HIGH-INTENSITY INTERVAL TRAINING'S EFFECT ON ACUTE PHYSIOLOGICAL STRESS RESPONSES AND ESPORTS PERFORMANCE

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TIIVISTELMÄ

Pakarinen, K. 2023. Korkeaintensiteettisen intervalliharjoituksen vaikutukset akuutteihin fysiologisiin stressivasteisiin ja eurheilusuoritukseen. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, liikuntafysiologian pro gradu tutkielma, 87 s. 5 liitettä.

Tämän pro gradu tutkielman tavoitteena oli tutkia korkeaintensiteettisen intervalliharjoituksen (HIIT) akuutteja vasteita fysiologisiin stressivasteisiin ja eurheilun suorituskykymarkkereihin. Eurheilu nähdään usein antagonistisena aktiviteettina liikuntaan nähden sen inaktiivisen luonteen vuoksi. Kuitenkin liikunnalla on havaittu alustavia edullisia vaikutuksia eurheilussa hyödyllisiin markkereihin, kuten toiminnanohjaukseen ja eurheilun suorituskykymarkkereihin, mikä voisi kannustaa eurheilijoita osallistumaan liikuntaan. Eurheilijat altistuvat myös korkeille stressivasteille, jotka kroonistuessaan voivat aiheuttaa terveyshaittoja. Liikunnalla, erityisesti aerobisella liikunnalla, vastaavasti on havaittu hyötyjä stressivasteisiin.

Pro gradu -tutkimus toteutettiin intraverrokkiasetelmalla, jossa tutkittavat osallistuivat liikunta- ja lepointerventioon. Tutkimus jaettiin kolmelle päivälle: alkumittauksiin, liikuntainterventioon ja lepointerventioon, johon kaikki koehenkilöt osallistuivat. Alkumittauksissa tutkittavilta tiedusteltiin sähköisillä Webropol-kyselyillä terveydentilaa, elintapa- ja pelitottumuksia, sekä mitattiin kehonkoostumusta bioimpedanssilla ja maksimaalista hapenottokykyä polkupyöräergometritestillä. Liikuntainterventiossa tutkittavat suorittivat 15 minuutin HIIT-suorituksen, ja suorittivat tunnetilakyselyn ja 20 minuutin lukemisen jälkeen simuloidun eurheilusuorituksen Counter Strike: Global Offensive -pelissä AimBotz tarkkuusharjoituskartalla. Tutkimuksen aikana koehenkilöiltä seurattiin sykettä, sykevälivaihtelua ja syljen stressimarkkereita (kortisolia, alfa-amylaasia ja dehydroepiandrosteronia). Samat tutkittavat lepointerventiossa istuivat polkupyöräergometrin päällä levossa 15 minuuttia, ja suorittivat identtisesti mittaukset liikuntainterventioon nähden. 7 päivää viimeisen interventiopäivän jälkeen koehenkilöille jaettiin sähköpostitse Webropol-kysely fyysisen aktiivisuuden nautinnollisuudesta. Syljen stressimarkkerianalyysit suoritettiin linkitetyllä entsyymi-immunoabsorptioanalyysitekniikalla ja sykevälivaihteluanalyysi Kubios-ohjelmistolla. Tilastollista merkitsevyyttä arvioimaan käytettiin RStudio-ohjelman epäparametrisiä menetelmiä (Friedman ja Wilcoxon).

Päivänsisäiset erot syljen kortisolissa liikuntainterventiossa oli tilastollisesti merkitseviä, samoin interventioiden- ja kontrollinvälisessä syljen alfa-amylaasissa. Liikuntainterventiossa kortisolipitoisuudet laskivat eurheilusuorituksen jälkeen. Vastaavasti alfa-amylaasipitoisuudet olivat korkeammat pelisuorituksen aikaan lepointerventiossa verrattuna liikuntainterventioon ja kontrolliin. Pelinaikainen syke erosi liikunta- ja lepointervention välillä, jossa liikuntainterventiossa havaittiin korkeampia sykkeitä. Sykevälivaihtelun lyhytaikaisissa markkereissa ei havaittu tilastollisesti merkitseviä eroja interventioiden välillä. Eurheilusuorituksissa ei havaittu tilastollisesti merkitseviä eroja liikunta- ja lepointervention välillä, ei myöskään tunnetiloissa tai tunnetilahäiriössä. Tulokset viittaisivat eroaviin vasteisiin eri stressivastejärjestelmissä: potentiaalisesti vaimentuneeseen hypotalamus-aivolisäke-lisämunuaiskuori -akselilla, sekä kohonneeseen aktiviteettiin sympatikus-lisämunuais-medulla -akselilla, samalla mahdollisesti vahvistaen eurheilun aiheuttamien stressivasteiden vaikutusta. Lisätutkimuksia tarvitaan tutkimaan eri liikuntamuotojen vaikutuksia stressivasteisiin eurheilussa ja eurheilusuorituskykyyn.

Asiasanat: HIIT, fysiologia, stressi, eurheilu, pelaaminen, suorituskyky

ABSTRACT

Pakarinen, K. 2023. High-intensity interval training's effect on acute physiological stress responses and esports performance. Faculty of Sport and Health Sciences, University of Jyväskylä, Master's thesis, 87 pp. 5 appendices.

This Master's thesis aimed to investigate the high-intensity interval training (HIIT) acute effects on physiological stress responses and esports performance markers. Esports and exercise are commonly seen as antagonistic activities due to esports's inactive nature. However, exercise has been seen potential beneficial effects in useful markers in esports, like cognitive function and esport performance markers, which could potentially encourage esports athletes to participate in physical activity. Esports athletes are also exposed to high stress responses, and if prolonged, can cause health defects. Exercise on the other hand has shown beneficial effects on stress responses.

This Master's thesis study was conducted as an intra-subject study, in which the subjects participated in exercise and resting models. The study was divided into three days: preliminary measurements, exercise model and resting model. The subjects participated in all phases. Preliminary measurements the subjects answered electrical Webropol questionnaires about their health, health habits and gaming habits, and participated in bioimpedance body composition measurement and maximal oxygen uptake cycle ergometer test. In the exercise model, the subjects conducted a 15-minute HIIT protocol, after which they answered to a mood state questionnaire and did a cognitive reboot reading a book for 20 minutes. After reading session, the subjects did a simulated esports performance in Counter Strike: Global Offensive game, within AimBotz aim training map. During the research, the subjects were monitored of heart rate, heart rate variability and salivary stress markers (cortisol, alpha-amylase and dehydroepiandrosterone sulfate). In resting model, the same subjects sat on an cycle ergometer in rest for 15 minutes, and conducted all the other measurements identical to exercise model. 7 days after the last intervention day, the subjects were forwarded a physical activity enjoyment questionnaire (Webropol) via email. Salivary stress marker analysis was conducted on enzyme-linked immunosorbentassay method, and heart rate variability analysis with Kubios. Statistical significance was assessed with RStudio using nonparametric methods (Friedman and Wilcoxon).

Within-day differences in salivary cortisol were found statistically significant. Moreover, differences between the exercise and resting models and control in salivary alpha-amylase were also found statistically significant. The differences between exercise and resting models in average in-game heart rate were found statistically significant. Esports performance, mood states and mood disturbances didn't show statistically significant differences. The results would indicate differentiated responses from different stress response systems, with an attenuated response from hypothalamic-pituitary-adrenal axis, and an incerased response from sympathoadrenal-medullary system with a potential enhancing effect to esports-induced stress responses. More studies are required to examine the effects of different exercise modalities in stress responses in esports and effects on esports performance.

Key words: HIIT, physiology, stress, esports, gaming, performance

ABBREVIATIONS

ANS	autonomic nervous system
BMI	body mass index
ACTH	adrenocorticotropic hormone
BDNF	brain-derived neurotropic factor
CRH	corticotropin-releasing hormone
CS:GO	Counter-Strike: Global Offensive
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulfate
ELISA	enzyme-linked immunosorbent assay
FPS	first-person-shooter
fMRI	functional magnetic resonance imaging
HIIT	high-intensity interval training
HPTLC	high-performance thin layer chromatography
HR	heart rate
HR_{max}	maximal heart rate
HRV	heart rate variability
IPAQ	International Physical Activity Questionnaire
MOBA	multi-online battle arena game
POMS	Profile of Mood States
PNS	parasympathetic nervous system
RIA	radioimmunoassay
sAA	salivary alpha-amylase
SAM-p	sympatho-adrenal-medullary pathway
SNS	sympathetic nervous system
TMD	total mood disturbance
VO_{2max}	maximal oxygen uptake
VO_{2peak}	peak oxygen uptake

TABLE OF CONTENTS

1 INTRODUCTION

Competitive esports has been a thriving business in the recent years with growing pool of professional players (Birch et al 2023). Video game play (especially action video games, such as first-person shooter (FPS) games) can be seen as a way to enhance perceptual abilities (e.g. attention), motor abilities (e.g. reaction time) and neurocognitive abilities (e.g. decision-making and working memory) of a player, which has emerged interest in the research communities of various fields (Kowal et al 2018). However, in sport sciences, esports has not yet been recognized in full scope despite the similarities between traditional sport and esports stress conditions, especially in tournament settings (Sadowska et al 2023; Sousa et al 2020; Nagorsky & Wiemeyer 2020). Nevertheless, sport sciences are seen as an important field to optimize coaching, training and for predicting performance of the esports players (Sadowska et al 2023). Without evidence-based practices, esports as an activity could lead to participation-limiting challenges (mental and physical) and even to premature career endings. (Baena-Riera et al 2023.)

Video gaming and exercise have commonly been seen as antagonistic, time-competing activities (De Las Heras et al 2020). De Las Heras et al (2020) study suggests, that introduction of short bouts of intense cardiovascular exercise before video game playing could enhance game performance, and promote improves in the physical and cognitive well-being within a growing population at risk of cardiometabolic and psychosocial challenges.

This master's thesis aims to put light on the stress responses esports players experience, and if a single, acute bout of high-intensity interval training affects the physiological acute stress markers (salivary steroids, heart rate and heart rate variability) the esports players' experience during a simulated esports performance taking the esports players' psychological status (mood states and physical activity enjoyment) into account.

In this thesis' evidential background, esports as a field is introduced, its demands to the player, and general evidence on the demographic of esports players. Moreover, the reader is introduced to the general physiology of the acute stress response (primary and secondary resposnes) and recent evidence on specialized stress responses in exercise and video game playing conditions. Lastly, the definition and acute responses of high-intensity interval training (HIIT) are being defined, and what previous evidence states about HIIT training's effect on video game playing.

2 DEFINITION OF ESPORTS

Pedraza-Ramirez et al (2020) suggest research professionals to use the following definition of esports:

”Esports is the casual or organized competitive activity of playing specific video games that provide professional and/or personal development to the player. This practice is facilitated by electronic systems, either computers, consoles, tablets or mobile phones, on which teams and individual players practice and compete online and/or in local-area-network tournaments at the professional or amateur level. The games are established by ranking systems and competitions and are regulated by official leagues. This structure provides players a sense of being part of a community and facilitates mastering expertise in fine-motor coordination and perceptual-cognitive skills, particularly but not exclusively, at higher levels of performance.”

Esports games are controlled by specific sensori-motor actions on interfaces and sensors through hand-mouse and finger-keyboard interactions. To operate the game avatars and to use items (e.g. weapons) in the game interface, esports includes goal-oriented physical interactivity adapted to a specialized sensori-motor condition of the virtual environment. (Nagorsky & Wiemeyer 2020.)

Esports shares some basic commonalities with traditional sports (Sousa et al 2020; Birch et al 2023; Nagorsky & Wiemeyer 2020). Tournaments involve players as individuals or teams, who face opponents in the game, that is divided into rounds and matches (Sousa et al 2020; Birch et al 2023). Esports game have an in-game ranking system, that has been suggested to be an indicator of player success (Birch et al 2023).

Esports players train regularly, usually in large quantities, and participate in professionally coached training. Esports players also can receive team and sponsor opportunities from third parties, and have potential to gain similar earnings to traditional sport athletes. (Sousa et al 2020; Baena-Riera et al 2023; Nagorsky & Wiemeyer 2020.)

2.1 Esport games – Counter Strike: Global Offensive

In general, different game types include action, adventure, strategy and management, role-playing games, simulation or board and card games (van der Vijgh et al 2015). By definition, not every game is an esports game (Pedraza-Ramirez et al 2020, Leis & Lautenbach 2020). Esports are played on different platforms with different control devices (Nagorsky & Wiemeyer 2020). Most common esports games can be divided into specific genres, such as multiplayer online battlefield arena (MOBA) games, first-person shooter (FPS) games, and real-time strategy (RTS) games and sports simulations. Different game types and games have different sets of competition rules, and different mechanics for playing. (Sadowska et al 2023; Nagorsky & Wiemeyer 2020.)

First-person shooter (FPS) games are part of an action video game umbrella genre (West et al 2018). Action video games are fast-paced in gameplay, and focus on movement, quick decision-making, strategizing, fast hand-eye coordination, and combat and reaction time performance characteristics (Kowal et al 2018; Sousa et al 2020). FPS games aim to stimulate the players' visual field during gameplay in real time (Sousa et al 2020).

In this study, the focus is on the most played FPS game in the Western world at the beginning of this study is Counter: Strike: Global Offensive (CS:GO) by Valve Corporation, with a peak of over 900 000 players. CS:GO involves two teams of five players. One round in CS:GO lasts maximum 1 min 55 s, where players aim to eliminate the opposing team or to complete the objective, which depends on the team position the player is in. In full games, the teams play altogether 30 rounds, and change positions after 15 rounds, with the outcome of a single game of best-of-30. (Sharpe et al 2023.)

Counter Strike: Global Offensive (CS:GO) is a tactical FPS game, in which also strategizing with team economy is in key role (Sadowska et al 2023; Sharpe et al 2023). Economy, in CS:GO context, refers to managing monetary balances, that each player possess, and with which players purchase items for every round to give advantages for the player himself and the team (Sharpe et al 2023).

2.2 Esports performance demands

Performance can be defined as the action or process of performing a task or function (Sharpe et al 2023). In general, playing computer games demands high perceptual, attentional, cognitive and fine motor skills (Kim et al 2023). High performance in esports demands the player to meet all skill requirements as effectively as possible (Nagorsky & Wiemeyer 2020). Currently, there is limited knowledge about the factors affecting gamers' expertise, performance and optimal structures of esports training (Baena-Riera et al 2023; Pedraza-Ramireze et al 2020). However, since diffrent esports games state different demands for the player, the factors for successful performance need to be analyzed separately in each esport (Nagorsky & Wiemeyer 2020; Pedraza-Ramirez et al 2020).

Performance in esports can be measured by the basis of results (e.g. win/lose), by in-game statistics (e.g. kills-deaths-assists rate (KDA), completion time) or by positioning (e.g. ranking). Different games may define different outcome measures: in FPS games, KDA). (Pedraza-Ramirez et al 2020.)

Indicators of performance have been suggested to be overall game score and kill/death ratio, however it has been argued to be a simplified approach. It has been suggested, that fundamentally contributions of various performance metrics in the contribution of outcome performance (i.e. winning a game) need to be determinded. Some skills have been suggested to directly contribute to the primary outcome without being evident in the game statistics (task performance) and some indirectly (contextual performance). (Sharpe et al 2023.) Figure 1 from Sharpe et al (2023) demonstrates esports performance types and their relationships.

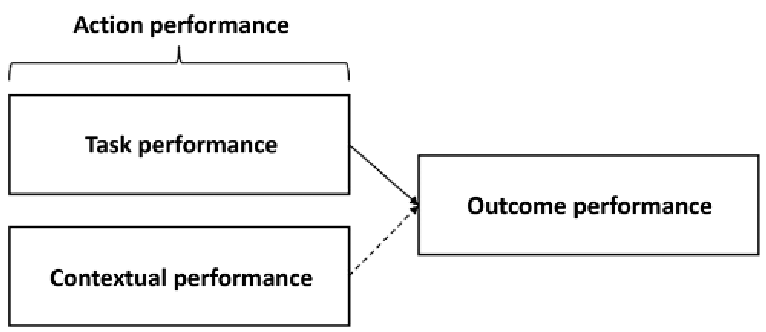

FIGURE 1 Performance types and their suggested relationships (dotted representing indirect relationship, straight line direct relationship) (Sharpe et al 2023)

In esports, task performance could be described as an individual's capacity to perform skills, that contribute to a performance outcome. Task performance parameters could be e.g. reaction time, mouse control and response time. Contextual performance skills, that do not directly contribute the outcome performance, such as personal initiative and sportsmanship. Task performance parameters may vary from different roles within the game, however contextual skills appear similar among roles. (Sharpe et al 2023.)

Nagorsky & Wiemeyer (2020) represented a model integrating a general sport competence model and game skill model to produce a fundamental model for esports performance demonstrated in Figure 2.

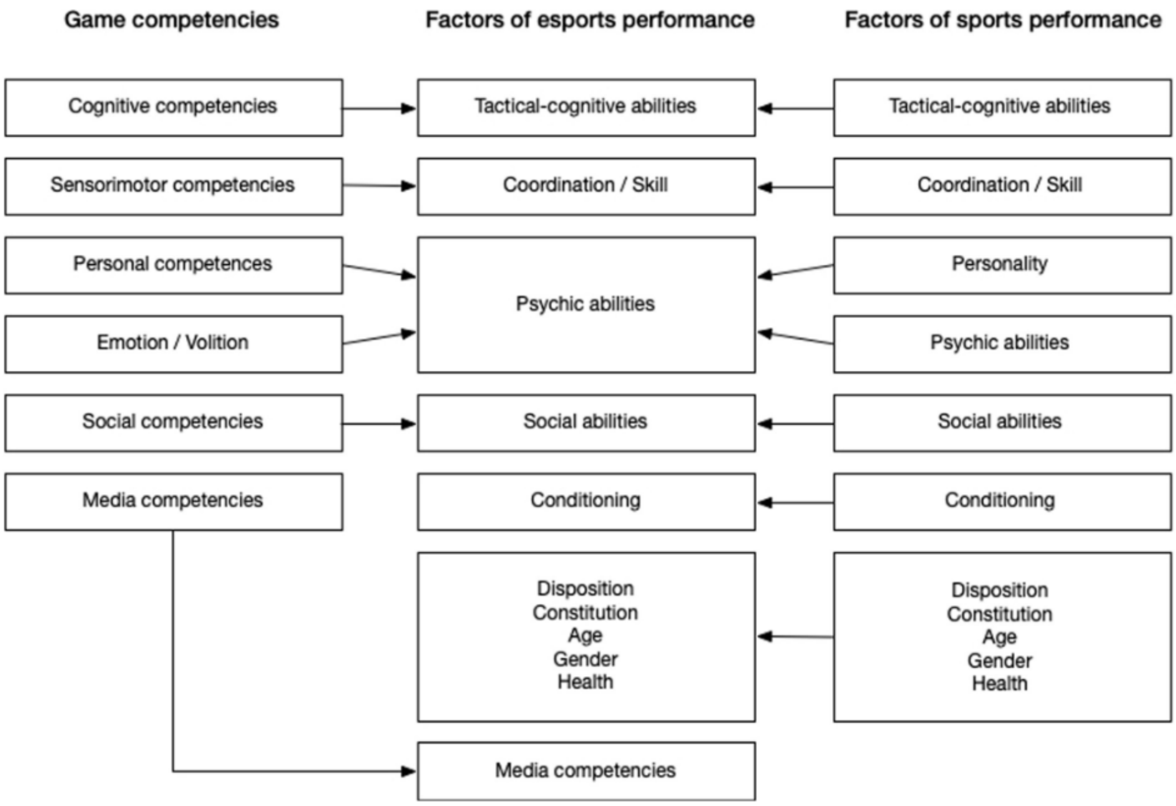

FIGURE 2. Competence model of esports performance (Nagorsky & Wiemeyer 2020)

As stated before, different esports game types and games demand different skillsets from the player. In this thesis, the focus is on Counter Strike: Global Offensive (CS:GO). In CS:GO, action performance skills could include carrying the bomb, providing utility and/or directing teammates (Sharpe et al 2023). Fast reaction times (speed to response to stimuli, speed to respond to a strategy and time to respond to a game-specific challenge) are mentioned in multiple studies to be a key factor in CS:GO (Sharpe et al 2023; Sadowska et al 2023). From an individual's perspective, key CS:GO performance indicators are concidered to be reaction time, response time, keyboard proficiency and mouse control (Sharpe et al 2023; Kim et al 2023). Figure 3 summarizes suggested CS:GO competence indicators from multiple studies.

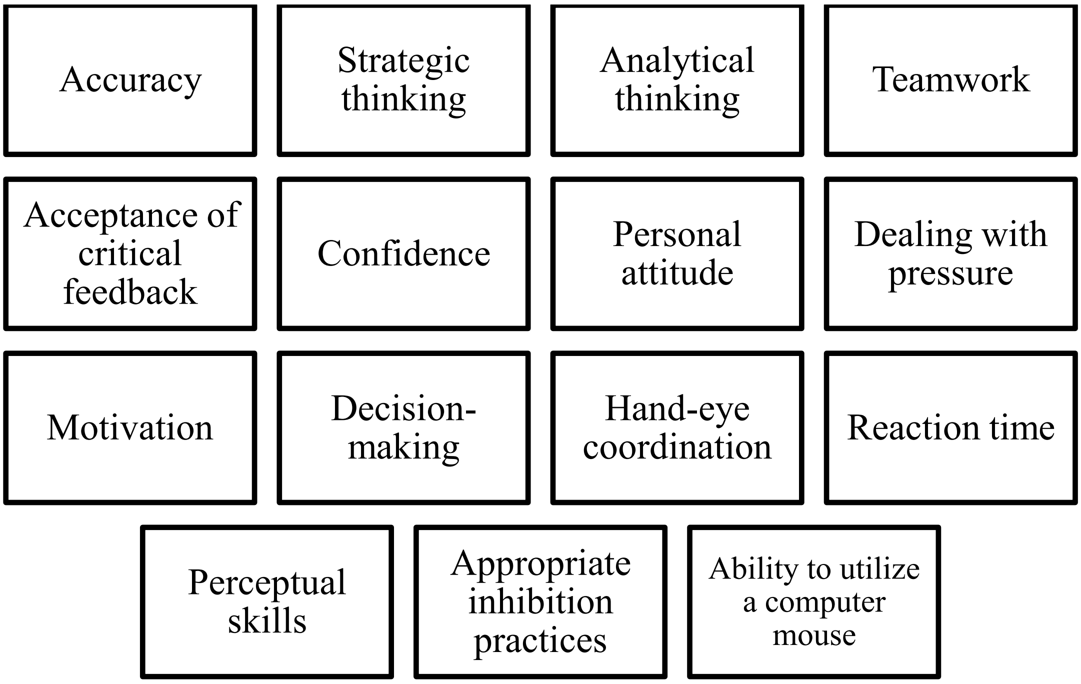

FIGURE 3. Components of CS performance. (Sharpe et al 2023; Sadowska et al 2023; Nagorsky & Wiemeyer 2020)

It has been in general interest to find methods to improve the performance of the players, and to seek competitive advantage (Kowal et al 2018).

2.2.1 Physical performance

Esports demands both acute and long-term physical conditioning from the players: controlling fast movements during the game, and also endurance to manage through long duration, multiple hours-lasting esports competitions. Therefore, local anaerobic-alactic endurance in upper extremities is required as well as global aerobic endurance. Force endurance of the trunk muscles to control posture is also required. (Nagorsky & Wiemeyer 2020.) Coordinative, synchronized and sequental upper extremity (finger, hand, arms) movement and eye-hand coordination as important skills are well established in current studies (Kim et al 2023;

Nagorsky & Wiemeyer 2020). In the context of fast reactions and movements, maximal strength is a key contributor of speed, which has potential to have an indirect effect to game performance (Nagorsky & Wiemeyer 2020).

In general, playing esports also causes physical strain to musculoskeletal system (DiFrancisco et al 2022). Suboptimal posture holding, overuse and repetitive strain injuries cause challenges for players' performance and threaten professional careers (Baena-Riera et al 2023). Most common injuries in esports are eye fatigue, neck pain, back pain, wrist pain and hand pain (Baena-Riera et al 2023).

In Counter Strike: Global Offensive (CS:GO), players engage the muscles of both upper limbs while utilizing a computer mouse and a keyboard simultaneously with precise and fast movements. Studies have demonstrated, that a novice player conducts approximately 50 action moves per minute, as high-level esport athletes produce 500-600 action moves per minute. (Sadowska et al 2023.) To demonstrate the level of strain, Sadowska et al (2023) study didn't find any increased accumulation of blood lactate after playing CS:GO, which was hypothesized to be due to the few muscle groups being activated during the game, and the tournament duration being short compared to a real tournament setting (duration approximately 3-6 hours).

2.2.2 Cognitive performance

Just like in traditional sports, tactical and cognitive skills affect the performance in esports (Nagorsky & Wiemeyer 2020; Kowal et al 2018). Functional magnetic-resonance imaging (fMRI) studies have showed, that activation in areas of the brain implicated in executive control was associated with better game performance (Sousa et al 2020). Therefore, high cognitive function (cognitive functions, that govern behavior, decision-making, abilities, organization, planning, goal-setting, time management and self-regulation) and executive function processes are in a key role in competitive video gaming, and cause a high cognitive demand on the user (Kowal et al 2018; Sousa et al 2020). Spatial abilities (knowing their own and other players' positions, perception of static and dynamic objects), anticipative thinking (e.g. anticipating opponent actions), ability to predict actions and events in the game are concidered important skills in esports context. (Nagorsky & Wiemeyer 2020; Kim et al 2023.)

Throughout the esports games, the players face sensory information from multiple origins: visual information (e.g. motions and actions of other players, changing brightness conditions) and auditory information (e.g. in-game sounds and communication with teammates) and haptic information (e.g. position of hands and fingers, force on keyboard and speed of finger movements) (Kim et al 2023; Nagorsky & Wiemeyer 2020; Sousa et al 2020). As the stimuli are exhibited, a successful player needs to control their body movement by appropriately integrating and interpreting sensory input as visual, auditory and tactile information. Experienced players demonstrate consistency, efficiency, stability, adaptability and coordination with inhibitory practices (to try to minimize the amount of erroneous choices) while engaging in rapid decision-making in a competitive environment to execute an action. (Kim et al 2023; Sousa et al 2020.)

In action video games (e.g. first-person shooter games, FPS games) cognitive skills needed are e.g. alertness, attention, memory functions, information handling and task-switching ability (Toth et al 2020; De Las Heras et al 2020; Pedraza-Ramires et al 2020; Sousa et al 2020).

2.2.3 Psychological performance

Esports are played in a competitive and demanding setting, that places demands on the players' mental capacity (Baena-Riera et al 2023). Pressure-related demands in esports can be distinguished as time pressure (e.g. actions within limited time), precision pressure (e.g. accurate movement of the mouse and using correct keys and buttons), complexity pressure (e.g. number of simultaneous and/or sequential coordinations of movements) and stress-strain pressure (e.g. physical and mental) (Nagorsky & Wiemeyer 2020). This elevates the risks for psychological and behavioral problems, such as anxiety, aggression and burnout. Esports players should be prepared psychologically for emotional stability to be able to face the pressures of competition while conducting complex, fast and precise movements. (Baena-Riera et al 2023.) Esports competitions have been shown to be accompanied by strong emotions, that may lead to physiological responses (Sadowska et al 2023; Birch et al 2023).

As esports games are often played as a team, team tactics and ability to work as a team play an important role in many esports games (Nagorsky & Wiemeyer 2020). Practising video games have been suggested to positively influence social skills (Pedraza-Ramirez et al 2020). In CS:GO, teamwork skills are demanded from successful players (Birch et al 2023).

3 STRESS RESPONSE

Stress can be defined as a state of threatened (or perceived as threatened) and dysregulated biological homeostasis, which can be caused intrisicly or extrinsicly (Charmandari et al 2005; Giacomello et al 2020). It is a complicated physiological, cognitive and psychological process (Leis & Lautenbach 2020). Physiological stress response aids humans adaptively to mobilize energy and respond to challenging challenging or threatening environmental, physical and external or internal psychosocial stimuli (Caplin et al 2021).

Allostatic load refers to a measure of the cumulative physiological burden to the body of accommodating multiple stressors over time. It is based on biological parameters, including DHEA-S, cortisol, epinephrine, norepinephrine, high density lipoproteins (HDL), total cholesterol, waist-to-hip-ratio, glycosylated hemoglobin (HbA1C), systolic blood pressure and diastolic blood pressure. (Maninger et al 2009.) Regenerative and degrading effects are concerned, when assessing allostatic load score: e.g. high cortisol concentrations and low DHEA-S concentrations contribute to increases in allostatic load score. (Maninger et al 2009.)

In psychology, stress is divided into two categories: positive stress (*eustress*) and negative stress (*distress*). Eustress enhances performance (mentally or physically), whereas distress is associated with detrimental effects. (Giacomello et al 2020.) Especially if prolonged, stress responses may have adverse effects on several physiologic and cognitive functions, and may lead to and/or intensify numerous diseases (Charmandari et al 2005; Molina-Hidalgo et al 2023; Giacomello et al 2020; Thayer et al 2012; Kudielka & Kirschbaum 2005). Detrimental dysregulation through continuous stress has been seen in hippocampal circuits (e.g. memory processes), prefrontal cortex (e.g. working memory processes) and endocrine and autonomic outputs (Molina-Hidalgo et al 2023; West et al 2018; Zschucke et al 2015; Thayer et al 2012).

Stress response system can be divided into central and peripheral components. Central components are located in the hypothalamus and the brainstem. Central components include: parvocellular neurons of corticotropin-releasing hormone (CRH), the arganine vasopressin (AVP) and the paraventricular nuclei (PVN) of the hypothalamus, the CRH neurons of the paragigantocellular and parabranchial nuclei of the medulla and the locus ceruleus (LC) and other mostly noradrenergic cell groups in the medulla and pons (LC/NE system). The peripheral components include peripheral limbs of the HPA axis, efferent SAM-p and parasympathetic nervous system (PNS) components. (Charmandari et al 2005.) Moreover, right prefrontal cortex has been suggested to demonstrate the strongest connection to stress-regulatory systems, and inferior frontal gyrus in inhibitory control (Zschucke et al 2015). Figure 4 demonstrates the anatomical model and the relations of the central and peripheral components.

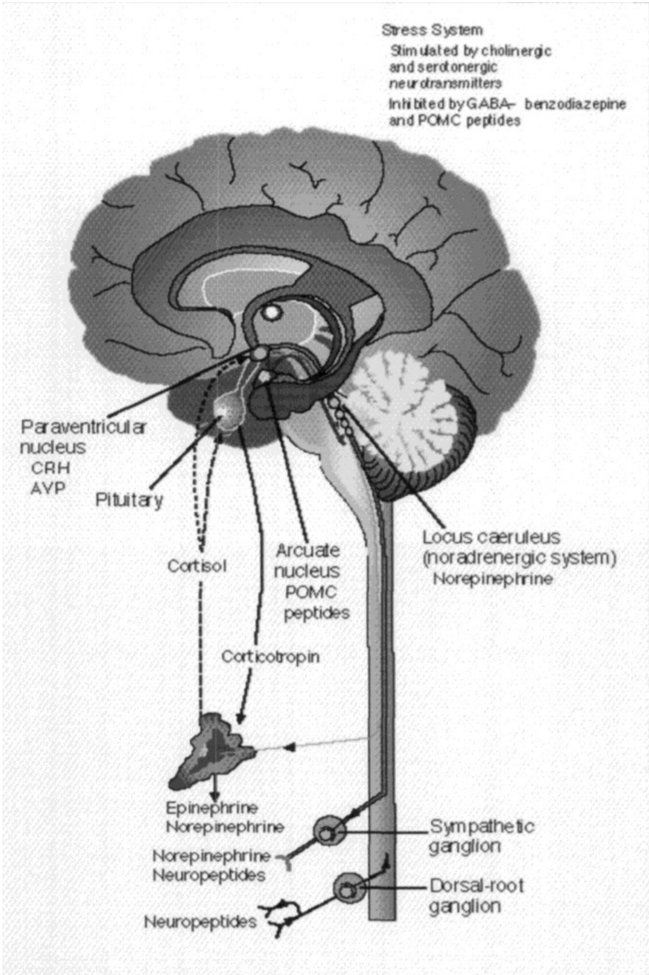

FIGURE 4. Central and peripheral components of the stress system with their relations to other nervous system components involved in the stress response (Charmandari et al 2005)

In response to a physical and psychosocial stressor, two neuroendocrine pathways are activated: the autonomic nervous system (ANS) (rapid responder) and hypothalamic-pituitary-adrenal (HPA) axis (delayed responder) (Molina-Hidalgo et al 2023; Schumacher et al 2013; Caplin et al 2021). A key branch of ANS, is the sympathetic nervous system (SNS), that through sympathoadrenal-medullary pathway (SAM-p) releases catecholamines (norpinephrine and epinephrine) in the adrenal medulla (Molina-Hidalgo et al 2023; Caplin et al 2021; Schumacher et al 2013). HPA axis activation leads to the release of glucocorticoids by the adrenal cortex

(Molina-Hidalgo et al 2023). It is recommended to approach stress responses as a multisystem, since both systems interact in order to generate the stress response (Schumacher et al 2013).

In immediate stress reaction, autonomic nervous system's SAM-p release of catecholamines (epinephrine and norpinephrine) modulate the immediate "*fight-or-flight*" response, which aims to optimize alertness and bodily functions for management of the physical or psychological stressor (Caplin et al 2021; Giacomello et al 2020; Kudielka & Kirschbaum 2005). The HPA axis connects the central nervous system (CNS) with the hormonal system (Kudielka & Kirschbaum 2005). After a time delay from the onset of the stressor, HPA axis activates a long transient hormonal cascade, that terminates with the release of glucocorticoids from the adrenal cortex (Caplin et al 2021; Schumacher et al 2013).

Depending on the quality of a stimulus (physical or psychological), different pathways of the HPA axis are activated (Kudielka & Kirschbaum 2005). Physiological stressors have been suggested to activate the HPA axis through a direct pathway to the paraventricular nucleus (PVN) of the hypothalamus, whereas psychological stressors stimulate PVN through the limbic system (medial prefrontal cortex, hippocampus, amygdala) (Zschucke et al 2015; Thayer et al 2012; Kudielka & Kirschbaum 2005). In parallel with HPA axis, further pathways are also activated: gonadal axis, adipose axis and immune system (Giacomello et al 2020).

The hypothalamus-pituitary-adrenal (HPA) axis involves hypothalamic corticotropin-releasing hormone (CRH), pituitary corticotropin and adrenal cortisol (Molina-Hidalgo et al 2023). CRH is the primary hypothalamic regulator of the pituitary-adrenal axis. CRH stimulates the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH's target is adrenal cortex, which regulates glucocorticoid and adrenal androgen secretion (in zona fasciculata and reticularis, respectively). (Giacomello et al 2020; Kudielka & Kirschbaum 2005; Charmandari et al 2005.) In non-stressful conditions, CRH and AVP are secreted in a circadian and pulsatile fashion. CRH and AVP pulse levels increase early in the morning, leading to diurnal variations in the pulsatile secretions of ACTH, and eventually cortisol. (Charmandari et al 2005.) Cortisol circulates into the peripheral blood where it mobilizes energy by releasing glucose from their storage sites (Caplin et al 2021). Behavior of cortisol is discussed in detail in later chapters.

At the end of the stressor, HPA axis activates several negative-feedback circuits, in which circulating glucocorticoids released from the upstream HPA pathway (from adrenal gland to PVN in hypothalamus, hippocampus, anterior cingulate cortex, prefrontal cortex pituitary, hippocampus and frontal cortex) in the cortex are shut down (Molina-Hidalgo et al 2023; Caplin et al 2021; Zschucke et al 2015; Kudielka & Kirschbaum 2005). Fast action inhibition (cell membrane) occurs in milliseconds on neurons and within minutes at the pituitary upon steroid exposure. HPA axis inhibition can last for hours. (Caplin et al 2021.) In prolonged (or chronic) stress, the response of the HPA axis is attenuated, and leads to higher basal steroid (e.g. cortisol) levels (Molina-Hidalgo et al 2023; Giacomello et al 2020). Overall, HPA axis response is expected to be strong and rapid, and coupled with a rapid recovery process: first response providing organism with the necessary energy to cope with an enfaced stressor with an adequate return to baseline conditions (Caplin et al 2021; Kudielka & Kirschbaum 2005). A delayed return to the basal state could indicate an underlying dysfunction of the overall stress system (Kudielka & Kirschbaum 2005). The stress response system not only helps to adapt to increased demands and maintain homeostasis after a stressor, but is also vital for supporting normal physiological functioning (Kudielka & Kirschbaum 2005).

In this thesis, the focus is on endocrinological and cardiovascular effects of the stress response system.

3.1 Endocrinological stress responses

Stress hormones are fundamental in mediating both adaptive and maladaptive stress responses by interacting with specific aspects of physiology of affected tissues (Molina-Hidalgo et al 2023). In this study, the focus is on three key biomarkers of stress: cortisol, alpha-amylase and dehydroepiandosterone sulfate (DHEA-S). Figure 6 represents most common stress-related biomarkers in studies.

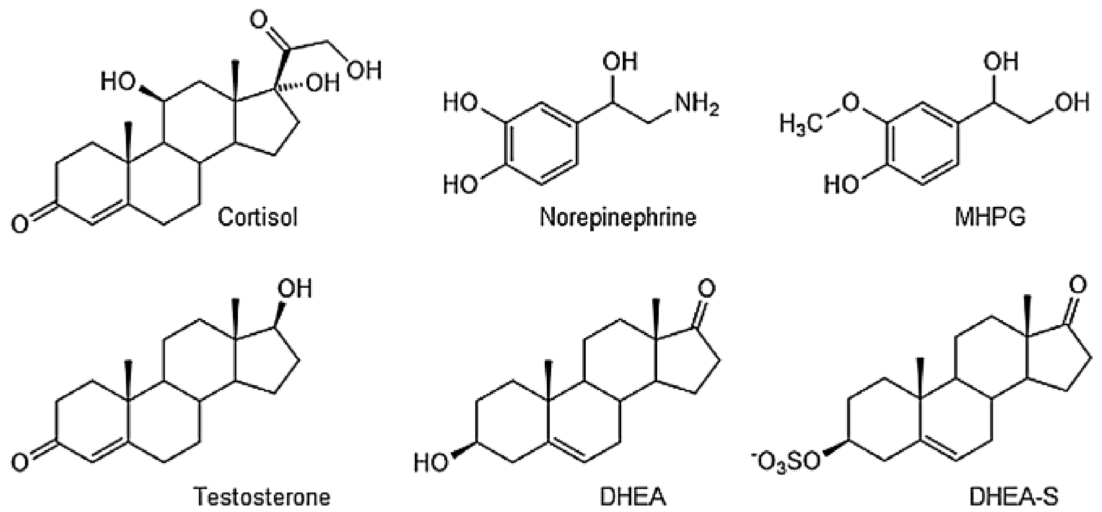

FIGURE 6. Chemical structures of small molecule stress-related biomarkers cortisol, norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), testosterone, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEA-S) (Giacomello et al 2020)

3.1.1 Cortisol

The most common biomarker for stress and important indicator of hypothalamic-pituitary-adrenal (HPA) axis signaling in humans is cortisol (Giacomello et al 2020). Cortisol is a glucocorticoid hormone (corticosteroid), that is released in response to stress via HPA axis and produced in the adrenal cortex. (Molina-Hidalgo et al 2023; Martínez-Díaz et al 2020; Monje et al 2020.) It is a catabolic hormone, that participates in immune function regulation and metabolism (Kilian et al 2016). Especially salivary cortisol has been shown to work as an effective indicator of HPA axis activity (Caplin et al 2021). It has been suggested that other hormones, cytokines, and neuronal information from the autonomic nerves of the adrenal cortex may also participate in the regulation of cortisol secretion (Charmandari et al 2005).

At pre-receptor level, 11-beta-hydrosteroid dehydrogenase 1 (11β-HSD1) modulates glucocorticoid exchange by activating cortisone to cortisol conversion to facilitate glucocorticoid receptor mediated action. At postreceptor level, the intracellular glucocorticoid receptor or mineralocorticoid receptor glucocorticoid activation (mainly cortisol) mediates hormone action, by translocation to the nucleus, binding to specific DNA sequences and modulating mRNA. Glucocorticoid receptor is expressed ubiquitously and its density is largerly regulated at tissue level. (Gatti et al 2009.) The description of cellular signaling and cortisol has been described in Figure 5.

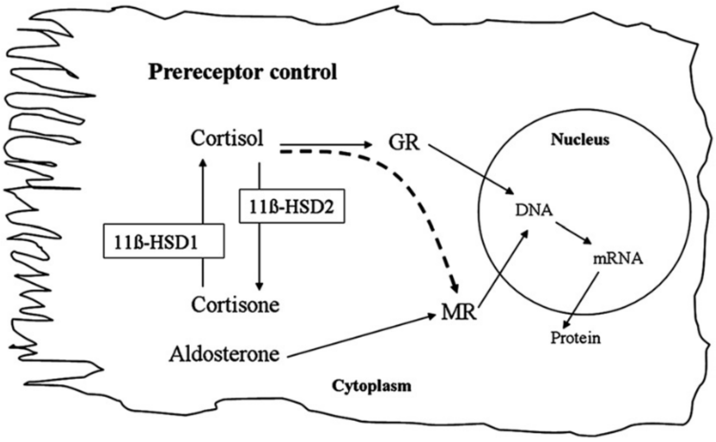

Fig. 2. Cortisol and cellular signalling. Glucocorticoid (GR) and mineral (MR) receptors, following binding of steroid hormones, translocate from cytoplasm into the nucleus and display their transactivation potential. The receptor linkage depends on steroid concentration controlled by the 11β-hydroxysteroid dehydrogenase type 1 and type 2 (11β-HSD1, 11β-HSD2) enzymes. 11β-HSD1 converts cortisone to cortisol with high affinity for both GR and MG. 11β-HSD2 inactivates cortisol into cortisone, which allows protection of MR expressing cells from activation of MR by glucocorticoid hormone cortisol.

FIGURE 5. Cortisol and cellular signaling (Gatti et al 2009)

Cortisol concentrations are influenced by several factors, e.g. sex, age and circadian rhythm (Molina-Hidalgo et al 2023, Giacomello et al 2020; Schumacher et al 2013; Gatti et al 2009). In normal circumstances, cortisol ranges are around 30-160 ng/mL in blood, and 1,0-1,6 ng/mL in saliva (Giacomello et al 2020). Being diurnal in nature, cortisol levels increase after awakening (approximately in 30 minutes) with a decrease during the day and facing its lowest levels around midnight, after which the levels begin to rise after first hours of sleep (Molina-Hidalgo et al 2023; Schumacher et al 2013; Gatti et al 2009). Altogether, the plasma cortisol diurnal rhythm consists of 10-15 pulses (Gatti et al 2009).

Facing acute stress and due to the delayed response of the HPA axis, cortisol levels peak at around 20-30 minutes after the stressor onset and remain elevated for approximately 2 hours until return to the baseline (Molina-Hidalgo et al 2023; Giacomello et al 2020; Schumacher et al 2013) During this acute stress response and when cortisol levels are being elevated, the negative feedback loop returns the cortisol levels to the homeostatic point (Molina-Hidalgo et al 2023; Giacomello et al 2020; Zschucke et al 2015).

The majority (90-95%) of cortisol circulates bound to the plasma protein corticosteroid-binding-globulin (CBG or transcortin) and to albumin (Gatti et al 2009; Kudielka & Kirschbaum 2005). This binding prevents the hormone from penetrating the membrane of the target cells. CBG works as a specific plasma transport glycoprotein for cortisol, and it has specific steroid binding sites. CBG regulates the bioavailability and metabolic clearance of the glucocorticoid. (Gatti et al 2009.)

The rest of the total plasma cortisol (3-10%) circulates as bioactive, unbound (“free”) cortisol (Gatti et al 2009; Kudielka & Kirschbaum 2005). Free cortisol is a lipophilic molecule and is able to pass through capillaries into tissues mainly by passive diffusion (Gatti et al 2009). Cortisol in saliva reflects the free cortisol, and correlates well with serum cortisol levels e.g. at rest and exercise conditions (Kilian et al 2016; Schumacher et al 2013; Gatti et al 2009). Salivary cortisol has been regarded as a valid marker of the activation of the HPA system (Schumacher et al 2013).

Cortisol has a wide range of physiological effects in the body: all body’s single nucleated cells are potential targets for cortisol (Kudielka & Kirschbaum 2005). Cortisol’s most important effect in metabolism is mobilizing resources to provide energy for increased metabolic demand due to cathecolamine release. (Giacomello et al 2020; Gatti et al 2009; Kudielka & Kirschbaum 2005.) It also participates in maintenance of electrolyte balance and blood pressure, immune system, cell proliferation and differentiation as well as regulation of cognitive functions (Gatti et al 2009). Moreover, moderate cortisol levels seem to be positively associated with executive functioning, while high cortisol levels have been shown to interfere with cognitive functions on prefrontal networks (e.g. inhibitory control, attention regulation and working memory) (Martínez-Díaz et al 2020).

3.1.2 Alpha-amylase

Alpha-amylase (α-1, 4-α-D-glucan 4 glucanohydrolase) is a salivary enzyme, that is part of the glycosyl hydrolase family (Schumacher et al 2013; Nater & Rohleder 2009). It is produced locally in the oral cavity by salivary glands in the response to autonomic nervous system (ANS) and beta-adrenergic (and potentially alpha-adrenergic) stimuli (Schumacher et al 2013; Nater & Rohleder 2009). It is associated with both plasma epinephrine and norepinephrine changes, which indicates it being regulated by sympathetic-adrenal-medullary pathway (SAM-p). Therefore, it is considered as an important indicator of SNS activity and physiological/psychological stress. (Molina-Hidalgo et al 2023; Rutherfurd-Markwick et al 2017; Kilian et al 2016; Schumacher et al 2013.)

Salivary glands can be divided into three different types: parotid, submandibular and sublingual glands. Salivary alpha-amylase (sAA) is mainly produced in the parotid glands. (Schumacher et al 2013.) Acinar cells of the salivary glands are innervated by both SNS and PNS. These autonomic nerves are adjacent to both acinar and ductal cells. The afferent pathways go through the facial and glossopharyngeal nerves to a solitary nucleus in the medulla. The parasympathetic efferent pathways for the parotid gland originate from the glossopharyngeal nerve via the otic ganglion. In response to stimuli, neurotransmitter norepinephrine binds to both alpha- and beta-adrenergic receptors of the acinar cell. Especially beta-receptor activation is linked to secretion of salivary proteins, that are stored in membrane-bound secretory granules. sAA consists of 40-50% of the total salivary gland-produced proteins, and in parotid gland, 80% of the total enzymes. (Nate & Rohleder 2009.)

The primary function of alpha-amylase is to digestion of macromolecules, especially carbohydates and starch. It splits carbohydrates into more digestible oligosaccharides, which is key in acute stress responses. (Kilian et al 2016; Schumacher et al 2013; Nater & Rohleder 2009.)

Normal range of salivary alpha-amylase (sAA) is between 90-250U/mL. (Giacomello et al 2020.) In conditions, where ANS activation is increased, sAA levels are shown to increase (Nater & Rohleder 2009). sAA also demonstrates a diurnal profile: 30 minutes from awakening sAA levels sharply decrease, and thereafter steadily rise reaching a peak in the late afternoon/evening (Molina-Hidalgo et al 2023; Schumacher et al 2013).

However, correlations between sAA and other SNS activity parameters (e.g. plasma epinephrine and norepinephrine, cardiovascular parameters and skin conductance levels) have been mixed and inconsistent in studies even though pathways that lead to secretion of sAA are confirmed to be sympathetic/parasympathetic in nature (Schumacher et al 2013; Nater & Rohleder 2009). Instead, it has been suggested, that sAA could function as an additional tool for the biological factors due to its lack of certain association with other markers (Schumacher et al 2013).

3.1.3 Dehydroepiandosterone sulfate (DHEA-S)

Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) are anabolic steroids, that function as sex steroid precursors (Dutheil et al 2021; Giacomello et al 2020). DHEA and DHEA-S are synthesized and secreted in the zona reticularis of the adrenal cortex in the response to ACTH via Δ5 pathway (Dutheil et al 2021; Maninger et al 2009). DHEA is synthesized from cholesterol by two steroid metabolizing enzymes. Cholesterol is converted into pregnenolone by mitochondrial enzyme cholesterol side chain cleavage (P450scc). Pregnenolone is converted further into DHEA by the enzyme cytochrome P450c17, that is expressed in adrenals, gonads, and the brain. P450scc catalyzes both 17-alpha-hydroxylation reaction, that converts pregnenolone to 17-OH-pregnenolone, and the 17,20-lyase reaction converts 17-OH-pregnenolone to DHEA. Finally, the sulfation of DHEA into its more stable sulfate ester DHEA-S is catalyzed by the enzyme hydroxysteroid sulfotransferase (DHEA sulfotransferase). Downstream conversion continues into androstenedione and further into other sex steroids. (Maninger et al 2009.) Figure 6 demonstrates the synthesis pathway of DHEA and DHEA-S.

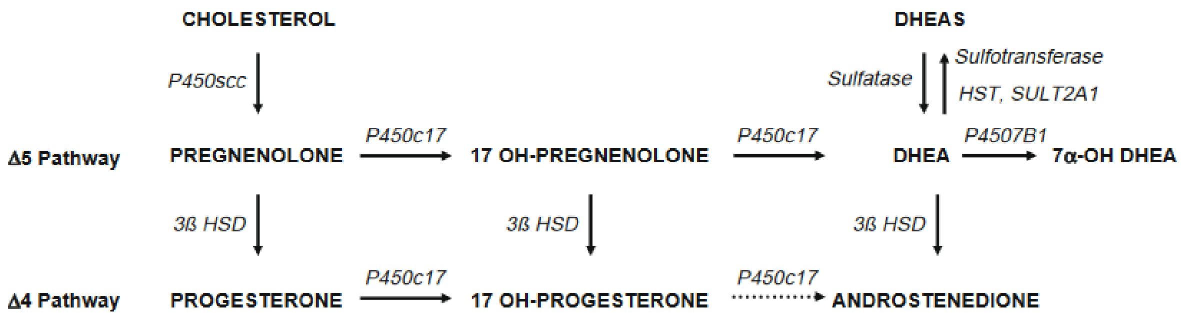

FIGURE 6. DHEA and DHEA-S synthesis (Maninger et al 2009)

DHEA and DHEA-S have been labeled as neurosteroids due to their high levels in the brain than in the plasma (Maninger et al 2009). Average concentrations in saliva are between 0,2-2,7 ng/mL, and is highly dependent on age, sex and time of the day (Giacomello et al 2020). DHEA-S has been suggested to be locally synthesized, and predominantly transported out of the brain across the blood-brain barrier due to its hydrophilic nature as a sulfated steroid. Consequently, DHEA-S is the most abundant circulating steroid hormone in the human body. (Maninger et al 2009.) DHEA-S is more prone to bind to albumin than DHEA, and as being more stable in nature, it has a longer biological half-life of 16 hours. Consequently, DHEA-S has been suggested a stable index of adrenocortical activity linked with chronic stress, whereas DHEA to reflect acute stress, however both are reported to increase in acute stress situations. (Dutheil et al 2021; Giacomello et al 2020.)

These neurosteroids (especially DHEA-S) have been suggested to have a regenerative role, that involves e.g. neuroprotection and antiglucocorticoid effects (Dutheil et al 2021; Maninger et al 2009). Moreover, ACTH has been suggested to stimulate the secretion of DHEA-S (Dote-Montero et al 2021b). However, the mechanisms, how DHEA and DHEA-S work in an anti-glucocorticoid manner, are not conclusive. It has been suggested in vitro studies, that they work through antagonistic effects in glucocorticoid receptor translocation, or in local cortisol catalyzer 11β -hydroxysteroid dehydrogenase (11β-HSD) type 1 and type 2. (Dutheil et al 2021; Maninger et al 2009.) Therefore, DHEA-S could play a protective role against other stress hormones (Dutheil et al 2021).

3.2 Cardiovascular stress responses

Heart has dual neural innervation, of which autonomic nervous system (ANS) is the most prominent determinant of cardiac functions (Végh et al 2016; Thayer et al 2012). Primary parasympathetic nerve (vagal nerve) with spinal sympathetic inputs balances the cardiac autonomic regulation (Végh et al 2016; Thayer et al 2012; Acharya et al 2006). Cardiac sympathetic nerves arise from the cervical and thoracic portions of the spinal cord, where they project two chains of sympathetic ganglia parallel to the spinal cord. From these ganglia, neurotransmitters forward signals from the CNS to postgangliotic nerves via acetylcholine. Acetylcholine functions as a pregangliotic neurotransmitter and norepinephrine as postgangliotic neurotransmitter. (Végh et al 2016.) Figure 6 demonstrates the cardiac innervation.

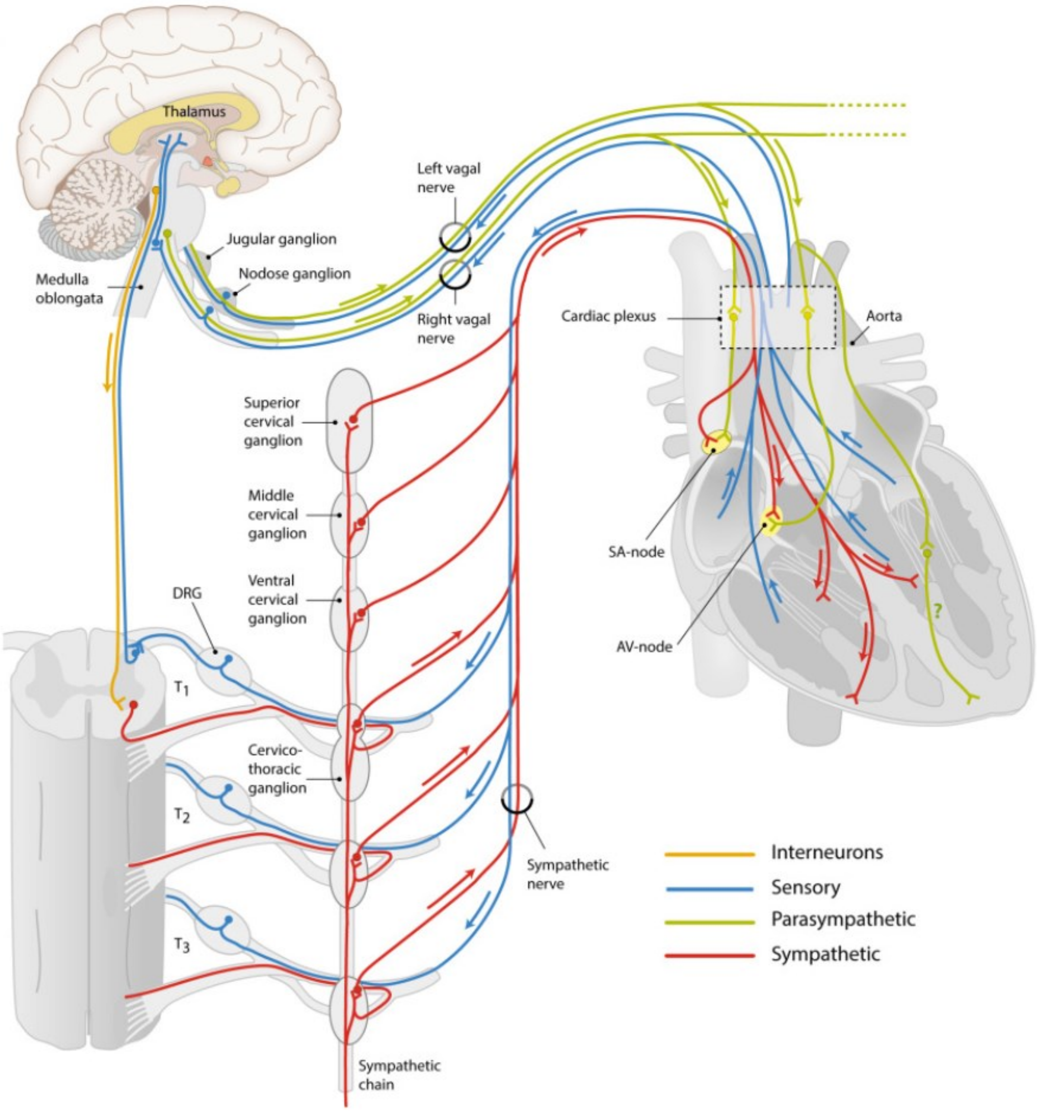

FIGURE 6. Overview of the neural innervation of the heart (Végh et al 2016)

Sympathetic nervous system (SNS) effects in the heart are slow in nature (in scale of seconds), whereas the parasympathetic effects are fast, implemented in milliseconds. Parasympathetic effects are therefore only capable of producing rapid changes, when regarding beat-to-beat effects of the heart. (Thayer et al 2012.) Sympathetic stimulation (and decreased parasympathetic stimulation) leads to increase in HR by increasing the firing rate of pacemaker cells in the heart's sinoatrial node (Thayer et al 2012; Acharya et al 2006). Parasympathetic activity (and decreased sympathetic stimulation) on the contrary decreases the firing rate (Acharya et al 2006). These rhythmic contributions modulate the heart rate intervals (RR) of the QRS-complex in the electrocardiogram (ECG) at different frequencies: sympathetic activity being associated with 0,04-0,15 Hz and parasympathetic with 0,15-0,4 Hz (Thayer et al 2012;

Acharya et al 2006). The degree of variability defines the functioning of the nervous control of the HR, and make the foundation of the HRV analysis for SNS and PNS (Acharya et al 2006).

Heart rate variability (HRV) has been suggested to give information about the sympathetic-parasympathetic autonomic balance, and the heart's ability to adapt to changing conditions. It is defined as the variation over time of the period between consecutive heartbeats and is dependent on the extrinsic regulation of heart rate (HR). The normal variability in HR happens due to the autonomic neural regulation of the heart and the circulatory system. (Acharya et al 2006.)

Resting heart rate variability (HRV) has been suggested to be a marker for flexible dynamic regulation of the autonomic activity. A low HRV is associated with risk of all-cause mortality and has been proposed as a marker for disease occurrences. It has been shown that individuals with low resting HRV show delayed recovery from psychological stressors of cardiovascular and endocrine responses compared to those with higher levels of resting HRV. This concludes that individuals with high resting levels of HRV could experience appropriate recovery after the stressor has ended. (Thayer et al 2012.)

Cardiovascular responses to stressful situations (e.g. competition) are influenced by cognitive (e.g. past experience), intrapersonal (e.g. personality traits), interpersonal (e.g. relationships) and biological factors (e.g. genetic factors) of an individual (Leis & Lautenbach 2020). In flight-or-fight responses, conduction system will be stimulated, and HR and contraction force elevated through stimulation of the ventricles (Végh et al 2016). In stressful situations, passive stressors have been recognized to induce primarily α-adrenergic reactions with more vascular perturbation, whereas active stressors have been found to affect primarily β-adrenergic pathways with increased myocardial responses (Chauntry et al 2022). These increases in cardiovascular tone contribute to promote availability of vital substrates (Charmandari et al 2005).

3.3 Specialized stress responses

In this thesis's context, insight to exercise-induced and video game playing/esports-induced stress responses are introduced to help understand the mechanisms and current evidence behind these specialized stress conditions.

3.3.1 Exercise-induced stress responses

Voluntary exercise as a stressor has been shown to promote both major neuroendocrine stress response systems: hypothalamic-pituitary-adrenal (HPA) axis and sympathoadrenal-medulla pathway (SAM-p) (Rutherfurd-Marwick et al 2017; Zschucke et al 2015; Dote-Montero et al 2021b). During exercise, autonomic nervous system (ANS) increases cardiac activity by sympathetic nervous system (SNS) activity and control hemodynamics by reducing parasympathetic nervous system (PNS) activity (Mao et al 2022; Nater & Rohleder 2009). After exercise, it regulates the cardiovascular system to promote recovery, and as a long-term adaptation, faster recovery. (Mao et al 2022; Zschucke et al 2015.)

As in other stressful conditions, sympathoadrenal-medulla pathway (SAM-p) activation functions as an immediate response to exercise, the HPA axis shows a delayed response, which demand hemodynamic, endocrine, and metabolic adaptations to restore homeostasis. (Rutherfurd-Marwick et al 2017; Zschucke et al 2015). SAM-p and HPA axis activation depend on the stressor intensity and duration (Zschucke et al 2015). As a physical stressor, exercise has been shown to activate HPA axis at the intensities of 60% VO_{2peak} (Caplin et al 2021; Kilian et al 2016). Beyond this threshold, a single bout of exercise induces a cortisol response, that is linearly proportional to the intensity at which the exercise is performed (Caplin et al 2021). Figure 7 demonstrates the simplified model of HPA stress response during exercise.

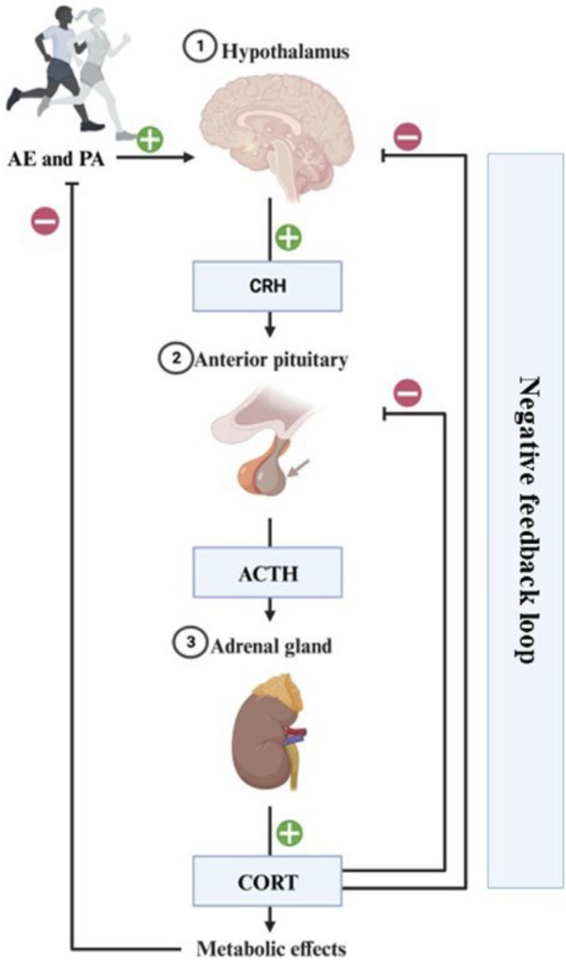

FIGURE 7. Simplification of the hypothalamic-pituitary-adrenal (HPA) stress response to aerobic exercise/physical activity (Molina-Hidalgo et al 2023)

Several positive effects of exercise to stress responses have been suggested. High cardiorespiratory fitness has been related to smaller systolic blood pressure (SBP) reactivity, faster heart rate (HR) recovery post-stress and larger HR stress reactivity. Moreover, high volumes of physical activity are related to attenuated HR and cortisol responses to stress. (Chauntry et al 2022.) Exercise has also been suggested to protect hippocampal neurons against cell damage usually caused by high level of glucocorticoid exposition. (Zschucke et al 2015.) However, even though voluntary exercise is generally not seen as a stressor (rather as positive eustress), regular exercise could promote the detrimental effects stress (Monje et al 2020; Zschucke et al 2015).

Salivary cortisol levels have been reported to increase in relation to the intensity and duration of continuous endurance exercise in men, as well as high intensity short duration endurance exercise in women (Monje et al 2020). If exercise stress is excessive, cortisol production increases and may cause overtraining symptoms (Dote-Montero et al 2021b). However, post-exercise increases in cortisol are essential, due to its catabolic effect onto exercise-induced damage on proteins, which leads a “pool” to synthesis for new amino acids. Increased energy and protein turnover are more likely to occur when intense training is conducted compared to high-volume training (Kilian et al 2016). It has been suggested that consistent exercising at vigorous intensities with adequate recovery might help promote anabolic steroid (e.g. DHEA-S) synthesis, normalize daily cortisol rhythms and mitigate abnormal cortisol responses among groups, who are particularly vulnerable to stressor exposures (Caplin et al 2021; Dote-Montero et al 2021b).

Salivary alpha-amylase (sAA) has been titled as the most sensitive stress response marker and an exercise-induced stress marker due its direct production in saliva (Monje et al 2020; Kilian et al 2016). Acute increases in sAA levels have been reported following strenuous activities such as short progressive tests to exhaustion (Monje et al 2020). The increases in protein levels in saliva following exercise may be due to increased adrenergic activity in the salivary glands and increases in energy-demand (causing hydrolysis of starch to glucose and maltose) during strenuous events (Monje et al 2020; Nater & Rohleder 2009). A linear increase in sAA with peak values at the end of the exercise and a full recovery after 30 minutes after the exercise have been documented. (Kilian et al 2016.)

Despite the physiological stress effects of exercise, it has been suggested that various modalities of acute exercise (e.g. ergometer cycling) can reduce the HPA axis salivary cortisol response to various (especially psychosocial) stressor tasks (Caplin et al 2021). In Zschucke et al (2015) study, overall cortisol output was reduced after a mild cognitive challenge task, when participants engaged in 30 minutes of moderate-to-vigorous aerobic exercise (60-70% VO_{2peak}) before the mental challenges compared to non-exercise controls. Moreover, acute exercise has been found to reduce self-reported distress and anxiety in response to stressful situations (Zschucke et al 2015). These findings may contribute to the factor, that exercise might play a protective role against allostatic load (Molina-Hidalgo et al 2023).

The underlying mechanisms for dampening stress responses of exercise are still unclear, but many theories have been made (Caplin et al 2021; Dote-Montero et al 2021b; Zschucke et al 2015). It has been theorized, that repeated challenge of one type of stressor (e.g. exercise) can result in adaptations, that are able to habituate or sensitize the physiologic stress system to dissimilar stressors, such as psychosocial stressor. Studies over this theory are mixed. (Molina-Hidalgo et al 2023; Caplin et al 2021; Zschucke et al 2015.) Secondly, it has been suggested aerobic exercise's effects on key brain areas (hippocampal and prefrontal cortex subregions), that are involved in the HPA axis negative feedback chain (Zschucke et al 2015). Thirdly, exercise-induced increases in regenerative dehydroepiandrosterone sulfate (DHEA-S) have been suggested to connect with increases in adrenocorticotropic hormone (ACTH), however studies are mixed (Dote-Montero et al 2021b). Lastly, one suggestion has been, that exercise (as a stressor) activates HPA/gonadal axis, but reaching an overwhelming threshold (e.g. injury or overtraining), the steroid synthesis pathway is directed towards the production and release of cortisol, whereas manageable stress levels would direct the synthesis pathway activation towards positive outcomes (e.g. DHEA-S). (Dote-Montero et al 2021b.)

3.3.2 Video game -induced stress responses

Esports presents a similar stress-environment as other competitive traditional sports and professional players are susceptible to high stress levels (Zimmer et al 2022; Leis & Lautenbach 2020). Even though esports does not involve the level of physical strain (stress) compared to other sports forms, esports may elicit a stress psychological and physiological environment, that leads in ANS alteration, and potentially to a risk of physical strain and injury (Sousa et al 2020; Leis & Lautenbach 2020). Competition, workload, socio-evaluative threat, and emotions are fundamental factors in esports, which has been suggested to relate to the psychophysiological state (Leis & Lautenbach 2020; van der Vijgh et al 2015). Understanding psychophysiological stress in esports is fundamental, so that players could maintain cognitive (e.g. strategic thinking) and motoric performance (e.g. fine motoric functions) in competitive and stressful situations (Sadowska et al 2023; Leis & Lautenbach 2020).

Despite the sedentary nature of the activity, esports have been shown to elicit physiological responses, especially increases in sympathetic nervous system (SNS) activation, including increased blood pressure (especially systolic blood pressure, SBP) and changes in heart rate (HR) and heart rate variability (HRV) (Sadowska et al 2023; Kraemer et al 2022; Sousa et al 2020; Toth et al 2020; Siervo et al 2018; van der Vijgh et al 2015). HR has been reported to vary between 40-70% of the age predicted HR_{max} range during a game session (Kraemer et al 2022). Moreover, Siervo et al (2018) reported an elevated SPB, HR and feelings of stress in relation to non-violent TV watching. The magnitudes of changes vary greatly between studies (van der Vijgh et al 2015). In HRV, studies suggest an increase in low- and high-frequency components compared to resting conditions (Leis & Lautenbach, 2020). A sympathetic drive elevations could origin from anticipatory stress, arousal, visual stimuli and emotional responses (Kraemer et al 2022).

In addition to SAM-p activation, esports are also likely to elicit physiological changes through neuroendocrine responses, especially stress hormones (e.g. cortisol and ACTH) (Sadowska et al 2023; Sousa et al 2020). Hormonal changes during esports games are generally mixed (Sadowska et al 2023). Cortisol levels have been reported to elevate during competitions, and to be higher in winners, which could indicate high levels of anxiety being beneficial for optimized gaming performance (Kraemer et al 2022; Toth et al 2020). However, Leis & Lautenbach (2020) suggest, there are no significant changes in cortisol before, during and after games, and Sadowska et al (2023) suggest, that low- to moderate elevations were found in well-performing esports athletes and decreases with lowest-performing esports athletes. In conclusion, salivary cortisol may also be related to the level of game play, experience and skill levels (Kraemer et al 2022). For sAA, Skosnik et al (2000) had found a significant increase in sAA after a 15-minute stressful video game.

Different games have been suggested to elicit differing physiological responses, which may in one way explain the variety of research findings for esports studies regarding stress physiology (Kraemer et al 2022). Action video games are hypothesized to induce an autonomic stress-related physiological excitement. This could reduce long-term plasticity in the hippocampus since the process is mediated by the amygdala. (West et al 2018.) Moreover, FPS games resulted in larger SNS responses, e.g. changes in low-to-peak HR and SBP increases when compared to MOBA games (Sadowska et al 2023; Kraemer et al 2022; Sousa et al 2020; Valladão et al 2020). Therefore, it could be stated, that FPS games elicit more SNS-based responses (Sousa et al 2020). In addition, studies have suggested, that aggressive or violent video games elicit cardiovascular responses and SBP increases, however overall studies are mixed (Sousa et al 2020; Siervo et al 2018). However, it seems that players' HR and electrodermal activity correlate with players' subjective game-playing experience (Sousa et al 2020). Figure 8 shows an example study's salivary cortisol responses to an FPS game "Overwatch".

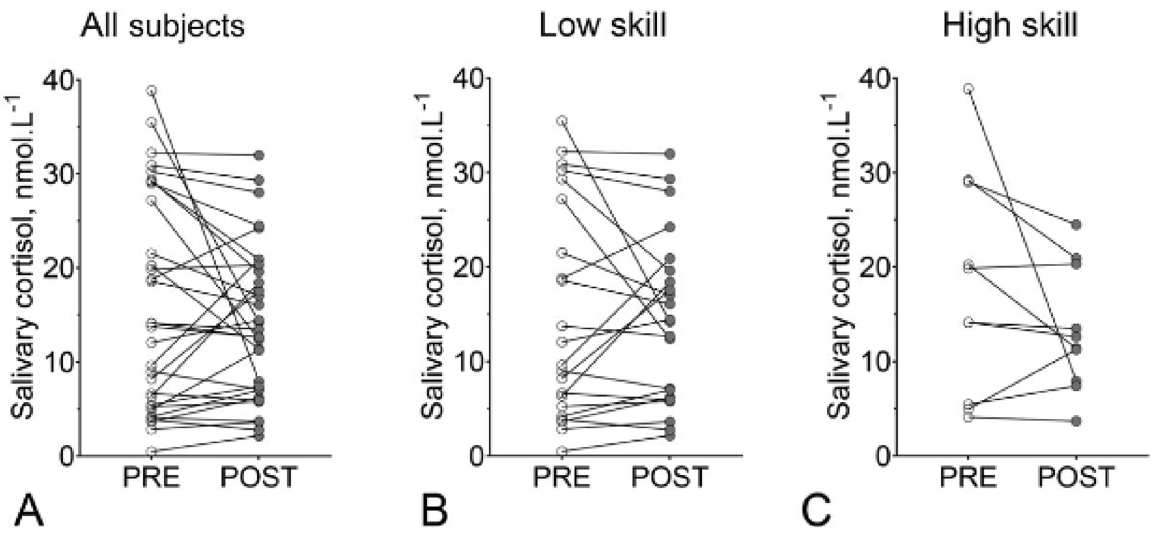

Figure 2. A) Individual responses in salivary cortisol for all players to pre to post game play. B) Individual responses of each player in the low skill group. C) Individual responses for each player in the high skill group.

FIGURE 8 Salivary cortisol responses to an FPS-game ”Overwatch”. No significant differences were observed (Kramer et al 2022)

Counter Strike: Global Offensive (CS:GO) has been shown to place high demands on players, with a variety of stressors (Birch et al 2023). CS:GO tournament conducted in Sadowska et al (2023) study, induced an increase in HR and SPB and blood cortisol. In their study, they also observed a decrease in HRV DFA-α1 values in CS:GO game, which indicates the games being stressful for the participants. (Sadowska et al 2023.) It could be concluded, that CS:GO game induces significant physiological arousal. (Sadowska et al 2023.)

Different conditions in the research setting might affect the results in esports studies. It is important, whether the testing conditions are in competitive setting (e.g. tournament), in a controlled setting (e.g. lab, simulation) or in a familiar setting (e.g. home) (Valladão et al 2020). Non-competitive settings have been reported to induce no significant changes in cortisol levels (Sadowska et al 2023). Moreover, the phase of the game could potentially be relevant. Some studies have reported significant increases in HR only during the recovery time after the second match of an tournament. SPB has been shown to increase during and after the game. Moreover, highest elevations in cortisol have been reported after 16-30 minutes of assimilation. (Leis & Lautenbach 2020.) Also facing a human counterpart or computer during the game resulted in different stress responses in HR and higher cortiol levels (Leis & Lautenbach 2020; van der

Vijgh et al 2015). Player skill level could also contribute to the players' ability to modulate stress: highly skilled players have been suggested to be able to facilitate cognitive and stress modulation compared to lower skilled and amateur players (Kraemer et al 2022).

4 HIGH INTENSITY INTERVAL TRAINING (HIIT)

High intensity interval training (HIIT) involves alternating periods of high-intensity exercise (equal or above to maximal lactate steady-state velocity) with low-intensity/passive recovery periods (Dote-Montero et al 2021b; Martínez-Díaz et al 2020; Mao et al 2022; Monje et al 2020). It has been suggested to be one of the most effective methods for improving cardiorespiratory and metabolic functions compared to traditional moderate-intensity continuous training (Dote-Montero et al 2021b; Martínez-Díaz et al 2020; Mao et al 2022). It has also been indicated, that HIIT could induce similar physiological adaptations to high-volume low-intensity training with less training times (Kilian et al 2016). Typical methods for HIIT training are either on treadmills or cycle ergometers, however resistance-type of HIIT exercises conducted with free weights, specialized equipment or body weight have drawn recent attention of general population and researchers (Mao et al 2022).

The main physiological responses produced by HIIT are improved substrate utilization, increased maximal oxygen uptake (VO_{2max}), improved cardiac and endothelial function and acute metabolic stress and hormonal responses (Monje et al 2020). The sympathetic nervous system (SNS) response to HIIT exercise can be seen as elevated hormonal (e.g. cortisol and sAA) levels after 20-30 minutes of HIIT session (Martínez-Díaz et al 2020; Monje et al 2020). Cardiac parasympathetic activity therefore becomes suppressed and returns fully recovered after at least 24 hours of single aerobic-based training session (Mao et al 2022).

Being intensity-dependent responder, circulating cortisol has been suggested to elevate after HIIT (Dote-Montero et al 2021a; Martínez-Díaz et al 2020; Quintero et al 2018). After a HIIT session, cortisol levels have been acutely (0-60 min) to elevate above resting levels, to fall below the resting levels in 120-180 minutes and to return to resting levels after 24 hours. Longer (≥60 s) intervals in a HIIT session would seem to produce a greater elevation in cortisol levels. Due to HIIT's intense nature, cortisol level decreases demand a longer time to recover. (Dote-Montero et al 2021a.) In long-term perspective, prolonged periods of intensified training have been shown to decrease sAA and increase salivary cortisol concentrations (Kilian et al 2016). Moreover, 12-week HIIT training program significantly improved steroid hormone status in Dote-Montero et al (2021b) study with inactive middle-aged men.

High-intensity interval training (HIIT) facilitates increases in circulating lactate, that is a key biomarker for neural metabolism and neural sensitization in the brain (Magistretti & Allaman 2018; Moreau & Chou 2019). It also has been suggested, that a single HIIT session could promote executive function (e.g. working memory) with changes in blood flow (leading to increased oxygen and nutrition supply), biochemical markers (e.g. brain-derived neurotropic factor BDNF), electrical neural activation and faster handling of stimuli (Martínez-Díaz et al 2020; Kao et al 2018; Hötting & Röder 2013). However, on the other hand, elevations in cortisol and catecholamine levels induced by HIIT have been suggested to be detrimental to cognitive performance (Quintero et al 2018; Martínez-Díaz et al 2020). Especially catecholamine level increases lead to activation of the limbic system at the expense of prefrontal lobes. This could lead to a decrease in executive control tasks. (Quintero et al 2018.)

5 STRESS PHYSIOLOGY METHODOLOGY

When concerning stress response study designs, Kudielka & Kirschbaum (2005) state important characteristics to take in concideration: sample selection/composition and sample size, characteristics of the applied stressor, studied outcome and implementation of the study protocol. Health status as well as age at testing are important concierations. (Kudielka & Kirschbaum 2005.)

Type of the stressor (e.g. psychological/physical/pharmacological stress and real-life/laboratory setting stressor) and duration of stress exposure (acute/chronic) are important in study design as well. For example, laboratory stress protocols offer the advantage of standardization across test sessions, but might lack the ecological validity of field studies. (Kudielka & Kirschbaum 2005.) Different methods can involve costly apparatus and labor-intensive complex techniques, e.g. impedance cardiography or electrocardiograms, which are inapplicable in field research settings (Schumacher et al 2013).

It has to be concidered, that there are several sources of inter-individual differences in stress responses in humans which should, if possible be held constant or controlled (e.g. health status, time of day, habituation effects due to repeated stress exposure). Moreover, time to recovery seems to be an important index in assessment of stress effects. (Kudielka & Kirschbaum 2005.)

The focus of this thesis is on cardiovascular and endocrinological response assessment methodology. Common methodology is introduced in heart rate (HR), heart rate variability (HRV) and salivary sampling and analysis methods on common stress hormones (cortisol, alpha-amylase, dehydroepiandrosterone sulfate (DHEA-S).

5.1 Stress hormones

Relevant stress hormones covering the hypothalamic-pituitary-adrenal (HPA) axis, the steroid hormones, the autonomic nervous system (ANS), the immune system, different metabolic processes and the antioxidant defence system have been suggested to be cortisol, DHEA-S, testosterone, sAA, CgA and sIgA. Multi-analyte methods allow an efficient simultaneous measurement, and helps to reliably assess possible stress-related risks in a multifactorial design, however a comprehensive detection of small molecules, like catecholamines and steroids together with larger proteins may remain challenging. (Giacomello et al 2020.) In this study, the focus is set on cortisol, sAA and DHEA-S sampling and analysis methods.

Common methods to measure cortisol are via blood, urine and saliva. Blood can provide information on the cortisol diurnal rhythm, as urine levels indicate the cortisol production over an interval of time (Gatti et al 2009). Blood sampling is an example of an invasive method, which are often accompanied with anxiety, that could (in stress response context) serve as a stressor itself, leading to catecholamine release and biased data (Schumacher et al 2013).

A strong positive correlation between several analytes and blood serum levels have been reported (e.g. cortisol and DHEA-S) (Giacomello et al 2020; Gatti et al 2009). Saliva as a product is less complex than blood serum and includes less proteins, therefore requiring less preparation for analysis (Rutherfurd-Marwick et al 2017). Salivary samples offer a practical, non-invasive and inexpensive method for different inner strain and recovery state biochemical marker analysis, and can be collected at all times without the assistance of trained staff (Giacomello et al 2020; Dutheil et al 2021; Djaoui et al 2017; Schumacher et al 2013; Gatti et al 2009; Kudielka & Kirschbaum 2005). Salivary sampling makes it also possible to collect multiple saliva samples in short time intervals (Schumacher et al 2013).

In stress response studies, saliva carries multiple markers of HPA and sympatho-adreno-medullary pathway (SAM-p) activation biomarkers (Rutherfurd-Markwick et al 2017). Salivary cortisol has been suggested to be a reflector the activation of the hypothalamus-pituitary adrenal (HPA) system, as salivary alpha-amylase (sAA) for sympathetic nervous system activity, therefore facilitating simultaneous investigation of the activity of the two major stress systems in ecologically valid settings (Schumacher et al 2013).

Saliva can be divided into whole saliva (derived from different salivary glands) and duct saliva (derived from a single salivary gland). Moreover, saliva can be divided into stimulated and unstimulated saliva, where under stimulation, 50% of total saliva is produced (via sympathetic innervation) in the parotid glands. (Schumacher et al 2013.)

Various devices can be utilized and the saliva can be collected by stimulation (e.g. cotton swabs) or by passive drooling (golden standard) (Rutherfurd-Markwick et al 2017; Gatti et al 2009). Despite being the golden standard method of salivary sampling, passive drooling may have challenges of low saliva flow rate. In exercise studies, exercise has been found to affect the salivary flow rate. (Rutherfurd-Markwick et al 2017.)

The studies are generally mixed about differences between stimulated and non-stimulated collection methods, as tthere have also been reports of cotton swabs (Salivette®) and passive drool collection having no significant differences in cortisol and DHEA-S collections (Giacomello et al 2020). However, Salivette® is the preferred method of saliva collection and simple for subjects to conduct without technical personnel. It is important to tak in account, that the collection method and the devices used (e.g. sampling area of the mouth with sAA being more effective in parotid and submandibular glands) has been found to also influences the accuracy of the result. (Giacomello et al 2020; Gatti et al 2009.) Figure 9 demonstrates Salivette® tube.

FIGURE 9. Salivette® tube (Salivette 2023)

Steroids have been found to be fairly stable biomarkers in saliva. Cortisol concentrations are stable at 5ºC for up to 3 months or at -20ºC and -80ºC for at least one year. It has also been reported, that cortisol samples resist at least five days between 15-38ºC, and after one month in room temperature variations have been detected. (Giacomello et al 2020.) Enzymatic activity in sAA remained stable for at least 8 hours at room temperature (some studies suggest up to three weeks) and after four weeks at -80ºC, however in 4ºC there was a decrease of approximately 15% (Giacomello et al 2020; Schumacher et al 2013). sAA samples have been recorded to be robust against repeated freeze-thaw cycles (Schumacher et al 2013). There are limited stability studies for DHEA-S in saliva (Giacomello et al 2020). The stability of the steroids benefits also study practicalities, as samples can be delivered to the laboratory without freezing (Gatti et al 2009).

The sampling procedure of saliva sampling needs precautions to be accurate and reliable. It is recommended not to eat, smoke, drink or brush teeth for at least one hour before the collection to prevent sample contamination. (Giacomello et al 2020.) Moreover, sampling time is fundamental for interpreting results due to steroids diurnal and stress-induced responsiveness as discussed in previous parts of this thesis. In summary, e.g. free salivary cortisol responses can be observed 5-20 minutes after stress with peak levels 10-30 minutes after cessation of the stressor, and 30-40 minutes after awakening (Giacomello et al 2020; Kudielka & Kirschbaum 2009).

Limitations for salivary sampling are potential insufficient quantity of saliva and different sleeping patterns in different individuals and within same individuals (e.g. altered sleep-wake cycles, jetlag) (Gatti et al 2009).

Radioimmunoassays (RIAs) used to be a common method to analyze biomarkers, but due to the use of radioactive reagents, are now recommended to be avoided. Currently, the most frequently used methods for stress marker analysis are enzyme-linked immunosorbent assays (ELISAs) and immune-based biosensors. Immunoassays are based on the chemical binding reaction between antibodies and an analyte. (Giacomello et al 2020.)

The advantages of immunoassays include their effective usability in clinical routine with several commercially available kits. They are simple to use, sensitive, rapid, and relatively cheap in costs. Classical immunoassays represent single analyte assays only. (Giacomello et al 2020.)

Disadvantages of immunoassays are potential cross-reactivities with structurally related compounds (e.g. cortisol – cortisone) may confound the analytical results, especially if differences in concentration between the analyte and cross-reacting agent are very high. Differences in the concentration levels could require cross-reactivities far below 1%. Moreover, it is often not possible to compare different immunoassay results, therefore conversion tables have been developed to obtain factor scores between results obtained with most common immunoassays. (Giacomello et al 2020.)

For cortisol detection, liquid chromatography tandem mass spectrometry (LC-MS/MS) is often performed for selectivity and sensitivity. Compared to immunoassays to LC-MS/MS methods, there was a non-linear relation, which may in terms of immunoassay's cross-reactivity with saliva matrix components. However, immunoassays have been stated as the golden standard in cortisol detection due to its simplicity, rapidness, economic use, sensitivity and reproducibility. (Giacomello et al 2020.)

The most common methods to detect salivary alpha-amylase (sAA) is measuring its activity (e.g. rate of degrading starch) and electrochemistry. Activity measures vary a lot throughout studies, which has made it challenging to compare results between studies. Electrochemistry allows the realization of simple, inexpensive and portable assays suitable for point-of-care testing. For example amperometric method with screen-printed carbon electrodes, which detected sAA indirectly: first reaction being hydrolysis of starch to maltose by alpha-amylase followed by the conversion of $[Fe(CN)6\ ]3-$ into $[Fe(CN)6\ ]4-$ due to the reducing sugar produced in the first step. (Giacomello et al 2020.)

Detection of salivary DHEA-S has been reported to utilize immunoassays or HPLC-based methods. Due to their selectivity, LC-MS/MS based methods are concidered appropriate for determination of salivary DHEA-S. It has been demonstrated a high imprecision of some kits for DHEA-S by comparison of different immunoassay kits and isotope-dilution-liquid chromatography tandem mass spectrometry (LC-MS/MS). (Giacomello et al 2020.)

5.2 Heart rate (HR) and heart rate variability (HRV)

Heart rate (HR) and blood pressure have been regarded to be the golden standard for autonomic nervous system assessment as they are not easily influenced by stress hormones, but also by other factors such as posture (Schumacher et al 2013).

Heart rate variability (HRV) is the phenomenon of the variation in the time intervals between sequential heartbeats (Sadowska et al 2023; Thayer et al 2012). HRV has been concerned a reliable mean to indirectly observe autonomic nervous system (ANS) status due to hearts dual innervation by ANS. (Sadowska et al 2023; Thayer et al 2012.) HRV has proved to be a valuable tool to investigate the sympathetic and parasympathetic function of the ANS. (Acharya et al

2006.) In exercise studies, HRV produces a rapid and non-invasive way to assess changes in cardiac parasympathetic activity (Mao et al 2022).

Studies have shown a variety of HRV recording times: 24-48 hour, 36-hour and shorter periods 2-12 hours and ultrashorts 5-10 min (Järvelin-Pasanen et al 2018). Short-term measures of HRV (e.g. standard deviation of 5-minute intervals) and beat-to-beat measures (e.g. root mean square of successive RR differences (RMMSD)) have all been commonly used in HRV analysis (Thayer et al 2012).

HRV measurements are easy to perform, noninvasive and have good reproducibility, if used in standardized conditions (Acharya et al 2006). HRV has been commonly been analysed using a Holter electrocardiogram (ECG) device and HR monitors (e.g. Polar) in studies. Holter ECG has been recommended as the golden standard in esports research, however in low-to-moderate activities, some conductance sensors (e.g. Polar) have shown similar qualities (Welsh et al 2023). In general, ECG should be preferred over HR monitors due to its ability of verify abnormal beat intervals and possible ectopic beats. Moreover, ECG can estimate respiratory rate in ECG data. In addition, continuous optical pulse measurement devices have become popular, but the accuracy of the method has bee nfound a challenge. (Järvelin-Pasanen et al 2018.) In esports settings, photoplethysmography is not recommended due to its location in finger or wrist, which could be inconvenient in esports settings (Welsh et al 2023).

HRV analysis can be divided into three categories: time domain analysis, frequency domain analysis and nonlinear methods. **Time domain** methods are calculation-wise simple, but lack the ability to discriminate between sympathetic and parasympathetic contributions of HRV, and might ignore subtle changes in HRV. In time domain analysis, two types of HRV indices can be distinguished. Short term variability (STV) reflect fast changes in HR and long-term variability (LTV) reflect slower fluctuations. Both are calculated from the heartbeat (RR) intervals in a certain time-window (commonly between 0,5-5,0 minutes). Figure 10 visualizes a RR interval in practice. From the RR intervals, a number of parameters can be calculated: SDNN (the standard deviation of the NN intervals), SENN (standard error of the mean, an estimate of the standard deviation of the sampling distribution of means based on the data), SDSD (standard deviation of differences between adjacent NN intervals), RMSSD (the root mean square successive difference of intervals) and pNN50% (the number of successive difference of intervals which differ by more than 50 ms expressed as a percentage of the total number of ECG cycles analyzed). (Acharya et al 2006.)

FIGURE 10. RR-interval (Firstbeat 2023)

Most widely used time domain index is the average HR, SDNN and RMMSD. All the time domain measure indices could be affected by artifacts and outliers, and these measures therefore require data from which artifacts and ectopic beats have been carefully eliminated. (Acharya et al 2006.)

Frequency domain analysis is a a unique, noninvasive tool for achieving a more precise assessment of autonomic function in both the experimental and clinical settings (Acharya et al 2006). The power spectrum analysis can be a useful tool to assess ANS function (Acharya et al 2006). In the power spectrum analysis, short-term time series contains two main components: a high frequency (HF) component (0,15-0,40 Hz) and low frequency (LF) component (0,01-0,15 Hz) reflecting cardiac vagal tone. (Thayer et al 2012.). LF power spectrum component is more complicated, since it is modulated by both sympathetic and parasympathetic outflows, and other factors (e.g. baroreceptor activity) (Acharya et al 2006).

The **nonlinear dynamical techniques** are based on chaos theory, and it has been applied to many areas including the areas of medicine and biology. The parameters like correlation dimension (CD), largest Lyapunov exponent (LLE), SD1/SD2 of Poincare plots, ApEn, Hurst exponent, fractal dimension, alpha-slope of DFA and recurrence plots have been recognized. Poincare plots (a quantitative-visual technique from nonlinear dynamics) can be used to portray the nature of RR interval fluctuations. In the plot, RR intervals are plotted as a function of the previous RR interval. It summarizes information and provides detailed beat-to-beat information of the heart. Quantitative analysis happens by calculating the standard deviations of the distances of the R-R(i) to the lines y=x and y=-x + 2R-Rm, where R-Rm is the mean of all R-R(i). The standard deviations are referred to as SD1 and SD2, respectively. SD1 related to the fast beat-to-beat variability of the data, while SD2 describes the longer-term variability of R-R(i). The ratio of SD1/SD2 can be used to describe the relationship between short interval to the long interval variation. (Acharya et al 2006.) Figure 11 demonstrates a poincare plot of a normal subject.

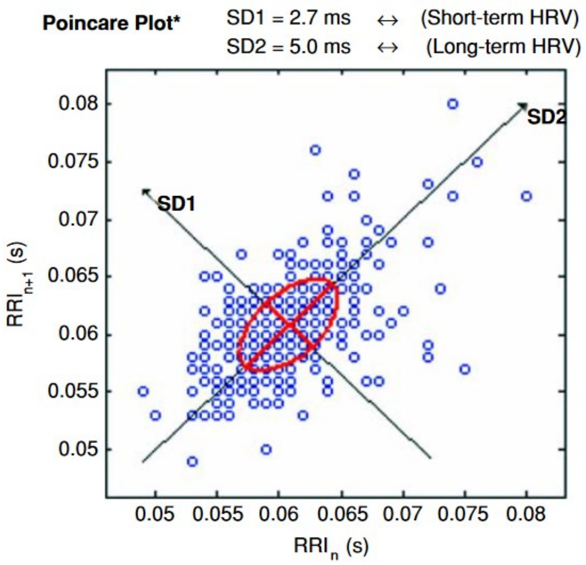

FIGURE 11. Poincare plot of a normal subject (Acharya et al 2006)

Main problems of the nonlinear analysis, when applying to biological signals can be a high level of random noise in the biological data, short experimental data sets due to the low frequencies of the biological signals, non-stationarity of the biological systems (e.g. ECG having different modulations indluenced by various external factors) and spatially extended character of the system. (Acharya et al 2006.)

6 PURPOSE AND AIM OF THE STUDY

The purpose of the study was to investigate, if an acute exercise session could have an effect on acute stress responses and/or performance in esports players. The aim of the study was to investigate, if a high-intensity interval training (HIIT) session had an effect on specific acute stress-biomarkers (cortisol, salivary alpha-amylase (sAA) and dehydroepiandrosterone sulfate (DHEA-S)) in addition to specific esports performance parameters (kills per second (KPS) and completion time) in first-person shooter (FPS) game Counter Strike: Global Offensive (CS: GO).

The main research questions of the study are:

1) Does a preceeding high-intensity interval training (HIIT) session affect acute physiological salivary stress markers (cortisol, alpha-amylase and dehydroandrosterone sulfate (DHEA-S), heart rate (HR) and heart rate variability (HRV)) induced by esports gaming task?
2) Does a preceeding high-intensity interval training (HIIT) session affect esports performance parameters (completion time and kills per second KPS)?

Due to the literature presented previously, this thesis states the following hypotheses:

1) **High-intensity interval training (HIIT) session enhances changes in salivary stress marker (cortisol, alpha-amylase and DHEA-S), HR and HRV responses.**

HIIT training is expected to elevate cortisol levels compared to changes in cortisol induced by esports gaming task alone. Both HIIT training and video gaming has been observed to elevate cortisol levels (Dote-Montero et al 2021; Toth et al 2020). Moreover, during video gaming sessions elevations in HR and high-frequency/low-frequency ratio in HRV have been observed (Toth et al 2020).

2) **Acute high-intensity interval training session improves esports performance parameters (completion time and kills per second KPS).**

De Las Heras et al (2020) had found a positive effect of 15-minute HIIT session on esports performance parameters (accuracy and targets eliminated) in multiplayer online battle arena

(MOBA) game League of Legends. Moreover, due to cardiovascular exercise's beneficial effects on cognitive function, it would be expected to see to improvements in video game performance (De Las Heras 2020; Martínez-Diaz et al 2020).

7 METHODS

This thesis study setting used De Las Heras et al (2020) study setting as the foundation. Additional measurements (e.g. physical activity inventory IPAQ, saliva sampling) were added to the setting to elaborate the subjects' physical activity status and physiological stress responses, and substitutes for others (e.g. Profile of Mood States for Positive and Negative Affect Schedule Scale) were made. Moreover, video game task was changed from a MOBA game League of Legends to an faster-paced FPS game (Counter Strike: Global Offensive). From initial evaluation, cognitive status with National Institutes of Health (NIH) cognitive toolbox wasn't used in this study setting.

7.1 Ethicality

The conduction of this study was approved with Jyväskylä University Ethics Committee. The study included measurements of the subjects' physical condition, cardiac function and stress state. The subjects are participating voluntarily, and they were informed about their right to cancel their participation at any point of the study. Moreover, they were informed their cancellation of participation wouldn't affect their position in a team. Before writing the agreement to participation, the subjects were informed about the study's subject, purpose, confidentiality and handling the data. The subjects were educated about the study's contents, benefits and potential risks. The voluntary subjects were asked to sign an agreement, in which they accepted to understand the voluntary participation and handling the personal data, and in which they guaranteed to participate as healthy subjects. As a part of the agreement, the subjects were given an abstract of the study and a data protection informing letter in printed and electronic forms. If the subjects preferred, they were informed about the scientific points of views, confidentiality procedures and publications of the results. The subjects were given enough time to familiarize with the documents before signing the participation agreement.

In the study, the subjects are going to play a game Counter Strike: Global Offensive, with an age restriction of 16 years. The content warnings of the game is violence. The subjects are recruited according to the age restriction, so that the exposure to violent content is producing the least developmental harm. All subjects are playing the mentioned game with a goal-setted mind, and are therefore not exposed to violent content more than in normal conditions.

In this study, information about the subjects' health is collected. With a health questionnaire, a sufficient health status is confirmed to participate in the study. Physical testing and physical exercise of esports players, who potentially are not participating in regular exercise, might lead to acute elevated risk of injury and health complications. With a health questionnaire, health issues with a risk of complications are excluded to perform safe physical testing. Regardless, the testing facilities have prepared for possible health complications and injuries with the University of Jyväskylä's safety protocols. The subjects are young adult men, so the risks are concidered to be relatively low. If any potential risk factors are found in the health questionnaire, the study staff will consult a doctor for safe participation. The participating study staff are educated in first aid, and research facilities have taken care of the first aid equipment.

The examination methods are chosen to be minimally invasive. In the VO2max test, a capillary blood sample is taken from a fingertip, which is an invasive method to assess the circulating lactate levels. The procedure is conducted in the assigned test facility, where accessibility and proper cleaning are taken care of during the measurements. In the measurements, the test personnel will use protective gloves to minimize infection risks, and after the procedure, the fingertip is protected with a plaster, if needed. As a method, capillary blood sampling is less invasive than venuous blood samples, and therefore produces the least possible harm for the subject. The capillary blood sampling may lead to a local bruise. Stress hormone measurements venuous bloos sampling has been replaced with salivary sampling. Salivary stress hormone sensitivity have been seen in literature to be sufficient for this study. Heart rate variability has a potential for local skin irritation due to the adhesive in EKG electrodes. Whether skin irritation occured, the subjects were informed to stop the measurement. Moreover, the measurement time has been asked to be as short as possible. All the potential harm for the subject are aimed to minimized.

In this study, the common scientific community protocols and methods were used. The research and its data handling and reporting are conducted with care and precision. The funding of the study is open and fair. Participation is not rewarded, and no potential traveling costs are covered, but the subjects are responsible for the costs. The study uses ethically sustainable and scientifical research criteria meeting data collection, research and evaluation methods. The reporting and design of the study respects the work of other scientists and are cited with the University of Jyväskylä's faculty of sport and exercise science's protocols. The study will follow the University of Jyväskylä ethical commitee's ethical principles. The research group has discussed the rights and responsibilities, and documentation handling with a common agreement.

The study staff had prepared for potential COVID-19 risks, and followed regional governmental and University of Jyväskylä health safety protocols during all the phases of the study.

7.2 Subjects

Subjects were collected by volunteering with invitation letters distributed throughout university campus, university website and university newsletter via email. The inclusion criteria were healthy, adult (above 18 years old) men, whose competitive ranking in the game Counter Strike: Global Offensive was above Gold Nova 1. Exclusion criteria were health issues, that would limit participation in the physical performance tests, and health issues that would effect gaming performance.

All voluntary subjects had a preliminary meeting remotely via Zoom with the study leader, who gave them a presentation of the general practicalities of the study (ethical rights of a subject and ethical advise regarding participation to a study), and contents of each stage and measurement of the study. The subjects were able to ask questions regarding the study, and were given timetables and contact information of the study leader. The subjects were also sent documents about the preparations for the study via email after their preliminary meeting. Moreover, subjects were asked to fill out an written agreement for participation to the study on the first day of the experimental phase.

7.3 Experimental setting

The experimental testing period was conducted between 27th January and 24th February 2023, for five weeks. The testing days were distributed to three days: preliminary tests and two model tests.

Preliminary tests were conducted on Friday in Jyväskylä University Exercise and Health Science's facilities, in the VO2max testing area. Preliminary tests included filling out questionnaires (health questionnaire, Edinburgh Handedness Inventory, International Physical Activity Questionnaire (IPAQ) Long form, body composition by bioimpedance, light reaction test and VO2max test with a cycle ergometer. The testing schedule was distributed between times 10.00 AM to 6.00 PM. After the preliminary measurements on Friday, the subjects were given supplies for heart rate variability (HRV) measurements (Firstbeat Bodyguard 2 appliance and electrodes), Salivette® salivary sampling tubes, Garmin Forerunner 245 activity watch with a charging cable, and a written documents with manuals for the appliances and detailed instructions how to conduct the measurements.

In between the preliminary tests and experimental tests, control period was conducted. During the control period, the subjects conducted HRV analysis for 3 consecutive days (Saturday-Monday). Moreover, the subjects conducted control saliva sampling on the day before the first experimental test day (Monday).

The experimental tests for each model (exercise and rest) were distributed into two days, Tuesday and Thursday. Each model was conducted on a different day: if the exercise model was conducted on Tuesday, Thursday had the rest model, and vice versa. It was randomized with a webtool (https://www.random.org/lists/), which model each subject started with. The exercise model included a 15-minute HIIT session with cycle ergometer, post-model saliva sampling, filling out a questionnaire (Profile of Mood States - POMS), 20-minute session of reading, 20 minute session of gaming in Counter Strike: Global Offensive AimBotz – aim training map, saliva sampling and light reaction test. The rest model was identical to exercise model, excluding the 15-minute HIIT session, and replaced with a 15-minute sitting on the cycle ergometer in rest.

After 7 days from the last experimental test day, the subjects were sent a link via email for the final Physical Activity Enjoyment Scale (PACES) questionnaire (Webropol), which the subjects were advised to answer within 7 days from receiving the link. A reminder was sent on the deadline day via email.

Due to the scope of this thesis, handling of the light reaction test was excluded from this thesis.

7.3.1 Questionnaires

Preliminary questionnaires included a health questionnaire (attachment 1), IPAQ Long form (attachment 2), Edinburgh Handedness Inventory (attachment 3). Mid-experimental sessions subjects filled Profile of Mood States (POMS) (attachment 4). 7 days after the final session the participants filled a Physical Activity Enjoyment Scale (PACES) questionnaire (attachment 5). All questionnaires on preliminary session and experimental sessions were filled electronically with a tablet device (Samsung®) on Webropol platform. The final PACES questionnaire the subjects filled with their own electronical device. All the questionnaires were translated in Finnish.

Health questionnaire included questions about the subject's general health state, medications and health issues, that could effect or prevent the subject from participation in physical performance tests. The questionnaire also had questions about health behavior (e.g. sleeping habits and gaming habits) relevant to the study. Table 1 demonstrates the results of the subjects' gaming habits: their ranking level in the particular game in the study (Counter Strike: Global Offensive), combined gaming hours in a week (esports training and leisure gaming) and esports training hours in a week.

TABLE 1. Gaming hours and ranking in Counter Strike: Global Offensive.

	Average (n=10)	SD
Ranking level	16,40	1,50
Combined/week	12,25	8,03
Training/week	3,20	6,23

Figure 12 visualizes the division between subjects' CS:GO skill levels compared to overall in-game ranking system.

FIGURE 12. Counter Strike: Global Offensive skill level of the subjects (n=10). The progression of the in-game level system is illustrated from left to right (Silver 1 = lowest, Global Elite = highest)

A well-validated Edinburgh Handedness Inventory was used to determine the handedness of the subjects (De Las Heras et al 2020). All subjects reported to be right-handed (all scores ≥80).

International physical activity questionnaire (IPAQ) Long Form was used to assess the physical activity and sedentary habits of the subjects. IPAQ has presented excellent concurrent and construct validity, when comparing data obtained with physical activity monitors (De Las Heras et al 2020). Table 2 demonstrates the results of the inventory.

TABLE 2. IPAQ Long Inventory

	Average (n=10)	SD
Vigorous (min/week)	1336,00	1213,29
Moderate (min/week)	564,00	614,35
Walking (min/week)	531,30	366,34
Combined (min/week)	2431,30	1749,06
Sitting time total (min/week)	3822,00	1406,88

Profile of mood states (POMS) was used to assess mood disturbances caused by the model (exercise/rest). It has been found, that higher self-reports of anxiety in Profile of Mood States (POMS) were positively associated with sAA reactivity to psychosocial stressor (Schumacher et al 2013).

Physical Activity Enjoyment Scale (PACES) was used to determine, if participants perceptions towards the activity potentially influeced their video game performance. Higher scores indicated greater enjoyment (De Las Heras et al 2020). It has been found to have high internal consistency (Kendzierski & DeCarlo 1991).

7.3.2 Body composition

Body composition was measured by bioimpedance with Inbody 720. The measurement was conducted in the university storage room next to the VO_{2max} testing area.

The subjects were advised to not eat or drink at least for 2 hours before the measurement. After the body composition measurement, the subjects were handed a copy of the results. The subjects were allowed to have a small snack (e.g. banana) before the VO_{2max} test.

7.3.3 Maximal oxygen uptake (VO_{2max}) test

In the original paper by De Las Heras et al (2020), a graded exercise test (GXT) was used to assess VO_{2max}. The setting of the GXT test was replicated in this setting for a VO_{2max} cycle ergometer test. The VO_{2max} test was performed in University of Jyväskylä endurance testing lab facilities in Liikunta building on Monark Ergomedic 839E cycle ergometer (Monark Exercise AB, Sweden). Oxygen consumption was measured using a Oxycon Pro (Viasys Healthcare GmbH, Germany) gas analyzer.

Before the testing, subject had a 15-minute warm-up on the ergometer with 25W resistance. The subject was instructed about the test protocol, and reminded of the permission to end the test at any point for any reason. The subject was also reminded to report changes in health during the test.

The starting level of resistance was set to 50W, which progressed with 30W elevations on each 3-minute stage. Between each stage, capillary lactate was measured, and perceived strain was monitored by Borg's Scale (6–20). Participants were advised to maintain the pedaling cadence of 80 rpm until exhaustion.

After the testing, VO_{2max}, HR_{max} and peak workload (W_{peak}) were recorded at each stage. Breathing gases were analyzed with Oxycon Pro, and the results were adjusted for known 7 % optimistic error in oxygen parameters. Lactate was analyzed post-testing by EKF Biosen (EKF Diagnostic). HR (Garmin Forerunner 245 and Garmin HR belt) was monitored continuously throughout the test.

7.3.4 Heart rate (HR) and heart rate variability (HRV) measurement

Heart rate (HR) was monitored with Garmin Forerunner 245 activity watch, and Garmin heart rate monitor belt. Heart rate variability (HRV) was monitored by using Firstbeat Bodyguard 2© (Firstbeat Technologies, Jyväskylä) devices.

The subjects were given the Firstbeat Bodyguard 2© equipment with verbal and written instructions on the preliminary test day. The subjects were instructed to do a control measurement by using the equipment for three days (Saturday-Sunday-Monday), and instructed to do their ordinary activities of daily living. The equipment were also used during the experimental days, and at least for two hours after waking up on the following day. In case the subject had any allergic reactions to adhesives of the equipment, they were advised to contact the staff of the study.

After the measurements, the results were analysed with free version of Kubios. The HRV data was corrected using a threshold of medium (0,25 seconds). If the corrections were higher than 5%, the threshold was set to low (0,5 seconds). If the corrections were still more than 5%, the threshold was lowered to very low. Whether the corrections were still higher than 5%, the data was excluded and declared as corrupted data. Moreover, if HRV recording was missing partly or completely (e.g. due to malfunction of the equipment), the data was also declared as corrupted or missing data.

This setting focused on acute physiological stress responses. Therefore, the main results are for short-term parameters root mean square of successive differences between normal heartbeats (RMSSD), low-frequency/high-frequency ratio (LF/HF) and standard deviation 1 (SD1).

7.3.5 Saliva sampling

Saliva sampling was conducted to assess the subjects' stress marker responses: cortisol, alpha-amylase and dehydroepiandrosterone sulfate. Saliva sampling was conducted using Salivette Cortisol® cotton swabs. The subjects were advised to do morning samples independently for five times: on a control day before preliminary testing, on the first experimental day, the following day on the experimental day, on the second experimental day and after the second experimental day. During the experimental days, the research staff would assist with saliva sampling four more times: on the first experimental day after the model, on the first experimental day after the video game task, after the second experimental day after the model and after the second experimental day after the video game task. Figure 14 demonstrates the saliva sampling schedule.

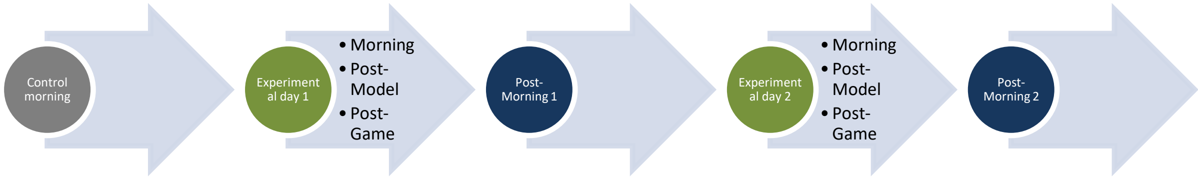

FIGURE 14. Saliva sampling schedule

At the end of the preliminary test session, the subjects were given verbal and written instructions of conducting saliva sampling with Salivette Cortisol® cotton swabs.

The saliva sampling was advised to be conducted right after waking up in the morning. The tube cap was advised to be opened with clean hands. The swab should be poured inside the mouth without touching the swab. The swab should be rolled inside the mouth for at least a minute or as long as the subject can't prevent swallowing extra saliva.

After covering the swab as much as saliva as possible, the subject was advised to guide the swab back inside the Salivette® tube without touching the swab and closing the cap. After sampling, the subject was advised to write the sampling date and time with their personal subject ID on the tube, and to contain the sample tube in the refrigerator until handing it to the research staff during the experimental sessions.

After the last experimental day, the subjects were advised to return the sample to University of Jyväskylä Viveca lobby, where they could contain it in a cold container box.

The samples were centrifuged (Megafuge 1.0R, Heraeus with 2400 rpm, 3 minutes in 20ºC), and stored in the freezer in -20ºC for analysis. For analysis enzyme-linked immunosorbent assays (ELISA) (Immulite 2000 XPi, Immunoassay System) was used.

7.3.6 Exercise and resting model

The exercise and resting model were conducted at Jyväskylä University of Applied Sciences building at the GamePit lobby. The area was separated from the lobby by a curtain.

Exercise model included a 15-minute high-intensity interval training (HIIT) session. De Las Heras et al (2020) suggest, that the time-efficiency of HIIT exercise could potentially be appealing to video gamers. The HIIT session was conducted on Monark LC4 (Monark Exercise AB, Sweden) cycle ergometer with an activity watch (Garmin Forerunner 245) and heart rate (HR) monitor belt (Garmin). The ergometer resistance was controlled by Monark computer application.

The high-intensity interval training (HIIT) session started with a 2,5 minute warm-up with 50 W resistance. HIIT intervals were 1-minute long with a resistance of 80 % P_{max} (W), that was calculated from each subject's VO_{2max} test results. After the HIIT interval, a 40% P_{max} (W) light interval followed. This was conducted until the 12,5 minute-time stamp, after which cool-down proceeded with 50W resistance for 2,5 minutes to fill the 15-minute session. The subjects were asked to maintain a cycling cadence of 80 of rpm. The intensity was monitored by Garmin Forerunner 245, HR monitor (Garmin) and RPE.

In the resting model, the subject was instructed to sit on the cycle ergometer (Monark LC4). The subject was instructed to refrain from talking during the resting session.

7.3.7 Reading session

Similarly to the original research of De Las Heras et al (2020), the participants were advised to read for 20 minutes to standardize any external stimulation effect before the assessment of the affective response to exercise, and the performance of the video game task.

The reading session was conducted at the Jyväskylä University of Applied Sciences building, in the GamePit lobby area. The reading area was separated from the lobby in a small-sized cabin with a closable door and had a window to the lobby. The subjects were offered and advised to read a Donald Duck comic book during the reading session. After the 20 minute time was over, the subjects were instructed and taken to the room with the video game task.

7.3.8 Video game task

The video game task was conducted at Jyväskylä University of Applied Sciences building, in the GamePit Pro gaming facility. The video game task included the following equipment:

- Processor: i7-12700K (12-core)
- Graphics card: Asus GeForce RTX 3080
- RAM: DDR4 16GB
- Hard drive: M2 SSD 1000GB
- Cooling: Noctua cooling
- AOC Gaming C27G2ZE/BK 27", 240 Hz screen
- Logitech G Pro -headset
- Logitech Gaming G203 mouse
- Logitech G512 keyboard
- Noblechairs EPIC Faux Leather Black gaming chair
- Xtrfy XG-GP2 XXL mouse pad
- Electrically adjustable table

Before the video game task started, the subjects were advised to adjust their individual preferences on mouse IP and mouse sensitivity inside the game Counter Strike: Global Offensive. This was to guarantee equal approach to the test, so the experimental setting didn’t favor a subject, who would have their control settings close to a chosen standard.

The gaming performance was assessed by using Counter Strike: Global Offensive custom aim training map AimBotz. Figure 15 shows general view of the AimBotz map (not adjusted to study settings).

FIGURE 15. AimBotz aim training map view (Rose 2022)

The subject was advised to move the character to the center of the control panel (under the T letter of the text ”ULLETICAL”) as close to the target area as possible. The view for the area was set to 90 degrees and targets to 100. The research assistants helped the subject set the preliminary settings correctly and checked the settings before beginning. These settings were set to reduce performance variability and add standardization to the gaming task.

The subjects were asked to eliminate 100 targets as quickly as possible, and it was reminded, that it would happen faster if aimed towards the target head area. The subject was advised to refrain from moving or doing additional activities besides shooting targets. Heart rate (HR) average assessment was started using HR monitor (Garmin) when the subject had eliminated 90 targets and ended, when the subject reached 100 targets. Video game task was overall monitored visually by at least two study staff member.

After finishing 100 targets, the gaming performance parameters (completion time, kills per minute KPM, kills per second KPS) and heart rate (HR) were recorded. The test was repeated for 5 times.

7.4 Statistical analysis

Statistical analyses were conducted using R Studio (version 4.3.1) using common system library packages (base, datasets, graphics, grDevices, methods, stats, utils). Outliers were checked in GraphPad Prism (10.1.0 (316)) outlier identification with ROUT method (Q=5%)

Significances were assessed by nonparametric tests: paired t-test with Wilcoxon's signed rank test and 2-way analysis of variance with Friedman's test. Friedman's test is used to evaluate, whether the parameter values has changes between different measurements (Uhari & Nieminen 2012, 180). Wilcoxon's test can calculate a test parameter's t value (Nummenmaa 2021, 371). Significance was set for $p<0,05$ and confidence intervals to 95 %.

8 RESULTS

10 healthy male subjects participated in the study. All subjects participated in all measurements in both exercise and resting models. Table 3 shows the mean results and standard deviations of the bioimpedance measurement and the maximal oxygen uptake (VO_{2max}) test.

TABLE 3. Subject antropometrics and maximal oxygen uptake (VO_{2max}) test (Inbody 720, OxyconPro, respectively)

	Average (n=10)	SD
Age	25,00	3,52
Height (cm)	184,27	5,36
Weight (kg)	88,38	9,91
BMI	26,04	2,86
Fat (%)	18,93	6,91
FFM (kg)	71,12	5,24
VO_2max (ml/kg/min)	41,49*	9,39*
VO_2peak (ml/kg/min)	51,24*	9,41*
HR_{max}	193,67*	6,15*

*(n=9)

One participant wanted to cancel the test performance after the first stage. For this subject, the HR_{max} was estimated by using Jones's formula: HRmax = 210 – age * 0,65 (Jones, 1988). Other participants completed the test until exhaustion with no adverse events.

The results were collected from acute physiological stress responses: salivary steroids (salivary cortisol, alpha-amylase (sAA) and dehydroepiandrosterone sulfate (DHEA-S)) and heart rate (HR), heart rate variability (HRV). Moreover, results were collected from esports performance parameters (completion time, kills per second (KPS), light reaction test) and psychological inventories (mood states, total mood disturbance (TMD) and physical activity enjoyment). In this thesis, the results from light reaction tests are excluded due to the focus on evidence-based esports performance parameters.

8.1 Salivary steroids

Salivary steroid values for cortisol, sAA and DHEA-S were collected from the subjects. One subject didn't produce enough saliva for reliable analysis, therefore timepoints, where n=9, have been marked with one asterisk (*) and significance is marked by two asterisks (**). Whether n was reduced to 9 in the data, the statsitical analysis was assessed with n=9, excluding other datapoints from that subject.

Salivary cortisol. Table 4 demonstrates the salivary cortisol values collected from the study. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

TABLE 4 Salivette® salivary cortisol values (nmol/l) in exercise and resting models with averages (AVE) and standard deviations (SD) (n=9-10)

	Control	PreE	PostE	PostGE	PostME	PreR	PostR	PostGR	PostMR
1	18,80	x [a]	24,40	14,00	20,10	11,8	36,10	10,80	x [a]
2	14,7	27,1	10,5	6,73	18,30	13,2	7,12	12,10	10,60
3	7,01	7,48	10,9	6,43	6,79	7,7	7,97	7,28	4,86
4	19,8	11,6	3,45	5,24	16,10	22,4	4,75	4,28	17,30
5	11,5	6,4	8,99	5,77	0,65	10,1	3,5	15,10	9,19
6	8,17	8,11	25	8,33	6,48	11,7	6,32	8,55	16,20
7	31,7	22,7	13,3	5,82	29,00	14,8	5,55	4,58	30,10
8	27,9	11,6	21,6	5,6	12,60	18,8	8,8	4,86	7,67
9	9,49	9,16	8,22	5,57	19,60	8,77	4,39	13,90	13,90
10	12,6	23,4	14,9	12,1	8,41	16,4	23,4	6,65	8,44
AVE	16,17	14,17 [b]	14,13	7,56	13,80	13,57	10,79	8,81	13,14 [b]
SD	7,93	7,49 [b]	6,93	2,90	7,98	4,37	10,02	3,76	7,13 [b]

[a] insufficient saliva [b] (n=9)

Figure 16 presents the changes of salivary cortisol values of each subject during each stage of the study, and salivary cortisol values with averages, respectively.

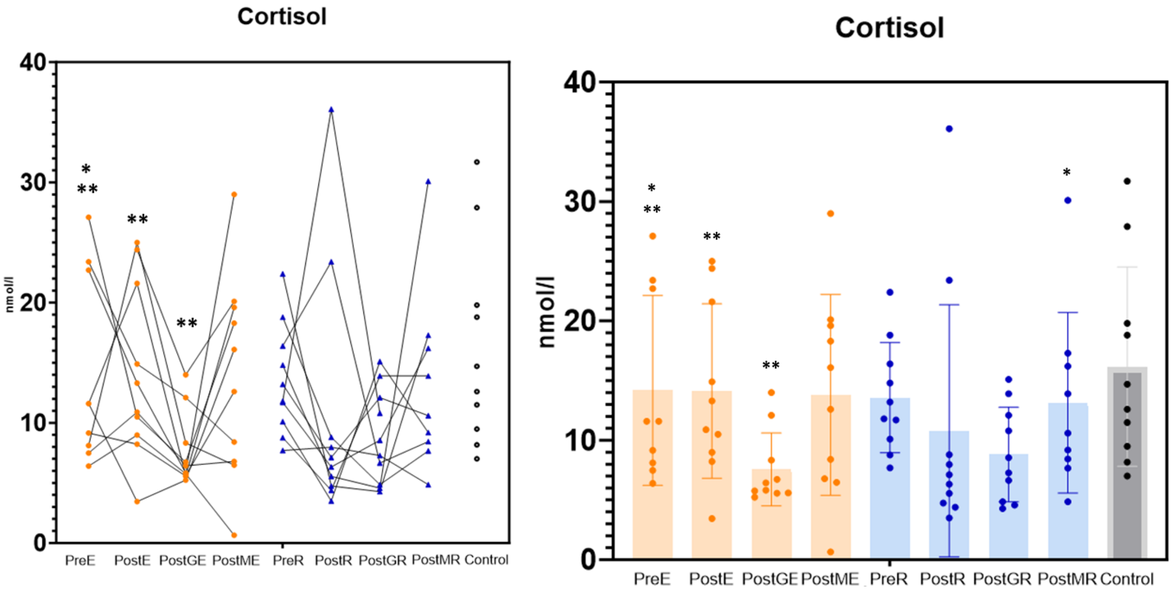

FIGURE 16. Salivette® Salivary cortisol with subject datapoints in different phases of the study (n=9-10). Orange refers to the exercise model and blue to resting model, and black to control values. Asterisks (*) mark n=9 and (**) mark significant differences.

A significant difference (p<0,05) between within-day measurements (test day morning, after HIIT protocol and after CS:GO gaming task) was found in the exercise group (n=9). No other significant differences were found between the models and control or within-day measurements in the rest model. Table 5 demonstrates the statistical relationships between phases of the study.

TABLE 5 Salivary cortisol statistical analysis (Friedman). Asterisk (*) marks significance.

	Friedman chi-squared	p-value
Control – PostGE – PostGR	4,222	0,1211
Control – PostE – PostR	4,667	0,09697
PreE – Exercise - PostGE	8,222	0,01639*
PreR – Rest - PostGR	4,200	0,1225
Control – PostME – PostMR	4,222	0,1211

Alpha-amylase (sAA). Table 6 demonstrates the salivary alpha-amylase (sAA) values collected during the study. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

TABLE 6. Salivette® salivary alpha-amylase (sAA) values (u/l) in exercise and resting models with averages (AVE) and standard deviations (SD) (n=9-10)

	Control	PreE	PostE	PostGE	PostME	PreR	PostR	PostGR	PostMR
1	4 761,46	x [a]	6 451,75	9 249,21	6 368,89	4 828,59	6 259,01	17 380,62	x [a]
2	39 774,34	26 992,28	267 116,30	170 443,20	50 816,78	173 650,40	188 946,60	43 570,66	72 858,34
3	54 858,31	68 171,20	337 908,30	223 481,00	64 567,33	62 982,27	152 775,30	181 993,00	44 990,34
4	37 146,52	295 972,60	288 101,10	280 408,80	299 901,60	51 024,83	132 550,40	262 777,40	263 726,00
5	41 668,46	26 354,13	22 221,34	19 012,19	18 275,58	15 939,48	17 781,78	7 752,81	26 409,43
6	14 540,83	24 409,83	15 581,92	84 973,84	81 475,86	14 382,91	52 923,09	157 818,80	31 794,37
7	91 831,23	133 883,00	334 995,10	333 525,40	106 130,40	57 371,37	259 705,50	699 821,40	136 975,10
8	17 247,45	51 340,27	40 557,66	118 728,00	54 194,49	35 652,89	157 851,00	153 100,40	87 991,12
9	38 966,56	54 877,06	68 334,01	54 580,18	75 441,30	87 351,57	70 676,73	60 965,52	37 429,55
10	108 964,90	51 180,56	56 132,28	185 773,20	213 150,30	36 476,12	104 650,10	178 139,60	147 184,30
AVE	44 976,01	81 464,55 [b]	143 739,98	148 017,50	97 032,25	53 966,04	114 411,95	176 332,02	94 373,17 [b]
SD	31 361,51	82 144,33 [b]	135 789,84	104 415,37	86 531,90	46 485,57	75 615,29	191 585,80	73 032, 99 [b]

[a] insufficient saliva [b] (n=9)

Figure 17 presents the changes of salivary alpha-amylase values of each subject during each stage of the study, and salivary cortisol values with averages, respectively. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

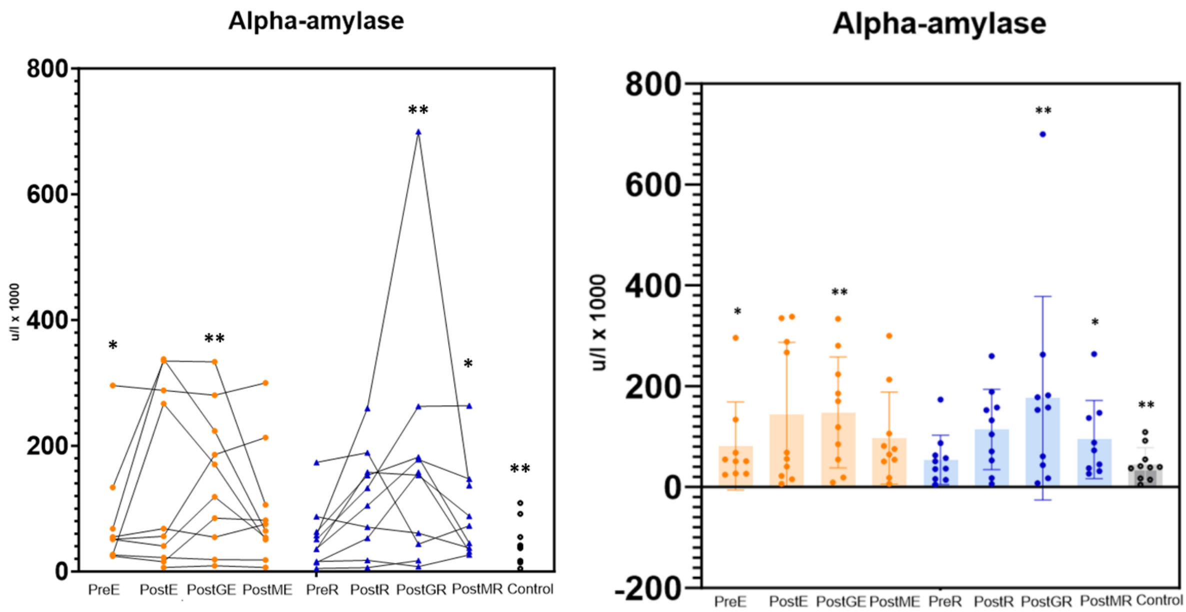

FIGURE 17. Salivette® salivary alpha-amylase with subject datapoints in different phases of the study (n=9-10). Asterisks (*) mark n=9 and (**) mark significant differences.

Significant differences (p<0,05) were found between the exercise model, resting model and control after CS:GO gaming task. Resting model seemed to induce lower elevations of sAA compared to exercise model. Both exercise model and resting model induced elevations to sAA levels to control values. No other significant differences were found. Table 7 demonstrates the statistical relationships between phases of the study.

TABLE 7. Salivary alpha-amylase statistical analysis (Friedman). Asterisk (*) marks significance.

	Friedman chi-squared	p-value
Control – PostGE – PostGR	9,600	0,00823*
Control – PostE – PostR	5,600	0,06081
PreE – Exercise - PostGE	0,889	0,6412
PreR – Rest - PostGR	5,400	0,06721
Control – PostME – PostMR	4,667	0,09697

Dehydroepiandrosterone sulfate (DHEA-S). Table 8 demonstrates the salivary dehydroepiandrosterone sulfate (DHEA-S) values collected during the study. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

TABLE 8. Salivette® salivary dehydroepiandrosterone sulfate (DHEA-S) values (ng/mL) in exercise and resting models with averages (AVE) and standard deviations (SD) (n=9)

	Control	PreE	PostE	PostGE	PostME	PreR	PostR	PostGR	PostMR
1	x [a]	x [a]	x [a]	x [a]	x [a]	x [a]	x [a]	x [a]	x [a]
2	12,97	11,748	12,775	9,427	11,272	11,034	8,333	11,601	11,296
3	7,601	6,298	8,22	10,827	6,48	10,118	8,01	12,288	5,821
4	17,511	7,234	11,81	10,40	15,027	16,30	11,348	14,103	11,168
5	7,354	6,331	7,19	5,784	7,735	16,747	12,602	8,101	11,092
6	9,141	14,338	10,411	9,416	9,412	7,887	8,391	9,119	15,647
7	18,708	23,294	24,867	23,789	20,626	16,086	18,614	20,46	22,552
8	7,081	6,33	7,235	6,788	7,961	5,867	5,07	7,103	7,292
9	16,937	21,105	11,498	10,697	15,438	11,911	13,662	17,136	20,843
10	22,464	17,062	20,367	14,34	14,48	13,069	16,147	11,154	10,15
AVE	13,31 [b]	12,64 [b]	12,71 [b]	11,27 [b]	12,05 [b]	12,11 [b]	11,35 [b]	12,34 [b]	12,87 [b]
SD	5,46 [b]	6,30 [b]	5,72 [b]	4,99 [b]	4,39 [b]	3,62 [b]	4,10 [b]	4,08 [b]	5,393 [b]

[a] insufficient saliva [b] (n=9)

Figure 18 presents the changes of salivary DHEA-S values of each subject during each stage of the study, and salivary cortisol values with averages, respectively. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

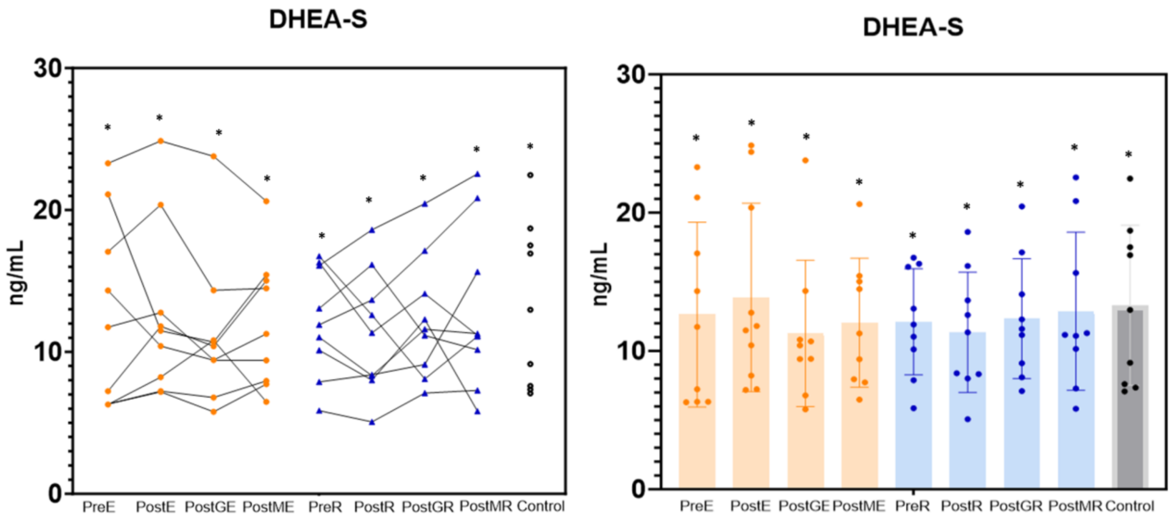

FIGURE 18. Salivette® salivary dehydroepiandrosterone sulfate (DHEA-S) with subject datapoints in different phases of the study (n=9-10). Asterisks (*) mark n=9 and (**) mark significant differences.

No significant differences were found in salivary DHEA-S changes between the models and control. Table 9 demonstrates the statistical relationships between phases of the study.

TABLE 9. Salivary dehydroepiandrosterone sulfate (DHEA-S) statistical analysis (Friedman). No significant differences were found.

	Friedman chi-squared	p-value
Control – PostGE – PostGR	1,5556	0,4594
Control – PostE – PostR	4,22222	0,1211
PreE – Exercise - PostGE	0,66667	0,7165
PreR – Rest - PostGR	2,8889	0,2359
Control – PostME – PostMR	0,22222	0,8948

Figure 19 visualizes the summary of the average changes of all salivary steroid values during each stage of the study. Different abbreviations are used: PreE (preliminary morning before exercise module), PostE (post exercise model), PostGE (post gaming task after exercise model), PostME (post morning after exercise model), PreR (preliminary morning before resting model), PostR (post resting model), PostGR (post gaming task after resting model), PostMR (post morning after resting model) and control.

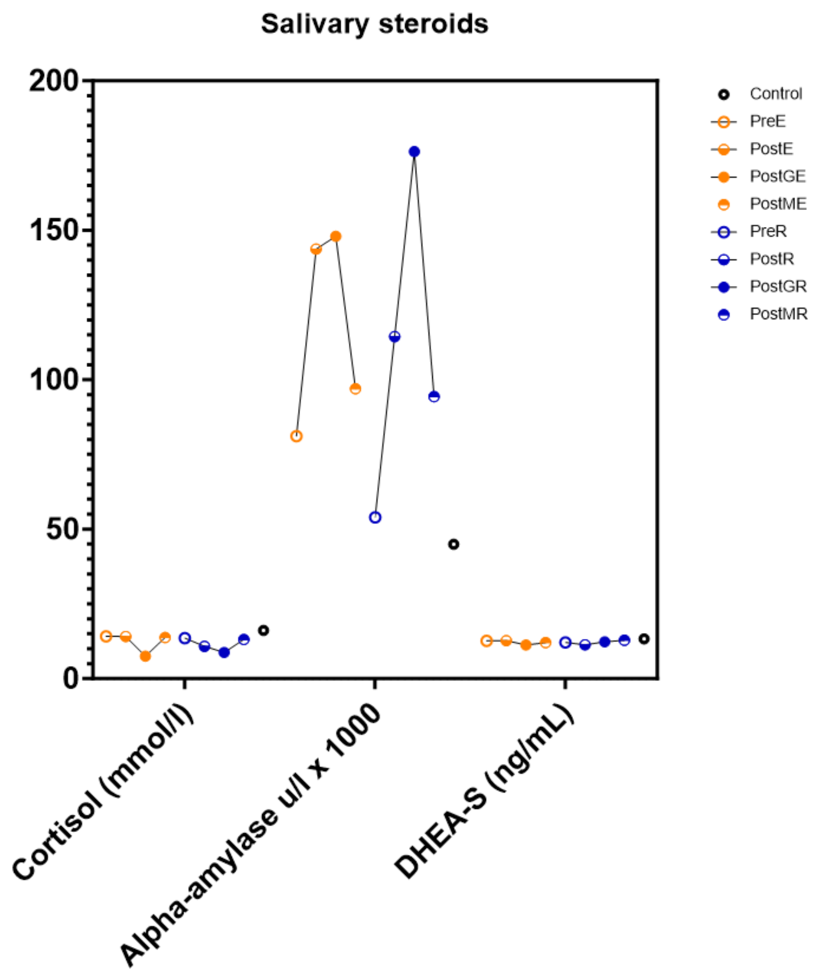

FIGURE 19. Average development of different salivary steroids in exercise and resting models with control values.

8.2 Heart rate (HR) and heart rate variability (HRV)

Heart rate variability (HRV). HRV short-term parameters (root mean square of successive differences between normal heart beats (RMSSD), low frequency/high frequency ratio (LF/HF) and standard deviation 1 (SD1)) were assessed from the subjects during gaming tasks and within 3-day control. Table 10 demonstrates the individual numerical values of different HRV parameters. Abbreviations used are the following: RMSSD_E = RMSSD during gaming task after exercise model, LF/HF_E = LF/HF during gaming task after exercise model, SD1_E = SD1 during gaming task after exercise model, RMSSD_R = RMSSD during gaming task after resting model, LF/HF_R = LF/HF during gaming task after resting model, SD1_R= SD1 during gaming task after resting model, RMSSD_C= RMSSD during 3-day control, LF/HF_C=LF/HF during 3-day control and SD1_C = SD1 during 3-day control.

TABLE 10. Firstbeat Bodyguard 2 gaming session heart rate variability (HRV) parameters between different models and 3-day control recording. (n=9-10)

	RMSSD_E	LF/HF_E	SD1_E	RMSSD_R	LF/HF_R	SD1_R	RMSSD_C	LF/HF_C	SD1_C
1	121,39	0,471	85,85	103,29	0,664	73,07	110,55	0,891	75,816
2	42,90	5,244	30,30	35,20	5,196	24,90	46,367	2,926	32,767
3	14,30	5,941	10,10	21,00	7,359	14,90	16,433	8,298	11,633
4	68,80	2,317	48,60	53,40	2,493	37,80	22,450	6,625	15,850
5	49,10	3,156	34,70	56,30	3,386	39,90	47,333	3,606	33,733
6	30,30	3,698	21,50	30,90	3,988	21,90	31,867	3,095	33,800
7	33,80	3,159	23,90	60,80	1,540	43,00	38,467	2,075	27,167
8	27,00	5,537	19,10	47,60	3,258	33,70	43,433	5,555	30,700
9	68,00	2,784	48,10	102,00	0,969	72,10	56,933	2,403	40,267
10	21,00	2,454	14,90	x [a]	x [a]	x [a]	x [a]	x [a]	x [a]
Average	47,66	3,48	33,71	56,72 [b]	3,21 [b]	40,14 [b]	45,98 [b]	3,94 [b]	33,53 [b]
SD	30,11	1,60	21,28	27,36 [b]	2,02 [b]	19,33 [b]	25,78 [b]	2,26 [b]	17,24 [b]

[a] corrupted data recording [b] (n=9)

Figure 20 visualizes the HRV responses (RMSSD, LF/HF, SD1) values of each subject with averages during gaming task after each model (exercise and rest) compared to 3-day control value. Abbreviations used are the following: GE = gaming task after exercise model and GR = gaming task after resting model.

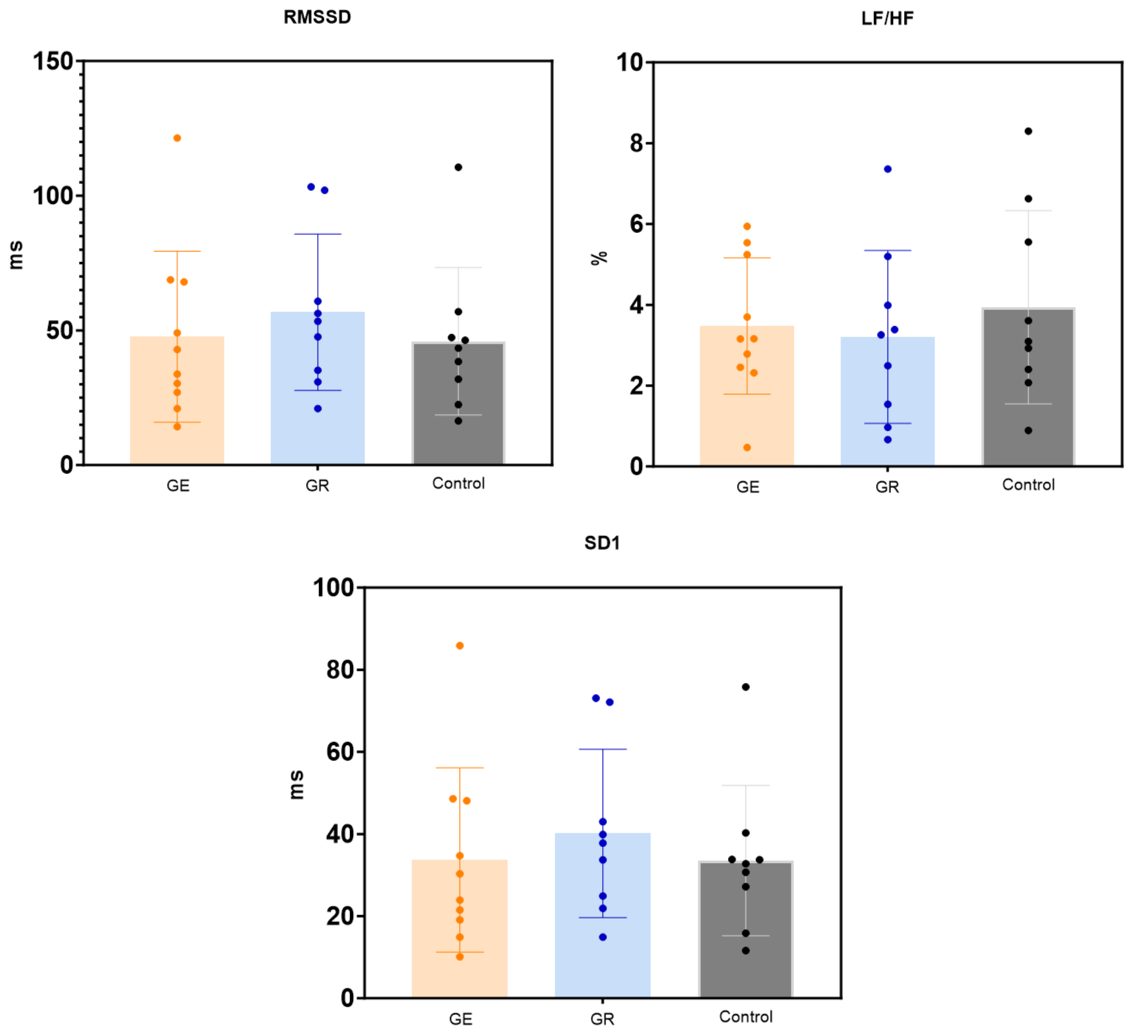

FIGURE 20. Firstbeat Bodyguard 2 heart rate variability (HRV) short-term parameters root mean square of successive differences between normal heart beats (RMSSD), low frequency/high frequency ratio (LF/HF) and standard deviation 1 (SD1) individual values with averages

No significant differences between either models and control were found in any HRV parameter. Table 11 demonstrates the statistical analysis of different HRV parameter relationships.

TABLE 11 Heart rate variability (HRV) statistical analysis (Friedman). No significant differences were found.

	Friedman chi-squared	p-value
RMSSD: Control – PostGE – PostGR	1,5556	0,4594
LF/HF: Control – PostGE – PostGR	1,5556	0,4594
SD1: Control – PostGE - PostGR	1,5556	0,4594

Heart rate (HR). In-game HR was recorded from the subjects after exercise and resting models. Table 12 demonstrates the HR values recorded during each model. Abbreviations used are the following: HRa_GE = average heart rate in 5-wave gaming task after exercise model, HRa_GR = average heart rate in 5-wave gaming task after resting model, HRpeak_GE= peak heart rate reached during the gaming task after the exercise model and HRpeak_GR = peak heart rate reached during the gaming task after resting model.

TABLE 12. Garmin Forerunner 245 in-game heart rate (HR) values after each model (n=10)

	HRa_GE	HRa_GR	HRpeak_GE	HRpeak_GR
1	91,6	88,8	95	97
2	97,6	78,4	102	82
3	108,2	92,0	113	96
4	85,8	75,4	91	80
5	69,6	69,6	72	71
6	91,2	97,4	107	127
7	98,8	88,0	103	94
8	75,8	65,4	84	74
9	84,6	87,4	87	90
10	90,4	75,6	94	81
AVE	89,39	81,8	94,8	89,2
SD	10,64	9,87	11,45	15,25

Figure 21 demonstrates the HR differences of each subject during gaming task after each model. Abbreviations used are the following: GE = gaming task after exercise model and GR = gaming task after resting model.

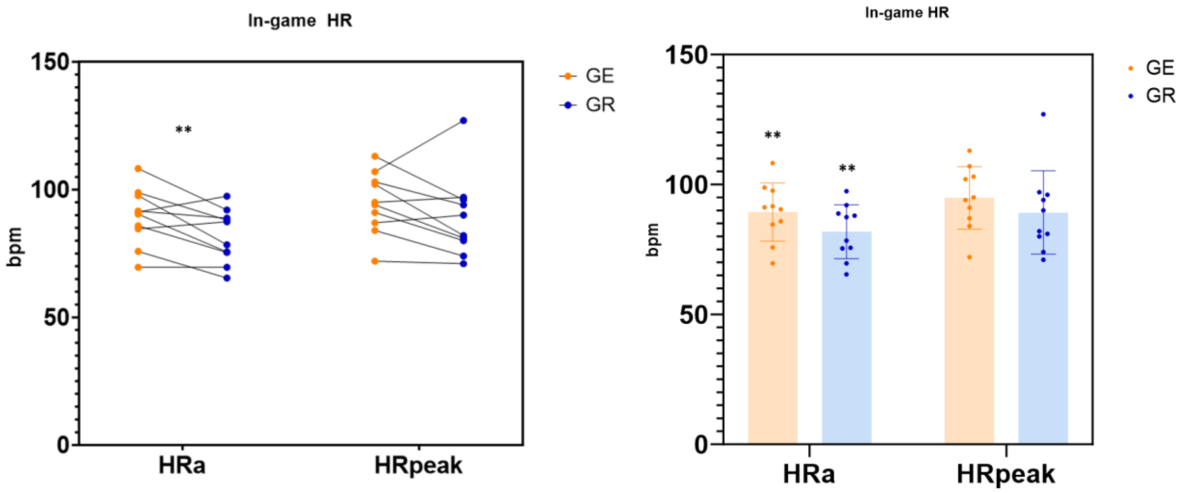

FIGURE 21. Garmin Forerunner 245 in-game heart rate (HR) average difference between exercise and resting models (n=10). Asterisks (**) mark significant difference.

A significant difference ($p<0,05$) was found between exercise and resting model within average HR responses, exercise model demonstrating a higher elevation compared to the resting model. No significant difference was found between models in HRpeak values. Table 13 demonstrates the statistical analysis of different HR relationships during gaming task.

TABLE 13 Heart rate (HR) parameters statistical analysis (Wilcoxon). Asterisk (*) marks significant difference.

	V	p-value	CI 95%
HRpeak R – HRpeak E	14,5	0,2023	-14,49991 – 3,499961
HRa R – HRa E	5,0	0,04383*	-15,000020 – -2,09992*

8.3 Esports performance

Esports performance in Counter Strike: Global Offensive (CS:GO) was assessed by game performance parameters completion time and kills per second (KPS). Table 14 demonstrates the overall results of esports performance after each model (exercise and rest). Abbreviations used are the following: Ta_E = completion time average in 5-wave gaming task after exercise model, Ta_R = completion time average in 5-wave gaming task after resting model, Tpeak_E = peak completion time after exercise model, Tpeak_R = peak completion time after resting model, KPSa_E = kills per second average in 5-wave gaming task after exercise model, KPSa_R = kills per second average in 5-wave gaming task after resting model, KPSpeak_E = peak kills per second after exercise model and KPSpeak_R = peak kills per second after resting model.

TABLE 14. AimBotz esports performance parameters (completion time and KPS) after each model (n=10)

	Ta_E	Ta_R	Tpeak_E	Tpeak_R	KPSa_E	KPSa_R	KPSpeak_E	KPSpeak_R
1	191,96	198,79	189,53	193,72	0,520	0,496	0,52	0,51
2	186,64	189,17	179,33	185,17	0,532	0,524	0,55	0,54
3	201,80	203,44	194,81	197,27	0,492	0,484	0,51	0,50
4	193,54	188,96	193,02	177,11	0,512	0,526	0,52	0,56
5	212,65	204,08	203,17	200,17	0,466	0,486	0,49	0,49
6	172,10	176,67	150,86	161,63	0,572	0,562	0,62	0,61
7	174,06	181,65	161,31	173,25	0,57	0,544	0,61	0,57
8	180,77	166,41	168,36	156,52	0,552	0,596	0,59	0,63
9	193,90	199,65	188,55	192,45	0,514	0,496	0,53	0,51
10	170,20	188,82	162,83	185,86	0,584	0,524	0,61	0,53
AVE	187,76	189,76	179,18	182,31	0,53	0,52	0,56	0,55
SD	13,03	11,64	16,47	14,14	0,04	0,03	0,05	0,04

Completion time. Figure 26 demonstrates the differences between completion time in different models. Used abbreviations are the following: GE (gaming task after exercise model) and GR (gaming task after the rest model).

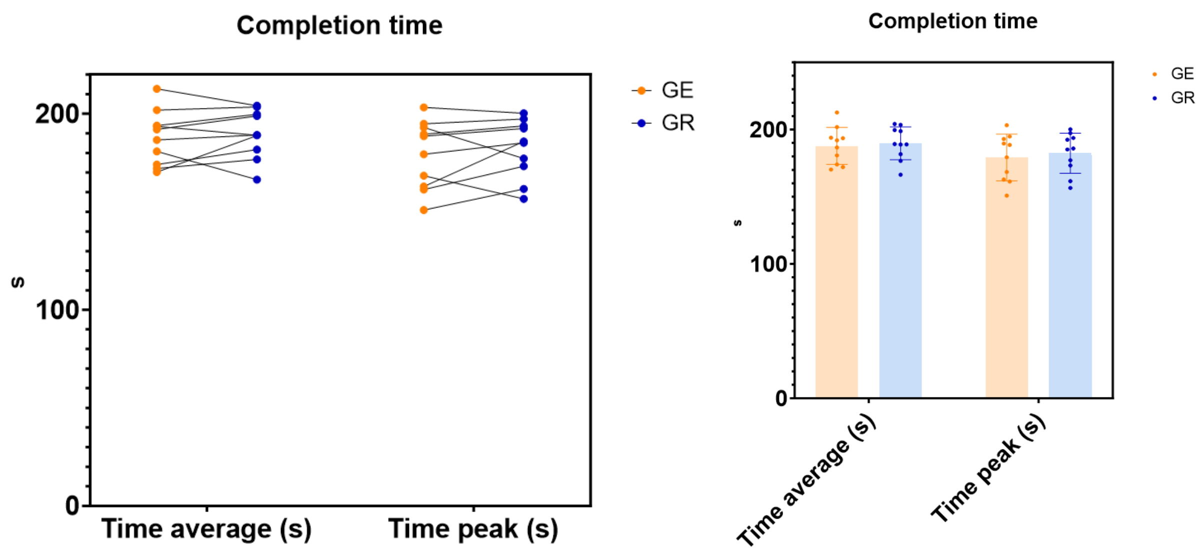

FIGURE 26. Aimbotz gaming task completion time (average and peak) between exercise and resting models.

No significant differences were found between exercise and resting models in completion time (avereage or peak). Table 15 demonstrates the statistical analysis of completion time values during gaming task.

TABLE 15. Statistical analysis (Wilcoxon) of Counter Strike: Global Offensive gaming performance parameter completion time.

	V	p-value	CI 95%
TimeR peak – TimeR peak	37	0,375	-5,032 – 11,351
TimeAR - TimeAE	34	0,5566	-4,5810 – 7,2155

Kills per second (KPS). Figure 27 demonstrates the differences between kills per second (KPS) in different models. Used abbreviations are the following: GE (gaming task after exercise model) and GR (gaming task after the rest model).

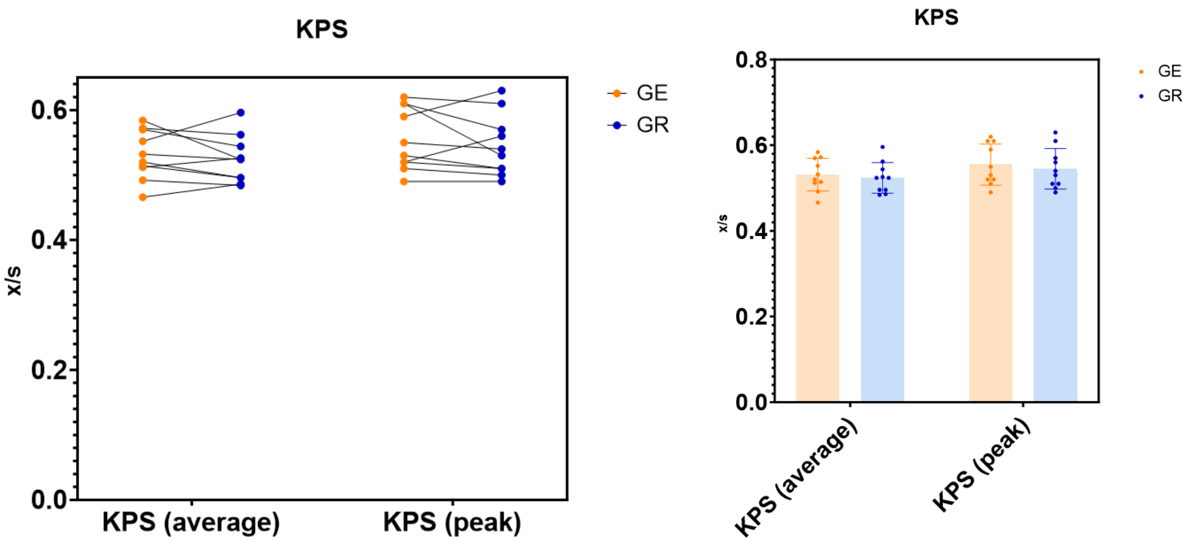

FIGURE 27. Aimbotz gaming task kills per second (KPS) values (average and peak) between exercise and resting models.

No significant differences were found between exercise and resting models in KPS values (average or peak). Table 16 demonstrates the statistical analysis of kills per second (KPS) values during gaming task.

TABLE 16 Statistical analysis (Wilcoxon) of Counter Strike: Global Offensive gaming performance parameter kills per second (KPS).

	V	p-value	CI 95%
KPSR peak – KPSE peak	14	0,3372	-0,04496908 – 0,015057
KPSa R – KPSa E	19	0,4145	-0,0259578 – 0,016999

8.4 Psychological stress and enjoyment

Psychological stress was evaluated by assessing mood state and total mood disturbance (TMD) changes between exercise and resting models. Enjoyment was assessed by overall Physical Activity Enjoyment Scale (PACES) scores.

Mood states. Table 17 demonstrates the Profile of Mood States (POMS) results (mood states and TMD) in exercise and resting models. Abbreviations E refer to exercise model and abbreviations R refer to resting model, respectively.

TABLE 17. Profile of Mood States (POMS) mood states in exercise and resting models (n=10)

	Tension _E	Tension _R	Depression_ E	Depression_ R	Anger _E	Anger_ R	Fatigue_ E	Fatigue_ R	Confusion _E	Confusion _R	Vigour_ E	Vigour_ R
1	7	10	5	12	2	6	8	4	8	5	20	21
2	10	6	1	0	0	0	6	1	5	1	19	21
3	7	6	6	7	4	2	6	7	4	6	22	20
4	5	4	2	0	0	0	3	4	3	4	18	15
5	5	1	0	0	0	0	6	4	4	3	21	25
6	2	2	0	2	0	1	0	0	0	1	25	27
7	3	6	0	0	0	0	1	2	3	6	23	19
8	9	3	0	0	0	0	3	2	4	4	27	18
9	1	2	0	0	0	0	0	1	0	1	32	27
10	9	7	4	3	1	2	7	12	5	5	20	17
AVE	5,8	4,7	1,8	2,4	0,7	1,1	4	3,7	3,6	3,6	22,7	21,0
SD	2,96	2,65	2,23	3,85	1,27	1,81	2,83	3,38	2,24	1,91	4,05	3,92

Figure 28 demonstrates the differences between Profile of Mood States (POMS) mood state scores in different models. Used abbreviations are the following: GE (gaming task after exercise model) and GR (gaming task after the rest model).

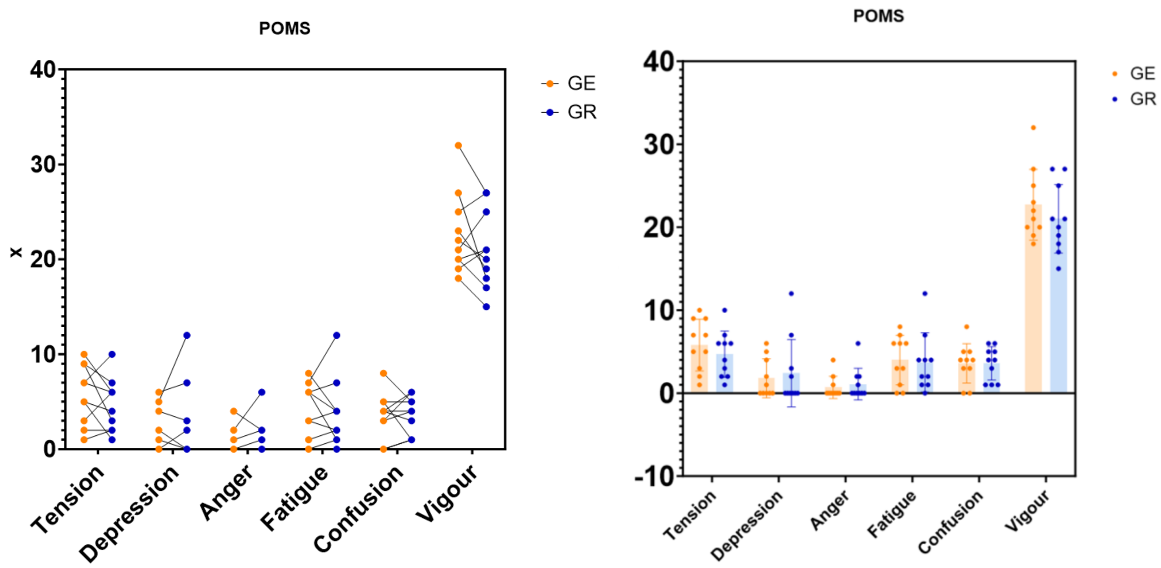

FIGURE 28. Profile of Mood States (POMS) mood state changes between exercise and resting models.

Exercise model showed a slight elevation in vigour compared to resting model, however no significant differences were found between the exercise and resting models. Table 18 demonstrates the statistical analysis of Profile of Mood States (POMS) mood state scores between different models.

TABLE 18 Statistical analysis of Profile of Mood States (POMS) mood state scores (Wilcoxon). No significant differences were found.

	V	p-value	CI 95%
POMS Tension E – POMS Tension R	32	0,2838	-1,000049 – 3,999983
POMS Depression E – POMS Depression R	8,5	0,7498	-4,000008 – 1,499997
POMS Anger E – POMS Anger R	3,0	0,5807	-2,5000447 – 0,5000447
POMS Fatigue E – POMS Fatigue R	24,5	0,8563	-1,999951 – 2,999952
POMS Confusion E – POMS Confusion R	17	0,9434	-2,000032 – 2,499966
POMS Vigour E – POMS Vigour R	40,5	0,2009	-1,5000058 – 4,499978

Total mood disturbance (TMD). Table 19 demonstrates the total mood disturbance (TMD) scores between different models. Abbreviations E refer to exercise model and abbreviations R refer to resting model, respectively.

TABLE 19. Total mood disturbance (TMD) between different models (n=10)

	TMD_E	TMD_R
1	10	16
2	3	-13
3	5	8
4	-5	-3
5	-6	-17
6	-23	-21
7	-16	-5
8	-11	-9
9	-31	-23
10	6	12
AVE	-6,8	-5,5
SD	12,79	13,06

Figure 29 demonstrates the differences between total mood disturbance (TMD) scores in different models. Used abbreviations are the following: GE (gaming task after exercise model) and GR (gaming task after the rest model).

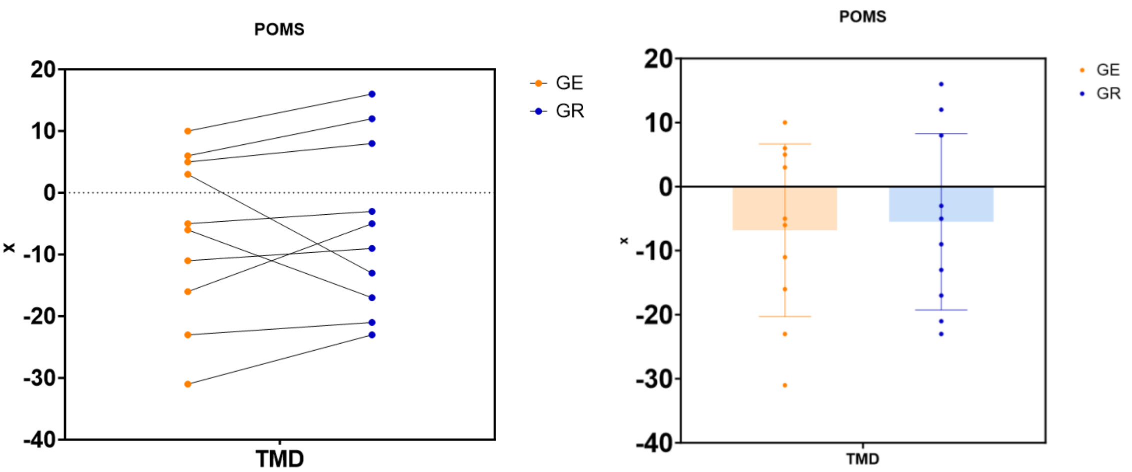

FIGURE 29. Total mood disturbance (TMD) changes between exercise and resting model.

No significant differences were found between exercise and resting models in TMD. Table 20 demonstrates the statistical analysis of POMS total mood disturbance (TMD) scores between exercise and resting models.

TABLE 20 Total mood disturbance (TMD) score statistical analysis (Wilcoxon)

	V	p-value	CI 95%
POMS TMDE - POMS TMDR	36,5	0,3844	-5,00065 – 6,999939

Enjoyment. Table 21 demonstrates Physical Activity Enjoyment Score (PACES) questionnaire results scores between different models.

TABLE 21. Physical activity enjoyment score (PACES) total scores and enjoyment category (1= lowest, 5= highest enjoyment, respectively).

	Score	Category (1-5)
1	70	3
2	76	3
3	78	3
4	59	2
5	83	3
6	109	5
7	80	3
8	75	3
9	116	5
10	101	4
Average	84,70	3,40
SD	17,18	0,92

Figure 30 illustrates the total scores of Physical Activity Enjoyment Scale (PACES) between different subjects. Colors represent the category of enjoyment (1-5, 1 representing the lowest and 5 the highest enjoyment score category).

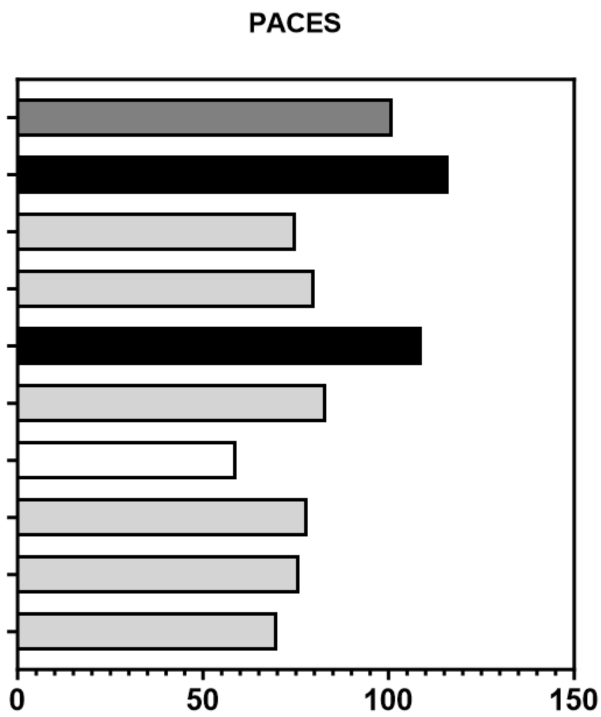

FIGURE 30. Physical Activity Enjoyment Scale (PACES) scores. Color representation: white=2, grey=3, dark grey=4 and black=5.

Physical activity enjoyment scale (PACES) scores indicate, that physical activity conducted in the study (HIIT) was mostly moderately or highly enjoyed.

9 DISCUSSION

The purpose of this study was to investigate the effect of an high-intensity interval training's effect on acute physiological responses and esports performance. The hypotheses for this study in this thesis were, that 1) high-intensity interval training (HIIT) session enhances changes in salivary stress marker, HR and HRV responses and 2) acute HIIT session improves esports performance parameters (completion time and kills per second (KPS)). In physiological stress responses, significant differences were found in wihin-day cortisol in exercise model, salivary alpha-amylase (sAA) between models (exercise and resting) and control, and in-game heart rate (HR). In cortisol, the levels showed a slight drop after the gaming session and after elevated levels from the HIIT session.

In cortisol, the levels showed a drop after the gaming session and after elevated levels from the HIIT session, whereas in resting model, the cortisol levels dropped in a more gradual manner. The elevated cortisol levels after the HIIT session are in line with the current literature (Dote-Montero et al 2021a; Martínez-Díaz et al 2020; Quintero et al 2018). However, when facing acute stress, cortisol levels remain elevated approximately 2 hours until the baseline (Molina-Hidalgo et al 2023; Giacomello et al 2020; Schumacher et al 2013). In the current results, the cortisol levels showed a drop after 35 minutes of initial acute stress (HIIT): after the HIIT session, subjects filled out a Profile of Mood States (POMS) questionnaire on an electronic tablet (duration approximately 15 minutes) and continued to reading session (duration 20 minutes). Altogether, subjects (in theory) were within the acute phase (0-60 min) of cortisol elevations after the HIIT session, when conducting the Counter Strike: Global Offensive gaming task (duration 20 minutes) (Dote-Montero et al 2021a). Therefore, it would be expected, that cortisol would remain elevated.

This could potentially be explained with the theorized potential dampening effect of exercise to stress responses (Caplin et al 2021; Dote-Montero et al 2021b; Zschucke et al 2015). As Zschucke et al (2015) found, overall cortisol output was reduced after a mild cognitive challenge task, when participants engaged in 30 minutes of moderate-to-vigorous aerobic exercise (60-70% VO2peak) before the mental challenges compared to non-exercise controls. This and other theories for dampened stress responses introduced in this thesis previously could potentially be applicable for the current study results as well, however no significant differences were found between the exercise and resting model to confirm this theory.

Salivary alpha-amylase (sAA) showed significant difference between control and both models after gaming task. Both models showed an increase compared to control levels of sAA. In the literature it was stated, that 20-30 minutes from HIIT session sAA levels show to elevate, and a stressful video game has been shown to elevate sAA levels after 15 minutes of play (Martínez-Díaz et al 2020; Monje et al 2020; Skosnik et al 2000). However, the subjects started gaming task approximately 35 minutes after the HIIT session with a duration of 55 minutes until sampling. Therefore, it would be expected, that sAA levels would remain elevated (or even be enhanced) after two consecutive sAA-elevating stressors. However, after the acute HIIT session, the elevations in sAA seem to be dampened in comparison to resting. This could also indicate potential functions of dampened sympathoadrenal-medulla pathway (SAM-p) stress responses. However, no other significant differences were found in sAA responses.

Dehydroepiandrosterone sulfate (DHEA-S) didn't show any significant changes. As stated before in the literature, DHEA could potentially be a better indicator for acute stress responses compared to DHEA-S due to its precursor origin (Dutheil et al 2021; Giacomello et al 2020). However, DHEA-S levels have also been reported to elevate during acute stress (Dutheil et al 2021; Giacomello et al 2020). In this study, a slight (but not significant) elevation were observed after HIIT (in exercise model) and after gaming (in resting model). These are different types of stressors, which could contribute to the findings, that different pathways (sympatho-andrenal-medullary pathway SAM-p and hypothalamus-pituitary-adrenal HPA axis) being activated during these models.

In the current study, average during-game heart rate (HR) showed a significant difference between the models. This is in line with current evidence, showing sympathetic activity to being activated in stressful situations, in this case, intense exercise in addition to Counter Strike: Global Offensive (CS:GO) (Sadowska et al 2023; Végh et al 2016). On the contrary to the De Las Heras (2020) findings, HR_{peak} didn't show significant differences between exercise and resting models. This may be due to a different game (League of Legends multiplayer online battle arena MOBA game vs. CS:GO a first-person shooter FPS game), as FPS games have been recorded to elicit a higher stress response compared to MOBA games (Sadowska et al 2023; Kraemer et al 2022; Sousa et al 2020; Valladão et al 2020). This could have potentially elicited a higher response in a game (such as a MOBA game), that didn't elicit higher HR responses. However, HRV responses didn't show significant changes. RMSSD and SD1 seemed slightly (but not significantly) lower in exercise model than in resting model. RMSSD reflects parasympathetic activation of ANS and SD1 short-term beat-to-beat variability of the data connected also to RMSSD (Järvelin-Pasanen et al 2018; Acharya et al 2006). Lower parasympathetic activation in exercise model could indicate a sympathetic dominance, which is in line with HR responses.

In the current study, neither of the chosen esports performance parameters (completion time and KPS) showed significant differences. This is in contrast with the original paper from De Las Heras et al (2020) study, where 15-minute HIIT exercise improved significantly the capacity to eliminate targets (9%) and increased accuracy 75% in MOBA game League of Legends. However, completion time was slightly (but not significantly) faster and KPS was slightly higher in exercise model compared to resting model. These findings might be due to the small sample size and general high skill-level of the players, however the overall strain from the HIIT session could have potentially been excessive: during the light-intensity (40% HR_{max}) periods HR levels showed to elevate above 50% HR_{max}. In some studies, highly intensive exercise has been shown also to impair cognitive functions (Quintero et al 2018; Martínez-Díaz et al 2020). This could be also an explaining factor for the current study findings. On the other hand, HIIT didn't show a significant decline in the esports performance parameters either. This could indicate, that the HIIT training's intensity wasn't on a level, that would cause catecholamines elevations, that could cause detrimental effects on cognitive function (Quintero et al 2018; Martínez-Díaz et al 2020). Therefore, it could be indicated, that there would potentially be no harm to execute this type of exercise before esports playing.

From the psychological point of view, no significant differences were found between exercise and resting models in mood states or total mood disturbances (TMD). However, resting model demonstrated to have slightly (but not significantly) higher depression and fatigue scores compared to exercise model. Moreover, exercise model demonstrated to have slightly (but not significantly) higher confusion and vigour compared to the resting model. This generally would indicate a more positive, ”energetic”, response to exercise, with resting indicating negative-related responses. To demonstrate the positive/negative effect in practice, Selänne et al (2001) have demonstrated a profile of mood states of an elite and unsuccesful athlete (in traditional sports), demonstrated in figure 31.

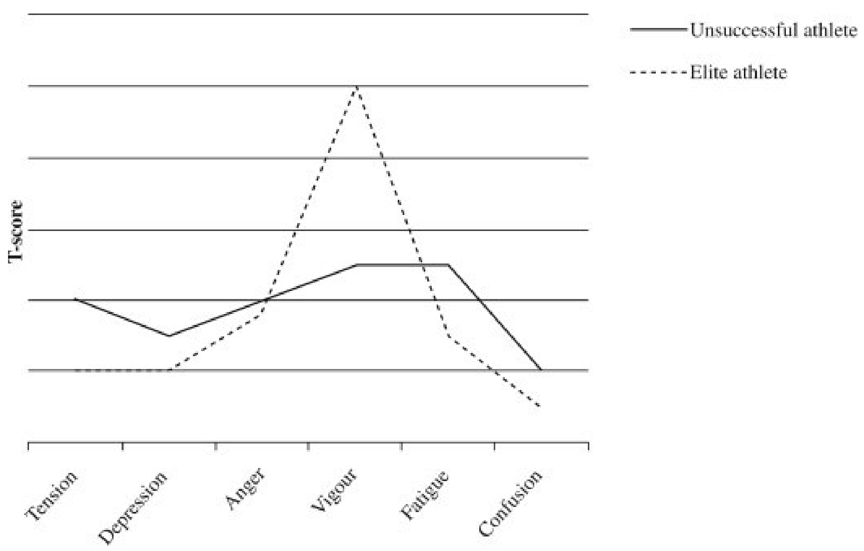

FIGURE 31. Profile of mood states (POMS) mood profile of an elite and unsuccessful athlete (Selänne et al 2001).

Figure 32 demonstrates the mood profiles of the current study in Selänne et al (2001) type of graph. Orange line demonstrating the results in the exercise model, and blue line demonstrating the results in resting model.

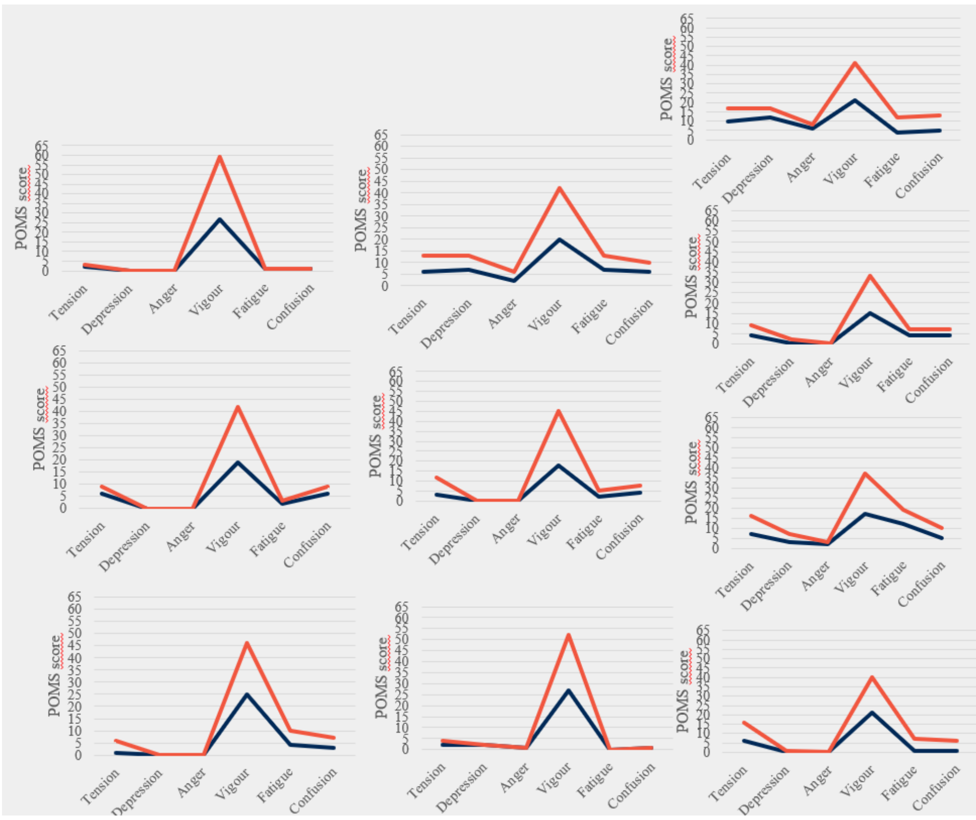

FIGURE 32. Individual mood profiles of the participants in the current study.

By graphical analysis of the graphs in figure 32, it would seem, that exercise model would provide a more favourable mood profile. This can be seen as a slightly (but not significantly higher peaks in vigour scores. Exercise is found to protect against pervasive effects of stress and anxiety (De Las Heras et al 2020). Even though the profile was created with traditional athletes, however could potentially be applicable to esports athletes as well. Moreover, Physical Activity Enjoyment Score (PACES) indicate, that the physical activity conducted in the study (HIIT) was at least moderately enjoyed among the subjects. Therefore, it could be assumed, that no excessive stress was caused to the subjects by participation in an activity, that they would find unenjoyable.

Overall, these results could indicate, that both sympatho-adrenal-medulla pathway (SAM-p) and hypothalamic-pituitary-adrenal (HPA) axis stress responses were affected during the models, manifesting in endocrinological and cardiac responses. However, these results are also mixed, whether the responses induced by high-intensity training are beneficial for the esports athlete acutely or long-term. In general, stress hormone cortisol is a catabolic hormone, that has been connected with health issues in long term (Kilian et al 2016; Charmandari et al 2005). Whether the catabolic hormone levels are enhanced by intense exercise (such as HIIT), the accumulating allostatic stress load could turn the positive effects of exercise into net negative. However, the results demonstrated a potential dampening effect of the stress pathways and a slight antiglucocorticoid response, which could indicate a protective response for allostatic stress load and therefore could have potential for health benefits in the esports players. However, more studies are needed to confirm these indications.

Finally, even though significant differences were not always found in the current study, clinical significance needs to be taken in consideration. The intra-individual changes showed great variation how the responses behaved in different models. This could indicate, that there could be an individual optimal arousal level (physiological and psychological) for the individual's best esports performance. This could be applied to individual esports coaching: whether an individual finds this type of exercise beneficial for their stress responses or esports performance, the type of exercise could be added into their regular esports training program. However, optimal arousal levels or stress responses in esports have been limitedly studied. More studies are still needed to understand better physiological stress responses with psychological considerations, and their effects on esports performance.

10 CONCLUSION AND PRACTICAL APPLICATIONS

Physical activity and video games are commonly regarded as antagonistic activities (De Las Heras et al 2020). This thesis aimed to enlighten the connections between the fields of exercise sciences and video game studies, and the potential benefits from utilizing exercise science field expertise on (commonly seen as sedentary) esports community through investigating esports stress environment and potential effects of an exercise intervention in relevant stress and performance parameters. To conclude the results of this thesis study, high-intensity interval training (HIIT) could be a potential modality to dampen stress responses in esports players, and this type of exercise didn't harm the esports performance in the subjects.

In the current thesis study, strengths and limitations were recognized, and future research considerations and recommendations are provided.

10.1 Strengths and Limitations

Strengths. Strengths of this study were 1) a solid foundation of De Las Heras et al (2020) well-constructed study setting. The study setting had clear instructions for HIIT intervention and GXT (VO_{2max}) test, that were simple to replicate. Moreover, study setting had versatility to add or change preferred measurements or parameters (e.g. saliva sampling, Profile of Mood States questionnaire). 2) the comprehensive subject recruitment and familiarization process. When asked, the subjects felt they were well informed about the study before participation. 3) AimBotz Aim training setting of 100 targets was easily standardized and it provided solid information of essential esports performance data. 4) analysing the salivary data. Salivary data was handled in University of Jyväskylä by trained professionals, therefore providing reliability to sample handling.

Limitations. Multiple limitations were recognized in this study. The most impactful limitation of this study was the small sample size. Due to the small sample size, potential statistical significance in multiple parameters might have been mitigated. Regarding saliva samples, some samples (e.g. alpha-amylase PostGR) results differed visibly from other subjects' values. This was considered as an outlier, however with the GraphPad Prism (10.1.0 (316)) outlier identification with ROUT method (Q=5%) didn't identify the values as outliers. Therefore, the values were included in the data. This affects the standard deviation, which may also be due to the small number of subjects.

Another important limitation to this study was the lack of funding, which limited the choices for methodology. Most of the study methodology was set by the original study setting, however specialized additions were made to the setting to apply to stress physiology measurements. For example, the golden standards for body composition measurement are dual x-ray absorption (DXA), and the analysis of salivary samples LC-MS/MS instead of immunoassays. Furthermore, salivary sampling golden standard is passive drooling instead of stimulated saliva, however it can be challenging to collect in exercise conditions due to the potential of decreased amount of saliva production. Therefore, the compromises for golden standard methodology were made due to the lack of funding (e.g. expensive methodology vs readily available methodology) and practicality (e.g. subjects conducting saliva sampling themselves) considered, which could affect the eventual results.

Moreover, the subjects were recruited via volunteering, which could have led to a bias: more physically active/fit and/or more skilled esports players could have participated in the study, biasing the data. Most participants of the study were highly ranked in Counter Strike: Global Offensive (CS:GO), which indicates, that this study focused on more on high-skill players. Moreover, some of the study subjects were more physically fit than others, which could explain some of the variability in the responses to a strenuous exercise. Therefore, the variability in the study subjects' backgrounds (e.g. highest skill and best physical fitness could have been a key confounding factor. The study also included only male participants. Female participants were excluded from the study due to the potential effect of sex-differences in physical performance tests and interventions.

The times for the measurements were chosen between 10 am and 6 pm. This gave a potential challenge to compare the results between the subjects in a study, where diurnally rhythmic biomarkers are on focus. Especially there could have been an effect between subjects, who started their measurements at 10 am and subjects, who started their measurements at 6 pm. Moreover, in the preliminary bioimpedance measurements, the subjects were given a 2-hour limit to not consume any food or liquids instead of full 8-hour fasting as the golden standard for bioimpedance body composition measurements recommend, which could have affected the reliability of the bioimpedance results.

Furthermore, the exercise and resting models were conducted in the GamePit lobby area, where there was an access to a classroom with occasional traffic of students of the university of applied sciences. Despite the cautionary informing (leaflets, student intranet) about the research hours in the area, there was occasional students moving from classroom through the lobby area. This could have affected the stress responses of a subject, especially in the resting protocol, during which the silence was broken with passing students' talking, opening doors, or viewing the research being conducted. Especially immediate stress responses could take place in these circumstances, affecting the true resting conditions.

In the literature review, the studies over esports and exercise were limited. The results of the different studies are generally mixed in quality, and some articles were excluded due to their publications in journals with low impact factors. This limited the quantity of usable articles in this thesis regarding esports.

The questionnaires used in the study were translated in Finnish. Especially in Profile of Mood States, the subjects state the stages of multiple emotions, they are currently feeling. There is potential for errors in similar terms or misunderstandings in the translations, which may lead to biased results. Moreover, the validity of the inventories doesn't directly apply to inventories translated in different languages. In addition, due to an error, Profile of Mood States questionnaire missed one inventory regarding Anger (the inventory "*deceived*"). Anger scores were relatively low among the subjects in both models (exercise and resting), however, this could affect the potential reliability and applicability of the results.

High-intensity interval (HIIT) session was potentially straining for the subjects. During the light-intensity intervals (<40% HR_{max}) between high-intensity intervals (80% HR_{max}) the subjects showed 55-85% HR_{max} heart rate responses. Therefore, some subjects didn't potentially have the variability of HIIT. This could have led to excessive strain, and to potentially biased responses in consecutive stress marker values.

In heart rate variability (HRV) data handling, no exact time stamps were recorded for the gaming session at the time of the esports performance test by error. Therefore, in the data handling phase with Kubios, to assess the correct time of the CS:GO gaming task, other data (e.g. Monark HIIT session data, Webropol questionnaire response data) was used to assess the durations of previous phases of the experimental days. This could have lead to inaccuracies in pointing the starting time of the gaming task in Kubios HRV analysis. Moreover, Firstbeat Bodyguard 2 data was partly or totally corrupted in some subjects, which lead to data exclusion. In addition, the HRV control data was collected from 3 day (>24 hour) data, whereas the gaming session data was collected from 20 minutes, which could lead to comparability issues and inaccuracies.

The subjects conducted the morning saliva sampling by their own with given instructions. Even though saliva sampling with Salivette® tubes have been reported as a simple method and salivary steroids being relatively stable in room temperature, the subjects might have not handled the sample appropriately (e.g. storing in fridge, avoiding touching the swab).

During the video gaming task, a repetitive pattern of 20 targets was found in AimBotz Aim training map. It could be seen from subjects of higher skill, or with subjects more familiar with the aim training map, that they recognized the pattern occurring. Moreover, the overall esport performance was conducted as a simulated manner. It has been shown that the responses to a simulated game compared to a game against another human elicits attenuated stress responses. This exclusion potential of socio-evaluative threat (Leis & Lautenbach 2020) could have affected the stress response results and is not potentially applicable for real-life competitive esports settings with multiple phases of the game.

Studying stress responses, trait or state anxiety were not controlled within subjects. Diagnosed mental health issues (e.g. depression) were controlled in the preliminary health questionnaire, however, undiagnosed and symptoms of anxiety were not controlled within the subjects. These could have potentially shown effect on stress responses, and potentially help differentiate the origins of inter-individual responses variability.

At the moment of this thesis to be finished in December 2023, Counter Strike: Global Offensive (CS:GO) has been updated in September 2023 by Valve Corporation to Counter Strike 2. The update included visual enhancements and quality of life additions to the game, while the ruleset and gameplay mechanics remained similar to the original CS:GO. Therefore, due to the visual changes, the study results might not directly indicate similar results in the present updated version of the game, and be instead, more preliminary.

Finally, the inexperience of the study staff affected the results. Using questionnaires and inventories outside the field of sport sciences and conducting a multidisciplinary study combining non-exercise related interventions and methods could have affected the reliable conduction of the overall study.

10.2 Future considerations

The importance of physical fitness and exercise for esports performance is not yet clearly determined. However, there is potential, that exercise interventions could help encounter the effects of sedentary nature of video gaming and enhance the beneficial adaptations, that are needed to conduct peak esports performance. Moreover, the connections between physical fitness (e.g. endurance, strength and speed) and esports performance are encouraged to be investigated further.

More studies are needed to examine these connections with different modalities of exercise (e.g. type, duration, intensity), different types of esports games (e.g. FPS, MOBA) and esports players with variable backgrounds (e.g. female esports players). Being multifaceted in nature, in the future research, psychological factors (e.g. state and trait anxiety), cognitive factors and potentially neuroimaging (e.g. fMRI) need to be taken in consideration and utilized in esports studies to help understand better beneficial practices for exercise interventions for esports athletes.

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APPENDICE 1. Health Questionnaire

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

Esitietolomake

On tärkeää, että tiedämme elintavoistasi ja aiemmista liikuntatottumuksista ennen kuin testaamme sinut. Vastaa seuraaviin kysymyksiin huolellisesti.

1. Nimi *

____

____

____

____

____

2. Syntymäaika *

pp.kk.vvvv

3. Paino (kg) *

____

4. Pituus (cm) *

____

5. Terveydentila

1. Onko sinulla ollut rintakipuja?

Oireet viimeisen 6 kk aikana *

○
○
○

Levossa?

Oireet viimeisen 6 kk aikana *

Rasituksessa?

Oireet viimeisen 6 kk aikana *

2. Onko sinulla ollut rasitukseen liittyvää hengenahdistusta?

Oireet viimeisen 6 kk aikana *

3. Onko sinulla ollut huimausoireita?

Oireet viimeisen 6 kk aikana *

4. Onko sinulla ollut rytmihäiriötuntemuksia?

Oireet viimeisen 6 kk aikana *

5. Onko sinulla ollut harjoittelua estäviä kipuja liikuntaelimissä?

Oireet viimeisen 6 kk aikana *

6. Oletko tuntenut ylikuormitus- tai stressioireita?

Oireet viimeisen 6 kk aikana *

- ○
- ○
- ○

6. Mikäli vastasit kohtaan 5.5 "Kyllä", missä liikuntaelimissä sinulla on ollut harjoittelua estäviä kipuja?

____

____

____

____

____

7. Todetut sairaudet: Onko sinulla tai onko ollut jokin/joitakin seuraavista? Voit valita yhden tai useamman vaihtoehdon. *

- ☐ Sepelvaltimotauti
- ☐ Sydäninfarkti
- ☐ Kohonnut verenpaine
- ☐ Sydänläppävika
- ☐ Aivohalvaus
- ☐ Aivoverenkierron häiriö
- ☐ Sydämen rytmihäiriö
- ☐ Sydämentahdistin
- ☐ Sydänlihassairaus
- ☐ Syvä laskimotukos
- ☐ Muu verisuonisairaus
- ☐ Krooninen bronkiitti
- ☐ Keuhkolaajentuma
- ☐ Astma
- ☐ Muu keuhkosairaus
- ☐ Allergia
- ☐ Kilpirauhasen toimintahäiriö
- ☐ Diabetes
- ☐ Anemia
- ☐ Korkea veren kolesteroli

- [ ] Nivelreuma
- [ ] Nivelrikko, -kuluma
- [ ] Krooninen selkäsairaus
- [ ] Mahahaava
- [ ] Pallea-, nivus- tai napatyrä
- [ ] Ruokatorven tulehdus
- [ ] Kasvain tai syöpä
- [ ] Leikkaus äskettäin
- [ ] Mielenterveyden ongelma
- [ ] Tapaturma äskettäin
- [ ] Matala veren K tai Mg
- [ ] Kohonnut silmänpaine
- [ ] Näön tai kuulon heikkous
- [ ] Urheiluvamma äskettäin
- [ ] Muu sairaus tai oire, mitä ________________
- [ ] Ei mitään sairauksia

8. Käytätkö jotain lääkitystä tai lääkeainetta säännöllisesti tai usein? *

- ( ) En
- ( ) Kyllä, mitä ________________

9. Käytätkö jotain ravintolisiä säännöllisesti tai usein? *

- ( ) En
- ( ) Kyllä, mitä ________________

10. Tupakoitko? *

- ( ) En
- ( ) Kyllä
- ( ) Olen lopettanut

11. Käytätkö nuuskaa? *

- ( ) En
- ( ) Kyllä

○ Olen lopettanut

12. Onko ollut seuraavia oireita viimeisen kahden viikon aikana: kuume, flunssainen olo, muuten poikkeava väsymys? *

○ Ei

○ Kyllä

13. Onko lähisuvussasi ennenaikaiseen kuolemaan johtaneita sydänsairauksia? *

○ Ei

○ Kyllä

14. Mikäli vastasit kysymyksessä 13 "Kyllä", onko kyseessä lähisukulainen? Minkä ikäisenä todettu?

____

____

____

____

____

15. Onko todettu synnynnäistä sydänvikaa? *

○ Ei

○ Kyllä, mikä ______________

16. Kuinka monta kertaa teet fyysistä harjoittelua viikossa? *

○ 0

○ 1

○ 2

○ 3

○ 4

○ 5

○ 6

○ 7 tai enemmän, kuinka monta:

17. Kuinka monta tuntia (h) teet fyysistä harjoittelua viikossa? *

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 7 tai enemmän, kuinka monta: ____________________

18. Arvioi oma kuntosi asteikolla 1-5 (1= heikko, 2= välttävä, 3= keskitasoinen, 4= hyvä, 5= erinomainen): *

	1	2	3	4	5	
Heikko	◯	◯	◯	◯	◯	Erinomainen

19. Kuinka monta tuntia (h) keskimäärin nukut yössä? *

____

20. Onko sinulla todettua uneen tai nukkumiseen vaikuttavaa sairautta? *

- ◯ Ei
- ◯ Kyllä, mikä ____________________

21. Onko sinulla ollut vaikeuksia nukahtamisen tai nukkumisen yhtäjaksoisuuden kanssa? *

- ◯ Ei
- ◯ Kyllä, millaisia: ____________________

22. Arvioi unesi laatua asteikolla 1-5 (1= heikko, 2= välttävä, 3= keskitasoinen, 4= hyvä, 5= erinomainen) *

	1	2	3	4	5	
Heikko	◯	◯	◯	◯	◯	Erinomainen

23. Kuinka palautuneeksi tunnet itsesi nukkumisen jälkeen? *

En lainkaan palautuneeksi 0 — 10 Erittäin palautuneeksi

24. Käytätkö kofeiinipitoisia juomia (esim. kahvi, energiajuomat)? *

- ◯ En
- ◯ Kyllä, mitä ____________________

25. Kuinka monta annosta (esim. kuppi, pullo, tölkki) kofeiinipitoisia juomia käytät päivässä? Jos et käytä, laita annokseksi 0. *

____

26. Käytätkö kofeiinivalmisteita (esim. kofeiinitabletit)? *

- ◯ En
- ◯ Kyllä, mitä ____________________

27. Kuinka monta annosta (esim. tablettia) kofeiinivalmisteita käytät päivässä? Jos et käytä, laita annokseksi 0. *

____

28. Kuinka monta kertaa pelaat videopelejä viikossa? (viihteeksi tai tavoitteellisesti) *

- ◯ 0
- ◯ 1
- ◯ 2

- 3
- 4
- 5
- 6
- 7 tai enemmän, kuinka monta: ________

29. Kuinka monta tuntia (h) pelaat videopelejä viikossa? (viihteeksi tai tavoitteellisesti) *

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7 tai enemmän, kuinka monta ________

30. Kuinka monta kertaa pelaat videopelejä tavoitteellisesti viikossa? *

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7 tai enemmän, kuinka monta ________

31. Kuinka monta tuntia (h) pelaat videopelejä tavoitteellisesti viikossa? *

- 0
- 1
- 2
- 3
- 4
- 5
- 6

◯ 7 tai enemmän, kuinka monta: ____________________

32. Kilpailullisen videopelaamisen taitotaso (eng. rank) Counter Strike: Global Offensive -pelissä: *

APPENDICE 2. International physical activity questionnaire IPAQ.

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

Fyysisen aktiivisuuden kysely IPAQ

Kyselyssä tiedustellaan viime viikoosi (7 edeltävää päivää) sisältynyttä fyysistä aktiivisuutta.

Vastaathan jokaiseen kysymykseen, vaikka et pitäisikään itseäsi liikunnallisena ihmisenä.

Ajattele kaikkia toimintoja, joita teit töissä, osana koti- ja pihatöitä, siirtyessäsi paikasta toiseen, sekä vapaa-aikanasi virkistyksen kuntoilun tai urheilun vuoksi.

Ajattele kaikkea fyysisesti raskasta toimintaa, jota teit viime viikon aikana.

Raskaalla fyysisellä aktiivisuudella tarkoitetaan toimintoja, jotka vaativat kovaa ruumiillista ponnistelua, ja jotka saavat hengityksesi kiihtymään paljon tavallista nopeammaksi.

Ota huomioon vain ne toiminnat, jotka kestivät vähintään 10 minuuttia kerralla.

Mikäli sinulla ei ollut lainkaan raskasta fyysistä aktiivisuutta, valitse kysymyksessä 3 "Siirry seuraavaan kysymykseen", ja paina Seuraava.

1. Kuinka monena päivänä viime viikon aikana fyysinen aktiivisuutesi oli raskasta, esim painavien taakkojen nostamista, kaivamista, aerobicia tai vauhdikasta pyöräilyä? (vastaus muotoa: ___ päivänä viikossa)

2. Kuinka paljon aikaa käytit keskimäärin tuollaisena päivänä raskaaseen fyysiseen aktiivisuuteen? (vastaus muotoa: ___ tuntia _____ minuuttia päivässä)

3. Ei lainkaan raskasta fyysistä aktiivisuutta

☐ Siirry seuraavaan kysymykseen

Ajattele kaikkea kohtalaisen rasittavaa fyysistä toimintaa, jota teit viime viikon aikana.

Kohtalaisen raskaalla fyysisellä aktiivisuudella tarkoitetaan toimintoja, jotka vaativat kohtalaista ruumiillista ponnistelua, ja jotka saavat hengityksesi kiihtymään jonkin verran tavallista nopeammaksi.

Ajattele jälleen vain niitä toimintoja, jotka kestivät vähintään 10 minuuttia kerrallaan.

Mikäli sinulla ei ollut lainkaan kohtalaisen raskasta fyysistä aktiivisuutta, valitse kysymyksessä 6 "Siirry seuraavaan kysymykseen", ja paina Seuraava.

4. Kuinka monena päiävnä viime viikon aikana fyysinen aktiivisuutesi oli kohtalaisen raskasta, kuten kevyiden taakkojen kantamista, tavallista pyöräilyä tai tenniksen nelinpeliä? Älä laske mukaan kävelyä. (vastaus muotoa: ___ päivänä viikossa)

5. Kuinka paljon aikaa käytit keskimäärin tuollaisena päivänä kohtalaisen raskaaseen fyysiseen aktiivisuuteen? (vastaus muotoa _____ tuntia _____ minuuttia päivässä)

6. Ei lainkaan kohtalaisen raskasta fyysistä aktiivisuutta.

☐ Siirry seuraavaan kysymykseen.

Ajattele aikaa, jonka käytit kävelemiseen viime viikon aikana. Tähän sisältyy kävely töissä tai kotona, kävely paikasta toiseen siirtyessäsi, ja kaikki muu kävely, jota teit pelkästään urheilun, kuntoilun tai virkistyksen vuoksi.

Mikäli sinulla ei ollut lainkaan kävelyä, valitse kysymyksessä 9 "Siirry seuraavaan kysymykseen", ja paina Seuraava.

7. Kuinka monena päivänä viime viikon aikana kävelit vähintään 10 minuuttia kerrallaan? (vastaus muotoa: ___ päivänä viikossa)

8. Kuinka paljon aikaa käytit keskimäärin kävelyyn tuollaisena päivänä? (vastaus muotoa: ___tuntia ___ minuuttia päivässä)

9. Ei lainkaan kävelyä.

☐ Siirry seuraavaan kysymykseen

Viimeinen osio koskee aikaa, jonka käytit istumiseen viime viikon aikana. Ota huomioon aika esim. töissä, kotona opiskellessasi ja vapaa-aikanasi sekä matkustaessa. Tähän sisältyy aika, jonka istuit työpöydän ääressä, ystävien luona käydessä tai televisiota katsellessa istuen tai maaten.

10. Kuinka paljon keskimäärin käytit aikaa istumiseen tavallisena arkipäivänä viime viikon aikana? (vastaus muotoa: ___tuntia ___ minuuttia päivässä)

11. Kuinka paljon keskimäärin käytit aikaa istumiseen viikonloppuna viime viikon aikana? (vastaus muotoa: ____ tuntia ___ minuuttia päivässä)

12. Kuinka paljon keskimäärin käytit aikaa nukkumiseen tavallisena arkipäivänä viime viikon aikana? (vastaus muotoa: ___ tuntia ___ minuuttia päivässä)

13. Kuinka paljon keskimäärin käytit aikaa nukkumiseen viikonloppuna (la-su) viime viikon aikana? (vastaus muotoa: ___tuntia ___ minuuttia päivässä)

APPENDICE 3. Edinburgh Handedness Inventory

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

ESPORT Edinburgh kätisyyskysely

Merkitse kunkin tehtävän (1-10) kohdalla rasti kohtaan, joka kuvaa parhaiten käsiesi käyttöä kysymyksessä olevan toiminnan tapauksessa. Rastita vain yksi vaihtoehto per kysymys.

Jotkin alla luetelluista tehtävistä vaativat molempien käsien käyttöä. Näissä tapauksissa, tehtävän tarkempi osa tai kohde on ilmoitettu sulkeissa.

1.

	Kätisyys				
	Vain vasemmalla	Useimmiten vasemmalla	Molemmilla yhtä paljon	Useimmiten oikealla	Vain oikealla
Kirjoittaminen	○	○	○	○	○
Piirtäminen	○	○	○	○	○
Heittäminen	○	○	○	○	○
Leikkaaminen saksilla	○	○	○	○	○
Hampaiden harjaus	○	○	○	○	○
Veitsen käyttö (ilman haarukkaa)	○	○	○	○	○
Lusikan käyttö	○	○	○	○	○
Lakaiseminen harjalla (ylempi käsi)	○	○	○	○	○
Tulitikun sytyttäminen (tulitikku)	○	○	○	○	○
Lasipurkin avaaminen (kansi)	○	○	○	○	○

Lähetä

APPENDICE 4. Profile of Mood States (POMS)

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

ESPORT Profile of Mood States (POMS; McNair, Lorr & Droppleman 1971)

1. Tutkimuspäivä

◯ 2. tutkimuspäivä (tiistai)

◯ 3. tutkimuspäivä (torstai)

2. Tutkimuspäivän suoritusmalli

◯ Lepomalli

◯ Liikuntamalli

3. Alla on luettelo tunnetiloista, joita kuka tahansa voi kokea.

Käy luettelo läpi huolellisesti, ja valitse kultakin riviltä osa, joka parhaiten kuvaa sitä, miltä Sinusta tuntuu juuri nyt.

	Tunnetilan aste				
	Ei lainkaan 0	Melko vähän 1	Jonkin verran 2	Melko paljon 3	Erittäin paljon 4
Ystävällinen	◯	◯	◯	◯	◯
Jännittynyt	◯	◯	◯	◯	◯
Suuttunut	◯	◯	◯	◯	◯
Uupunut	◯	◯	◯	◯	◯
Onneton	◯	◯	◯	◯	◯
Selväjärkinen	◯	◯	◯	◯	◯
Pirteä	◯	◯	◯	◯	◯
Hämmentynyt	◯	◯	◯	◯	◯
Katuva	◯	◯	◯	◯	◯
Epävarma	◯	◯	◯	◯	◯
Haluton	◯	◯	◯	◯	◯
Ärtynyt	◯	◯	◯	◯	◯
Huomaavainen	◯	◯	◯	◯	◯
Surullinen	◯	◯	◯	◯	◯
Toimelias	◯	◯	◯	◯	◯
Kireä	◯	◯	◯	◯	◯
Pahantuulinen	◯	◯	◯	◯	◯
Alakuloinen	◯	◯	◯	◯	◯
Tarmokas	◯	◯	◯	◯	◯
Pakokauhun vallassa	◯	◯	◯	◯	◯
Toivoton	◯	◯	◯	◯	◯
Rentoutunut	◯	◯	◯	◯	◯
Kelvoton	◯	◯	◯	◯	◯
Ilkeä	◯	◯	◯	◯	◯
Myötätuntoinen	◯	◯	◯	◯	◯
Vaivautunut	◯	◯	◯	◯	◯
Levoton	◯	◯	◯	◯	◯
Keskittymiskyvytön	◯	◯	◯	◯	◯
Väsynyt	◯	◯	◯	◯	◯
Avulias	◯	◯	◯	◯	◯
Harmistunut	◯	◯	◯	◯	◯
Masentunut	◯	◯	◯	◯	◯
Pahastunut	◯	◯	◯	◯	◯
Hermostunut	◯	◯	◯	◯	◯
Yksinäinen	◯	◯	◯	◯	◯
Kurja	◯	◯	◯	◯	◯
Sekava	◯	◯	◯	◯	◯
Iloinen	◯	◯	◯	◯	◯
Katkera	◯	◯	◯	◯	◯
Lopen uupunut	◯	◯	◯	◯	◯

Tuskainen	○	○	○	○	○
Riidanhaluinen	○	○	○	○	○
Kiltti	○	○	○	○	○
Synkkä	○	○	○	○	○
Epätoivoinen	○	○	○	○	○
Saamaton	○	○	○	○	○
Kapinoiva	○	○	○	○	○
Avuton	○	○	○	○	○
Kyllästynyt	○	○	○	○	○
Pyörällä päästään	○	○	○	○	○
Tarkkaavainen	○	○	○	○	○
Raivostunut	○	○	○	○	○
Tehokas	○	○	○	○	○
Luottavainen	○	○	○	○	○
Täynnä puhtia	○	○	○	○	○
Huonotuulinen	○	○	○	○	○
Arvoton	○	○	○	○	○
Muistamaton	○	○	○	○	○
Huoleton	○	○	○	○	○
Kauhistunut	○	○	○	○	○
Syyllisyydentuntoinen	○	○	○	○	○
Elinvoimainen	○	○	○	○	○
Epäröivä	○	○	○	○	○
Nääntynyt	○	○	○	○	○

Lähetä

APPENDICE 5. Physical Activity Enjoyment Scale (PACES)

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

Physical Activity Enjoyment Scale (PACES)

Pakolliset kysymykset merkitty tähdellä (*)

1. Arvioi, mitkä ovat yleiset näkemyksesi kestävyystyyppisestä harjoittelusta: samanlaisesta, jonka suoritit tutkimuksessamme. *

	1	2	3	4	5	6	7	
Nautin siitä	◯	◯	◯	◯	◯	◯	◯	Vihaan sitä
Se on mielestäni tylsää	◯	◯	◯	◯	◯	◯	◯	Se on mielestäni kiinnostavaa
En pidä siitä	◯	◯	◯	◯	◯	◯	◯	Pidän siitä
Se on miellyttävää	◯	◯	◯	◯	◯	◯	◯	Se on epämiellyttävää
Olen erittäin paljon mukana tässä toiminnassa	◯	◯	◯	◯	◯	◯	◯	En ole lainkaan mukana tässä toiminnassa
Se ei ole mielestäni lainkaan hauskaa	◯	◯	◯	◯	◯	◯	◯	Se on mielestäni erittäin hauskaa
Saan siitä energiaa	◯	◯	◯	◯	◯	◯	◯	Se rasittaa minua
Se masentaa minua *	◯	◯	◯	◯	◯	◯	◯	Se tekee minut iloiseksi
Se on erittäin mukavaa	◯	◯	◯	◯	◯	◯	◯	Se ei ole lainkaan mukavaa
Se tuntuu fyysisesti hyvältä	◯	◯	◯	◯	◯	◯	◯	Se tuntuu fyysisesti epämiellyttävältä
Se on erittäin elvyttävää	◯	◯	◯	◯	◯	◯	◯	Se ei ole lainkaan elvyttävää
Olen erittäin turhautunut siihen	◯	◯	◯	◯	◯	◯	◯	En ole lainkaan turhautunut siitä
Se on erittäin palkitsevaa	◯	◯	◯	◯	◯	◯	◯	Se ei ole lainkaan palkitsevaa
Se on erittäin piristävää	◯	◯	◯	◯	◯	◯	◯	Se ei ole lainkaan piristävää
Se ei ole lainkaan stimuloivaa	◯	◯	◯	◯	◯	◯	◯	Se on erittäin stimuloivaa
Tunnen vahvoja saavutuksen tuntemuksia	◯	◯	◯	◯	◯	◯	◯	Se ei anna lainkaan saavutuksen tuntemuksia
Se on erittäin virkistävää	◯	◯	◯	◯	◯	◯	◯	Se ei ole lainkaan virkistävää
Teen mielummin jotain muuta kuin tätä	◯	◯	◯	◯	◯	◯	◯	Teen mieluiten juuri tätä

Lähetä