

training on the four different times of measurement ($M_{a-amylase1}$ = 36.27, SD = 28.94, $M_{a-amylase2}$ = 28.05 , SD = 20.14, $M_{a-amylase3}$ = 59.40, SD = 34.44, $M_{a-amylase4}$ = 65.94, SD =

38.10) was found to be statistically significant $F_{(1.85, 53.76)}$ = 64.98, p < .0001, η^2 =

.69. Paired-samples t-tests were also conducted to investigate post-hoc comparisons

among the different times of salivary collection as shown in Table 3.13b (Appendix

B.2). In this table, it is shown that all possible pairwise comparisons derived from the

above mentioned repeated-measures ANOVA result in statistically significant

differences; however, as also shown in Figure 3.3, while statistically significant

differences appear to be in the expected direction in the case of pair 1 (i.e. $t_1{(29)}$ =

3.19, p < .003), in the rest of post-hoc comparisons, significant differences appeared in

the opposite direction (i.e. t_2 (29) = -7.53, p < .0001, t_3 (29) = -8.43, p < .0001, t_4 (29) = -9.26, p < .0001, t_5 (29) = -9.14, p < .0001, t_6 (29) = -3.46, p < .002).

Nevertheless, the above analyses suggest that we can reject the null hypothesis and accept the alternative hypothesis, according to which a-amylase levels are significantly lower- at least -immediately after a 20-minute HRV.

3.3.3.4 Overall effects detected across the seven weeks of the experiment.
Furthermore, the development of the biochemical markers across the seven sessions would be worth investigating; the analyses, discussed in this section, investigates how each biochemical marker (i.e. cortisol and a-amylase) changes at the same time of collection across the 7 weeks of the experiment (i.e. baseline plus five weeks of HRVT plus post-intervention measurement), for the experimental group only- see Figure 3.1 and Figure 3.2 above).

3.3.3.4.1 Cortisol. Four repeated-measures ANOVA's were conducted to explore the change in cortisol over the 7-week collection period at each of the time points, that is, before the HRVT session (collection no. 1), immediately after (collection no. 2), 45 minutes after the HRVT (collection no. 3) and 90 minutes after (collection no. 4) the HRVT session. Table 3.14a (Appendix B.2) displays the results of the ANOVA conducted for cortisol 1 (i.e. cortisol detected before the stress profile in the pre- and post- intervention sessions and before the HRVT in the five weeks of training). There was a statistical difference ($M^{cortisol1}_{pre-intexp}$ = 0.26, SD = 0.18, $M^{cortisol1}_{HRVT1}$ = 0.37 , SD = 0.27, $M^{cortisol1}_{HRVT2}$ = 0.33, SD = 0.25, $M^{cortisol1}_{HRVT3}$ = 0.19, SD = 0.14, $M^{cortisol1}_{HRVT4}$ = 0.22,

SD = 0.15, $M^{cortisol1}_{HRVT5}$ = 0.18, SD = 0.13, $M^{cortisol1}_{post-intexp}$ = 0.29, SD = 0.21) in the levels

of cortisol 1 over time, $F(4.02, 116.53)$ = 7.32, $p < .0001$, η_p^2 = .20. Paired-samples t-

tests were also conducted to investigate post-hoc comparisons among the different

weeks of salivary cortisol 1 as shown in Table 3.14b (Appendix B.2). There were nine

statistically significant differences between the various pairs towards the desirable

direction (i.e. cortisol was lower in the second collection within the pairwise

comparison) at the .05 level of significance, whereas many of these significant

differences result from comparisons of the first 2 weeks of training with the following

weeks (see also Figure 3.1 for a diagrammatic presentation of those differences).

Table 3.15a (Appendix B.2) displays the results of the ANOVA conducted for

cortisol 2 (i.e. cortisol detected immediately after the stress profile in the pre- and

post- intervention sessions and immediately after the HRVT in the 5 weeks of training).

There was a statistical difference ($M^{cortisol2}_{pre-intexp}$ = 0.19, SD = 0.11, $M^{cortisol2}_{HRVT1}$ = 0.31 ,

SD = 0.21, $M^{cortisol2}_{HRVT2}$ = 0.32, SD = 0.27, $M^{cortisol2}_{HRVT3}$ = 0.16, SD = 0.12, $M^{cortisol2}_{HRVT4}$ =

0.17, SD = 0.10, $M^{cortisol2}_{HRVT5}$ = 0.11, SD = 0.07, $M^{cortisol2}_{post-intexp}$ = 0.13, SD = 0.10) in the

levels of cortisol 2 over time, $F(2.83, 81.98)$ = 12.67, $p < .0001$, η_p^2 = .30. Paired-

samples t-tests were also conducted to investigate post-hoc comparisons among the

different weeks of salivary cortisol 2 as shown in Table 3.15b (Appendix B.2). Again, the

results, presented in this table, indicate that there were twelve statistically significant

differences in different pairwise comparisons of cortisol 2 towards the desirable

direction, at the .05 level of significance; significant differences are mostly derived

from the comparison of the 1st week and 2nd week with the subsequent weeks. Figure 3.1 shows diagrammatically how cortisol 2 develops over the period of 7 weeks.

Table 3.16a (Appendix B.2) displays the results of the ANOVA conducted for cortisol 3. There was a statistical difference ($M^{cortisol3}_{pre-intexp}$ = 0.15, SD = 0.08, $M^{cortisol3}_{HRVT1}$ = 0.21 , SD = 0.12, $M^{cortisol3}_{HRVT2}$ = 0.14, SD = 0.07, $M^{cortisol3}_{HRVT3}$ = 0.09, SD = 0.05, $M^{cortisol3}_{HRVT4}$ = 0.07, SD = 0.03, $M^{cortisol3}_{HRVT5}$ = 0.05, SD = 0.03, $M^{cortisol3}_{post-intexp}$ = 0.14, SD = 0.09) in the levels of cortisol 3 over time as well, $F_{(3.44, 99.76)}$ = 19.81, $p <$ .0001, η_p^2 = .41. Therefore, cortisol 3 levels did differ significantly across the 7 weeks of the protocol. Table 3.16b (Appendix B.2) presents post-hoc comparisons of this ANOVA and indicates that 13 out of 21 pairwise comparisons were significantly different with the difference being towards the desirable direction, at the .05 level of significance, suggesting a declining trend of cortisol 3 across the 7 weeks, as shown clearly in Figure 3.1.

Furthermore, Table 3.17a (Appendix B.2) displays the results of the ANOVA conducted for cortisol 4. As shown in that table, there was a statistical difference ($M^{cortisol4}_{pre-intexp}$ = 0.15, SD = 0.10, $M^{cortisol4}_{HRVT1}$ = 0.19 , SD = 0.29, $M^{cortisol4}_{HRVT2}$ = 0.09, SD = 0.04, $M^{cortisol4}_{HRVT3}$ = 0.09, SD = 0.09, $M^{cortisol4}_{HRVT4}$ = 0.08, SD = 0.05, $M^{cortisol4}_{HRVT5}$ = 0.04, SD = 0.02, $M^{cortisol4}_{post-intexp}$ = 0.13, SD = 0.07) in the levels of cortisol 4 over time, $F_{(1, 29)}$ = 4.69, $p <$ .05, η_p^2 = .14. Table 3.17b (Appendix B.2) presents post-hoc comparisons of this ANOVA in which the results indicate that there were eight statistically significant differences with the difference being towards the desirable direction at the .05 level of significance, especially, between pre-intervention cortisol 4

with most of the intervention weeks, but also between each of the first 4 intervention

weeks with the 5th HRVT week. Figure 3.1 clearly depicts the declining trend of cortisol

4.

Figure 3.1 also shows the declining trend of both Cortisol 3 and Cortisol 4,

starting from the 1st week onwards, whereas an increase is observed from the 5th

week to the post-intervention session. Cortisol 1 and Cortisol 2 start declining after the

2nd week, whereas a slight increase is observed from the 3rd to the 4th week and then

again from the 5th week to the post-intervention session. It is interesting that Cortisol

2, Cortisol 3 and Cortisol 4 all converge at the post-intervention measurement.

3.3.3.4.2 A-amylase. Four repeated-measures ANOVA's were also conducted to

explore the change in a-amylase over the 7-week collection period at each of the time

points, that is, before the HRVT session (collection no 1), immediately after (collection

no 2), 45 minutes after the HRVT (collection no 3) and 90 minutes after (collection no

4) the HRVT session. Table 3.18a (Appendix B.2) displays the results of the ANOVA

conducted for a-amylase 1 (i.e. a-amylase detected before the stress profile in the pre-

and post- intervention sessions and before the HRVT in the 5 weeks of training). There

was a statistical difference ($M^{\text{a-amylase1}}_{\text{pre-intexp}}$ = 61.94, SD = 42.64, $M^{\text{a-amylase1}}_{\text{HRVT1}}$ =

45.31, SD = 44.56, $M^{\text{a-amylase1}}_{\text{HRVT2}}$ = 39.59, SD = 41.04, $M^{\text{a-amylase1}}_{\text{HRVT3}}$ = 35.51, SD =

33.94, $M^{\text{a-amylase1}}_{\text{HRVT4}}$ = 26.28, SD = 20.02, $M^{\text{a-amylasel1}}_{\text{HRVT5}}$ = 34.64, SD = 34.08, $M^{\text{a-amylase1}}_{\text{post-intexp}}$ = 75.65, SD = 64.17) in the levels of a-amylase over time, F (3.13, 90.81)

= 10.67, p < .001, η_p^2 = .27. Paired-samples t-tests were also conducted to investigate

post-hoc comparisons among the different weeks of salivary a-amylase 1 as shown in

Table 3.18b (Appendix B.2). There were seven significant differences between the various pairs towards the desirable direction (i.e. a-amylase was lower in the second collection within the pairwise comparison), at the .05 level of significance, whereas most of these significant differences result from comparisons between the pre-intervention week and all of the intervention weeks (see also Figure 3.2 for a diagrammatic presentation of those differences). Figure 3.2 showed how a-amylase develops over the period of 7 weeks; a decline of a-amylase 1 is observed from the pre-intervention measurement to the rest of the HRV training weeks, whereas it appeared to have a steep increase from the 5th week to the post-intervention session.

Table 3.19a (Appendix B.2) displays the results of the ANOVA conducted for a-amylase 2 (i.e. a-amylase detected immediately after the stress profile in the pre- and post- intervention sessions and immediately after the HRVT in the 5 weeks of training). There was a statistical difference ($M^{\text{a-amylase2}}_{\text{pre-intexp}}$ = 61.02, SD = 42.89, $M^{\text{a-amylase2}}_{\text{HRVT1}}$ = 31.81, SD = 28.78, $M^{\text{a-amylase2}}_{\text{HRVT2}}$ = 28.92, SD = 23.67, $M^{\text{a-amylase2}}_{\text{HRVT3}}$ = 31.13, SD = 31.19, $M^{\text{a-amylase2}}_{\text{HRVT4}}$ = 20.83, SD = 15.88, $M^{\text{a-amylasel2}}_{\text{HRVT5}}$ = 27.55, SD = 24.03, $M^{\text{a-amylase2}}_{\text{post-intexp}}$ = 68.16, SD = 53.86) in the levels of a-amylase 2 over time, $F_{(3.41,99.01)}$ = 17.20, $p < .0001$, η_p^2 = .37. Paired-samples t-tests were also conducted to investigate post-hoc comparisons among the different weeks of salivary a-amylase 2 as shown in Table 3.19b (Appendix B.2). There were eight significant differences in different pairwise comparisons of a-amylase 2 towards the desirable direction, at the .05 level of significance; significant differences are obvious when comparing the pre-intervention

a-amylase 2 with subsequent weeks. Figure 3.2 shows diagrammatically how a-amylase 2 develops over the period of 7 weeks.

Table 3.20a (Appendix B.2) also displays the results of the ANOVA conducted for a-amylase 3. There was a statistical difference ($M^{\text{a-amylase3}}_{\text{pre-intexp}}$ = 100.46, SD = 57.34, $M^{\text{a-amylase3}}_{\text{HRVT1}}$ = 72.93, SD = 58.83, $M^{\text{a-amylase3}}_{\text{HRVT2}}$ = 65.47, SD = 40.83, $M^{\text{a-amylase3}}_{\text{HRVT3}}$ = 65.31, SD = 43.86, $M^{\text{a-amylase3}}_{\text{HRVT4}}$ = 43.51, SD = 27.93, $M^{\text{a-amylasel3}}_{\text{HRVT5}}$ = 49.79, SD = 35.68, $M^{\text{a-amylase3}}_{\text{post-intexp}}$ = 102.27, SD = 57.74) in the levels of a-amylase 3 over time as well, $F_{(3.46, 100.29)}$ = 13.23, $p < .001$, η_p^2 = .31. Therefore, a-amylase 3 levels did differ significantly across the 7 weeks of the protocol. Table 3.20b (Appendix B.2) presents pairwise post-hoc comparisons of this ANOVA and indicates that 11 out of 21 pairwise comparisons were significantly different with the difference being towards the desirable direction, at the .05 level of significance suggesting a declining trend throughout the weeks with the exception of the post-intervention session at which a-amylase 3 showed a steep increase, as also shown in Figure 3.2.

Furthermore, Table 3.21a (Appendix B.2) displays the results of the ANOVA conducted for a-amylase 4. As shown in that table, there was a statistical difference ($M^{\text{a-amylase4}}_{\text{pre-intexp}}$ = 97.41, SD = 51.43, $M^{\text{a-amylase4}}_{\text{HRVT1}}$ = 85.22, SD = 52.34, $M^{\text{a-amylase4}}_{\text{HRVT2}}$ = 74.91, SD = 72.51, $M^{\text{a-amylase4}}_{\text{HRVT3}}$ = 69.31, SD = 53.26, $M^{\text{a-amylase4}}_{\text{HRVT4}}$ = 41.45, SD = 26.30, $M^{\text{a-amylasel4}}_{\text{HRVT5}}$ = 58.81, SD = 36.29, $M^{\text{a-amylase4}}_{\text{post-intexp}}$ = 108.24, SD = 70.73) in the levels of a-amylase 4 over time, $F_{(3.61, 104.58)}$ = 9.47, $p < .001$, η_p^2 = .25. Table 3.21b (Appendix B.2) presents pairwise post-hoc comparisons of this ANOVA in which the

results indicate that there were seven statistically significant differences, with the difference being towards the desirable direction, at the .05 level of significance.

Figure 3.2 depicts the progress of a-amylase across the 7 weeks for all four measurements. This figure clearly depicts the declining trend of all four a-amylase indices up to the 4th week of intervention, whereas an increase is observed from the 4th week to the post-intervention session in all four a-amylase indices. It is interesting that a-amylase 1, a-amylase 2, a-amylase 3 and a-amylase 4 all move in parallel from the 5th week to the post-intervention measurement.

3.3.3.5 Additional analyses. In addition to the HRV-related measurements and the salivary samples taken during the stress profiles (i.e. in the pre- and post-intervention sessions), other psychophysiological variables were also recorded; in order to investigate long-lasting effects on those variables as well, more analyses of covariance were conducted as explained below:

3.3.3.5.1 Electrical activity on trapezius muscles (left + right). An analysis of covariance was conducted to investigate whether there were significant differences in the total electrical activity measured in both of the trapezii of the participants of the two groups during the post-intervention session, controlling for the activity measured in the pre-intervention session ($M_{exp-post}$ = 3.85, $SD_{exp-post}$ = 3.86 and $M_{ctrl-post}$ = 7.59, $SD_{ctrl-post}$ = 8.77 compared to $M_{exp-pre}$ = 7.43, $SD_{exp-pre}$ = 13.37 and $M_{ctrl-pre}$ = 6.04, $SD_{ctrl-pre}$ = 7.28). As Table 3.22 (Appendix B.2) shows, total electrical activity (i.e. on both of the trapezii) measured in the post-intervention session was measured to be significantly

different for participants of the experimental group than for those in the control group, controlling for electrical activity measured for both groups in the pre-intervention session, $F(1, 53) = 5.14$, $p = .028$, $\eta_p^2 = .09$.   From Table 3.3 (descriptive statistics), it is also concluded that experimental group participants had significantly lower electrical activity on their trapezii, in the post-intervention session, than control group participants.

3.3.3.5.2 Skin conductance. An analysis of covariance was conducted to investigate whether there were significant differences in the skin conductance measured during the post-intervention session, controlling for the skin conductance (SC) measured in the pre-intervention session ($M_{exp-post} = 1.73$, $SD_{exp-post} = 1.46$ and $M_{ctrl-post} = 3.21$, $SD_{ctrl-post} = 3.27$ compared to $M_{exp-pre} = 2.93$, $SD_{exp-pre} = 1.84$ and $M_{ctrl-pre} = 2.71$, $SD_{ctrl-pre} = 2.62$). As Table 3.23 (Appendix B.2) shows, SC in the post-intervention session was measured to be significantly different for participants of the experimental group than for those in the control group, controlling for SC measured for both groups in the pre-intervention session, $F(1, 53) = 9.35$, $p = .003$, $\eta_p^2 = .15$.   From Table 3.3 (descriptive statistics), it is concluded also that experimental group participants had significantly lower skin conductance, in the post-intervention session, than control group participants.

3.3.3.5.3 Peripheral skin temperature. An analysis of covariance was conducted to investigate whether there were significant differences in the peripheral skin temperature (TEMP) measured during the post-intervention session, controlling for the TEMP measured in the pre-intervention session ($M_{exp-post} = 32.30$, $SD_{exp-post} = 3.26$ and

$M_{\text{ctrl-post}} = 30.36$, $SD_{\text{ctrl-post}} = 3.84$ compared to $M_{\text{exp-pre}} = 30.29$, $SD_{\text{exp-pre}} = 3.94$ and $M_{\text{ctrl-pre}} = 29.22$, $SD_{\text{ctrl-pre}} = 4.03$). As Table 3.24 (Appendix B.2) shows, TEMP in the post-intervention session was not found to be significantly different for participants of the experimental group from TEMP recorded in the control group, controlling for TEMP measured for both groups in the pre-intervention session, $F (1, 53) = 3.28$, $p = .076$.

The above results suggest that we can reject the null hypothesis referring to the long-lasting effects as well; biochemical and psychophysiological variables are significantly different after a period of five controlled and five uncontrolled HRV training sessions.

To sum up the findings of this study, when comparing with a control group, long-lasting effects were found in such major HRV variables as RMSSD and LF but also in the cortisol levels detected immediately after the stress profile conducted in the post-intervention session. Acute effects of a 20-minute HRV-training session were also found in the a-amylase levels detected immediately after the session compared to a-amylase detected before the session. Additionally, both a-amylase and cortisol appear to have a declining trend as sessions of HRV training progress during the 5-week protocol which is an indication of some effects created with the regular repetition of HRV training. More psychophysiological variables showed long-lasting effects too, as for example, electrical activity recorded on the trapezius muscles as well as skin conductance. These results will be further discussed in the next section.

3.4 DISCUSSION

The present study investigated the effects of an HRV-training protocol on stress-related variables. Its main purpose was to investigate the acute and long-lasting effects of heart rate variability training (HRVT) on the stress levels of Middle-Eastern adolescent student-athletes of a sports academy. The experimental group completed a 5-week HRVT protocol which consisted of five weekly lab-based and five weekly home-based 20-minute HRVT sessions, whereas the control group went through five weekly educational sessions. There were acute effects of HRVT on a-amylase levels within each session with a-amylase levels decreasing over the course of the session. Both cortisol and a-amylase levels reduced over the 5 weeks of the HRVT protocol. The data collected after the training protocol, by means of the stress profile and salivary samples, showed significantly lower cortisol and skin conductance levels in the experimental group compared to the control group. These findings suggest that HRVT may be an effective way of reducing psychological stress in Middle-Eastern adolescent student-athletes.

Consistent with the research hypotheses according to which, (i) the proposed 5-week HRVT protocol would result in significant effects on psychophysiological indices but also on biochemical markers of stress, and (ii) a single HRVT session would have acute effects on the a-amylase levels measured immediately after the session, the results of this study are aligned with previous literature; previous literature has suggested that electrodermal activity (i.e. skin conductance) fluctuates in parallel with sympathetic activity (e.g. Pakarinen et al., 2016), and is related to emotional arousal

(e.g. Sabatinelli et al., 2001), whereas it is also related to HRV since they both correlate with self-reported stress and reflect sympathetic activity (Pakarinen et al.; Peper et al., 2008). This is the first study to report statistically significant reductions in EDA after a 5-week HRVT protocol followed by a 2-week wash-out period. Furthermore, this is the first study to show a lasting biochemical downward trend during the introduced 5-week protocol, whereas this trend indicates reduced arousal. To the best of our knowledge, this is also the first study to provide evidence that a 5-week HRVT protocol followed by a 2-week wash-out period resulted in diminished cortisol levels detected in the salivary sample collected immediately after the stress profile in the post-intervention session, compared to the levels detected in the control group.

As mentioned above, a-amylase is considered as a practical, non-invasive biomarker of sympathetic activity (Nater & Rohleder, 2009), whereas, due to its nature, it responds immediately to any fluctuation of the SNS (Maruyama et al., 2012). As the intervention used in this study is training of heart rate variability, an increase in HRV was followed by a decrease in salivary a-amylase given that it is inversely correlated with heart rate variability (De Vries-Bouw et al., 2012) (NB Apart from whether HRV indices eventually changed significantly or not, RSA, which is a component of HRV, was maximized during the HRVT, hence the increase in HRV). Although there have been studies investigating the effects of a single HRVT session (e.g. Prinsloo et al., 2011; Prinsloo et al., 2013a; Prinsloo et al., 2013b), this is the first study that explored acute biochemical effects after a single HRVT session and showed significant reductions in a-amylase.

The measures of central tendency and dispersion indicated that some key indices moved towards the desirable direction during the post-intervention measurement. As such, the root mean square of successive differences (RMSSD), the mean total power of low frequency range (LF) and the standard deviation of all NN intervals (SDNN) were found to be higher in the post-intervention measurement for the experimental group and slightly elevated for the control group; in the case of non-HRV-related psychophysiological variables measured during the stress profile, the total electrical activity on the trapezii (EMG), the skin conductance and skin temperature also moved towards the desirable direction in the post-intervention measurement, though it became worse for the control group. As for the measured biochemical markers, levels of a-amylase were detected to be higher in the post-intervention session whereas levels of cortisol were lower.

Statistical significance was investigated within three different directions: (a) the first direction of investigation referred to whether there were long-lasting effects detected in the post-intervention session (after the two-week wash-out period), (b) whether there were significant differences in the biochemical markers collected over the course of 5 (i.e. actual intervention) to 7 weeks (i.e. intervention, pre- and post-intervention assessment) at a given time on the day of the session, for the experimental group only, and (c) the third was related to whether there were significant differences in the biochemical markers collected from the experimental group at different times within the same HRVT session (i.e. acute effects).

3.4.1 Long-Lasting Effects

The ANCOVA analyses showed that whereas the standard deviation of NN intervals (SDNN) along with the proportion of the number of pairs of successive NNs that differ by more than 50ms divided by the total number of NNs (pNN50) were not proved to be significantly different between the two groups, statistically significant differences between the control and experimental groups were found for the root mean square of successive differences between adjacent NNs (RMSSD) and the absolute power of the low-frequency band (LF). These findings build on previous literature that supported that LF was significantly different after the intervention (e.g. Paul et al., 2012; Paul & Garg, 2012), whereas in another study the LF/HF ratio was also found to be significantly different after the HRV training intervention (Choudhary et al., 2016); additionally, HRV indices became better in the case studies with the golf players discussed in the literature review (Lagos et al, 2008; Lagos et al., 2011).

However, the effect sizes of the differences were both below 0.1, which does not really allow for reaching concrete conclusions or substantive recommendations based on these results, given that the two effect size values are even below the level of a small effect size (Cohen, 1992). In other words, the investigation on the above-mentioned HRV-related parameters did not yield any promising findings as, on the one hand, the probability that the data came out as they did purely by chance was less than 5% but, on the other, the calculated effect sizes indicated that the results for RMSSD and LF were of very low practical significance and minimal practical meaningfulness. It might be that whereas the suggested intervention aims at the training of HRV, the frequency of the controlled sessions did not result in any practically meaningful

improvement in HRV per se, but the sympathovagal balance achieved by means of the suggested protocol results in the enhancement of other variables (e.g. skin conductance, biochemical markers of stress) which ultimately may positively affect performance. It might be that the suggested frequency of two HRVT sessions per week for 5 weeks was borderline adequate to create some beneficial effects, though these effects were not clearly reflected in HRV-related indices. An increase of the frequency might result in a bigger effect size of the positive effect on HRV indices. Nevertheless, as the present study is designed within a context of high performance and elite athleticism, even a small change might contribute to a fractional enhancement of performance which may, eventually, lead to success.

The total electrical activity of the trapezii yielded statistical significance, but, again, the calculated effect size indicated that those results may not have any practical significance, as it did not even meet the standard of a low effect size. This is in line with previous literature that supports that electrical activity on the muscles might be an index of stress, on the one hand (e.g. Schleifer et al., 2008; Peper et al., 2008) and, therefore, electrical activity is related to arousal, as well as with previous literature supporting that surface electromyography responses correlates with cardiac autonomic modulation (Saraiva et al., 2016), though, in that study, EMG was recorded on the vastus lateralis and not on the trapezii, which is the case in this study. Again, the fact that the effect size was not above 0.1 is something that should be taken into consideration, though this small change might contribute to the success of an elite athlete within the appropriate context.

Likewise, the skin conductance levels recorded for the experimental group in the post-intervention session were significantly different from those recorded for the control group with a small effect size. This means that the recorded effect was small here, but not so small to be trivial (Cohen, 1992). So, the statistically significant differences measured between the two groups appear to be practically meaningful, though the magnitude of the effect of the intervention on hand sweat was measured to be small.

Differences between the experimental and control groups in skin temperature were not found to be statistically significant. Therefore, from all non-HRV related psychophysiological variables recorded during the stress profile protocols in the post-intervention session, it was only the skin conductance which yielded practically meaningful results since, on the one hand, there were statistically significant differences between the two groups and, on the other, the calculated effect size was found to be within the acceptable range as defined by Cohen (1992). Therefore, the suggested HRV training protocol appeared to create beneficial long-lasting effects on the levels of hand sweat of the student-athletes, which is one of the physiological stress-related indices (Peper et al., 2008).

Long-lasting effects were also investigated in the analyzed biochemical markers, as there was regular collection of salivary samples from both groups, before, during and after the intervention (both groups gave salivary samples in the pre-intervention session, in the 3rd week of the HRV-training protocols and the post-intervention session). The participants of the experimental group gave four salivary samples every

time they came to the mindroom, whereas the control group gave two samples (i.e. immediately before and immediately after the session).

The results showed that the levels of salivary cortisol were found to differ significantly between the two groups after the post-intervention session. A downward trend was recorded for both groups, with the mean cortisol levels of the experimental group reaching a much lower level immediately after the post-intervention session compared to the levels of the control group. This builds on previous literature that supported that cortisol is inversely related to HRV (Sgoifo et al., 2003; Stalder et al., 2011), whereas to the best of our knowledge, this is the first study that measures the development of salivary cortisol within a protocol of multiweek HRVT. Additionally, the calculated effect size indicates that the statistical difference has low practical significance, though it is still worth considering, since it is within the acceptable range as per Cohen (1992) and, as discussed above, a small improvement in elite athleticism may result in a desirable result. Again, as the magnitude on cortisol levels collected after a two-week wash-out period was borderline practically meaningful due to the low, but acceptable, effect size, it might be that an HRVT protocol consisting of more frequent sessions could have resulted in a higher effect size.

3.4.2 Trends of Biochemical Markers

Furthermore, the development of the biochemical markers during the total duration of - at least - seven weeks (i.e. Pre-Int$_{exp}$ + RBFtest +5 weeks of HRVT + Post-Int$_{exp}$) was investigated; the biochemical trends emerging from the results are worth

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discussing, as shown in Figure 3.1 and Figure 3.2. Figure 3.1 shows how the levels of

the four collected cortisol samples developed during the HRVT protocol and to which

extent these trends persisted in the post-intervention session. As shown in the figure,

there was a general downward trend for all cortisol levels from week 1 onwards

(cortisol 1 slightly increased after week 1 but then started declining as well). The

downward trend ended in week 5 of the HRVT and all cortisol levels started increasing

during the wash-out period till the post-intervention session. Figure 3.1 shows that,

apart from cortisol 1 which ended up at a higher level than its initial measurement,

cortisol 2, 3 and 4 were recorded at lower levels in the post-intervention session

compared to the pre-intervention session. As also mentioned in the previous chapter

and above, cortisol levels detected in the samples collected immediately before the

session, in the experimental group, did not differ significantly in the post-intervention

session. Although, those levels were recorded to have a – generally - declining trend

during the 5 weeks of the HRVT with its lower levels having been recorded in week 5 of

the intervention, the final levels of it were not significantly different from the baseline.

Nevertheless, the declining trend of cortisol 1, which signifies the cortisol levels

detected in the salivary samples collected before each session of the protocol - always

at the same time to control for circadian rhythms - indicates that those levels might

have been affected by some factor; this factor cannot be anything else but the protocol

the participants engaged in for 5 weeks and the overall magnitude of this effect (i.e.

partial eta squared) was calculated at a small-to-medium level.

Additionally, the levels of cortisol detected immediately after each session (cortisol 2) reached significantly lower levels later on in the protocol with a small exception from week 1 to week 2 that they slightly increased. However, the value measured in the post-intervention session was significantly lower than the baseline, whereas the magnitude of the overall effect was recorded at a medium level. The same trend was recorded for cortisol 3 (collected 45 minutes after the end of the session), which, unlike the other cortisol measurements, kept continuously decreasing from week 1 to week 5 and its overall effect size was recorded at medium to high levels, though, cortisol levels were not recorded at significantly different levels in the post-intervention session compared to baseline. Last but not least, cortisol levels detected in the salivary samples collected 90 minutes after the end of the session (cortisol 4) also had a declining trend from week 1 to week 5 and an increase from week 5 to the post-intervention session. Although the overall effect size appeared to be small-to-medium the levels recorded in the Post-Int$_{exp}$ were not significantly different from those detected in Pre-Int$_{exp.}$

Likewise, the trends recorded for a-amylase were similar, though, in this case, the downward trend started from week 1 and ended in week 4; after that, all detected a-amylase levels started increasing even slightly. The overall effect size of the significant differences in the levels of a-amylase detected in the salivary samples collected before the sessions was borderline medium-to-large. What is interesting here is that there was a significantly lower value in week 1, compared to baseline, although the participants of the experimental condition had not been subjected to the actual

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protocol yet. It may be that positive expectations resulted in some decrease of the levels of a-amylase, which is what we often refer to as placebo effects (e.g. Foroughi, Monfort , Paczynski , McKnight , & Greenwood, 2016; Petrie, & Rief, 2019). This phenomenon was not observed in the case of cortisol due to its delayed response compared to a-amylase (e.g. Maruyama et al., 2012).

It also appears that the downward trend of a-amylase stopped in week 4, after which a-amylase started increasing again. This means that in week 4, a-amylase reached the lowest possible value and, then, started moving upwards till the post-intervention session. So, whereas in the case of cortisol, the lowest recorded level for the four measurements was achieved in the 5th week, and, then, cortisol started increasing again as expected, in the case of a-amylase the lowest level was achieved in the 4th week and, then, it increased slightly in the 5th week and much more in the post-intervention session.

The most important finding in this part of the study has been that the introduced protocol of five controlled and five uncontrolled HRVT sessions induced a downward trend of both measured stress-related biochemical markers; this downward trend may have been a type of plasticity acquired within the ANS as a result of the plasticity acquired in the baroreflex system through the HRV biofeedback training (Wheat & Larkin, 2010; Lehrer et al. , 2003) and/ or the hippocampus of the brain (e.g. Kotozaki, Takeuchi, Sekiguchi, Yamamoto, Shinada, Araki, Takahashi et al., 2014).

3.4.3 Acute Effects

Furthermore, acute effects in the biochemical markers were investigated only in salivary a-amylase, since this is related to the sympathetic adrenomedullary system (SAM) and the ANS and, as a result of this, responds rapidly, whereas salivary cortisol is related to the HPA axis and responds with a delay (e.g. Maruyama et al., 2012). As shown earlier, the effect size of the statistically significant differences of the repeated measures ANOVA was large, indicating that the acute effects of HRVT sessions on a-amylase collected at different times of the days of the sessions were of large magnitude. The conducted pairwise comparisons showed that whereas a-amylase (sAA) detected in the sample before the session was significantly higher than the sAA detected immediately after the session, the sAAs collected subsequently (i.e. a-amylase 3 and a-amylase 4) were recorded at higher levels than a-amylase 1 and 2 with a-amylase 4 being even higher than 3; in other words, a-amylase 3 and a-amylase 4 followed the expected diurnal trajectory as shown in Figure 3.0 in which the concentration levels of a-amylase (but also cortisol), as recorded under normal circumstances during a day due to circadian rhythms, are depicted (Adam et al., 2011).

Figure 3.0 also indicates that the diurnal range of sAA is from approximately 40U/ ml to 100 U/ml, with its levels lying between 65 to 80 U/ml in the morning (though that study's [Adam et al.,2011] third salivary collection took place at 10:30 am, which, in this study, was the time of the last morning collection). The difference however, between our salivary collections and the collections conducted by Adam et al. (2011) is that, in our case, we did not collect any sample at wake-up or even 30 minutes later, as the boys had already woken up at least 1 hour before coming to the

mindroom. Figure 3.3 provides a graphical representation of the development of the mean sAA in the present study.

As the analysis of diurnal patterns of the biochemical markers of stress (Adam et al., 2011) does not state specific times of collection before 10:30 a.m. and the lower levels of sAA, before 10:30 a.m., may have been merely related to wake-up, we cannot compare the sAA levels early in the morning, as, in the present study, participants had already woken up long before the first salivary collection. What we can conclude, however, is that whereas it was expected that the levels of a-amylase would keep progressively increasing in the samples collected during the morning, they decreased significantly at 9:00 a.m., which was the salivary collection immediately after a 20-minute HRVT session (i.e. a-amylase 2). This is because of the statistically significant acute effects detected immediately after the 20-minute intervention; a-amylase started increasing from that moment onwards and reached the expected levels (as per Adam et al., 2011). In other words, contrary to expectations, a-amylase declined after the 20-minute HRVT session and started increasing again soon after that.

As the main purpose of the thesis is to inform practice, the main benefits of the acute effects of the HRVT are related to the preparation of an athlete for a competition and his/her pre-competition routine. Since the ultimate level of the athlete's mental preparation in view of a competition is to reach an ideal level of arousal and composure, including a 20-minute HRVT exercise before the competition may be beneficial, as there will be immediate effects on the levels of a-amylase and, as a result of this, on the ANS itself.

Consequently, it might be advisable for an athlete to rehearse HRVT regularly (i.e. as instructed in this project, at least twice a week by 20 minutes each time) in order to acquire the above-mentioned plasticity but also include HRVT in their pre-competition routine, with a view to causing acute desirable effects and be able to control their emotions more efficiently. Besides, the essential part of this project is to enrich the athletes' arsenal of mental strategies used for the development of their mental readiness in view of a competition which, in turn, will lead to performance enhancement and, possibly, success.

Last, the above advice should be given to an athlete once they have finished the suggested 5-week protocol in which their resonant breathing frequency has been identified successfully and s/he (i.e. the athlete) has familiarized themselves with the slow-breathing strategy to an extent that they can rehearse their resonant breathing frequency without difficulty. After having finished the 5-week protocol, it is advisable that the athlete consults with the practitioner as to which portable HRV portable device they should use when they are alone practicing HRVT. In case that they cannot afford buying such a device, though it is not expensive, they could alternatively use a smartphone-based app, similar to the one they used for this study's protocol. However, they should bear in mind that the HRV portable device can give more accurate feedback.

3.4.4 Shortcomings

Despite the numerous variables that this study examined which were related to physiological responses to stress but also biochemical markers, there were not any performance-related variables in this study. So, although Study 1 investigated acute effects of an HRVT session on performance under pressure and, as discussed in the literature review, previous research studies conducted with HRV biofeedback as a main intervention, used sport-performance variables to measure the effects of HRVT on performance (e.g. Choudary et al., 2016; Paul et al., 2011), this study did not investigate any performance- related variable. The main reason why reactive stress tolerance test was included in Study 1 was to induce stress. On reflection, as the performance variable (i.e. included in Study 1) was not considered to relate to sports performance, and given the range of sports included in Study 2, performance data was not included.

Indeed, each of the studies discussed in the literature review focused on a single sport, in which context, it is easier to use/develop one or more performance variables and investigate the HRVT effects on them. However, the current study dealt with participants coming from completely different sports such as athletics field events, athletics track events, football, table tennis, squash, football referees, gymnastics, shooting, golf and fencing. Therefore, it was practically impossible to develop a performance variable for each of those sports and normalize the measurements so that they could be used in a meaningful way in the statistical analyses.

3.4.4.1 Future research. The 5-week protocol initially introduced by Lehrer et al. (2013) is quite different from the 5-week protocol suggested in this study, as the former instructed their clients to practice HRV breathing at home for 20 minutes twice daily, which seemed unrealistic in the case of ASPIRE student-athletes who have a really overloaded daily schedule at ASPIRE academy and go home late in the evening, every day. On the other hand, the suggested frequency in this study might not be ideal as shown by some results. Future research should investigate what a minimum frequency of rehearsing HRVT is, within the framework of 5 weeks of training, in order to produce some practically meaningful results. Considering the effect sizes that resulted from this study for the HRV indices (i.e. 0.9, 0.7) the target minimum frequency will not be very different from the suggested frequency in this study, though, to have some robust evidence on that, this needs to be further researched.

Additionally, future research should find a way to normalize performance measures across different sports with a view to including such measures in the investigation of the effects of the proposed 5-week protocol.

3.4.5 Conclusion

This study was the first to investigate the effects of a 5-week protocol of five controlled (i.e. lab-based) and five uncontrolled (i.e. home-based) HRVT sessions on such stress-related psychophysiological responses as electrical activity on trapezius muscles, electrodermal activity, skin temperature and HRV, in addition to key biochemical markers of stress such as salivary cortisol and a-amylase, given that all

these indices are related to performance (e.g. Landers, 2007), whereas it was the first study to explore acute biochemical effects after a single HRVT session and show significant reductions in a-amylase. Second, this study is the first to explore the viability of such a 5-week training programme in adolescent Middle-Eastern athletes and to report statistically significant reductions in EDA and salivary cortisol - collected immediately after the stress profile in the post-intervention session - after a 5-week HRVT protocol, followed by a 2-week wash-out period. Furthermore, to the best of our knowledge, this is the first study to show a lasting downward trend for both cortisol and a-amylase during the introduced 5-week protocol with this trend indicating reduced arousal.

In conclusion, this study addressed Aim 2 and Aim 3 of the thesis and showed that the suggested 5-week HRVT of five weekly controlled (lab-based) and five weekly uncontrolled (home-based) sessions resulted in long-lasting effects in skin conductance but, also, in the levels of cortisol detected immediately after the post-intervention session; additionally, a downward trend of both cortisol and a-amylase was observed during the course of the protocol which might be attributed to some progressively acquired plasticity as the protocol developed during the 5 weeks, whereas acute effects on a-amylase levels were also detected immediately after each 20-minute HRVT session. The results suggest that adolescent athletes should practice 20-minute HRVT sessions regularly (i.e. at least twice a week) and include HRVT within their pre-competition routine, with a view to training their ANS and, thus, develop some

plasticity in it, on the one hand, and benefit from the acute biochemical and

psychophysiological effects prior to competition, on the other.

CHAPTER 4: HEART RATE VARIABILITY TRAINING: A QUALITATIVE ANALYSIS

OUTLINING NEEDS FOR ADAPTATION OF THE HRVT PROTOCOL

4.1 INTRODUCTION

The purpose of this study is to investigate the experiences of the participants who took part in the HRV training protocol in Study 2. Whereas in the previous chapters the efficacy of HRVT was explored, in this chapter the participants' experiences are considered. This will provide information on the delivery of HRV interventions to enhance the delivery of future work in this area. This study addresses Aim 4 of the thesis as participants' reflections on their experience during the 5-week HRVT protocol are investigated.

4.1.1 Using Qualitative Research

The use of qualitative research has expanded in the last decade of the 20th century, when a growing volume of research papers in sport psychology started using qualitative analysis. This indicates that researchers started realizing the benefits of qualitative research, as opposed to the strict framework of numerical analyses that a quantitative investigation can provide (e. g. Côté , Salmela, Baria, & Russell, 1993). It is true that qualitative research creates a different dimension of analysis for a discussed construct or research question, which cannot be provided by any type of quantitative method. The uniqueness in the nature of findings resulting from qualitative analysis lies in one of four broad categories of outcomes, namely, description, interpretation,

verification and evaluation (Strean, 1998). These categories are not independent of each other, as many times they overlap to a significant extent.

Description is the process in which researchers provide the readers with information on how people make sense of their own context and the world, in general. By means of observational data and interviews, researchers can provide information on processes, as, for example, how events and actions occur, or a description may refer to contexts and various aspects of them, as, for instance, the contextual factors contributing to the success of an elite performer in sport. Another form of description is the biographical account of successful individuals, though, this is an underdeveloped area research-wise, as the experiences and views of successful coaches and athletes have not been always recorded in the way they should have been done, since many important questions have not been adequately addressed (Strean, 1998). Capturing the experience requires an advanced level of self-presentation skills and dexterity that will help the researcher capture the data more profoundly.

Interpretation is a broad category that has contributed substantially to the researchers' understanding of sport. Exploring the lives of performers in sport by means of interpretive investigation has helped researchers extend their knowledge of sport, acquired through conventional understandings of particular sport-related phenomena (Denzin, 1989). Besides, the main objective of qualitative researchers is to interpret the lives of sport performers as accurately as possible, which may lead to generalizations made by the reader, closer to what one would consider as naturalistic generalizations. That is, based on the reader's experience and perception, as opposed

to generalizations made on statistical grounds (Lincoln & Guba, 1985). By means of interpretation, one can identify issues or problems for a new intervention and work on them accordingly but can also come up with new constructs, or factors not considered in earlier examinations of the research question (Strean, 1998). Last but not least, interpretation helps researchers understand the complexity of a discussed phenomenon (Peshkin, 1993).

The third category of outcomes resulting from qualitative analysis is verification, which is related to testing the extent to which an idea is actually true, or can help researchers investigate various aspects of sport performance at an individual level, which often informs practice.

The last category of outcomes refers to evaluation, as is the case when a new intervention, used in performance enhancement, is evaluated by means of qualitative work. For instance, the efficacy of newly-introduced sport psychological interventions is often investigated by means of qualitative-evaluative analyses, given that such interventions are often implemented in the complex environment of sports. Indeed, the complexity of this environment allows room for qualitative-descriptive analyses, whereas it is a good opportunity for insight as well; at the same time, evaluation may be supported by direct engagement and prolonged observation as well as other ways of qualitative analysis such as focus group discussions. Therefore, the contribution of evaluation focuses on developing support for the newly-introduced intervention, rather than testing whether the new intervention works effectively or not (Strean, 1998).

4.1.2 Need for Adaptation of an Existing Intervention

An intervention is designed initially for a particular cultural context or cohort;

proving that this intervention is effective for this context or group of people should not

create the illusion that this specific intervention can be equally effective in any cultural

context and/or for any other cohort. HRV training has been used in different

professional contexts and for different reasons (e.g. Lehrer et al., 1997; Lehrer et al.,

2004; Lehrer et al., 2006; Cowan, Pike, & Budzynski, 2001; Del Pozo, Gevirtz, Scher, &

Guarneri, 2004; Nolan et al., 2005) and the truth is that using the intervention in a

different way from what is initially tested and proven may require further testing and

possibly adaptation of it (Rosen, Kuo, Gobin, Peabody, Wechsberg, Zlotnik, & Johnson,

2018). Practically speaking, in reality, what happens, with the implementation of an

intervention in a new cultural context without investigating its effectiveness within this

new context as well as its need for adaptation to the new context, is that the

intervention will be altered anyway, due to the different sociodemographic, cultural

and contextual variables. By investigating the need for adaptation of the initially-

designed intervention and applying such an emerging need, a researcher may be in

position to ensure that the intervention will continue being efficacious even in this new

context (Rosen et al., 2018).

Qualitative approaches focus on the subjective lived experience of individuals,

and can contribute to the value that an intervention may have in a context it has been

recently introduced in. They can provide information on the way the context of an

intervention or its delivery mode need to be refined in order to be delivered to a new

group of people (Alderfer & Sood, 1996). For instance, in an initiative to minimize

harmful alcohol use in young people, a multiphase qualitative study was conducted by

researchers to define and evaluate various stages of the used intervention (de Visser,

Graber, Hart, Abraham, Scanlon, Watten & Memon,, 2015). In another study, a

psychoeducation sleep management intervention, used by practitioners, for children

with neurodevelopmental disabilities and its evaluation were investigated by means of

a qualitative analysis (Beresford, Stuttard, Clarke, & Maddison, 2016). Additionally, the

development of family-based intervention for pediatric cancer (Hocking, Kazak,

Schneider, Barkman, Barakat & Deatrick, 2014) as well as the development of coping

tools in a medical setting (Marsac, Klingbeil, Hildenbrand, Alderfer, Kassam-Adams,

Smith-Whitley, & Barakat, 2014), were both analyzed by means of qualitative

interviews. Another example of use of qualitative analysis and, more specifically,

thematic analysis is the investigation of the effectiveness of group mindfulness for

people with intellectual disabilities (Yildiran & Holt, 2014).

Besides, in a study conducted to adapt a psychological skills training (PST)

program, already used for performance-enhancement purposes in the sport domain,

to music performers (Hatfield & Lemyre, 2015), semi-structured interviews were used

along with observation and logs to evaluate the perceived effects of the PST program;

the main purpose of the semi-structured interviews was to investigate the efficacy of

the intervention tools, to examine the participants' need for mental training as well as

to evaluate the usefulness of the discussed skills. The main findings of this study were

that the combination of single and group delivery of the PST program can maximize its

effects and increase the participants' motivation as well. Additionally, the participants'

personal interest and engagement for the intervention was also of high importance

(Hatfield & Lemyre, 2015). Using alternately a deductive way of communication

discussing rationales for involvement in the PST program and an inductive way of

communication was also reported as a recommended way for a practitioner to

optimize the involvement of participants.

The perceived effectiveness of a life-skills program was assessed by means of

focus group discussions and semi-structured interviews in another study (Hardcastle,

Tye, Glassey, & Hagger, 2014). The themes formed by the participants' responses were

achieving balance and *managing stress*, *time management*, *goal-setting*, *confidence*

and *control*, *information overload* and *repetition*, *credible role-models*, *coach*

reinforcement and *follow up*. The results of this study showed that such a program can

be improved in terms of effectiveness as long as the practitioners put emphasis on the

practical application and engagement of the participants rather than spending more

time on giving information on its theoretical background (Hardcastle et al., 2014).

Finally, a study was also conducted with volleyball players to investigate the

effectiveness of heart rhythm variability biofeedback on the players' athletic

performance (Tanis, 2012), which was actually a study of mixed design (i.e. with a

quantitative but also qualitative part). The qualitative part involved interviews, the

analysis of which revealed three major themes; overall experiences, intervention

benefits and intervention detriments (Tanis, 2012). The first theme discussed the

participants' perceptions about attending such a six-week protocol, whereas the theme

"intervention benefits" focused on discussing physical and mental stress reduction, improved academics as well as sleep quality, improved performance, energy levels, healthy relationships and composure. Lastly, in terms of intervention detriments, unwanted relaxation along with disrupted skill performance and reliance on the HRV device were reported (Tanis, 2012).

4.1.3 The Present Study

This study will build on the findings of Tanis's (2012) study which investigated participants' perceptions of HRV biofeedback training within a cohort of western female collegiate volleyball players. To date, there have been no qualitative investigations of HRVT (i.e. heart rate variability training) within a Middle-Eastern context discussing associated challenges and ways to optimize this. This study will provide qualitative data regarding the perceived efficacy of HRVT and how this needs to be tailored to meet the needs of Middle-Eastern male adolescent student-athletes studying in the Qatar-based ASPIRE academy. As such, the present study comprises semi-structured one-to-one interviews conducted to cover the above-mentioned topics while allowing the freedom to discuss the wider topic.

The present study addresses Aim 4 of the thesis and will focus on the athletes' reflections on their experience during the HRV training protocol in Study 2 and will inform future HRVT programmes. It builds on the work of Tanis (2012) by interviewing individuals with different athletic background, that is, fencers, squash players, track and field athletes, football players, football referees, gymnasts, shooters and golfers;

additionally, a different cohort consisting of a bigger number of athletes was

interviewed in the present study. It also extends previous literature by asking more

questions related to different aspects of HRVT, as will be discussed later in this chapter.

4.2 METHOD

4.2.1 Participants

Twenty-two participants from the experimental group of study 2 volunteered to participate in this study. Participants were recruited in the Doha-based ASPIRE academy. They were eligible for inclusion if (a) they were student-athletes of ASPIRE academy, (b) they had already taken part in the experimental group of Study 2 of this project and (c) they were aged between 11 and 18 years. The mean age of the 22 participants who completed the study was 16.32 years (SD= 1.46). All of the participants were male adolescents (100%) who practiced a sport regularly. The sports represented were fencing (13.64%), squash (9.09%), middle-distance running (22.73%), sprinting (4.54%), football (18.18%), football referees (13.64%), gymnastics (9.09%), shooting (4.54%) and golf (4.54%).

4.2.2 Procedure

Following institutional ethical approval and the approval of the IRB committee of the ADLQ (i.e. Anti-Doping Lab Qatar), participants were recruited within the ASPIRE academy's school.

The researcher met the participants who had already participated in the experimental group of Study 2 and informed them about the nature of the study. A mutually convenient time was agreed with each of the participants, and the interviews were conducted face-to-face. The researcher had already developed a good rapport with each of the participants in order to ensure that their views would be expressed openly without any hesitation. To achieve this and minimize, at the same time, any

apprehension, the interviewer/researcher discussed the background of the project,

how it may inform practice and tried to initiate a casual discussion before starting with

the actual interview. Anonymity and confidentiality were discussed with the

participants; they also held the right to ask the researcher to just voice-record the

interviews, should they not wish to be video-recorded during their interview. The

average duration of the interviews was approximately 20 minutes.

4.2.3 Semi-structured interviews

Qualitative interviews often take the form of semi-structured interviewing.

Semi-structured interviews can give information to the interviewer on the experience

that the interviewee may have had in a specific context and under specific

circumstances. The quality of the interview depends, to a large degree, on the rapport

built between the interviewer and interviewee. However, this rapport may be

disrupted due to the fact that, at certain points, the interviewer may need to make his/

her role more salient at the expense of the casual style that such a setup may have,

given the open-ended questions which usually result in an informal conversation. The

rapport is really sensitive in a way; the informal setup of the conversation should not

result in the interviewee being driven to reveal more than what he would feel

comfortable with (Willig, 2009).

In terms of analyzing the data collected from the interview, Braun and Clarke

(2006) maintain that qualitative methods can be divided into two main categories; the

first category includes all those methods that result from a specific theoretical or

epistemological position, such as conversation analysis or IPA (i.e. interpretive

phenomenological analysis). The second category refers to such methods that are not dependent on a theory and epistemology, and can be used in a wide spectrum of approaches. One of the methods included in this second category is thematic analysis, due to the fact that it is a flexible, realist and experiential method, and is used to identify, analyze and report patterns, known as themes, within the collected data. What is unique in this type of analysis is that there is no universal consensus on how one goes about doing it, whereas, it does not appear to be so well-defined as other qualitative methods, despite the fact that it organizes data in such a way that it interprets various aspects of the research topic (Boyatzis, 1998). In other words, there is no rigid procedure and terminology to follow in thematic analysis, yet, it has been recognized as a strategy used for interpreting different aspects of qualitative data quite effectively.

   4.2.3.1 Interview guide used in this study (see Appendix C.1). The semi-structured interview used in this study was constructed following seven main research questions which resulted from either practical issues discussed in the literature (e.g. Lehrer et al. 2000; Lehrer et al., 2013) or discussions with experts in the field of HRVT and covered the following areas:

- level of difficulty in adjusting one's breathing pattern to a resonant breathing frequency;

- length of time of familiarization phase with the resonant breathing frequency;

- practical difficulty in breathing at one's resonant frequency without the help of a breathing pacer;

- the extent to which one can breathe at their resonant frequency in a non-resting state (e. g. while moving vigorously);

- the extent to which one can adjust their breathing frequency to a resonant breathing frequency without taking deep breaths (i. e. by just decreasing their breathing pace);

- perceived "side-effects" of HRV training;

- the participants' perception of the level of effectiveness of an HRV-training intervention;

- recommendations on making such an intervention more practical.

At the beginning of the interview, there was an introductory discussion which, facilitated the rapport between interviewer and interviewee. This introductory discussion mainly focused on the participants' understanding of the nature of the project and the reason why they think they had gone through those sessions of 20 minutes of slow breathing. After that, the interviewer reminded the interviewees of the process and the rationale for the study in order to aid recall.

The main part of the interview explored the main research questions discussed above (see also Appendix C.2). Semi-structured interviewing was selected as a method of interviewing, as it allows participants to be flexible while answering and develop a

conversation with the interviewer on the discussed topic; this maximizes the possibility that the conversation will reflect the views of the interviewees (Guest, MacQueen, & Namey, 2012).

4.2.3.2. Reflexivity. My motivation for conducting this research was an interest in the strategies used by those, who have chosen to compete at an elite level, to minimize any negative thought and cope with stress effectively. I find this really intriguing, as I have never aspired to be an elite athlete, though, I have worked in high performance sport for more than 15 years, and started this research with a view to recording student- athletes' response and level of commitment to the process of learning and finetuning a newly introduced strategy for emotional control. Of course, I knew that other people supporting athletes could be quite dismissive of a -probably- completely incomprehensible (to them) intervention, such as people from the coaching staff but I wanted to try this. Personally, I think that athletes can be taught how to control their emotions more efficiently, if they manage to "fill in" their arsenal with evidence-based strategies and, when the time comes, use that strategy that suits them most. I have never competed at an elite level at any sport, though I have experienced a different level of high performance as I myself used to be a classical violinist when I was younger. With all the participants of this study, I have a good relationship as they are/ were student-athletes of the academy I have worked in; however, those boys would not say something in the interview to satisfy me, as most of them are boys who express their opinion frankly and without any fear. Nevertheless, although I have

known all these boys very well, during the interviews, I realized that a couple of the interviewees may hve not given 100% accurate answers.

After each of the interviews I conducted in this study, I used reflection to review my technique as an interviewer; for example, there were many times that I asked myself "Did I discuss this or that issue in depth?", or "Did I cover all aspects of that issue or I should have persisted more in that?".

4.2.4 Data Analysis

Interviews were transcribed verbatim by the author and were analyzed using applied thematic analysis (Guest et al., 2012). During this process, the author read each transcript several times and developed structural codes, each of which answered one of the main research questions. These codes had been linked with the main research questions a priori and, in each of these questions, there was an exchange between the interviewer and the interviewee until the moment the interviewer felt that the question had been answered thoroughly, at which point he proceeded to the next interview question.

Within each of the structurally coded parts of the interview, the researchers conducted a line-by-line analysis in order to collect information about potential themes; this resulted in the isolation of the coded themes, whereas more specific themes were further identified within each section. By means of deductive analysis and the formation of manageable units, the author was in position to shed light on the experience of the student-athletes during Study 2 as well as their interpretation of the instructed heart rate variability protocol (Braun & Clarke, 2006).

Each interview was scrutinized for references to positive, negative or neutral effects of HRV training on the participants' self-regulation, as those experiences were in line with the rest of the answers that the participants had given during the interview (e.g. a student-athlete who claimed that he experienced positive effects after or during the protocol is more likely to have said that they have felt very well while breathing at their resonant breathing frequency or that they did not breathe deeply during the protocol or they breathed deeply to a minimal extent, etc.). The approach followed during this qualitative analysis was a flexible approach meaning that the above-mentioned steps were followed in an interactive fashion. This approach has been based on Braun and Clarke's (2006) approach, according to which, thematic analysis involves a continuous moving back and forward until solid conclusions are reached.

A frequency analysis was also conducted to investigate the percentages of those who had a positive experience versus those with neutral or negative experience as well as the number of occurrences of the different effects discussed with the participants.

4.3 RESULTS AND DISCUSSION

The findings of this study are presented under the main structural codes which correspond to the seven major questions of the semi-structured interview. These are as follows: (a) whether the instructed slow pace of breathing was good or not, (b) how long it took the participant to get used to the agreed resonant breathing frequency, (c) whether one can breathe alone at their resonant breathing frequency without the help of a pacer, (d) whether one can breathe at their resonant breathing frequency while moving vigorously, (e) whether one can do HRV training without taking deep breaths, (f) positive and negative outcomes of HRV training, and (g) suggested modifications for the HRV training protocol.

The above main research questions, asked in the interviews, helped the researcher consider the experience of the participants within a framework that determined a need for customization of the introduced strategy to meet the needs of the male adolescent student-athletes of ASPIRE academy. Within this framework, positive experiences were weighed along with less positive or - even - negative experiences and suggestions on how the HRVT protocol can create only positive experience in a similar cohort were put forward.

4.3.1 Whether the Instructed Slow Pace of Breathing was Good or Not

The participants of this study were classified into three main categories within this structural code: (A) those who reported that their resonant breathing frequency was ideal and did not experience any serious difficulty in adjusting their breathing at the instructed pace, (B) those who reported that breathing at their resonant breathing

frequency caused some problems to them and, therefore, they do not think that the instructed pace was ideal at all, and (C) those who believed that the instructed slow pace of breathing was good but they experienced some issues. From the participants, twelve (54.54%) belonged to Category A, three (13.64%) belonged to Category B and seven (31.82%) belonged to Category C.

Participants in Category A reported that they could slow down their breathing pace to reach the instructed resonant breathing pace without any serious difficulty and could sustain this for 20 consecutive minutes without any serious problem. On the other hand, the issues reported by participants in categories B and C form three main themes. The first theme refers to the fact that the instructed resonant breathing frequency might not have been the ideal frequency for the discussed participants, mainly discussed by those in Category B (i.e. the ones having had only a negative experience in Study 2). This is aligned with that what Vaschillo et al. (2006) suggested, that lack of familiarity with paced breathing might sometimes result in deep breathing, hyperventilation, perhaps anxiety which, in turn, leads to erroneous conclusions as to which breathing pace produces maximal effect and, therefore, creates a negative experience for the individual. In such cases, it is advisable that more time and attention be spent on determining the individual's RBF (Vaschillo et al., 2006).

'Breathing slowly was a bit harder for me to do it – it was way harder than breathing faster...' and he added later *'I think it was very difficult. Most of the times it was very difficult...'.* This was a characteristic statement of those participants who reported that their entire experience was not pleasant, and they struggled to finish

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every HRV training session. That means that despite the fact that their resonant breathing frequency had been measured with a valid and reliable protocol and that the emerging breathing frequency was later tested with them, it turned out that the agreed frequency resulted in a negative experience for those participants.

The second theme formed by the responses of the participants was related not to the pace itself but to the extent to which one can continue breathing at the instructed pace for a long time. It appeared that although it was feasible to breathe effortlessly at their resonant breathing frequency, those participants reported that this was not possible for 20 consecutive minutes. The original manual by Lehrer et al. (2000) proposed 20-minute HRVT sessions, which was supported by the newer 5-week version of the protocol as well (Lehrer et al., 2013). However, there have also been studies that discussed shorter durations for an HRVT session; as for example, Deschodt-Arsac et al.(2018) where participants practiced 5-minute HRVT sessions twice daily for 5 weeks, or in studies investigating acute effects where participants had to practice a single 10-minute HRVT session (Prinsloo et al. , 2011; Prinsloo et al. , 2013a; Prinsloo et al. , 2013b).

'The last 3 to 4 minutes of a session I felt a bit discomfort. I could not really continue at the same ease during the whole duration. It was approx 15 to 16 minutes without any issue.'

For this participant, if the session had been 5 minutes shorter, then it would have been much easier for him to finish the session without any issue at all. Others, felt that an even a shorter session would have been ideal:

'*I could control the slow breathing from the first session without any issue. However, there were ups and downs. Especially I could not control it easily in the last half of the session.*'

In other words, this participant claims that while he felt at ease in the first half of the session, it was in the second half that the problems appeared. Therefore, a 10-minute session could have been completed by him without any issue.

The measured resonant breathing frequency (RBF) was calculated following the same procedure for all those student-athletes who took part in Study 2; however, as there are individual differences, whereas for the majority of them the RBF appeared to make them feel comfortable while breathing at the instructed slow breathing pace, and seemed to be feasible to breathe at this pace for twenty consecutive minutes, some participants reported having difficulty with their RBF. This is in line with what Lehrer et al. (2000) and Lehrer et al. (2013) suggested, that, in some cases, more than a couple of sessions may be needed in order for the RBF to be identified. Indeed, very few of them said they could not breathe at their RBF while others reported that, on the one hand, the suggested RBF made them slow down their breathing pace effortlessly and naturally, but, on the other, they did not feel they could sustain this for as long as it was required. In other words, the breathing cycle that the measured RBF suggested

could be supported effortlessly by those participants, however, as time went by this effortlessness phased out for some reason.

Another interesting theme formed by the responses of the participants refers to the duration of inhalation versus duration of exhalation of the instructed resonant breathing frequency.

'In a perfect world I think that the inhale was perfect, I think that if the exhale was shorter … I was just trying to put out as much air as possible and sometimes it was unnatural, sometimes I had to try to really… But I mean if the exhale was a little shorter or just the ball moved a little faster on the exhalation, I think it would have been perfect because the inhale I felt great but the same distance on the exhale and that extra bit every time was quite a bit of effort ….'

This theme raises the importance of customizing the duration of the inhalation and the duration of the exhalation after identifying one's resonant breathing frequency, as the default durations might not be ideal for all participants. This is in line with what has been discussed in the literature (Strauss-Blasche, Moser, Voica, McLeod, Klammer, & Marktl, 2000) according to which the inhalation/ exhalation ratio is always worth considering in HRVT-related studies. In other words, whereas there is a procedure to identify one's resonant breathing frequency and some HRV-related parameters can strongly indicate what the ideal RBF could be, within the same cycle of breathing, one might need to customize the duration of inhalation at the expense of the duration of exhalation and vice versa, to better meet the needs of the client.

'...I think that if the exhale was shorter because one line is this big, the exhale was just taking so long... I was just trying to put out as much air as possible and sometimes it was unnatural, sometimes I had to try to really... '

As this participant reports, in order to be able to support the long exhalation, he tried to take in as much air as possible during the inhalation, which is not ideal, as this sounds like a deep breath and may lead to hyperventilation after a few minutes, which might be actually the cause of the fact that some of the participants felt that they could not continue breathing at their RBF for twenty consecutive minutes. So, for instance, someone whose RBF is six breaths per minute might be more comfortable with a ratio of 4 seconds inhalation to 6 seconds exhalation than 3 seconds inhalation to 7 seconds exhalation; such a difference in the inhalation to exhalation ratio would not also affect HRV autonomic indices significantly (Wang, Kuo, Lai, Chu, & Yang, 2013). The customization should be done on the basis that the exhalation is always longer than the inhalation.

4.3.2 How Long it Took the Participant to Get Used to the Agreed Resonant Breathing Frequency

The participants of this study were classified into two main categories within this structural code: (D) those who reported that they managed to familiarize themselves with the instructed resonant breathing frequency, after some time, and, eventually, did not experience any serious difficulty in adjusting their breathing pace at the instructed pace, and (E) those who reported that breathing at their resonant

breathing frequency caused some problems to them and, therefore, they answered

negatively as to whether they managed to familiarize themselves with the instructed

pace of breathing, which is what has been discussed above and explained by Vaschillo

et al. (2006). From the participants, 17 (77.27%) belonged to the D category, whereas

the remaining five (22.73%) belonged to Category E.

The answers of the participants of Category D form two major themes: 1. the

first theme was the actual objective of the creation of this question and refers to the

number of sessions, within the 5-week protocol, after which the participant felt that he

had already got used to the slow-breathing strategy; after this session, the participant

started feeling at ease with his resonant breathing frequency and the 20-minute

training sessions. It is interesting to mention here that, out of the 22 interviewed

participants 17 (77.27%) said that they managed to feel at ease after sometime within

the 5-week protocol; from those, one (5.88%) said that he felt comfortable with the

slow-breathing strategy after 4 weeks of controlled training sessions (i.e. they felt

comfortable in the fifth session), whereas five (29.41%) said that they felt at ease after

3 weeks of training (i.e. they felt comfortable in the fourth session).

'The 2nd time I started to control the pace longer, be on the pace longer. The

3rd time it was much easier, we say in Arabic...I had more breath. I feel I had more

breath. The 4th time it was the best for me. As in my mind I only see the monitor not

get distracted and do it.'

Additionally, six (35,29%) said that they felt they mastered the strategy after 2 weeks

(they felt comfortable in the third session), and four (23.53%) of them stated that they

could master slow breathing from the second session onwards. This is in accordance with previous literature which suggests that some individuals may have difficulty in following paced breathing and, therefore, in some cases, it may take - at least - three sessions to learn breathing at one's RBF (e.g. Vaschillo et al., 2006; Lehrer et al., 2000). As far as familiarization with one's RBF is concerned, there are also two parameters that should be taken into consideration; the first is that the amount of time spent on HRVT by each participant varied, since a different level of commitment has been recorded during the lab-based (controlled) sessions and, presumably, the same is true for the home-based (uncontrolled) sessions. The second is the extent to which one was following the instructions about pursed lips and abdominal (i.e. diaphragmatic) respiration, which are two factors that contribute substantially to the sustainability of a 20-minute breathing session at one's RBF, as also explained by Lehrer et al. (2000). Within this theme, two sub-themes were also formed from the responses of the participants:

➢ this sub-theme refers to the strategy one uses to learn the slow-breathing technique faster and master it, as, characteristically, one participant said:

'…because it is easier for you to find a pattern. Or something, like in a computer trying to program something because I do a little bit of programming. You try to find a pattern which you are familiar with. Even in gymnastics, you try to find a pattern or a routine. So, I try to find patterns everywhere I go to even study. When I study for maths or physics or something like this, I am trying to find a pattern…'

Or with regards to the software used and the sinusoidal nature of the respiration curve

and the way one can use its peak-to-valley amplitude to master the slow-breathing

strategy, the same participant said:

'…I will stop breathing and I will try to make the lines as straight as possible and I will

try to keep them like to not go over this one and not go down, like below this one. I try

to keep them in between. I don't care about the pink (i.e. the curve depicting the

fluctuation of the HR)*, I didn't know how to figure that out…'*

In other words, this participant claims that, with the correct strategy, one can be more

efficient in mastering HRV training. This is in line with the therapist's instructions in the

original manual for training HRV by Lehrer et al. (2000), which advises trainees to use

abdominal breathing, pursed lips for a long exhalation, whereas they should find a way

to establish a comfortable breathing pace.

> ➢ The second sub-theme refers to the gradual increase of familiarity with the
>
> slow-breathing strategy one acquires week-by-week as s/he goes through the
>
> 5-week protocol.
>
> *'A learning process that is getting better and better as sessions proceed…'*, as

one participant said, which eventually, results to a plasticity of the ANS and a good

level of control of emotions which is the ultimate objective of this strategy; this is also

aligned with Lehrer et al. (2003) that demonstrates the existence of neuroplasticity in

the baroreflex system due to chronic increases in baroreflex gain as a result of

biofeedback practice.

2. Some participants also enriched the collected information by discussing how long it took them to adapt to slow breathing, in each individual session. So, the second theme refers to the time needed for the participants to get accustomed to the slow-breathing strategy within the same session. There were a few participants who also gave an insight into the length of time needed, within the same session, in order for someone to get used to the slow breathing:

'Within the same session, it took me 5 minutes to do it without mistakes...'

As such, it appears, that even within the same session, one needs a bit of time to really practice their resonant breathing frequency at an optimal level, as, in the beginning of it, there is some likelihood that some imperfections in the extent to which one can accurately follow the instructed pace may occur. Lehrer et al. (2013) pointed out that by rehearsing HRVT regularly, one may eliminate such undesirable symptoms.

4.3.3 Whether One Can Breathe Alone at Their Resonant Breathing Frequency Without the Help of a Pacer

The third structural code is the extent to which one can breathe at their resonant breathing frequency without the help of a pacing stimulus. Twelve (54.54%) of the participants answered positively whereas 10 participants (45.45%) said that they do not think they can do it. The reasons behind these responses are worth discussing:

'...I think maybe I can with more training not like just twice per week. So, I can know exactly what I am doing and how long it takes to breathe and to inhale and how long to exhale, when to hold it and when to exhale. For me maybe after a few times of training I think I can manage, yes...'

As per their answers, all the participants had the tendency to say that if they had one, or a couple, or a few more sessions they would be more able to do this (i.e. breathe alone). Further, most of them agreed that twice a week was borderline low in terms of frequency of practice in order for them to be able to breathe alone, without a pacer, whereas those who said that they could do it, were unsure before stating this, which is in line with what is discussed above. This, on the one hand, is opposed to what Gross et al. (2016) showed in their study in which elite sport support and management staff following a five-session HRV training protocol were able to rehearse slow breathing at their RBF for up to 5 minutes when they were asked to do so starting from the third session of the protocol which, in terms of slow breathing-related exercise, had been only preceded by two RBF estimation exercises, a 5-minute paced breathing exercise and a home-based practice (Gross et al., 2016). On the other hand, what the participants said here (i.e. that they would need a higher frequency of paced-breathing exercises to practice slow breathing at their RBF without a paced stimulus) is aligned with what was discussed by Vaschillo et al. (2006) according to which, in some cases, individuals may have difficulty in following paced breathing and, therefore it may take them - at least - three sessions to learn to breathe at their RBF, as an increased frequency would result in a high number of total sessions within the 5 weeks.

Indeed, the major theme being formed by the answers of those participants who said that it is possible for them to breathe at their resonant frequency without a pacer, is that they would need to practice it more often. This has been expressed in different ways, though, all these ways converge to the idea that those who answered

positively believe that, in order for them to breathe at their resonant breathing frequency without a pacer, they would need more practice than two sessions per week for 5 weeks (NB The 5-week protocol included one weekly supervised [controlled] session in the lab and one weekly 20-minute practice as homework [uncontrolled session] with the phone app). The main reason why they would like to have more practice is to fully understand the proportion of breath cycle needed for exhalation as opposed to inhalation. The inhalation-exhalation ratio is again discussed here, in the sense that participants felt that due to the prolonged exhalation, they believed they were not in position to rehearse it without a pacing stimulus, as they felt they would tend to breathe out for a shorter time, which in turn, would result in a wrong cycle and, therefore, wrong RBF. This is aligned with previous literature, which maintains that the inhalation/exhalation ratio is an important factor that determines the autonomic and subjective effects of such a respiratory exercise (Van Diest, Verstappen, Aubert, Widjaja, Vansteenwegen, & Vlemincx, 2014).

This is also linked with the next theme analyzed within the same structural code, according to which, breathing at one's RBF without the help of a pacer can be possibly sustained for less than 20 minutes, which may stem – again - from the difficulty in rehearsing the default inhalation-exhalation ratio. So, in brief, all of these participants maintain that if they practice this more often, eventually, they will be able to do it without a pacer (as also discussed by Vaschillo et al., 2006). Lastly, the degree to which they think they will be close to their resonant frequency without a pacer was - for most of the participants of this category - 75% and higher.

As for those who claimed that they cannot sustain slow breathing without the help of a pacing stimulus there are two themes being formed by their responses:

1. The first theme refers to the fact that participants believe that they cannot estimate correctly the duration of inhalation as opposed to the duration of exhalation, despite their exposure to the protocol. This is not aligned with what Gross et al. (2016) showed, that participants could practice their RBF quite soon after the beginning of the protocol, but it is aligned with what Van Diest et al. (2014) claimed, according to which, the inhalation-exhalation ratio does affect subjective effects of a taught ventilatory pattern.

'Athlete: I think the breathing in part is easy for me, but the breathing out is a bit difficult.
Interviewer: But why does this happen?
Athlete: I think because when I breathe in, I do not get too much air to ensure a long exhalation. I may end up breathing out for 4 seconds not for 6 seconds.'

This athlete raises an issue that is discussed many times in this interview and should be taken into consideration when we finally reach conclusions on the practical side of the HRV training strategy; that is, the default duration of exhalation may create issues in the extent to which certain athletes can sustain the breathing cycle for as long as it is required.

2. The second theme refers to the fact that athletes maintain that they cannot breathe at their resonant breathing frequency, for 20 consecutive minutes, without a pacer.

'…I don't think so. It is really hard to rehearse the pace. I do not think that I can do it for 20 minutes even if I manage for a short while…'

Therefore, it appears that participants, who answered negatively to the question on whether they think they would be able to do this, could possibly have answered positively had it been for a shorter duration (i.e. less than 20 minutes), which would be in accordance with what Gross et al. (2016) discussed in their study. It is mainly the fixed duration of the session that discouraged them to even think about doing so.

A second part of the interview question which comprises the main structural code was under which circumstances the participants believed they would need to practice HRV training without the help of a pacing stimulus. The answers given to this question formed three main themes which, actually, correspond to the different contexts that HRV training should be practiced without a pacer, that is, before exams, before competition, and before sleep.

'…Maybe in competitions. Because in gymnastics when the competition starts the phone is not allowed. And in competition you try your best not to overthink, not to stress…'

This statement says that HRV training could be rehearsed before competition to help the athlete minimize his stress in view of the competition.

'…Maybe before exams, where I can't use my phone…'

This athlete suggests that he would practice HRV training - without a pacer - before the school exams, when smartphones or any other gadgets are not allowed, to manage his stress more efficiently. The above statements are again aligned with Gross et al. (2016) who say that it is usually during moments of challenge or stress that on-demand practice of RBF is needed with a view to reaching a state of emotional regulation.

'...Last time, I was gonna sleep and I feel I get dizzy to sleep. So, I do it...'

This statement implies that, by means of HRV training and slow breathing, one may reach a drowsy state, which will lead to falling asleep; therefore, slow breathing may help individuals reach a state of deep relaxation more efficiently, which, under certain circumstances, may be the desirable result. This is opposed to what Prinsloo et al. (2011) suggested that HRVT results in a state of alert relaxation rather than a state of sleepy relaxation. It might have been that those participants that reported this may have engaged in deep breathing which resulted in a hyperventilation and dizziness (e.g. Vaschillo et al., 2006).

As such, the reason why one would need to practice HRVT without the help of a gadget or smartphone would be, according to the participants, for a preparation in view of a competition, or an exam, or even for relaxing and falling asleep, which actually, adds value to HRVT, given that it is considered as a strategy that can help individuals reach a desirable state of arousal in different contexts of life and for different purposes.

4.3.4 Whether One Can Breathe at Their Resonant Breathing Frequency While Moving Vigorously

The answers to this question were more straightforward than to any other question during this interview. Twenty out of 22 interviewees (90.9%) responded that under no circumstances do they think it would be possible for them to practice slow breathing while moving vigorously, whereas, most probably, the other two participants might have not understood the question well and, for this reason, they did not really give a meaningful answer, as for example, one of those participants said that this is because he would not be able to use a timer while moving vigorously, not because it is impossible to breathe slowly while e.g. running.

One of the participants that clearly said that he cannot do it added:

'…No, it is impossible. Because you are moving around, the muscles work and you need more oxygen - if you are taking less oxygen the outcome is not good…'

This participant suggests that one needs more oxygen intake while moving vigorously, implying that less oxygen intake is not advisable. To the best of our knowledge, in all of the studies researching HRVT, participants are in a resting state (e.g. Lehrer & Gevirtz, 2014).

4.3.5 Whether One Can Do HRV Training Without Taking Deep Breaths

This question was asked to investigate whether participants managed to avoid deep breathing during the 5-week protocol, as the instructed slow-breathing strategy is based on effortless breathing and, therefore, deep breathing would not be advisable.

Only eight out of 22 (36.36%) interviewees answered positively to this major question (i.e. they could do HRVT without taking deep breaths). The rest of the participants claimed that they would be able to avoid breathing deeply, under certain circumstances; in other words, they provided different reasons because of which, they could not avoid breathing deeply during the HRVT protocol in Study 2, which has been explained by Vaschillo et al (2006); until participants familiarize themselves with slow breathing, they may breathe deeply, often causing hyperventilation.

The answers given by the participants formed the following themes:

1. the duration of a single session does not help an individual avoid deep breathing as it is longer than it should be.

'... *It was easy in the beginning but in the end after 18 or 17 min I began to be more dizzy or something...*'

This theme suggests that avoiding deep breathing cannot be sustained for as long as an HRV-training session lasts for. While one interviewee maintained that after the 10th minute things started being harder, most of the reports converge that it is after the 15th and especially the 17th minute that avoiding breathing deeply seemed inevitable. Most of the studies on HRVT, however, promote 20-minute sessions (e.g. Lehrer et al., 2000; Lehrer et al., 2013) with some exceptions (e.g. Prinsloo et al., 2011; Gross et al., 2016). Nevertheless, it is worth pointing out that the themes formed by the responses of the student-athletes here are again related to what had been discussed earlier in the interview; it appears that the duration of the session was longer than expected, with

some of the participants claiming that, in the last few minutes, they could not avoid breathing deeply. But again, as everything discussed in this context is inter-related, one should consider what causes deep breathing, or discomfort in the last minutes of an HRVT session.

2. It is because of the duration of the exhalation that participants cannot avoid breathing deeply, which is aligned with what Strauss-Blasche et al. (2000) suggested that the inhalation-exhalation ratio has an effect on RSA but also on the subjective effects of an HRVT exercise (Van Diest et al., 2016). This theme suggests that only with a shorter exhalation can one sustain this pace of breathing without breathing deeply. Due to the prolonged exhalation, one may need to take a deep inhalation in order to be able to support the long exhalation, hence the deep breathing. When asked if it was possible to avoid breathing deeply during the 20 minutes of the training session or not, an athlete said:

I tried at first but then I took some deeper breath so I would be able to support the long exhalation.'

Whereas another athlete said:

'…It was …most of the exhalation was perfect - it was fine. It was maybe the last second - Well one second makes a big difference, is not a small part…'

In his interview, this athlete also maintained that he definitely needed to take a deep breath every minute to support the whole cycle of slow breathing, given the prolonged exhalations. Therefore, it appears that the main idea in this theme is that breathing out

was much longer than it should have been, which resulted in deep, fast inhalations in order for the prolonged exhalations to be supported. It might have been that, due to lack of familiarity with paced breathing at low frequencies, some participants ended up taking deep breaths (Vaschillo et al., 2006). As such, the fact that some participants felt unable to avoid deep breathing for 20 consecutive minutes might have been related to that they had not been in position to breathe at their RBF effortlessly anyway, since they had to support the long exhalation somehow. So, while they believed that, for the first - approximately - 15 to 17 minutes of the session, they had been able to breathe effortlessly and without taking deep breaths, it might have been that a fast (or deep) inhalation had not been perceived by them as an element of deep breathing and as a factor that interfered with the effortless and natural slow breathing they should have actually engaged in during the HRVT protocol.

3. The third theme refers to the fact that slow breathing may result in sleepiness which, in turn, may result in yawning or other symptoms which do not help a lot. This, however, is discordant with what Prinsloo et al. (2011) claimed about the state of alert relaxation caused by HRVT. Additionally, one may not be able to follow the pacing stimulus continuously if s/he is in a drowsy/sleepy state.

'…Sometimes I did, sometimes I lost focus but then I got back again to the pacing thing.'

Or another athlete said

'…Yes, I will get it all the time. I don't know when I try to control my breath I will do it and I get a lot of yawning…'

The responses forming this theme suggest that slow breathing has caused sleepiness and loss of focus on the pacing stimulus.

4. Last but not least, the last theme formed by the participants' answers refers to that participants were not so familiar with the slow breathing strategy in the first three controlled sessions, and, therefore, they were not in position to completely avoid breathing deeply (as also discussed by Vaschillo et al., 2006). As they were approaching, though, to the end of the protocol (i.e. the fifth controlled session) they felt more comfortable with the idea that they had to breathe effortlessly and naturally, without taking deep breaths, which means they became more familiar with the slow-breathing strategy and could practice it more easily.

'…After the third one I was ok. At the beginning of the third one I felt comfortable but then it was better. Fourth and fifth were perfect…'

In the above statement, it is implied that it was not until the fourth controlled (weekly) session that the athlete felt at ease and, as a result of this, did not need to take deep breaths.

Within this structural code, the participants were also asked whether they had understood the reason why they had been instructed not to breathe deeply during the slow-breathing protocol. Ten of them (45.45%) stated that yes, they were aware of the fact that deep breathing would result in dizziness, whereas the remaining twelve of them (54.54%) said they did not know why they had been instructed not to do so, as

they had not been in position to create a mental link between hyperventilation and dizziness, as explained by the main researcher, during this project.

4.3.6 Positive and Negative Outcomes of HRV Training

The next structural code comprises the sixth major question of the interview and refers to the positive and negative outcomes of the HRV training as experienced by the interviewees. Under this structural code, there appear four codes covering four different - and to a certain extent overlapping - aspects of the question which, in turn, refer to the perceived positive/ negative outcomes of the HRV training as well as what is perceived as benefits resulting from the HRVT, and possible uses of it in everyday life.

4.3.6.1 Positive outcomes of the HRV training protocol. The responses given here by the participants formed five themes:

(P1) The first theme is related to that many of the participants reported that while, on the one hand, they felt more relaxed with the slow-breathing strategy, they felt that they still had a good level of arousal that allowed them to be aware of what was going on around them (as also suggested by Prinsloo et al., 2011).

'… I felt very well. Relaxed, easy breathing (I can breathe very easily), very active. I can now finish the training with my coach more easily now. Before it was not always the case. Positive result…'

The above answer indicates that the interviewee managed to reach an ideal level of arousal (alert relaxation) by means of slow breathing (e.g. Prinsloo et al., 2013b).

(P2) Some participants reported that the slow-breathing strategy could be used as assistance for deep relaxation and, eventually, to fall asleep, which could be a positive outcome for someone who cannot sleep easily. It might be that, by practicing HRVT participants managed to minimize cognitive anxiety, thus it was easier for them to fall asleep (e.g. Lehrer et al. 2000).

(P3) A major theme resulting from the interviewees' responses is that HRV training results in a better control of stress, a better control of anger and nervousness and, generally, a better control of emotions, which is in agreement with previous literature (e.g. Lehrer & Gevirtz, 2014; Lehrer et al., 2000; Gross et al., 2016).

'…I can control my emotions more. I will not get angry or nervous that easily any more. It was positive…'

The statement above implies that one can use HRV training to control emotions not only before or during a competition but in different contexts of everyday life as well (as also discussed by e.g. Jimenez-Morgan & Molina-Mora, 2017; Lehrer & Gevirtz, 2014).

(P4) A clearer mind has been also reported to result from breathing slowly at one's resonant breathing frequency.

'…I think there was an improvement. After the second session, whenever I did something that required mental effort, I managed to do it effortlessly…'

Throughout the discussion, it was understood that the above statement referred to a clearer mind which led to a better decision-making ability and cognitive performance in general (as also discussed by Prinsloo et al., 2011) and/or a state of alert relaxation which helped participants to complete the task at hand more efficiently (as also discussed by Prinsloo et al., 2013a).

(P5) The intervention had also an educational dimension since it taught the participants how they can slow down their breathing pace and breathe slowly effortlessly, and naturally.

4.3.6.2 Negative outcomes of the HRV training protocol. Again, five themes were formed by the responses of the participants on the negative aspects of the HRV training protocol:

(N1) The first theme which has been discussed earlier under different structural codes and will be further discussed in the last major question of the interview refers to that, for many participants, the session should have been shorter than 20 minutes, as this cannot be sustainable without a break, or may create other issues as the below-pasted statement suggests:

'…Maybe looking at the monitor for a long time hurts my eyes…'

Again, this is not supported by literature, to the best of our knowledge, as, in general terms, 20-minute sessions are proposed by researchers (e.g. Lehrer et al., 2013), though there are studies discussing shorter sessions as well (Gross et al., 2016; Prinsloo et al., 2011).

(N2) Prolonged sleepiness was another symptom reported as a negative effect of the slow breathing strategy; some participants implied that they were far more relaxed than expected which may not help in a competition context.

(N3) Dizziness is the symptom discussed more than any other symptom; some of the participants reported that they felt dizzy at the beginning of the 20-minute HRV-training session, whereas others said that it was in the last five minutes that they felt dizzy. This is probably related to that participants might have not been familiar with paced breathing within the LF area, as explained by Vaschillo et al. (2006).

'…You could feel dizzy if you are not careful and you take deep breaths. Sometimes I felt at the beginning of the session…'

This participant creates a link between breathing deeply and dizziness which is also discussed more later on.

(N4) An unexpected level of fatigue was also reported as a result of the HRV training session; it might have been that this fatigue resulted from practicing slow breathing in a wrong way.

(N5) The last theme refers to a general discomfort reported by a few participants, not having been able to specify what this discomfort was about:

'…I felt kind of discomfort in the first session, at the beginning…'

It might have also been that this discomfort turned out to be one of the above-mentioned symptoms as time went by, as participants were more in position to

identify those symptoms either later on within the same 20-minute session or later on within the 5-week protocol.

The truth is that a session of 20 minutes would not feel as being too long, if the suggested slow pace of breathing had been ideal (e.g. Lehrer et al., 2000; Vaschillo et al., 2006). So, either the measured RBF had not been estimated in the best way and, as a result of this, it caused discomfort in some of the participants, or there was another reason behind that. The present study sheds light on a parameter of the RBF that has emerged many times within this project and seems to be as important as the calculation of the RBF itself. This is again the inhalation-exhalation ratio and its impact on the feasibility of an HRVT session (as also explained by Van Diest et al., 2014) as well as the extent to which an individual can sustain the RBF for 20 minutes.

4.3.6.3 Benefits of the HRV training. As discussed earlier in this chapter, themes formed by the responses given to different codes (e.g. positive outcomes vs benefits) tend to overlap sometimes. There were six themes formed within the code "Benefits" discussed with the participants under this major question.

(B1) The first theme refers to the benefit of being less stressed in competitions, having better control of emotions and a good level of arousal after the HRV training protocol (as also discussed by Lehrer & Gevirtz, 2014).

'…I felt more relaxed. I did not feel angry. You can think properly, clearly…'

The above statement indicates a good level of emotional control which is what the training is about and will be further discussed in the next section.

(B2) The second theme refers to the state of mental clarity, as discussed above, that an individual may achieve by means of the slow breathing strategy. This may be related to the better cognitive functioning researched in the literature as a beneficial effect of HRVT (Johnsen et al., 2003; Thayer et al., 2009) or the better attentional capacity reported as a positive effect of HRVT ((e.g. Hansen et al., 2003).

(B3) The next formed theme referred to the deep relaxation that one may reach and, eventually, manage to fall asleep, which is perceived as a benefit by some users.

(B4) An educational dimension of the protocol has emerged as well, with some participants stating that it is a benefit for them that they have learnt about their body and the benefits of slow breathing.

(B5) the fifth theme refers to the fact that certain participants mentioned that they felt more energized after the HRV training session, and as a result of this, they felt more motivated to do things or to complete the task at hand, as shown below:

Interviewer: Are there any benefits that you see in this type of training?

Athlete: Yes, energized and motivated.

Again, this is aligned with the concept of alert relaxation discussed by Prinsloo et al. (2011).

(B6) Last but not least, it was also reported that one of the benefits of the slow breathing strategy was that participants felt that after the protocol they felt they could breathe better, they were in position to breathe more efficiently, which might be

related to the fact that exhaling through pursed lips may decrease airway turbulence (Lehrer et al. , 2000) and will be further discussed later on.

All benefits are inter-related to a high extent, as a better level of arousal results from an ideal balance between the sympathetic and parasympathetic branches of the autonomous nervous system and is related to the athlete's ideal zone of optimal functioning (IZOF) (Hanin, 2000); it also results, as discussed in the interviews, in better anger and stress management, thus, a better level of emotional control (Gross et al., 2016; Lehrer & Gevirtz, 2014) but, also, better level of cognitive functioning, in the sense that many of the student-athletes claimed that they experienced a clearer mind (i.e. higher level of mental clarity).

4.3.6.4 Uses of the HRV training protocol. The last code within the sixth structural code of the interview is related to the different uses of the protocol suggested by the participants who took part in the second study. Five themes were formed by their responses which are as follows:

(U1) The first theme refers to the use of HRV training before the school exams in order to help students reduce their stress level and think clearly.

'…I can use it before exams. Because it will reduce my stress and I will think more clearly…'

The above statement combines the positive effects of HRV training mentioned earlier, that is, emotional control and mental clarity, and discusses how those can help a student in the context of school exams.

(U2) The second theme refers to the use of HRV training before a competition, apparently, for the same reasons (i.e. better emotional control and mental clarity). A better emotional control may help the athlete minimize their stress to a desired level and may help them have better anger management as well, as in, for example, team sports, as reported by participants. Again, this builds on existing literature (Gross et al., 2016) which says that it is usually during moments of challenge or stress that on-demand practice of RBF is needed with a view to reaching a state of emotional regulation.

 (U3) Another interesting use has emerged from the responses given, which is shown below:

'At night, because at night I come back from training, I keep on thinking about the elements that I haven't done, what should I do tomorrow and my homework….also I need to help my parents in the house, we are a lot of kids in the house, I need to help my father so I get stressed a lot and this would help me…'

In other words, this statement suggests that, after a quite busy day, HRV training can be used to help an individual relax and reach a good level of composure (as also discussed by e.g. Lehrer et al, 2000, Lehrer & Gevirtz, 2013).

(U4) This theme refers to the acquired ability of an HRV-training user to regulate their heart rate accordingly when they are under stress. The longer exhalation always helps individuals to gradually lower their average heart rate which results in a better level of arousal (e.g. Lehrer & Gevirtz, 2014).

(U5) Last but not least, the last theme formed here was related to the use of HRV

training in one's preparation for an important sport training session. This, of course,

will result in the same benefits as the ones already mentioned within the context of a

competition.

4.3.7 Suggested Modifications for the HRV Training Protocol

The last structural code of the interview aimed to investigate whether the participants

believed that the suggested HRV training protocol would suit them better in their life

as students and athletes, or anywhere in their life, had they been able to modify it.

Therefore, within this major question, they were asked how they would change the

protocol to make it suit better their needs as a student and as an athlete. The obtained

responses formed five themes.

1. The first theme constituted the majority of the responses as it referred to the fact

that many of the participants found the duration of a single HRV training session as

being too long. Different types of suggestions were made with the most common one

being that a desirable duration would be around 10 minutes, whereas one participant

mentioned that 5 to 7 minutes would only be possible to be included in an athlete's

pre-competition routine. Of course, a 10-minute session is supported by the literature

(e.g. Prinsloo et al, 2011) but as mentioned above, the big majority of theorists/

practitioners talk about 20-minute sessions (Lehrer et al 2000; Lehrer et al (2013).

Other interviewees suggested that a break every 5 minutes would help an individual

finish the total duration of 20 minutes.

'…I would like it to be divided, not to sit 20 min in one session. Maybe 5 min in the morning, 5 min in the afternoon, 5 min before training, 5 min after training. I think this is the best…'

This statements implies that conducting a 20-minute session of slow breathing at the suggested resonant breathing frequency might not be feasible, perhaps, both because of the fact that it might not be always possible to physically sustain 20 consecutive minutes of slow breathing but also, it might be the duration itself that makes it look a difficult task to complete (NB This will be discussed further in the next chapter). Another interviewee said:

*'…Twenty minutes is too much because you cannot dedicate 20 minutes of your time (*i.e. in the pre-competition routine) *to do this. Ten minutes is okay…'*

The above statement suggests that, practically, speaking, a practice of around 10 minutes would be more possible to be included in a pre-competition routine, which stresses the importance of the practical side of the strategy and will be discussed more in the next chapter.

2. The second theme formed by the answers referred to the fact that many participants complained that the duration of the exhalation was much longer than expected in a cycle of slow breathing; therefore, interviewees suggested that a slightly shorter duration of exhalation, which would still make it slightly longer than the inhalation, could possibly resolve many issues related to the sustainability of the strategy as well as the feasibility of rehearsing it for as long as it is required (e.g. in this

case, an individual could possibly avoid deep breathing which causes dizziness and could complete the 20-minute session with less difficulty [Van Diest, 2014]) .

'…Maybe not so long, but a bit longer than the inhalation…'

3. The third theme is related to a suggestion made by participants concerning the complexity of the biofeedback equipment used during the HRV training.

'…Not on a laptop, on my phone and instead of having all these wires attached to me maybe I could put a watch or something with a sort of sensor…'

This statement is related to the practical side of the strategy and the extent to which one can run a proper HRV training session before a competition or an important training session. (The participants had been told to practice HRV training at home as homework, using a smartphone app, but this app did not actually include any hardware for monitoring physiological responses). The theme here refers to whether one can use some simplified valid hardware to rehearse the slow breathing strategy.

4. The fourth theme raises an important aspect of the strategy; it was suggested that lighting in the room where HRV training is practiced should not be dimmed but, rather, it should be turned up to maximum intensity in order to help the client avoid entering a drowsy state, a state of sleep relaxation, as opposed to alert relaxation (see Prinsloo et al., 2013a).

'…It would be cool if the athlete could make this kind of testing in the area where they…, like if there was a chair on the track and they would do it. It's just in here the chairs are comfortable and the lights were dimmed so I would definitely feel sleepy…'

The above response says that anything that could trigger a drowsy state should be eliminated in order to achieve a state of alert relaxation (e.g. Prinsloo et al., 2011) and an optimal level of arousal.

5. The fifth theme refers to that sound feedback could be provided during the HRV training session which could act as background music, as "relaxation music" that some participants mentioned; this music would change when negative feedback should be given to the individual in case that s/he gets drifted from his resonant breathing frequency.

'…Maybe the negative feedback that you gave us from the microphone could be codified with different sounds (or music) *for positive or negative feedback…'*

(NB Actually, there is a software [HRV suite by Thought Technology] that automatically gives feedback to the client if they deviate from their RBF, but this software was not used in this project).

As such, the most common response given here was that the duration of the session was longer than it should have been. Some of the participants said that had it been around 10 minutes, the HRVT session would be really much more practical. This is different from what has been discussed above which refers to an ideally shorter session due to discomfort experienced from a certain point onwards, and is purely

related to the practical side of the HRVT. That is, to which extent an individual can include a 20-minute session in their preparation in view of a competition or an exam, and whether there is enough time for that or not before a competition or an exam. Additionally, participants mentioned the issue of the need for customization of the inhalation-exhalation ratio which has been extensively discussed above.

Therefore, the interviews, conducted in this study, gave information about the experience of the participants in the second study, according to which, HRV training is a strategy that can be used to achieve better emotional control not only within an academic or sport-related context but within any stressful context of everyday life. A 5-week protocol appears to provide an individual with sufficient time and exposure so that they can get well familiarized with the slow-breathing strategy and rehearse it efficiently when this is required. Some modifications on the duration of a single HRV-training session as well as the duration of exhalation compared to inhalation should be further investigated.

4.4 CONCLUDING POINTS

The five-week heart rate variability training (HRVT) protocol appeared to provide the participants with sufficient time to familiarize themselves with their resonant breathing frequency and reach a level of optimal arousal, which results from a balance achieved between the sympathetic and parasympathetic branches of the human autonomous nervous system (ANS). Consistent with the psychophysiology of HRVT (e.g. Lehrer & Gevirtz, 2014), the interviewees shared their experience during the protocol with the interviewer, and raised a couple of topics for further investigation, which have been already considered in the previous chapter. Concluding remarks are also discussed in this section.

Within this framework, the difficulty that the participants have experienced in adjusting their breathing pace to follow the instructed RBF has been attributed to that the prolonged exhalation did not make participants feel comfortable (Van Diest et al., 2014). As discussed during the interviews, due to the long exhalation in every breathing cycle, participants often felt that they had to take in as much air as possible during the inhalation, in a very short period of time, to the extent that sometimes it felt unnatural; they actually felt they had to make a big effort just to support a very long exhalation; this may account for the reason why some participants reported that they could not sustain their RBF for 20 consecutive minutes.

However, the ratio used during the protocol for every RBF was what had been given as default ratio from the physiology suite that was used during the current

project. The rule of thumb in HRVT says that the exhalation should be longer than inhalation (Strauss-Blasche et al., 2000) to increase the vagal tone and create a state of alert relaxation (Prinsloo et al., 2013a). However, as long as the breath cycle is not affected and inhalation is shorter than exhalation (Strauss-Blasche et al., 2000), it would be wise, in certain cases, to slightly modify the inhalation-exhalation ratio, to make a 20-minute session more pleasant and sustainable for the participants and, also, minimize hyperventilation. Besides, studies have shown that if this ratio is slightly modified, there is no significant change in the levels of HRV (e.g. Wang, Kuo, Lai, Chu & Yang, 2013).

The time taken for the participants to feel more comfortable with the RBF and the 20-minute session itself varied with most of them claiming that they felt much better with the HRVT protocol when they were half-way through, that is, in their third week of training (as also suggested by Vaschillo et al., 2006). Nevertheless, the main message obtained from the analyses of the interviews was that most of the participants were in position to familiarize themselves after having completed three lab-based sessions (i.e. from the 4th week onwards).

Moreover, the responses given to whether one could practice HRVT without the help of a pacer formed themes that were worth discussing. The first issue emerging was that a frequency of training twice a week is not an adequate frequency to make the participants confident to practice HRVT without a pacing stimulus; this is not aligned with what was shown by Gross et al. (2016) in which study participants could practice their RBF without a pacer for a few minutes, just after two RBF estimation

exercises, a 5-minute paced breathing exercise and a home-based practice. Another

issue discussed again here is the inhalation-exhalation ratio; the participants of the

present study felt they would tend to breathe out for a shorter time, as the prolonged

exhalation feels a bit unnatural, which in turn, would result in a wrong cycle and,

therefore, wrong RBF. It might be the case that, with a different inhalation to

exhalation ratio, participants would probably feel more confident to rehearse the cycle

without a pacing stimulus.

Another topic discussed with the interviewees in this study was related to

whether they felt that they were in position to breathe at their RBF without taking

deep breaths during the HRVT protocol. Again, the issue of a fast inhalation, which is

often referred to in the interview as a deep inhalation, to support the prolonged

exhalation, may have minimized the feeling of effortlessness in slow breathing, which

is required to conduct HRVT efficiently (Van Diest et al., 2014). In other words, it might

have been that it is because of the – default - prolonged exhalation of the software

that, on the one hand, some participants reported that they were not in position to

continue slow breathing for 20 consecutive minutes without taking any deep breath,

and, on the other, in other parts of the interview, it has also been reported that a 20-

minute HRVT session cannot be sustainable due to discomfort caused especially in its

last minutes.

Besides, the negative outcomes of the HRVT protocol discussed at the end of

the interview were actually related to the above-mentioned issues; the main points

mentioned here as negative outcomes were dizziness, discomfort in the last minutes of the session, that a session of 20 minutes was too long, sleepiness and fatigue.

All of the symptoms discussed within the framework of the negative outcomes of the HRVT, are symptoms that can be caused due to deep breathing (i.e. dizziness, drowsy state/sleepiness, discomfort, etc.), which might stem from the fact, as already discussed, that a very long exhalation requires a fast and deep inhalation in order to be sustained. After a few minutes of deep inhalations, it is very likely that the client may start feeling dizzy, very sleepy and generally tired and in a drowsy state.

Nevertheless, HRVT appears to be mainly associated with various benefits, which were discussed in detail with the interviewees. The ones that became more prominent during the interviews were: (a) the ideal level of arousal achieved by means of HRVT; the participants felt ready to perform, therefore, they were neither too relaxed nor too energized (i.e. they were in a state of alert relaxation as explained by Prinsloo et al., [2013a]); (b) a better level of stress/ anger management and, generally, better emotional control achieved during the protocol; and (c) the fact that many participants reported having experienced a state of better mental clarity (Johnsen et al., 2003; Thayer et al., 2009). It is assumed that what was described as *clearer mind* by the interviewees referred to a level of composure that allows an individual to be future-oriented and task-oriented rather than engage in negative self-talk which may result in a lot of sympathetic activity, tension and anxiety.

What is really interesting is that the interviewees suggested HRVT as a method that can be used in different contexts of life such as in school/academic life in view of exams, and in sport-related activities to prepare someone in view of a competition or important training; the ultimate objective of using HRVT would be to achieve composure or reduce sympathetic activity and increase parasympathetic activity, thus, regulating the level of arousal (as also proposed by Gross et al., 2016).

Furthermore, the interview closed with any suggested modifications the participants had for the proposed HRVT protocol. The present project puts emphasis on that aspect of the interview, as it is believed that, as long as the exhalation is longer than the inhalation, by modifying this ratio a lot of the issues discussed above will be resolved (Wang et al., 2013). Therefore, it is suggested that, apart from the assessment of the individualized resonant breathing frequency, the ideal slow breathing cycle for each individual, the assessment of the individualized inhalation-exhalation ratio within that cycle is highly recommended, ensuring that exhalation is always longer than the inhalation (Strauss-Blasche et al., 2000).

Last but not least, another interesting point raised by the student-athletes was that as long as a practitioner wants their client to achieve a state of alert relaxation (i.e. the state of optimal arousal which is related to increased cognitive performance as well), they should be careful with the intensity of the lighting of the room they are doing the HRVT in; as such, a dimly-lit lab will most probably prompt a client to reach a sleepy relaxation state, whereas a well-illuminated room can help the athlete reach the desirable state of arousal, a state of alert relaxation (Prinsloo et al., 2011).

To optimize the experience of practicing HRVT in view of an important event, an athlete should spend so much time on identifying and finetuning their resonant breathing frequency as it is needed in order to achieve a better level of self-regulation in a timely manner, as all of the issues, discussed above, are mostly related with the experience of the participants. Once this experience is optimized, the individual feels comfortable and can focus more efficiently on the task at hand (i.e. breathing slowly following a pacer). As a result of this, the positive effects of HRVT are maximized.

4.4.1 Shortcomings of the Study

The main shortcoming of the study was that, although ASPIRE academy is an English-speaking institute, occasionally there are a few student-athletes whose level of English does not allow them to engage in really profound discussions. Two of the participants of this study could, on the one hand, speak English, communicate to a satisfactory extent in English, but, on the other, they were not able to elaborate as much as this study required. If the interviews with those two boys had been conducted in Arabic, a better insight could have been possibly obtained.

4.4.2 Conclusions

In conclusion, heart rate variability training has been perceived as being beneficial in terms of reaching an ideal level of optimal arousal due to a balance achieved between the sympathetic and parasympathetic branches of the ANS. This state of alert relaxation results in better stress and anger management, thus, better emotional control and increased cognitive performance which is often discussed as

"better mental clarity" by the participants. This study managed to shed light on an aspect of HRVT that has not been discussed that extensively before; this is the inhalation-exhalation ratio and the need to customize it within the estimated resonant breathing cycle of each individual, where necessary. Other practical suggestions were made with a view to maximizing effectiveness of HRVT and practicality within a realistic context of preparation.

CHAPTER 5: OVERALL DISCUSSION

The present thesis explored the effects of HRVT on emotional control, and how HRVT can be used for the better preparation of an athlete in view of an important event. It builds on previous literature by exploring how HRVT affects different stress-related psycho(physio)logical and biochemical indices. There were no acute effects of an HRV session on performance under pressure, measured by means of a reactive stress tolerance test, after a single 20-minute heart rate variability training session. The acute effects of HRVT were found in biomarkers of stress and specifically in the concentration levels of salivary a-amylase. There were acute effects of HRVT on a-amylase levels within each session with a-amylase levels decreasing over the course of the session. Both cortisol and a-amylase levels reduced over the 5 weeks of the HRVT protocol. The data collected after the training protocol, by means of the stress profile and salivary samples, showed significantly lower cortisol and skin conductance levels in the experimental group compared to the control group. These findings suggest that HRVT may be an effective way of reducing psychological stress in Middle-Eastern adolescent student-athletes. HRVT programmes should also consider the importance of customizing the inhalation-exhalation ratio and the need to customize it within the estimated resonant breathing cycle of each individual, where necessary.

This programme of research, investigating acute and long-lasting effects of heart rate variability training on emotional control, has reached conclusions that build on the work of Lehrer et al. (2013) by introducing a different version of a 5-week

protocol tailored to the needs of Middle-Eastern male adolescent student-athletes and the reality of the Qatar-based ASPIRE academy, where this study was conducted. Study 2 has been the first study to investigate the effects of such a protocol on such psychophysiological variables as electrical activity on the trapezius muscles, electrodermal activity and HRV indices, providing statistically significant results for the long-lasting effects of the protocol on all those parameters with varying effect sizes, whereas it is the first study to show that both cortisol and a-amylase displayed downward trends during the course of the protocol. To the best of our knowledge, Study 2 has been the first study to investigate the acute effects of a single HRVT session on salivary a-amylase in parallel with Study 1, which has been also the first study to investigate the acute effects of a single HRVT session on performance under pressure, whereas Study 2 also showed that there are significant effects on a-amylase, which builds on the findings of Prinsloo et al. (2011), Prinsloo et al. (2013a) and Prinsloo et al. (2013b). Last but not least, Study 3 built on the study of Tanis (2012) by exploring the experiences of the participants in Study 2 and contributed to the existing literature by giving emphasis to the importance of the inhalation-exhalation ratio as a factor affecting RSA (as also discussed by Strauss-Blasche et al., 2000) that also affects the subjective effects of HRVT (as also discussed by Van Diest et al., 2014). The present section addresses Aim 5 of the thesis and discusses the main findings of the three studies making recommendations on how HRVT can be better used in an athlete's preparation in view of an important event.

5.1 Acute Effects

It may be confusing that, while acute effects on performance were not proved to be statistically significant in Study 1, they were found to be statistically significant in the biochemical markers of stress in Study 2, and, actually, with a large effect size. One may wonder why the two studies led to contradictory conclusions. To answer this question a closer investigation into the two studies would be of paramount importance.

Study 1 measured performance in a task requiring fast responses to rapidly incoming visual and acoustic stimuli. Performance in this task was recorded during a test in which the main construct (i.e. reactive stress tolerance) is related to the ratio of correct responses to the total number of generated stimuli within the four minutes of the test and is also reversely related to the ratio of the total number of wrong responses (i.e. incorrect plus omitted responses) divided by the total number of generated stimuli. In other words, acute effects, in Study 1, were investigated by means of measuring performance in a task requiring the participant to respond to rapidly incoming stimuli.

It might have been that results were not significant because there was a 9 to 12 minutes delay due to the time elapsed as participants had to walk to a different wing of ASPIRE academy in order to move from the mindroom to the labs where the reactive stress tolerance test could be administered. In other words, the process of removing the sensors from the participants (approx. 2 minutes), walk from the mindroom to the other labs of ASPIRE (6-7 minutes) and start the reactive stress

tolerance tests for them (1-3 minutes) was time-consuming and might have been the reason why differences were not statistically different.

Study 2 built on Study 1 by extending the investigation of acute effects to the biochemical responses to HRVT and, specifically, salivary a-amylase. Additionally, the duration of acute effects, if any, was further considered in Study 2, by collecting another two salivary samples every 45 minutes after the end of the session. Consequently, while, in Study 1, acute effects were measured by means of performance in a test measuring reactive stress tolerance that commenced within an average of 10.5 minutes after the end of an HRVT session, in Study 2, acute effects were measured upon the completion of the HRVT session by means of measuring salivary a-amylase. Additionally, Study 2 showed that, whereas in the salivary sample collected immediately after the session, a-amylase was found to be significantly lower compared to before the session, the samples collected subsequently showed a significant upward trend of a-amylase, which, in practical terms, means that it started increasing from a certain point within the time interval between the collection immediately after the HRVT session and the sample collection which took place 45 minutes after the end of the HRVT session, onwards. Of course, it would not be appropriate to compare the two different investigations conducted to measure HRVT acute effects as those were different in terms of nature. But as Study 2 shows the detected effects did not last for long, which could be one of the reasons why Study 1 failed to detect them in performance. Or it might have been that the physical activity involved between the moment the HRVT session finished and the commencement of

the reactive stress tolerance test (i.e. the participants had to walk for at least 6 minutes to move from one lab to the other) covered any effects; the fact that acute effects exist does not necessarily mean that those should be measurable and practically meaningful in every performance-related task that an individual may carry out.

As explained in Study 2, salivary a-amylase has attracted the interest of experts, as it is a non-invasive way for measuring the ANS response to stress (e.g. Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011) and is a consequence of the secretion of catecholamines resulting from the activation of the SNS (e.g. Nater & Rochleder, 2009). Additionally, the rapid reaction of salivary a-amylase to fluctuations of psychological stress has been discussed in literature (e.g. Takai, Yamaguchi, Aragaki, Eto, Uchihashi, Nishikawa, 2004; Maruyama et al., 2012). Consequently, any fluctuation in the SNS activity is immediately reflected in the levels of a-amylase, hence the collection of saliva immediately after each of the HRVT sessions in Study 2.

Therefore, it is quite promising that a-amylase showed significant reduction after a single 20-minute HRVT session, as controlling sympathetic activity is what one would like to achieve by means of a stress management strategy. This is an important finding and can be really useful in the applied field; its practical applications will be discussed further.

5.2 Long-lasting Effects

The initial research question, as conceived at the beginning of this project, discussed chronic effects (as also discussed in Lehrer et al., 2003; Karavidas et al.,

2007); later on, this changed to long-lasting effects, since there is a fine line between chronic and long-lasting effects; the former refers to - somehow - permanent changes, whereas the latter refers to some effects that last for some time, on the one hand, but phase out after a while, in the absence of the intervention. Study 2 focused on the extent to which the suggested 5-week protocol resulted in long-lasting effects or not, whereas, due to the nature of the recorded parameters, additional research questions were subsequently added as, for example, the analysis of acute effects discussed above.

Long-lasting effects on the control of emotions were measured by means of parameters of different nature that are associated with the ANS and reflect fluctuations in the SNS or PNS activity. It is true that, as discussed in the "Discussion" section of Study 2, performance-related variables were not used to investigate long-lasting effects on the control of emotions, as this would seriously complicate the design of the study and would probably lead it to a deadlock in terms of reaching reliable and dependable conclusions, since student-athletes of various sports were recruited, and measuring performance in a standardized way but also normalizing these scores - coming from different sports - would be really challenging, if not impossible. Nevertheless, the variables used were, as discussed, variables reflecting SNS activity which affects arousal and, as a result of this, also affects performance (e.g. Gould & Udry, 1994).

Additionally, the title of the research project suggests that heart rate variability will become better (i.e. higher) due to the introduced HRVT; as mentioned again in

Study 2, the results indicated that, whereas the standard deviation of NN intervals

(SDNN) along with the proportion of the number of pairs of successive NNs that differ

by more than 50ms divided by the total number of NNs (pNN50) were not proved to be

significantly different between the two groups, statistically significant differences

between the control and experimental groups were found for the root mean square of

successive differences between adjacent NNs (RMSSD) and the absolute power of the

low-frequency band (LF). These are two indices expected to yield some practically

meaningful results, since the former is considered as a reliable HRV index for short-

term recordings, whereas the latter reflects existence of both SNS and PNS activities.

The data were statistically significant, on the one hand, but they were not practically

meaningful, on the other, due to the fact that the calculated effect size was lower than

what is considered as a low effect size (i.e. 0.1, Cohen 1992). Nevertheless, as the

present study is designed within a context of high performance and elite athleticism,

even a small increase of a - perceived as - very small effect size might contribute to a

fractional enhancement of performance which may, eventually, lead to success.

In both cases, the calculated effect size was borderline lower than 0.1, which is

a low effect size (Cohen, 1992), so both effect sizes were small. This is the effect of a

protocol in which clients had to practice a 20-minute HRVT exercise at their RBF almost

every 3 to 4 days. This frequency is much lower than the initial protocol as described

by Lehrer et al. (2000), and all the studies that used this protocol (Raymond et al.,

2005; Lagos et al., 2008; Lagos, et al., 2011; Choudhary et al., 2016), which, included

apart from the weekly HRVT sessions, daily homework consisting of two 20-minute

HRVT exercises for 10 weeks, and is also lower than the frequency used by Paul et al. (2012) and Paul and Garg (2012) in their studies with basketball players, in which they both had daily 20-minute HRVT sessions for 10 consecutive days. It might have been that, as the level of the reported effect sizes suggests, the proposed frequency of repetition of the HRVT exercise was borderline low. A slightly higher frequency could have probably resulted in a better effect size, as far as the HRV variables are concerned.

Despite the limited training, cortisol levels in the experimental group were shown to be statistically different from those in the control group in the sample collected immediately after the stress profile in the post-intervention session, while, at the same time, these results had a larger effect size (i.e. $\eta_p^2 = .13$). This shows that the 5-week protocol had a practically meaningful effect on the levels of cortisol measured after a 2-week wash-out period, which indicates some impact on the hypothalamic-pituitary-adrenal (HPA) axis activity, which is one of the two biological systems involved in the stress response (e.g. Chroussos & Gold, 1992). Therefore, the introduced 5-week protocol resulted in lowered stress response as recorded by means of cortisol, often considered as a measure of emotional distress (Appelhans & Luecken, 2006a). This was detected in the salivary sample collected immediately after the stress profile in the post-intervention session. This is the first study to provide evidence that a 5-week HRVT protocol results in significantly lower cortisol levels and, consequently, a lowered stress response, within a context of athletes.

The same was true for the measured skin conductance; that was the second psychophysiological index which was recorded as significantly different between the experimental and control groups, with this difference being also practically meaningful ($\eta_p^2 = .15$). As discussed earlier, skin conductance is another index of SNS activity, as it gets proportionally increased when sympathetic activation occurs, which also results in the secretion of sweat by the sweat glands (Peper et al., 2008).

5.3 Plasticity, Expectations or Both?

Figures 3.1 and 3.2 in Study 2 suggest that there are some other effects detected by observing the biochemical trends during the weeks of the suggested protocol. On the one hand, as already discussed, Figure 3.1 shows the downward trend of salivary cortisol during the protocol and, on the other, Figure 3.2 outlined what the trend looks like for a-amylase. The downward trend for cortisol continues till the 5th week of HRVT, whereas, for a-amylase, it reaches its lowest value in week 4 of HRVT. In the case of cortisol, it looks more reasonable that its levels started moving upwards during the wash-out period, as one would expect that when the HRVT is over, cortisol levels would start, little by little, moving back to their initial levels. In the case of a-amylase though, an upward trend began after the 4th week of intervention, which is worth discussing further.

So, it might have been that a-amylase did not continue to decline after the 4th week of HRVT as, it had already reached the lowest possible levels (Adam et al., 2011) in all four of the samples collected at different times of the school day. Perhaps, this is

the reason why a slight increase is observed from the 4th to the 5th week of HRVT, whereas this increase becomes steep during the wash-out period towards the post-intervention session.

Another explanation for the differences observed between the trend of salivary cortisol and that of salivary a-amylase may be related to the different response patterns showed by the hypothalamic-pituitary-adrenal axis compared to those displayed by the sympathetic adrenomedullary system (Maruyama et al., 2012). However, this explanation presupposes that cortisol responded to the introduced HRVT strategy with a one-week delay, since it moved upwards from the pre-intervention session to the first week of HRVT and then kept decreasing until the 5th week, as shown in Figure 3.1, whereas a-amylase started decreasing from the pre-intervention session until the 4th week of HRVT. This does not seem to be the most plausible explanation, though, as it – somehow - implies that cortisol should have decreased from the pre-intervention session to the 1st week of HRVT, had the delay in its response not been the case, despite that HRVT had not taken place before the 1st week of intervention.

This is the first study to show that an HRVT protocol results in such a decline in both biochemical markers of stress, whereas this is reversed when the training stops. There are two phenomena that need to be discussed here: The first is the fact that both biochemical markers appear to have a declining trend during the HRVT protocol, and the second is the above-mentioned difference in response patterns between the two biochemical markers of stress. So, at first, Figure 3.1 and 3.2 depict, as already

discussed, a downward trend of both markers which indicates that both the

hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenal medullary

(SAM) system were affected by some factor; this factor could be either an actual effect

caused by HRVT, or it might be purely related to positive expectations that participants

had for the intervention. Last but not least, figures 3.1 and 3.2 also demonstrate that

the trend is becoming steeply upward from the 5th week onwards, which provides

evidence on that the beneficial effects cease to exist in the absence of a regular

rehearsal of HRVT.

To understand the above-mentioned trends better, let's consider a-amylase 1 in

comparison with a-amylase 2, which has been proved to decrease significantly after

the HRVT session (i. e. as shown in Study 2); Figure 3.2 shows the declining trend of

both a-amylase 1 and 2 up to the 4th week of the protocol, whereas the two values

appear to be exactly the same at the pre-intervention level. Then, in the 1st week of

HRVT, a-amylase 1 appears to be lower, although the time of collection was exactly the

same as in the pre-intervention session. This may be attributed to the positive

expectations participants had in view of the proposed HRVT intervention (Petrie & Rief,

2019). Even more surprisingly, Table 3.18b shows that there was a statistically

significant reduction in a-amylase 1 from the pre-intervention session to the 1st week

of intervention. So, the only way to interpret this statistically significant difference is by

attributing it to positive expectations towards the expected intervention, which are,

actually, positive cognitions about future events, in this case, the beneficial effects of

HRVT (Petrie & Rief, 2019). Such expectations are thought to be closely associated with

placebo effects; in other words, as participants have positive expectations for this specific treatment, positive effects on stress-related metrics might have occurred (Petrie & Rief, 2019). In practice, this might have been caused by the fact that although a-amylase 1 was measured, in both instances, at the same time of the day, as participants of the experimental group had been informed about the nature of the study and the intervention itself, they developed positive expectations towards the intervention which resulted in lower a-amylase levels the second time they were measured before any actual intervention taking place.

A-amylase 2, on the other hand, was much lower than a-amylase 1 in the 1st week, which indicates that there was – objectively - some direct effect from the HRVT session on it which, although it had started from the same level as a-amylase 1 in the pre-intervention session, it ended up in the 1st week at much lower levels than a-amylase 1 which indicates that, apart from the positive expectations, there might have been some actual impact of the 20-minute HRVT session. This is aligned with what is discussed above about the effects of the protocol on the SAM system but also in accordance with previous literature (e.g. Nater & Rohleder, 2009) according to which salivary a-amylase is a sensitive biomarker of stress-related fluctuations in the human body. The declining trend in the following weeks might have been a constellation of factors, that is, positive expectations, actual impact of the intervention but also some familiarity that the ANS acquired with the process so that it (i.e. the ANS of the participants) reorganized itself during the intervention in order to respond to the intervention better (e.g. Gallen & D'Esposito, 2019). Besides, as discussed earlier in this

project, HRVT has been classified as a mindfulness-type meditation strategy (Jimenez-Morgan & Molina-Mora, 2017); mindfulness practices, on the other hand, have been found to result in neuroplasticity (also discussed by Lehrer et al., 2003) which, in other words, means that such practices help the brain to adjust better, which, in turn, supports an introduced mental training strategy more effectively (Allen, Dietz, Blair, van Beek, Rees, Vestergaard-Poulsen, Lutz, & Roepstorff, 2012). Of course, it is worth mentioning that plastic changes are always related to relevance, intensity and/ or frequency of intervention (Kolb & Muhammad, 2014). These are factors that correlate to the extent to which a plastic change occurs, which means that an intervention may result in better ANS changes if the person involved perceives it as relevant; additionally, the more frequent and intense an event is (i.e. in this case HRVT) the better the outcome (Kolb & Muhammad, 2014). In other words, the observed phenomenon in Figure 3.2 (downward trend from the pre-intervention session to the 4th week of HRVT) might be an adjustment of the ANS to the introduced intervention.

However, the fact that this phenomenon is observed until the 4th week might be attributed either to that a-amylase across the different times collected (i.e. a-amylase 1, 2, 3, 4) has reached very low levels, or it might have been that, as intensity cannot be really modified in this context of heart rate variability training, the proposed frequency of five controlled and five uncontrolled weekly HRVT sessions could not sustain the downward trend for longer.

In the case of cortisol, similar conclusions may be drawn for its declining trend, as shown in Figure 3.1. However, cortisol levels, appear to increase in all four samples

from the pre-intervention session to the 1st week of HRVT and start declining after

that until the 5th week, at which point they start increasing again. Again, the same

constellation of factors may be responsible for the downward trend from the 1st to the

5th week of HRVT, although in the case of cortisol positive expectations might be less

responsible due to its delayed response (e.g, Maruyama et al., 2012). Consequently, an

adjustment of the ANS to the proposed protocol might be responsible for the trend

which is inter-related to the actual impact of it. As discussed in the previous chapters,

whereas a-amylase primarily shed light on acute effects, salivary cortisol mostly

reflected long-lasting effects due to its nature, whereas this finding adds a different

dimension to the effects of the HRVT protocol; It shows the change over time, with

HRVT effects getting stronger as time goes by, which is a very important finding and is

worth considering when drawing final conclusions about the proposed intervention.

To sum up the findings on the biochemical trends during the HRVT protocol, it

appears that a-amylase and cortisol have been affected by the proposed HRVT

protocol to the extent that a progressively developing downward trend is observed

during the course of the HRVT protocol. This is actually the first study to show this and

provides evidence on that repeated rehearsal of the HRVT exercise has beneficial

effects on the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic-adrenal-

medullary system (SAM) which are physiologic stress response systems. Additional

causes for these trends might be an ANS plasticity created due to the repeated HRVT

sessions (also discussed by Lehrer et al, 2003), as explained above, as well as positive

expectations towards the proposed intervention. Perhaps, with an increased

frequency, the downward trend of a-amylase could have continued even after the 4th week of HRVT.

5.4 Comparison Between the Proposed Protocol and Other HRVT Protocols Used in Sport

A comparison between the proposed 5-week protocol and other HRVT protocols used mainly in sport leads to the conclusion that the protocol presented in this thesis differs from any other protocol in many different respects. Therefore, athletes have been subjected to different amounts and patterns of HRVT across different studies in the sport domain and any effort to compare their results might lead to erroneous conclusions as intensity and/or frequency of intervention (as per Kolb & Muhammad, 2014) may differ.

At first, the initial protocol, as introduced by Lehrer et al. (2000), was a 10-week protocol. In that protocol, there were 10 weekly lab-based HRVT sessions, whereas the homework that the clients were asked to do consisted of two 20-minute HRVT exercises per day for the whole duration of the protocol; additionally, special HRV instrumentation was recommended for home practice (NB approximately, in this original protocol, the total number of unsupervised, home-based, HRVT exercises is estimated around one hundred twenty 20-minute HRVT exercises plus 10 lab-based sessions [considering that on the day of the lab sessions, clients do not do any homework]).

The above protocol was used in different studies, in its original form or variations of it, as discussed in previous chapters, as, for example, in the study with ballroom dancers (Raymond et al., 2005) in which the group who did HRVT participated in 10 sessions, on the one hand, as instructed by Lehrer et al. (2000), though they were not instructed to do homework as was the case in the original protocol. The volume of practice in this variation of the original protocol is much less, if we consider that the participants did not do any home-based exercises; therefore, in total, they were subjected to ten 20-minute HRVT sessions.

In the case study conducted with the golfer by Lagos et al. (2008), the athlete was subjected to the original protocol, as this was designed by Lehrer et al (2000). Therefore, the golfer was subjected to 10 weeks of weekly lab-based sessions along with the twice daily 20-minute HRVT exercises. That means that again the athlete conducted a total of one hundred twenty 20-minute HRVT exercises in addition to the lab-based sessions he was asked to attend. It is worth adding that the participant used a special HRVT device for his home-based sessions. Likewise, in the case study conducted by Lagos et al. (2011), another golfer was subjected to the same amount of HRVT lab-based sessions as well as home-based exercises whereas for the latter the same HRV device was used (i.e. portable Stress Eraser device). The original protocol by Lehrer et al. (2000) was used in the study conducted with the athletics track athletes as well (Choudhary et al., 2016), who were exposed to the same amount of HRVT as the above-mentioned golfers and were also given a special HRV device so that they could practice HRVT more efficiently at home.

As discussed earlier, there have also been variations of the existing protocol, which is the variation used in both studies with the basketball players (Paul & Garg, 2011; Paul et al., 2011), in which the players had to go through daily supervised (i.e. lab-based) 20-minute HRVT sessions for 10 consecutive days. Therefore, in those studies, in total, the players were subjected to 10 HRVT sessions. Last but not least, in the study with the volleyball players (Tanis,2012), another approach was used, and the players attended six weekly HRVT sessions in addition to some HRV exercise they did alone with the Heart Math's emWave in order to learn how to self-regulate with or without the device, though it has not been specified how often they did this (NB the PC-based emWave was used for the weekly sessions, whereas the portable version of it was used for the individual self-regulation exercises).

The initiators of the original protocol (Lehrer et al., 2000) reconsidered the length of time needed for teaching HRVT and suggested, later on, a shortened 5-week version of it in which clients are subjected to five weekly lab-based sessions, in addition to home-based (unsupervised) 20-minute HRVT exercises using a portable HRV device, which should take place twice a day for the whole duration of the protocol. So, considering that on the day of the lab-based session, a client does not do any homework, in this shortened version of protocol, an individual is subjected to 60 sessions of HRVT exercise in addition to the lab sessions.

There have been other protocols used for HRVT in different domains of life (e.g. Gross et al., 2016); the protocols mentioned above present the variability of the different approaches used for HRVT which, as discussed above, result in considerable

differences in the amount of time spent on HRVT; as already shown this may vary from ten 20-minute sessions in total, to 130 sessions of HRVT. The protocol suggested in Study 2 of the present thesis consists of, totally, five weekly lab-based sessions (controlled sessions) plus five weekly home-based (unsupervised/uncontrolled sessions) in addition to one extra session dedicated to the estimation of a client's resonant breathing frequency; so, in total the participants were subjected to ten 20-minute HRVT sessions which took place over a period of five weeks, whereas the home-based sessions were conducted without the help of a portable device but with the help of a simple smartphone-based breathing pacer configured at the client's RBF.

To sum up, different protocols result in different effect sizes of statistically significant results or in non-significant results, as the different amounts of time spent to learn HRVT as well as the different frequencies by means of which HRVT is practiced may result in a different impact (which may be from very small to really worth considering). Considering the above-mentioned protocols, the introduced HRVT protocol in Study 2, is closer to the lower end in terms of time.

5.5 Practical Implications

The ultimate objective of this research project is, as it has been designed within the area of applied sport psychology, to inform practice and contribute to the existing literature.

As such, the present thesis has introduced and researched a psychophysiological strategy that can help athletes achieve a better level of emotional

control or, in more practical terms, better stress and anger management when they are under pressure, which, eventually, results in better performance (e.g. Landers, 2007). This psychophysiological strategy, which is HRVT at one's resonant breathing frequency, has been found to have acute but also long-lasting effects. However, as already discussed, acute effects have been found only immediately after a 20-minute HRV training session and not 45 minutes or 90 minutes later. Additionally, Study 1 investigated the existence of acute effects on performance under pressure, by means of a reactive stress tolerance test which commenced approximately 9 to 12 minutes after the completion of an HRVT session and ended 15 to 18 minutes after the HRVT session (since the test lasted for 6 minutes). Considering, at the same time, that the instructions/practice phase, in the reactive stress tolerance test, lasts for almost 2 minutes, the actual testing phase commenced 11 to 14 minutes after the completion of the HRVT session, in Study 1. Therefore, it is safe to conclude that there were no acute effects from the 11th minute after the end of the session onwards.

Although Study 1 and Study 2 have explored acute effects in different ways, with the former focusing on possible effects on performance under pressure, and the latter on effects on salivary a-amylase, one could conclude that should an athlete include HRVT in their pre-competition routine, acute effects should be expected either immediately after rehearsing HRVT or within 11 minutes after this. Of course, one should always bear in mind that the two studies measured acute effects on different constructs and such a conclusion may not be accurate. Therefore, the present thesis can guarantee acute effects only immediately after HRVT and not after that.

Consequently, including such a strategy in the routine prior to competition

would be beneficial because of these acute effects that may result in a good start of

the competition and will possibly reinforce the progressively reduced sympathetic

activity caused by the regular rehearsal of the HRVT exercise suggesting also the

development of an ANS plasticity mentioned earlier (as also discussed by Lehrer et al.,

2003) and is a unique finding of Study 2. It would be also interesting to consider how

the above-mentioned acute effects relate to sport events that have short duration as,

for example, athletics jumping and throwing events. In this context, an athlete can

rehearse 20 minutes HRVT prior to the first attempt and then practice it again between

the attempts just for a couple of/a few minutes, if possible. Other events of short

duration are sprint events in athletics and swimming, archery, golf, weightlifting, some

canoe sprint events, some disciplines of shooting (e.g. trap events) and, generally,

sports where the actual performance of an athlete is completed within seconds or –

maximum - a couple of minutes. However, all sports could be included in this list, as

athletes can always benefit from the acute effects to ensure a good start in other

sports as well, regardless of the duration of the event. Again, the fact that lower

sympathetic activity can last for a few seconds/a few minutes is undoubtedly

something that athletes of short - in terms of duration - events should always take into

consideration in conjunction with the progressively reducing sympathetic activity

caused by regular (i.e. at least twice per week as shown in Study 2) repetitions of HRVT

exercises, which is the greatest advancement in this thesis that considerably informs

practice. A combination of these two beneficial outcomes may result in a good start in

the competition guaranteed by the acute effects and, also, better emotional control due to the lasting biochemical trends.

The suggested HRVT protocol consisted of two 20-minute exercises per week which resulted in some long-lasting effects. In terms of practical implications of those long-lasting effects, it appears that rehearsing the 20-minute HRVT exercise regularly has a long-lasting effect on certain psychophysiological variables such as skin conductance but also cortisol levels. This builds on previous literature, according to which cortisol is inversely related to HRV (Sgoifo et al., 2003; Stalder et al., 2011), whereas researchers maintain that stress-related psychophysiological and biochemical variables are related with arousal which is highly related to performance (e.g. Landers, 2007). This indicates that regular practice of HRVT exercises can have a strong impact on biochemical markers of stress but also on skin conductance. It is, therefore, suggested that athletes get introduced into the HRVT protocol in order for them to learn more about their own resonant breathing frequency, rehearse it with the help of the practitioner and biofeedback to check if this is - indeed - the frequency that their baroreflex system resonates, and continue rehearsing HRVT regularly even after the protocol with a view to causing a lasting change in stress-related parameters and, as a result of this, in their performance.

So, the progressively reducing sympathetic activity caused by regular repetitions of HRVT exercises ideally suggests that athletes (but also performers in general) should include HRVT in their weekly programme with a frequency of - at least - two 20-minute HRVT exercises per week using an HRV portable device, whereas they

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should also plan their pre-competition routine in such a way that HRVT should be closer to the end of it, if possible, in order to gain a maximal advantage of its acute effects on stress-related parameters.

Considering the findings of Study 2 and Study 3 together, the latter showed that partcipants started feeling at ease while practicing HRVT sometime within the 5-week protocol, with the higher number of participants having said that starting from week 3 of the protocol they felt at ease; the former showed that plasticity was actually present in the case of cortisol starting from week 3 onwards, as week 1 was higher than the pre-intervention session whereas cortisol in week 2 was reduced, which was actually the first indication of a remarkable effect. After that, cortisol in week 3 was much lower and so on, which indicates that it was from week 3 onwards that plasticity started developing in the case of cortisol. Though plasticity was obvious in a-amylase as well, one cannot tell at which point it started developing, as in the case of cortisol, since the downward trend in a-amylase started right from the beginning of the protocol. If we add the author's personal experience as well, the findings from Study 2 and 3 appear to be accurate as, in practice, when an athlete is halfway through the 5-week protocol (i.e. in the 3rd week of the HRVT protocol), they seem to be much more efficient in rehearsing slow breathing at their resonant breathing frequency and, also, it is- usually- at that time onwards that they (i.e. the athletes) start discussing any positive effects that they themselves experience. Additionally, to achieve a state of alert relaxation and minimize the possibility of reaching a state of sleepy relaxation, one should rehearse HRVT in a well-illuminated room (as opposed to a dimly-lit room),

as discussed in Study 3. Last, to avoid any interruptions/ disruptions during the practice of HRVT, an athlete should practice HRVT in a quiet room/ corner depending on the nature of their sport and competition venue, on competition days.

5.6 Further Recommendations

The suggested 5-week protocol includes a lot of interaction between the practitioner and the individual. Apart from anything else, the 5-week period of the protocol is the actual familiarization period of the client with HRVT, and any issues related to that should be resolved within those lab-based sessions. The two most important components of this familiarization period are (a) the identification of the client's resonant breathing frequency and (b) the customization/ configuration of the inhalation-exhalation ratio to ensure that the client feels comfortable with their own RBF.

Study 3 shed light on different aspects of the HRVT protocol; the main lesson learnt in Study 3 was that an athlete should not continue in the actual protocol unless they are sure that they are entirely comfortable with the estimated resonant frequency. In that study, there were few participants that reported that they had difficulty with their RBF. Although the RBF had been estimated both by means of quantitative analysis but also qualitative investigation, it seems that for a very small percentage of participants in Study 3, that was not enough. As the experience of a client may not be optimal while breathing at their resonant breathing frequency, it is of paramount importance that the practitioner ensure that the client feels comfortable

with the pace and practices HRVT being able to follow easily the instructions given to them during this familiarization period (i.e. they are able to breathe at their RBF without taking deep breaths, they inhale from the nose and exhale through pursed lips and can sustain this for 20 consecutive minutes without any issue). The RBF of the majority of the participants in Study 2 was estimated within Session 2, with some RBFs being finalized at the beginning of Session 3. However, the time dedicated to estimating RBF did not seem to be adequate for a small percentage of participants which became more obvious later on, when they were deviating from their RBF more than others during the HRVT sessions.

Heart rate variability training cannot be a pleasant experience unless the RBF has been estimated carefully and both parties (i.e. practitioner and client) are sure that this is the slow breathing frequency that maximizes the measured indices (e.g. LF power, peak-valley difference [HRV Max-Min], phase angle of HR vs Respiration patter [Lehrer et al., 2013]) and, beyond anything else, the client's comfort is at a very good level. As such, the assessment of RBF should take as long as it is needed before proceeding to the actual HRVT sessions. Besides, breathing slowly, but not on one's RBF, may result in continuous deep breathing as also pointed out in Study 3, which in turn, will result in hyperventilation and, then, dizziness (e.g. Lehrer et al., 2000).

Deep breathing and hyperventilation may be also caused, as discussed extensively in Study 3, by an unsuitable inhalation-exhalation time ratio. In other words, a very quick inhalation matched with a very long exhalation may result in a very deep inhalation as reported in Study 3, in order for the long exhalation to be

supported. Considering that this pattern continues for 20 consecutive minutes,

lightheadedness and dizziness may occur due to hyperventilation and discomfort. For

this reason, attention should be paid to the inhalation - exhalation time ratio and

ensure that the client feels comfortable with it, though, it is important to note that the

ratio should be customized in a way that inhalation should be always kept shorter than

the exhalation (Strauss-Blasche et al., 2000).

Furthermore, the results of Study 2 showed that some important

psychophysiological parameters were found to differ significantly in the post-

intervention session between the control and experimental groups, but this statistical

difference was not practically meaningful as the effect size was borderline lower than

what is considered as a low effect size (i.e. 0.1). Such parameters were RMSSD (η_p^2 =

0.09), LF (η_p^2 = 0.07) and total EMG activity (η_p^2 = 0.09). Given that RMSSD and LF are

HRV-related variables and most appropriate to use in this type of HRV recording

(Shaffer & Ginsberg, 2017), the fact that their effect sizes were borderline lower than

0.1 might have been due to the frequency of the planned HRV sessions (lab-based and

home-based). In other words, the frequency of two 20-minute HRVT session per week

has resulted in those figures which might become better and more meaningful if this

frequency is increased, though this should be researched further.

Additionally, Study 2 instructed the participants to do their homework using a

smartphone application as a breath pacer, which had been configured to their own

resonant breathing frequency. There might be clients, however, who would like to

know more about their HRV while practicing HRVT; for such purposes, a portable HRV

device would be advisable, as there are really practical, valid and reliable devices in the market that could be carried easily wherever an athlete (or a performer in general) would like to use it. As such, something like that could be useful, should someone wish to have more information about their HRV levels in real time.

5.7 Future Research

Future research should explore the frequency by means of which an athlete should rehearse HRVT after the completion of the 5-week protocol in order for the beneficial effects to be always present and the ANS plasticity to be retained at a desirable level. In other words, how often the athletes need to practice 20-minute HRVT exercises in order for the decline in the biochemical markers to be present and, generally, the stress-related psychophysiological indices to be within acceptable limits.

Another dimension, mentioned in Study 2, that would add value to findings of the present thesis would be the investigation of a 'threshold' frequency of HRVT sessions above which key psychophysiological variables (e.g. HRV parameters, electrical activity on the muscles, EDA) are significantly affected by HRVT. Different durations of a single session could be tested (e.g. 15-minute or 10-minute HRVT sessions) and could be explored always in relation with the frequency of repetition.

Last but not least, generalizing from the findings of the present thesis should be done with caution as there might be sampling bias due to the particularity of the cohort used in all three studies. Future research should try to replicate the research design in other cultural contexts as well, with a view to building on the present

research studies and providing evidence that these results are replicable in other contexts as well.

5.8 Final Conclusions

In conclusion, heart rate variability training was researched extensively in these three studies. Acute effects were investigated both within a framework of performance under pressure in a pre-and post- intervention design of randomized control trials with a crossover but also by means of measuring salivary a-amylase, a biochemical marker of stress which reflects immediate changes in sympathetic activity. Acute effects were shown to be statistically significant, with the a-amylase levels detected immediately after a 20-minute HRVT session being significantly lower than the levels detected before that. Salivary a-amylase was not found to be lower in the samples collected 45 and 90 minutes after the end of a HRVT session, whereas study 1 did not yield statistically significant results as well, which may be attributed either to the nature of the construct monitored in it (i.e. reactive stress tolerance), or to the time elapsing between the end of the single HRVT session until the actual measurement of the construct.

Furthermore, Study 2 looked into whether a 5-week protocol consisting of five weekly lab-based (controlled) and five weekly home-based (uncontrolled) 20-minute HRVT sessions had long-lasting effects on psychophysiological and biochemical markers of stress and the results showed that, indeed, there were long-lasting effects on cortisol and skin conductance levels detected in the post-intervention session which was planned after a two-week wash-out period after the last HRVT exercise.

Additionally, an interesting downward trend of the two monitored biochemical markers of stress was detected which could be attributed to positive expectations towards the proposed intervention as well as the actual impact of it, which is also correlated with some ANS plasticity created due to the repeated HRVT sessions. This is a pioneering finding of the present thesis that contributes substantially to existing literature, as it suggests that regular practice of HRVT results in lasting reduced sympathetic activity.

Study 3 also pointed out other interesting aspects of HRVT and showed that the customization/ configuration of the inhalation- exhalation default time ratio within the estimated resonant breathing frequency is substantial and is highly related to the positive experience of a client.

The 5-week training protocol itself does not appear to bring about a relatively permanent change in the individual; it is about teaching the individual a strategy they can use as and when they want before a psychologically important competition. Any more long-lasting or even relatively permanent change may appear as long as HRVT is practiced at least twice a week, though this should be further researched with a more appropriate design. What this research project suggests is that athletes of self-paced sports who complete their performance within seconds or a couple of minutes may be benefited to a maximum possible extent from this strategy due to the acute effects, found to be statistically significant immediately after an HRVT exercise, which can be included in their routine prior to a competition. Additionally, other long-lasting effects (i.e. lower cortisol and skin conductance levels) and the downward trend of cortisol

and a-amylase indicate that HRVT can help individuals control their sympathetic activity, thus, achieving a better level of emotional control, by regularly practicing HRVT.

Heart rate variability training is an upcoming psychophysiological strategy used in different contexts and was thoroughly researched and discussed in this thesis. Evidence from the thesis suggests that it is a slow-breathing exercise that should be included in one's weekly routine with a view to maintaining emotional control. HRV training changes the physiology of athletes in such a way that psycho(bio)physiological parameters move towards a positive direction which reflects a better level of stress management and composure.

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APPENDIX A.1

Step-by Step Presentation of the Procedure Followed in Study 1 for Both Groups

Randomly assigned 18 participants	**1E:** Stress Tolerance Test + Resonant Breathing Frequency Assessment (RBF)	**2E:** Intervention: HRV training + Stress Tolerance Test (immediately after the intervention)	Wash-out period (2 weeks) leading to **crossover**	**3E:** General Discussion about Sport Psychological Services + Stress Tolerance Test (immediately after the discussion)
		Two weeks break for both groups		
Randomly assigned 18 participants	**1C:** Stress Tolerance Test	**2C:** General Discussion about Sport Psychological Services + Stress Tolerance Test+ RBF		**3C:** Internention: HRV training + Stress Tolerance Test (immediately after the intervention)

Experimental Condition
Control Condition

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APPENDIX A.2

Results tables of Study 1

Table 2.2

Investigation of Differences Between the Experimental and Control Conditions on the RCR, While Controlling for Baseline RCR

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
RCR	.00015	1	.00015	.35	.56	.01
RCR*baseline RCR	.000046	1	.000046	.11	.75	.003
Error (RCR)	.015	34	.00043			

Table 2.3

Investigation of Differences Between the Experimental and Control Conditions on the RWR, While Controlling for Baseline RWR

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
RWR	.000057	1	.000057	.39	.85	.001
RWR*baseline RWR	.004	1	.004	2.94	.095	.08
Error (RWR)	.05	34	.001			

Table 2.4

Investigation of Differences Between the Experimental and Control Conditions on the ZV, While Controlling for Baseline ZV

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
ZV	11.03	1	11.03	0.16	.90	.00
ZV*baseline ZV	26.50	1	.0	0.04	.85	.01
Error (ZV)	23960.61	34	704.72			

Table 2.5

Investigation of Differences Between the Experimental and Control Conditions on the
F, While Controlling for Baseline F

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
F	122.59	1	122.59	0.85	.36	.02
F*baseline F	47.73	1	47.73	0.33	.57	.01
Error (F)	4896.71	34	144.02			

Table 2.6

Investigation of Differences Between the Experimental and Control Conditions on the A, While Controlling for Baseline A

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
A	2.31	1	2.31	0.12	.74	.003
A*baseline A	52.88	1	52.88	2.67	.11	.073
Error (A)	674.23	34	19.83			

Table 2.7

Investigation of Differences Between the Experimental and Control Conditions on the MDRT, While Controlling for Baseline MDRT

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
MDRT	0.004	1	0.004	0.73	.40	.02
MDRT*baseline MDRT	0.001	1	0.001	0.20	.65	.006
Error (MDRT)	0.195	34	0.06			

Table 2.8

Investigation of Differences Between the Experimental and Control Conditions on the
S, While Controlling for Baseline S

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
S	61.88	1	61.88	0.07	.79	.002
S*baseline S	220.08	1	220.08	0.25	.62	.007
Error (S)	29357.73	34	863.46			

APPENDIX B.1

Table Summarizing the Variables Measured in Study 2

	Pre -Intervention	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	Post- Intervention
Experimental	A) Stress profile (psychophysiological variables: EMG, EDA, TEMP, HR, RESP) B) 4 samples X 2 biochemical variables C) HRV (X 5 variables)	A) 4 salivary samples (X2)* B) HRVrelated variables	A) 4 salivary samples (X2)* B) HRVrelated variables	A) 4 salivary samples (X2)* B) HRVrelated variables	A) 4 salivary samples (X2)* B) HRVrelated variables	A) 4 salivary samples (X2)* B) HRVrelated variables	A) Stress profile (psychophysiological variables:EMG, EDA, TEMP, HR, RESP) B) 4 samples X 2 biochemical variables C) HRV (X 5 variables)
Control	A) Stress profile (psychophysiological variables: EMG, EDA, TEMP, HR, RESP) B) 2 samples X 2 biochemical variables C) HRV (X 5 variables)			15 of the control participants gave 2 salivary samples (X 2 biochemical variables)			A) Stress profile (psychophysiological variables:EMG, EDA, TEMP, HR, RESP) B) 2 samples X 2 biochemical variables C) HRV (X 5 variables)

*(X2) refers to the two researched biochemical variables: 1) cortisol, 2) a-amylase

Statistical tables in Study 2

Table 3.2

Demographic Information of the Participants of the Study

Variable		(%)
Gender	Male	100.0
	Female	0.0
Sport	Athletics	21.1
	Football	19.3
	Shooting	14.0
	Table Tennis	12.3
	Fencing	8.8
	Football Referees	8.8
	Gymnastics	7.0
	Squash	5.3
	Golf	3.5
Age	14	10.5
	15	21.1
	16	54.4
	17	7.0
	18	7.0

Table 3.4

Investigation of Differences Between the Experimental and Control Conditions on

the SDNN Measured in the Post-Intervention Session, While Controlling for SDNN

Measured in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	6347.518a	2	3173.76	6.35	0.00	0.20
Intercept	8786.02	1	8786.02	17.59	0.00	0.25
PRE_SDNN_EO	5903.83	1	5903.83	11.82	0.00	0.19
Group	822.24	1	822.24	1.65	0.21	0.03
Error	25970.65	52	499.44			
Total	338533.43	55				
Corrected Total	32318.17	54				

Table 3.5

Investigation of Differences Between the Experimental and Control Conditions on

the pNN50 Measured in the Post-Intervention Session, While Controlling for pNN50

Measured in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	0.04	2	0.02	2.14	0.13	0.08
Intercept	0.24	1	0.24	26.18	0.00	0.33
PRE_pNN50_50_EO	0.03	1	0.03	3.72	0.06	0.07
Group	0.00	1	0.00	0.37	0.55	0.01
Error	0.48	52	0.01			
Total	2.62	55				
Corrected Total	0.52	54				

Table 3.6

Investigation of Differences Between the Experimental and Control Conditions on

the RMSSD Measured in the Post-Intervention Session, While Controlling for RMSSD

Measured in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	13815.937a	2	6907.97	12.00	0.00	0.32
Intercept	7477.99	1	7477.99	12.99	0.00	0.20
PRE_RMSSD_EO	11482.83	1	11482.83	19.95	0.00	0.28
Group	2855.82	1	2855.82	4.96	0.03	0.09
Error	29932.65	52	575.63			
Total	323216.37	55				
Corrected Total	43748.59	54				

Table 3.7

Investigation of Differences Between the Experimental and Control Conditions on

the LF Measured in the Post-Intervention Session, While Controlling for LF Measured

in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	1730071.62	2	865035.81	2.62	0.08	0.09
Intercept	5443287.67	1	5443287.67	16.52	0.00	0.24
PRE_LF_EO	514939.95	1	514939.95	1.56	0.22	0.03
Group	1334300.23	1	1334300.23	4.05	0.05	0.07
Error	16806474.83	51	329538.72			
Total	40777211.59	54				
Corrected Total	18536546.45	53				

Table 3.8

Investigation of Differences Between the Experimental and Control Conditions on

the LF/HF Measured in the Post-Intervention Session, While Controlling for LF/HF

Measured in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	3.61	2	1.81	1.41	0.25	0.05
Intercept	54.23	1	54.23	42.37	0.00	0.45
PRE_LFratHF	0.10	1	0.10	0.07	0.79	0.00
Group	3.60	1	3.60	2.81	0.10	0.05
Error	65.29	51	1.28			
Total	163.70	54				
Corrected Total	68.90	53				

Table 3.9

Investigation of Differences Between the Experimental and Control Conditions on the A-amylase Detected in the First Sample (A-amylase 1), While Controlling for A-amylase 1 in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	128823.11	2	64411.55	23.53	0.00	0.48
Intercept	13222.10	1	13222.10	4.83	0.03	0.09
PRE_Aamylase_1	124701.66	1	124701.66	45.56	0.00	0.48
Group	2715.15	1	2715.15	0.99	0.32	0.02
Error	136860.56	50	2737.21			
Total	644967.36	53				
Corrected Total	265683.67	52				

Table 3.10

Investigation of Differences Between the Experimental and Control Conditions on

the A-amylase Detected in the Second Sample (A-amylase 2), While Controlling for

A-amylase 2 in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	46058.081[a]	2	23029.04	12.35	0.00	0.33
Intercept	30897.24	1	30897.24	16.56	0.00	0.25
PRE_Aamylase_2	43234.76	1	43234.76	23.18	0.00	0.32
Group	1130.89	1	1130.89	0.61	0.44	0.01
Error	93263.69	50	1865.27			
Total	441354.42	53				
Corrected Total	139321.77	52				

Table 3.11

Investigation of Differences Between the Experimental and Control Conditions on

the Cortisol Detected in the First Sample (Cortisol 1), While Controlling for Cortisol 1

in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	0.74	2	0.37	8.96	0.00	0.26
Intercept	0.50	1	0.50	12.13	0.00	0.20
PRE_Cortisol_1	0.67	1	0.67	16.21	0.00	0.24
Group	0.00	1	0.00	0.00003	1.00	0.00
Error	2.08	50	0.04			
Total	8.31	53				
Corrected Total	2.82	52				

Table 3.12

Investigation of Differences Between the Experimental and Control Conditions on

the Cortisol Detected in the Second Sample (Cortisol 2), While Controlling for

Cortisol 2 in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	0.44	2	0.221	10.37	.000	0.30
Intercept	0.22	1	0.224	10.50	.002	0.18
PRE_Cortisol_2	0.11	1	0.113	5.31	.025	0.10
Group	0.16	1	0.161	7.57	.008	0.13
Error	1.04	49	0.021			
Total	3.54	52				
Corrected Total	1.49	51				

Table 3.13a

Investigation of Acute Effects of HRVT on Different-Time Collection of Salivary A-

amylase

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
A-amylase	29586.58	1.854	15959.87	64.98	.0001	.691
Error	13204.14	53.76	245.61			

Table 3.13b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among Averaged A-

amylase Detected at Different Times Across the 5 Weeks of HRVT

	Paired Diff		t	df	Sig .
	Mean	SD			
Pair 1 A-amylase 1- A-amylase 2	8.22	14.12	3.19	29	.003
Pair 2 A-amylase 1- A-amylase 3	-23.13	16.83	-7.53	29	.000
Pair 3 A-amylase 1- A-amylase 4	-29.67	19.29	-8.43	29	.000
Pair 4 A-amylase 2- A-amylase 3	-31.35	18.55	-9.26	29	.000
Pair 5 A-amylase 2- A-amylase 4	-37.89	22.71	-9.14	29	.000
Pair 6 A-amylase 3- A-amylase 4	-6.54	10.34	-3.46	29	.002

Table 3.14a

Investigation of Effects of HRVT on Salivary Cortisol 1 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Cortisol 1	0.90	4.02	0.22	7.32	.0001	0.20
Error	3.55	116.53	0.03			

Table 3.14b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among Cortisol 1

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	$Cortisol_{pre-int1}$ -$Cortisol_{1stweek1}$	-0.11	0.27	-2.21	29	.035
Pair 2	$Cortisol_{pre-int1}$ -$Cortisol_{2ndweek1}$	-0.07	0.27	-1.47	29	.150
Pair 3	$Cortisol_{pre-int1}$ -$Cortisol_{3rdweek1}$	0.07	0.16	2.40	29	.023
Pair 4	$Cortisol_{pre-int1}$ -$Cortisol_{4thweek1}$	0.04	0.20	1.05	29	.303
Pair 5	$Cortisol_{pre-int1}$ -$Cortisol_{5thweek1}$	0.08	0.17	2.45	29	.021
Pair 6	$Cortisol_{pre-int1}$ -$Cortisol_{post-int1}$	-0.03	0.20	-0.74	29	.468
Pair 7	$Cortisol_{1stweek1}$ -$Cortisol_{2ndweek1}$	0.04	0.26	0.78	29	.440
Pair 8	$Cortisol_{1stweek1}$ -$Cortisol_{3rdweek1}$	0.18	0.23	4.27	29	.000
Pair 9	$Cortisol_{1stweek1}$ -$Cortisol_{4thweek1}$	0.15	0.21	3.92	29	.000
Pair 10	$Cortisol_{1stweek1}$ -$Cortisol_{5thweek1}$	0.19	0.24	4.32	29	.000
Pair 11	$Cortisol_{1stweek1}$ -$Cortisol_{post-int1}$	0.08	0.21	2.19	29	.037
Pair 12	$Cortisol_{2ndweek1}$ -$Cortisol_{3rdweek1}$	0.14	0.25	3.13	29	.004
Pair 13	$Cortisol_{2ndweek1}$ -$Cortisol_{4thweek1}$	0.11	0.22	2.72	29	.011
Pair 14	$Cortisol_{2ndweek1}$ -$Cortisol_{5thweek1}$	0.15	0.19	4.29	29	.000
Pair 15	$Cortisol_{2ndweek1}$ -$Cortisol_{post-int1}$	0.05	0.20	1.23	29	.228
Pair 16	$Cortisol_{3rdweek1}$ -$Cortisol_{4thweek1}$	-0.03	0.14	-1.22	29	.231
Pair 17	$Cortisol_{3rdweek1}$ -$Cortisol_{5thweek1}$	0.01	0.14	0.34	29	.740
Pair 18	$Cortisol_{3rdweek1}$ -$Cortisol_{post-int1}$	-0.09	0.17	-2.98	29	.006
Pair 19	$Cortisol_{4thweek1}$ -$Cortisol_{5thweek1}$	0.04	0.14	1.61	29	.119
Pair 20	$Cortisol_{4thweek1}$ -$Cortisol_{post-int1}$	-0.06	0.14	-2.57	29	.016
Pair 21	$Cortisol_{5thweek1}$ -$Cortisol_{post-int1}$	-0.10	0.15	-3.68	29	.001

Table 3.15a

Investigation of Effects of HRVT on Salivary Cortisol 2 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Cortisol 2	1.25	2.83	0.44	12.67	.0001	0.30
Error	2.86	81.98	0.04			

Table 3.15b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among Cortisol 2

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{1stweek2}$	-0.12	0.21	-3.16	29	.004
Pair 2	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{2ndweek2}$	-0.13	0.25	-2.80	29	.009
Pair 3	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{3rdweek2}$	0.03	0.12	1.31	29	.201
Pair 4	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{4thweek2}$	0.02	0.11	1.17	29	.251
Pair 5	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{5thweek2}$	0.08	0.10	4.41	29	.000
Pair 6	$Cortisol_{pre\text{-}int2}$ - $Cortisol_{post\text{-}int2}$	0.06	0.10	3.25	29	.003
Pair 7	$Cortisol_{1stweek2}$ - $Cortisol_{2ndweek2}$	-0.01	0.24	-0.22	29	.831
Pair 8	$Cortisol_{1stweek2}$ - $Cortisol_{3rdweek2}$	0.15	0.19	4.32	29	.000
Pair 9	$Cortisol_{1stweek2}$ - $Cortisol_{4thweek2}$	0.14	0.19	4.12	29	.000
Pair 10	$Cortisol_{1stweek2}$ - $Cortisol_{5thweek2}$	0.20	0.17	6.24	29	.000
Pair 11	$Cortisol_{1stweek2}$ - $Cortisol_{post\text{-}int2}$	0.18	0.23	4.24	29	.000
Pair 12	$Cortisol_{2ndweek2}$ - $Cortisol_{3rdweek2}$	0.16	0.25	3.45	29	.002
Pair 13	$Cortisol_{2ndweek2}$ - $Cortisol_{4thweek2}$	0.15	0.24	3.51	29	.001
Pair 14	$Cortisol_{2ndweek2}$ - $Cortisol_{5thweek2}$	0.21	0.24	4.61	29	.000
Pair 15	$Cortisol_{2ndweek2}$ - $Cortisol_{post\text{-}int2}$	0.19	0.26	3.94	29	.000
Pair 16	$Cortisol_{3rdweek2}$ - $Cortisol_{4thweek2}$	-0.01	0.12	-0.24	29	.813
Pair 17	$Cortisol_{3rdweek2}$ - $Cortisol_{5thweek2}$	0.05	0.11	2.37	29	.025
Pair 18	$Cortisol_{3rdweek2}$ - $Cortisol_{post\text{-}int2}$	0.03	0.11	1.69	29	.102
Pair 19	$Cortisol_{4thweek2}$ - $Cortisol_{5thweek2}$	0.05	0.07	4.13	29	.000
Pair 20	$Cortisol_{4thweek2}$ - $Cortisol_{post\text{-}int2}$	0.04	0.13	1.62	29	.116
Pair 21	$Cortisol_{5thweek2}$ - $Cortisol_{post\text{-}int2}$	-0.01	0.11	-0.74	29	.466

Table 3.16a

Investigation of Effects of HRVT on Salivary Cortisol 3 across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Cortisol 3	0.55	3.44	0.16	19.81	.0001	.41
Error	0.80	99.76	0.01			

Table 3.16b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among Cortisol 3 Detected

in Salivary Samples of the Experimental Group Across the 7 Weeks of the Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	$Cortisol_{pre-int3}$ - $Cortisol_{1stweek3}$	-0.06	0.12	-2.51	29	.018
Pair 2	$Cortisol_{pre-int3}$ - $Cortisol_{2ndweek3}$	0.01	0.08	1.01	29	.320
Pair 3	$Cortisol_{pre-int3}$ - $Cortisol_{3rdweek3}$	0.07	0.09	4.16	29	.000
Pair 4	$Cortisol_{pre-int3}$ - $Cortisol_{4thweek3}$	0.08	0.08	5.47	29	.000
Pair 5	$Cortisol_{pre-int3}$ - $Cortisol_{5thweek3}$	0.10	0.08	7.26	29	.000
Pair 6	$Cortisol_{pre-int3}$ - $Cortisol_{post-int3}$	0.01	0.12	0.40	29	.694
Pair 7	$Cortisol_{1stweek3}$ - $Cortisol_{2ndweek3}$	0.07	0.12	3.19	29	.003
Pair 8	$Cortisol_{1stweek3}$ - $Cortisol_{3rdweek3}$	0.12	0.13	5.31	29	.000
Pair 9	$Cortisol_{1stweek3}$ - $Cortisol_{4thweek3}$	0.14	0.11	6.95	29	.000
Pair 10	$Cortisol_{1stweek3}$ - $Cortisol_{5thweek3}$	0.16	0.11	8.25	29	.000
Pair 11	$Cortisol_{1stweek3}$ - $Cortisol_{post-int3}$	0.06	0.15	2.35	29	.026
Pair 12	$Cortisol_{2ndweek3}$ - $Cortisol_{3rdweek3}$	0.05	0.07	3.96	29	.000
Pair 13	$Cortisol_{2ndweek3}$ - $Cortisol_{4thweek3}$	0.06	0.07	4.90	29	.000
Pair 14	$Cortisol_{2ndweek3}$ - $Cortisol_{5thweek3}$	0.09	0.08	6.00	29	.000
Pair 15	$Cortisol_{2ndweek3}$ - $Cortisol_{post-int3}$	-0.01	0.09	-0.39	29	.696
Pair 16	$Cortisol_{3rdweek3}$ - $Cortisol_{4thweek3}$	0.01	0.05	1.30	29	.205
Pair 17	$Cortisol_{3rdweek3}$ - $Cortisol_{5thweek3}$	0.03	0.05	3.69	29	.001
Pair 18	$Cortisol_{3rdweek3}$ - $Cortisol_{post-int3}$	-0.06	0.10	-3.24	29	.003
Pair 19	$Cortisol_{4thweek3}$ - $Cortisol_{5thweek3}$	0.02	0.03	4.12	29	.000
Pair 20	$Cortisol_{4thweek3}$ - $Cortisol_{post-int3}$	-0.07	0.09	-4.18	29	.000
Pair 21	$Cortisol_{5thweek3}$ - $Cortisol_{post-int3}$	-0.09	0.10	-5.36	29	.000

Table 3.17a

Investigation of Effects of HRVT on Salivary Cortisol 4 Across the7 Weeks of the Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Cortisol 4	0.45	1	0.45	4.69	.039	0.14
Error	2.81	29	0.10			

Table 3.17b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among Cortisol 4 Detected

in Salivary Samples of the Experimental Group Across the 7 Weeks of the Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	$Cortisol_{pre-int4} - Cortisol_{1stweek4}$	-0.03	0.03	-0.56	29	.582
Pair 2	$Cortisol_{pre-int4} - Cortisol_{2ndweek4}$	0.06	0.10	3.55	29	.001
Pair 3	$Cortisol_{pre-int4} - Cortisol_{3rdweek4}$	0.06	0.13	2.65	29	.013
Pair 4	$Cortisol_{pre-int4} - Cortisol_{4thweek4}$	0.08	0.12	3.49	29	.002
Pair 5	$Cortisol_{pre-int4} - Cortisol_{5thweek4}$	0.12	0.10	6.21	29	.000
Pair 6	$Cortisol_{pre-int4} - Cortisol_{post-int4}$	0.03	0.10	1.43	29	.162
Pair 7	$Cortisol_{1stweek4} - Cortisol_{2ndweek4}$	0.09	0.29	1.77	29	.088
Pair 8	$Cortisol_{1stweek4} - Cortisol_{3rdweek4}$	0.09	0.31	1.66	29	.108
Pair 9	$Cortisol_{1stweek4} - Cortisol_{4thweek4}$	0.11	0.30	1.99	29	.056
Pair 10	$Cortisol_{1stweek4} - Cortisol_{5thweek4}$	0.15	0.29	2.78	29	.009
Pair 11	$Cortisol_{1stweek4} - Cortisol_{post-int4}$	0.06	0.30	1.09	29	.283
Pair 12	$Cortisol_{2ndweek4} - Cortisol_{3rdweek4}$	0.01	0.09	0.01	29	.993
Pair 13	$Cortisol_{2ndweek4} - Cortisol_{4thweek4}$	0.01	0.07	1.14	29	.265
Pair 14	$Cortisol_{2ndweek4} - Cortisol_{5thweek4}$	0.05	0.04	7.78	29	.000
Pair 15	$Cortisol_{2ndweek4} - Cortisol_{post-int4}$	-0.03	0.06	-2.99	29	.006
Pair 16	$Cortisol_{3rdweek4} - Cortisol_{4thweek4}$	0.01	0.10	0.78	29	.444
Pair 17	$Cortisol_{3rdweek4} - Cortisol_{5thweek4}$	0.05	0.09	3.48	29	.002
Pair 18	$Cortisol_{3rdweek4} - Cortisol_{post-int4}$	-0.03	0.11	-1.78	29	.085
Pair 19	$Cortisol_{4thweek4} - Cortisol_{5thweek4}$	0.04	0.05	4.29	29	.000
Pair 20	$Cortisol_{4thweek4} - Cortisol_{post-int4}$	-0.05	0.10	-2.72	29	.011
Pair 21	$Cortisol_{5thweek4} - Cortisol_{post-int4}$	-0.09	0.07	-6.66	29	.000

Table 3.18a

Investigation of Effects of HRVT on Salivary A-Amylase 1 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
a-amylase 1	54042.62	3.13	17258.43	10.67	.0001	.27
Error	146953.20	90.81	1618.25			

Table 3.18b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among A-Amylase 1

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	A-amylase$_{pre-int1}$ -A-amylase$_{1stweek1}$	16.64	43.36	2.10	29	.040
Pair 2	A-amylase$_{pre-int1}$ -A-amylase$_{2ndweek1}$	22.35	32.60	3.75	29	.001
Pair 3	A-amylase$_{pre-int1}$ -A-amylase$_{3rdweek1}$	26.43	30.27	4.78	29	.000
Pair 4	A-amylase$_{pre-int1}$ -A-amylase$_{4thweek1}$	35.66	31.99	6.11	29	.000
Pair 5	A-amylase$_{pre-int1}$ -A-amylase$_{5thweek1}$	27.30	29.46	5.08	29	.000
Pair 6	A-amylase$_{pre-int1}$ -A-amylase$_{post-int1}$	-13.71	56.14	-1.34	29	.191
Pair 7	A-amylase$_{1stweek1}$-A-amylase$_{2ndweek1}$	5.71	49.08	0.64	29	.529
Pair 8	A-amylase$_{1stweek1}$-A-amylase$_{3rdweek1}$	9.79	41.60	1.29	29	.208
Pair 9	A-amylase$_{1stweek1}$-A-amylase$_{4thweek1}$	19.02	38.91	2.68	29	.012
Pair 10	A-amylase$_{1stweek1}$-A-amylase$_{5thweek1}$	10.67	43.70	1.34	29	.192
Pair 11	A-amylase$_{1stweek1}$-A-amylase$_{post-int1}$	-30.35	63.57	-2.61	29	.014
Pair 12	A-amylase$_{2ndweek1}$-A-amylase$_{3rdweek1}$	4.08	23.56	0.95	29	.351
Pair 13	A-amylase$_{2ndweek1}$-A-amylase$_{4thweek1}$	13.31	30.24	2.41	29	.022
Pair 14	A-amylase$_{2ndweek1}$-A-amylase$_{5thweek1}$	4.96	24.01	1.13	29	.268
Pair 15	A-amylase$_{2ndweek1}$-A-amylase$_{post-int1}$	-36.06	57.52	-3.43	29	.002
Pair 16	A-amylase$_{3rdweek1}$-A-amylase$_{4thweek1}$	9.23	25.09	2.02	29	.053
Pair 17	A-amylase$_{3rdweek1}$-A-amylase$_{5thweek1}$	0.87	10.69	0.45	29	.658
Pair 18	A-amylase$_{3rdweek1}$-A-amylase$_{post-int1}$	-40.14	47.61	-4.62	29	.000
Pair 19	A-amylase$_{4thweek1}$-A-amylase$_{5thweek1}$	-8.36	25.19	-1.82	29	.080
Pair 20	A-amylase$_{4thweek1}$-A-amylase$_{post-int1}$	-49.37	58.70	-4.61	29	.000
Pair 21	A-amylase$_{5thweek1}$-A-amylase$_{post-int1}$	-41.01	50.16	-4.48	29	.000

Table 3.19a

Investigation of Effects of HRVT on Salivary A-Amylase 2 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
a-amylase 2	60298.81	3.41	17662.33	17.20	.000	.37
Error	101664.48	99.01	1026.86			

Table 3.19b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among A-Amylase 2

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed
		Mean	SD			
Pair 1	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{1stweek2}$	29.21	38.91	4.11	29	.000
Pair 2	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{2ndweek2}$	32.09	32.51	5.41	29	.000
Pair 3	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{3rdweek2}$	29.89	43.73	3.74	29	.001
Pair 4	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{4thweek2}$	40.19	37.88	5.81	29	.000
Pair 5	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{5thweek2}$	33.47	33.81	5.42	29	.000
Pair 6	A-amylase$_{pre\text{-}int2}$ -A-amylase$_{post\text{-}int2}$	-7.15	44.35	-0.88	29	.385
Pair 7	A-amylase$_{1stweek2}$-A-amylase$_{2ndweek2}$	2.88	25.16	0.63	29	.535
Pair 8	A-amylase$_{1stweek2}$-A-amylase$_{3rdweek2}$	0.67	30.99	0.12	29	.906
Pair 9	A-amylase$_{1stweek2}$-A-amylase$_{4thweek2}$	10.98	20.52	2.93	29	.007
Pair 10	A-amylase$_{1stweek2}$-A-amylase$_{5thweek2}$	4.26	29.49	0.79	29	.436
Pair 11	A-amylase$_{1stweek2}$-A-amylase$_{post\text{-}int2}$	-36.36	45.72	-4.36	29	.000
Pair 12	A-amylase$_{2ndweek2}$-A-amylase$_{3rdweek2}$	-2.21	25.18	-0.48	29	.635
Pair 13	A-amylase$_{2ndweek2}$-A-amylase$_{4thweek2}$	8.09	17.60	2.52	29	.018
Pair 14	A-amylase$_{2ndweek2}$-A-amylase$_{5thweek2}$	1.37	17.76	0.42	29	.675
Pair 15	A-amylase$_{2ndweek2}$-A-amylase$_{post\text{-}int2}$	-39.24	40.38	-5.32	29	.000
Pair 16	A-amylase$_{3rdweek2}$-A-amylase$_{4thweek2}$	10.30	22.32	2.53	29	.017
Pair 17	A-amylase$_{3rdweek2}$-A-amylase$_{5thweek2}$	3.58	28.13	0.70	29	.491
Pair 18	A-amylase$_{3rdweek2}$-A-amylase$_{post\text{-}int2}$	-37.03	48.08	-4.22	29	.000
Pair 19	A-amylase$_{4thweek2}$-A-amylase$_{5thweek2}$	-6.72	19.62	-1.88	29	.071
Pair 20	A-amylase$_{4thweek2}$-A-amylase$_{post\text{-}int2}$	-47.33	45.53	-5.69	29	.000
Pair 21	A-amylase$_{5thweek2}$-A-amylase$_{post\text{-}int2}$	-40.61	39.84	-5.58	29	.000

Table 3.20a

Investigation of Effects of HRVT on Salivary A-Amylase 3 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
a-amylase 3	93522.49	3.46	27042.14	13.23	.000	.31
Error	205077.09	100.29	2044.77			

Table 3.20b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among A-Amylase 3

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	A-amylase$_{pre-int3}$ -A-amylase$_{1stweek3}$	27.53	59.70	2.53	29	.017
Pair 2	A-amylase$_{pre-int3}$ -A-amylase$_{2ndweek3}$	34.99	52.30	3.67	29	.001
Pair 3	A-amylase$_{pre-int3}$ -A-amylase$_{3rdweek3}$	35.16	47.73	4.04	29	.000
Pair 4	A-amylase$_{pre-int3}$ -A-amylase$_{4thweek3}$	56.96	52.35	5.96	29	.000
Pair 5	A-amylase$_{pre-int3}$ -A-amylase$_{5thweek3}$	50.68	37.87	7.33	29	.000
Pair 6	A-amylase$_{pre-int3}$ -A-amylase$_{post-int3}$	-1.81	56.03	-0.18	29	.861
Pair 7	A-amylase$_{1stweek3}$-A-amylase$_{2ndweek3}$	7.45	36.65	1.11	29	.274
Pair 8	A-amylase$_{1stweek3}$-A-amylase$_{3rdweek3}$	7.62	44.50	0.94	29	.356
Pair 9	A-amylase$_{1stweek3}$-A-amylase$_{4thweek3}$	29.42	59.05	2.73	29	.011
Pair 10	A-amylase$_{1stweek3}$-A-amylase$_{5thweek3}$	23.14	42.30	3.00	29	.006
Pair 11	A-amylase$_{1stweek3}$-A-amylase$_{post-int3}$	-29.34	74.72	-2.15	29	.040
Pair 12	A-amylase$_{2ndweek3}$-A-amylase$_{3rdweek3}$	0.17	36.43	0.03	29	.980
Pair 13	A-amylase$_{2ndweek3}$-A-amylase$_{4thweek3}$	21.97	35.86	3.36	29	.002
Pair 14	A-amylase$_{2ndweek3}$-A-amylase$_{5thweek3}$	15.69	32.37	2.66	29	.013
Pair 15	A-amylase$_{2ndweek3}$-A-amylase$_{post-int3}$	-36.80	58.37	-3.45	29	.002
Pair 16	A-amylase$_{3rdweek3}$-A-amylase$_{4thweek3}$	21.80	40.67	2.94	29	.006
Pair 17	A-amylase$_{3rdweek3}$-A-amylase$_{5thweek3}$	15.52	28.34	3.00	29	.006
Pair 18	A-amylase$_{3rdweek3}$-A-amylase$_{post-int3}$	-36.97	58.34	-3.48	29	.002
Pair 19	A-amylase$_{4thweek3}$-A-amylase$_{5thweek3}$	-6.28	33.92	-1.01	29	.319
Pair 20	A-amylase$_{4thweek3}$-A-amylase$_{post-int3}$	-58.76	49.27	-6.53	29	.000
Pair 21	A-amylase$_{5thweek3}$-A-amylase$_{post-int3}$	-52.48	54.05	-5.32	29	.000

Table 3.21a

Investigation of Effects of HRVT on Salivary A-Amylase 4 Across the 7 Weeks of the

Protocol

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
a-amylase 4	93469.72	3.61	25919.24	9.47	.000	.25
Error	286317.34	104.58	2737.80			

Table 3.21b

Paired-Samples t-Tests Investigating Post-Hoc Comparisons Among A-Amylase 4

Detected in Salivary Samples of the Experimental Group Across the 7 Weeks of the

Protocol

		Paired Diff		t	df	Sig . (2-tailed)
		Mean	SD			
Pair 1	A-amylase$_{pre-int4}$ -A-amylase$_{1stweek4}$	12.19	47.51	1.41	29	.171
Pair 2	A-amylase$_{pre-int4}$ -A-amylase$_{2ndweek4}$	22.49	70.76	1.74	29	.092
Pair 3	A-amylase$_{pre-int4}$ -A-amylase$_{3rdweek4}$	28.49	55.78	2.76	29	.010
Pair 4	A-amylase$_{pre-int4}$ -A-amylase$_{4thweek4}$	55.95	37.80	8.11	29	.000
Pair 5	A-amylase$_{pre-int4}$ -A-amylase$_{5thweek4}$	38.59	38.77	5.45	29	.000
Pair 6	A-amylase$_{pre-int4}$ -A-amylase$_{post-int4}$	-10.83	51.97	-1.14	29	.263
Pair 7	A-amylase$_{1stweek4}$-A-amylase$_{2ndweek4}$	10.30	75.55	0.75	29	.461
Pair 8	A-amylase$_{1stweek4}$-A-amylase$_{3rdweek4}$	15.91	61.32	1.42	29	.166
Pair 9	A-amylase$_{1stweek4}$-A-amylase$_{4thweek4}$	43.77	46.24	5.18	29	.000
Pair 10	A-amylase$_{1stweek4}$-A-amylase$_{5thweek4}$	26.41	47.36	3.05	29	.005
Pair 11	A-amylase$_{1stweek4}$-A-amylase$_{post-int4}$	-23.02	68.42	-1.84	29	.076
Pair 12	A-amylase$_{2ndweek4}$-A-amylase$_{3rdweek4}$	5.60	52.52	0.58	29	.563
Pair 13	A-amylase$_{2ndweek4}$-A-amylase$_{4thweek4}$	33.46	61.15	3.00	29	.006
Pair 14	A-amylase$_{2ndweek4}$-A-amylase$_{5thweek4}$	16.10	55.48	1.59	29	.123
Pair 15	A-amylase$_{2ndweek4}$-A-amylase$_{post-int4}$	-33.32	84.16	-2.17	29	.038
Pair 16	A-amylase$_{3rdweek4}$-A-amylase$_{4thweek4}$	27.86	44.48	3.43	29	.002
Pair 17	A-amylase$_{3rdweek4}$-A-amylase$_{5thweek4}$	10.49	41.71	1.38	29	.179
Pair 18	A-amylase$_{3rdweek4}$-A-amylase$_{post-int4}$	-38.93	68.53	-3.11	29	.004
Pair 19	A-amylase$_{4thweek4}$-A-amylase$_{5thweek4}$	-17.36	28.04	-3.39	29	.002
Pair 20	A-amylase$_{4thweek4}$-A-amylase$_{post-int4}$	-66.78	68.30	-5.36	29	.000
Pair 21	A-amylase$_{5thweek4}$-A-amylase$_{post-int4}$	-49.42	63.61	-4.26	29	.000

Table 3.22

Investigation of Differences Between the Experimental and Control Conditions on the

Total Electrical Activity (EMG) Measured on the Trapezii of the Participants in the Post-

Intervention Session, While Controlling for the Total Electrical Activity (EMG) Measured

in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	265.42	2	132.71	3.01	.058	0.10
Intercept	1115.29	1	1115.29	25.27	.000	0.32
PRE_EMG_EO	53.39	1	53.39	1.21	.276	0.02
Group	226.80	1	226.80	5.14	.028	0.09
Error	2339.22	53	44.14			
Total	4458.82	56				
Corrected Total	2604.64	55				

Table 3.23

Investigation of Differences Between the Experimental and Control Conditions on the

Skin Conductance (SC) Measured on the Trapezii of the Participants in the Post-

Intervention Session, While Controlling for the Skin Conductance (SC) Measured in the

Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	134.92	2	67.46	15.06	.000	0.36
Intercept	12.13	1	12.13	2.71	.106	0.05
PRE_EO_SC_Mean	100.23	1	100.23	22.37	.000	0.30
Group	41.90	1	41.90	9.35	.003	0.15
Error	237.47	53	4.48			
Total	703.45	56				
Corrected Total	372.39	55				

Table 3.24

Investigation of Differences Between the Experimental and Control Conditions on the Peripheral Skin Temperature (Temp) Measured in the Post-Intervention Session, While Controlling for the Peripheral Skin Temperature (Temp) Measured in the Pre-Intervention Session

	Type III Sum of Squares	df	Mean Square	F	Sig.	η_p^2
Corrected Model	153.95	2	76.98	7.64	.001	0.22
Intercept	423.96	1	423.96	42.09	.000	0.44
PRE_EO_Temp_Mean	99.12	1	99.12	9.84	.003	0.16
Group	32.99	1	32.99	3.28	.076	0.06
Error	533.86	53	10.07			
Total	56622.66	56				
Corrected Total	687.81	55				

APPENDIX C.1

Interview Guide Used in Study 3

Hello…………………………..As you know, I am Babis from Sport Psychology. Now that we finished the project, I would like to ask you a few questions on your experience during this project.

First of all, as far as I know, you are ……………years old, a student of the………..grade, and you are an athlete of

....

How many years have you been practicing this sport?

....

(Athletes often experience a difficulty in achieving a good level of emotional control. This is the reason why we have introduced this new psychophysiological strategy [i.e. HRV training]. One of the reasons we have done all the measurements in the mindroom is to see the extent to which HRV training [slow breathing] helps you to control your stress in a more efficient way). During the project, as you remember, you were asked to breathe at a slow pace of breathing- ***Do you believe that this pace of breathing was ideal for you or not?*** (We have to discuss here what ideal breathing means- it always depends on which angle you are examining it from. We have to direct the participant to that angle where ideal breathing pace would be a pace that one can breathe easily and effortlessly).

It can take some time to the athlete to get used to this slow pace of breathing. ***How long did it take to you to get used to this breathing pace?***

(Here, a deeper investigation should take place- If it took a long time to the client, then we have to investigate why. What made it so hard to the client to get accustomed to slow breathing).

I understand that you had five sessions of twenty minutes here and another five you did at home with the application on your mobile. ***Do you think you would be able to breathe alone at this breathing pace without the help of a breathing pacer? How***

closely can you breathe to the resonant breathing frequency? Under which circumstances could you do this?

Do you think that you could breathe at this pace while moving vigorously? (Here we have to discuss/ define vigorous activity. A few examples can be used to better describe this, e.g. a table tennis player when playing a rally, or a fencer when attacking, etc. The expected answer, here, is "no", so if someone says that they can, we have to make sure that he has understood what vigorous activity is).

You may remember me telling you over and over again during the training phase of the project that you should not breathe deeply. Do you remember this? *Did you manage to avoid breathing deeply during the 20 minutes of the training sessions or not? Did you understand the reason why I insisted on you not breathing deeply? Could you just slow down your breath and breathe naturally and effortlessly?*

Let's go to how you felt after the twenty minutes of HRV training (slow breathing). *What do you think was the outcome of the HRV training?* Was the outcome positive or negative? Or was there no effect? Did you feel more energized or more relaxed? Was there any negative aspect of the training that you would like to report? *Do you believe that this type of training had some kind of side-effect and if so, what? Are there any benefits that you see in this type of training? How could it be used in your life as a student-athlete*? At which times and days do you think you could practice that and for what reason?

How would you change this type of training to make it suit better your needs as a student? How would you change this type of training to make it suit better your needs as an athlete? *Is there anything in particular that you would definitely change to make it more practical?*

Do you have any questions on any aspect of the project? Do you have any questions or recommendations on the slow-breathing strategy?

Thank you very much for your participation in the study.

APPENDIX C.2

Main Research Questions/Topics Discussed in Study 3

Q1. Whether the instructed slow pace of breathing was good or not.

Q2. How long it took the participant to get used to the agreed resonant breathing frequency.

Q3. Whether one can breathe alone at their resonant breathing frequency without the help of a pacer.

Q4. Whether one can breathe at their resonant breathing frequency while moving vigorously.

Q5. Whether one can do HRV training without taking deep breaths.

Q6. Positive and negative outcomes of HRV training.

Q7. Suggested modifications for the HRV training protocol.