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Exercise, Stress, and Recovery: Psychophysiological Responses and Training Adaptations

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# Abstract

Allostatic load — the “wear and tear” of cumulative stress regulation — has well-documented effects on health and performance. Physical exercise acutely increases physiological load, yet it is also a potent modulator of stress. Several interrelated studies were conducted during the dissertation project to examine the reciprocal relationship between stress and physical exercise. This cumulative thesis first presents a theoretical framework to provide a common context for this line of research and then includes three of these empirical studies, which constitute the project's cornerstone.

Study 1 compared stress responses to two laboratory paradigms in trained athletes, leveraging a broad panel of psychophysiological outcomes. Despite the small sample size, clear sex-specific patterns emerged, with a larger endocrine response in men than in women. The Trier Social Stress Test (TSST; psychosocial) elicited a significantly stronger cardiac-autonomic response than the Maastricht Acute Stress Test (MAST; psychophysical), underscoring the importance of stressor type.

Study 2 tested whether physical fitness buffers stress responses to different stress-induction paradigms in a mixed-sex sample. Participants completed a graded exercise test, the TSST, and the MAST in randomized, counterbalanced order. Higher cardiorespiratory fitness (VO2peak) was associated with a reduced heart rate response in both paradigms. No buffering effects emerged for hormonal or subjective outcomes, suggesting domain-specific benefits of fitness.

Study 3 was a randomized controlled trial that evaluated slow-paced breathing as a recovery aid during a 4-week sprint-interval training program. While the training robustly improved performance, adding slow-paced breathing conferred no additional benefits for performance or cardiac-autonomic regulation, despite its known acute vagal effects.

Together, these studies show that TSST and MAST are not interchangeable, that cardiovascular fitness selectively buffers cardiac-autonomic responses with limited transfer to endocrine or subjective domains, and that the timing and context of an intervention may constrain the benefits of slow-paced breathing. The findings further highlight the need for sex-sensitive designs and more nuanced theoretical models of stress to guide experimental work.

# Zusammenfassung

Allostatische Last – die Belastung durch kumulative Stressregulation – hat gut belegte Auswirkungen auf Gesundheit und Leistung. Körperliche Aktivität erhöht akut die physiologische Belastung, ist zugleich aber ein potenter Modulator von Stress. Im Rahmen des Dissertationsprojekts wurden mehrere zusammenhängende Studien durchgeführt, um die wechselseitige Beziehung zwischen Stress und körperlicher Aktivität zu untersuchen. Diese kumulative Dissertation bietet zunächst einen theoretischen Rahmen, der die Studien konzeptionell verbindet und präsentiert anschließend drei dieser Studien, die den Kern des Projekts bilden.

Studie 1 verglich die Stressreaktionen trainierter Athletinnen und Athleten bei zwei laborbasierten Verfahren zur Induktion von Stress, wobei ein breites Spektrum psychophysiologischer Messgrößen erfasst wurde. Trotz der geringen Stichprobengröße zeigte die Studie klare geschlechtsspezifische Unterschiede, mit stärkeren endokrinen Reaktionen bei Männern als bei Frauen. Der Trier Social Stress Test (TSST; psychosozial) löste im Vergleich zum Maastricht Acute Stress Test (MAST; psychophysisch) eine signifikant stärkere kardial-autonome Reaktion aus, was die Bedeutung des Stressors unterstreicht.

Studie 2 untersuchte, ob körperliche Fitness die Reaktion auf unterschiedliche Stresstests in einer gemischtgeschlechtlichen Stichprobe moduliert. Die Teilnehmenden absolvierten einen stufenförmigen Belastungstest sowie den TSST und den MAST in randomisierter, gegenbalancierter Reihenfolge. Höhere kardiorespiratorische Fitness (VO2peak) war mit einer reduzierten Herzfrequenzreaktion in beiden Stressinduktionstests assoziiert. Bei hormonellen oder subjektiven Markern zeigten sich hingegen keine Puffereffekte, was auf domänenspezifische Effekte von Fitness hindeutet.

Studie 3 evaluierte in einer randomisierten kontrollierten Studie den Einsatz langsamen Atmens (Slow-Paced Breathing) als Regenerationsmaßnahme während eines vierwöchigen Sprint-Intervall-Trainings. Das Training führte zu einer deutlichen Leistungsverbesserung. Die zusätzliche Atemübung brachte jedoch trotz bekannter akuter vagaler Effekte keine weiteren Vorteile für die Leistungsentwicklung oder kardial-autonome Regulation.

Zusammenfassend zeigen die vorliegenden Studien, dass TSST und MAST nicht als austauschbare Stressmodelle betrachtet werden können, dass kardiorespiratorische Fitness vor allem kardial-autonome Stressreaktionen beeinflusst, jedoch nur bedingt auf endokrine oder subjektive Parameter wirkt und dass Zeitpunkt und Kontext einer Intervention den

potenziellen Nutzen von langsamem Atmen begrenzen können. Die Ergebnisse unterstreichen darüber hinaus die Bedeutung geschlechtssensitiver Studiendesigns und differenzierter theoretischer Modelle als Grundlage zukünftiger experimenteller Stressforschung.

# 1. Introduction

The word *stress* is widely used in both science and colloquial contexts. Yet, it was only in the last century that stress was famously introduced to physiology and medicine by the Austro-Hungarian physician Hans Selye (1950). Precursor concepts had been in place in psychology and philosophy (Robinson, 2018). However, Selye borrowed the term from classical physics (Ayers & Steptoe, 2007; Robinson, 2018; Szabo et al., 2012). There was considerable debate on the terminology (Hinkle, 1974; Lazarus & Folkman, 1986; Mason, 1975; Robinson, 2018; Serban, 1976). This was made evident in Selye's influential book *The Stress of Life*, in which he devoted several pages to these "semantic difficulties" (Selye, 1956, p. 30). Later, he acknowledged that his physiological definition of *stress* would better fit with the definition of *strain* from classical mechanics. However, after twenty years, the term was already widely accepted (Selye, 1976, p. 50). While Selye's nomenclature has largely prevailed, the theoretical concept of stress has continued to evolve.

The concept of stress disclosed its importance due to its wide practical and theoretical applications spanning biology, medicine, psychology, sociology, and even general system theory. The direct lineage from Bernard, Cannon, and Selye not only influenced behaviorism and the development of cybernetics but also the early biomedical models of exercise training (Chiu & Barnes, 2003; Goldstein & Kopin, 2017; Gross, 1998; Kiely, 2015).

## 1.1. Structure of the thesis

Chapter 1 traces the development of stress models and summarizes key psychophysiological expressions of the stress response. It then examines the bidirectional relationship between stress and physical activity and exercise and concludes by outlining the aims of the dissertation project.

Chapter 0 provides an overview of the methods used across the studies, including the procedures applied to induce stress (Maastricht Acute Stress Test and Trier Social Stress Test), the training intervention, and the slow-paced breathing exercise implemented to support recovery and relaxation.

Chapter 3 presents three central studies conducted in the course of this PhD project. Their aim was to broadly characterize the complex interplay between stress and exercise and to establish a foundation for further scientific investigation and practical application. The included papers focus on (1) stress responses in athletes, (2) the stress-buffering role of

physical fitness, and (3) slow-paced breathing as a recovery and stress-management strategy during a demanding training protocol.

In addition to the three studies presented, the PhD project comprised further investigations that have not yet been published and are, therefore, not included in the present cumulative dissertation. One pilot project examined hormonal and autonomic responses to an acute stressor followed by resistance exercise. This study was presented as a poster at the annual conference of the European College of Sport Science in 2023 (Raidl et al., 2023), and informed a follow-up project that is currently in the final phase of data acquisition. This latter study compares the hormonal, autonomic, and subjective responses to a resistance exercise session after either a stress-induction (Maastricht Acute Stress Test) or a sham protocol, thereby shedding light on how exercise interacts with the baseline stress state. Furthermore, data collection is nearing completion for a project addressing the effects of stress on motor coordination and physiological synchrony during a small-ball game. The pre-registration of this study is available on the Open Science Framework (https://osf.io/5dcg8). Jointly, these studies provide a broad psychophysiological perspective on the interaction between stress and exercise.

Finally, Chapter 0 synthesizes the findings and discusses limitations as well as directions for future research.

## 1.2. Development of the Concept of Stress

*„I have found it increasingly obvious that those who talk about or even study stress may use the same language but do not necessarily share the same operational definition of stress“ (Robinson, 2018, p. 334)*

### 1.2.1. Early developments – Milieu Intérieur

While the word stress was popularized in the mid-20th century, the intellectual roots of stress research can be traced back to the French physiologist Claude Bernard (1813–1878). Bernard was a strong proponent of applying modern scientific methods to medicine. He implemented experiments to infer his clinical reasoning and, thereby, broke with the medical tradition of relying on instinct and metaphors to treat patients (Gross, 1998). Bernard's writings engaged empiricist and mechanistic epistemologies while acknowledging emergent properties in biological systems. (Gross, 1998). His *Milieu* Intérieur – a relative constant internal environment regardless of changes in the external environment (Robinson, 2018) – laid the ground for the model of homeostasis, which profoundly shaped physiological theory.

### 1.2.2. Homeostasis

Walter Cannon (1871–1945), a major advocate of Bernard's work, recognized that the proposed model was too static and did not align with his observations. Cannon was interested in adaptive processes in living organisms, especially mammals (Cooper, 2008). So, his model of homeostasis described the continuous adjustments necessary to keep internal metrics within a physiological range (Cannon, 1929; Cooper, 2008). His work on the "involuntary" (i.e., autonomic) nervous system, the adrenal gland and related blood sugar regulations made way for a response of "running or fighting" (Cannon, 1929, p. 423, 1939, p. 249), which was later popularized as the "fight-or-flight response", as the first modern encounter of what later became part of the stress response. At Harvard, Cannon collaborated with Arturo Rosenblueth to investigate the role of adrenaline as a messenger of the autonomic nervous system ANS (Cannon & Rosenblueth, 1933; Schwerdtfeger, 2025). When Norbert Wiener introduced Cybernetics[1] (Wiener, 1948, 1951), his ideas were formed in collaboration with Rosenblueth and the engineer Julian Bigelow, and extended the concept of homeostasis to encompass more general-purpose information processing. The regulation via feedback and feedforward control would be seminal for future development (Goldstein & Kopin, 2017; Rosenblueth et al., 1943). The importance of homeostasis as a *meta-theory* was well articulated by Karl Friston decades later, whose work will be important for the contemporary conceptualization of stress. He noted that "[…] indeed, the physiology of biological systems can be reduced almost entirely to their homeostasis" (Friston, 2010, p. 127).

### 1.2.3. Arousal and the Inverted-U shape

Before addressing Selye's introduction of the biomedical definition of stress, it is worth examining a parallel development in the science of psychology. Robert Yerkes, also based at Harvard but apparently without direct connection to Cannon (Robinson, 2018), and John Dillingham Dodson were interested in the formation of habits in relation to the intensity of an aversive stimulus. They trained mice to choose a bright box over a darker one by delivering electrical shocks of three different intensities (Yerkes & Dodson, 1908). Against their expectations, they found that the mice took longer to form the habit of avoiding the dark box when the shock was very mild, as well as when it was "decidedly disagreeable" (Yerkes & Dodson, 1908, p. 467f). This effect depended on the brightness difference between the

[1] The word cybernetics was Wiener's creation. However, the concepts outlined in his equally named book were based on the interdisciplinary collaboration between Rosenblueth, Wiener, and Bigelow.

boxes and was described by the authors as “preliminary results” (Yerkes & Dodson, 1908, p. 472).

It is worth noting that the original study made no reference to performance, arousal, or stress. Their paper was descriptive and aligned with the mechanistic *zeitgeist* of functionalism and pre-behaviouristic psychology. Replications of their paper yielded mixed success with notable boundary conditions (Teigen, 1994). Nevertheless, their work remains part of psychology textbooks to this day (e.g., Maderthaner, 2021; McFarlane et al., 2024; Schüler et al., 2020; Spielman et al., 2020) and has been reintegrated into more nuanced theories around conditioning, learning, performance, and motivation. To some extent, it has been taken as an axiom and integrated into more speculative interpretations as well (Fricchione, 2023; Wallace & Fricchione, 2024). The Yerkes-Dodson experiments, however, aligned with similar studies of the time (e.g., Freeman, 1940; Schlosberg, 1954), leading Donald Hebb to define the theory of optimal arousal – a systems-oriented account linking neural activation to performance and motivation (Hebb, 1949, 1955).

While Hebb did not reference Yerkes and Dodson in his seminal paper, their work is regularly presented together. Fundamentally, Hebb went beyond a purely behaviourist understanding and the drive theory of motivation. Therein, he laid the path for a neuroscientific-informed psychology, eloquently expressing his philosophy of science in the introduction to his 1949 book, *Organization of behaviour* (Hebb, 1949). By doing so, Hebb bridges the gap between psychology and the biomedical informed stress theory of Hans Selye. Like Selye, Hebb was at McGill University in Canada when he formulated the new theory of motivation.

### 1.2.4. The General Adaptation Syndrome

Hans Selye (1907–1982) was influenced by Walter Cannon, whom he cited in many of his publications (Selye, 1946, 1950, 1971, 1975b). Selye discovered that different noxious agents lead to very similar response patterns, including decreased organ size, fat loss, and gastrointestinal ulceration, followed by adrenal hypertrophy. In his rodent studies, prolonged exposure ultimately led to the animals' deaths (Selye, 1936). This observation, combined with his early interest in the general symptoms of sickness (Selye, 1975b), led Selye to describe the *General Adaptation Syndrome (GAS; see* Figure 1*)* as a three-stage model of adaptation to harmful stimuli. The first stage of the GAS was termed the “Alarm Reaction”, followed by the “Stage of Resistance”, and finally the “Stage of Exhaustion” (Selye, 1936, 1946). While Cannon introduced the ANS and the catecholamines as the master regulators, Selye focused much of his work on the hypothalamic-pituitary-adrenal (HPA) axis and the

role of glucocorticoids, which he later called the “Pathways of Stress” (Selye, 1975a, p. 627). Selye noted that Cannon never touched on the adrenal cortex or the pituitary and, therefore, missed the common denominator of all stress responses (Selye, 1975b, p. 33). At the time, the word stress was not as frequently used in medical sciences and physiology[2], and did not appear in his first description of the GAS theory (Selye, 1936). The term stress was borrowed from Newtonian mechanics. Also, his depiction of the GAS (Selye, 1946) resembles the stress-strain curve of elastic materials. As an operationalization in biomedicine, glucocorticoid release remains central, though not exhaustive, in measuring stress responses.

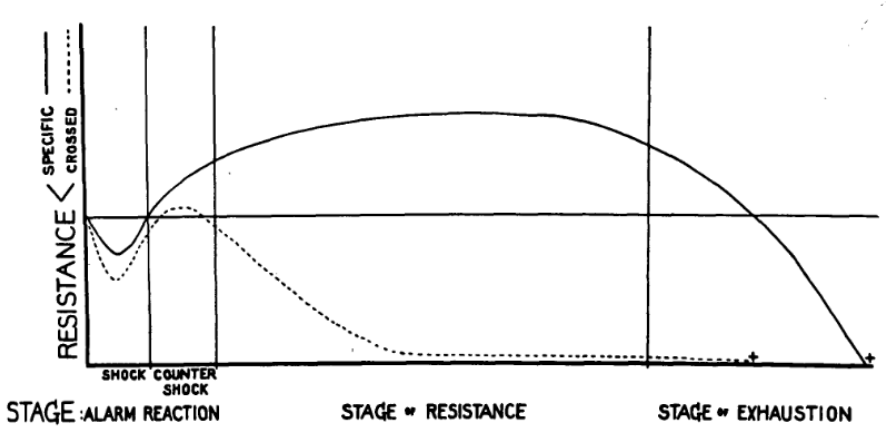


*Figure 1: Representation of the General Adaptation Syndrome (GAS). Three stages of resistance across time are shown for specific (solid) and crossed (dotted) patterns. According to this model, resistance to the agent (i.e., stressor) decreases in the very acute shock phase, before increasing during the resistance stage. Repeated or prolonged exposure leads to reduced resistance to that agent, called exhaustion. The crossed line refers to resistance against other, unrelated agents. Crossed resistance is marked by a rapid drop after the acute countershock phase (Selye, 1946, p. 123)*

### 1.2.5. Early criticism of Selye

Selye emphasized that the alarm response should be understood as a specific bodily reaction, while stress denoted the general, non-specific response[3]. In this definition, the stress response is always systemic and mediated by glucocorticoids (and Cannon’s catecholamines). The non-specificity of this definition was criticized by others. Especially his conflict

[2] Cannon titled one of his papers “Stress”, but did not use the word throughout the article. He also used the word to refer to psychological distress in some early works (Cannon, 1936). William Osler also used “stress and strain” as a metaphor for worry and psychological load (Osler, 1910). But before Selye, no one systematically introduced the concept of stress with defined physiological patterns.

[3] A clear differentiation between „stressor“, “stress response”, and “stress” did not appear in his early works and was probably only the result of external criticism (Lazarus et al., 1952; Mason, 1975).

with John Wayne Mason, who showed that the stress response was not uniform across different hormonal systems (Mason, 1971), is of interest. According to Mason, Selye did not realize that all his experiments were confounded by the psychological arousal as a first mediator of the stress response. He showed that psychosocial challenges elicited representative activation of the HPA axis, while purely physical challenges did not (Mason, 1975). The exchange reached a peak at the *Third International Symposium of the Kittay Scientific Foundation* in *1975.* Both Mason and Selye presented at the conference. Richard Lazarus wrote a discussion on the matter, and while he acknowledges Selye for his investigations on the HPA axis, he also criticizes him for a circulatory definition of stress and not adapting his theories to more recent data (Lazarus, in Serban, 1976).

A final observation should be made on the philosophical framework of Hans Selye. Selye quickly gained fame and influence with his work inside and outside of academia (Szabo et al., 2012). His books *The Stress of Life* (1956) and *Stress without Distress* (1975b) were translated into multiple languages and cited extensively. In both books, Selye not only developed a biomedical description of stress but also created a moral landscape by extrapolating his work from the cell and organ to the person and, ultimately, to society. This ontological reductionism brought him some criticism within the field (Lazarus, in Serban, 1976). However, those prolific writings are also likely part of the reason Selye remains relevant and is mentioned in many papers on stress. Furthermore, while his definition of stress is outdated (see following paragraphs), the most salient operationalization of stress in biomedical research is via the release of glucocorticoids.

### 1.2.6. Integrating Psychology with Physiology

Richard Lazarus brought stress research into the domain of psychology. While Cannon also held a degree in psychology and combined his understanding of the autonomic regulation and his observations during World War I to describe the fight-or-flight response (Cooper, 2008; Robinson, 2018), it was Lazarus who developed a theoretical framework for the psychological phenomenon of stress. While much of the earlier developments were described as reductionistic, Lazarus explicitly described his philosophy as phenomenological and interactive (Lazarus, 1993, 2015). Stress could not be viewed without the mediating cognitive processes of the individual (Folkman et al., 1986; Lazarus, 1993). Lazarus and others realized early that the experiential stress was not directly caused by external stressors. Individual differences in motivation and cognition were found to be the relevant mediators (Lazarus & Eriksen, 1952). Much of his work not only provided knowledge from laboratory and field experiments but also argued, on theoretical grounds, against oversimplifying scientific

models to suit the available methodological toolkit rather than the theory and the reality they are intended to represent (Lazarus, 1990, 1993).

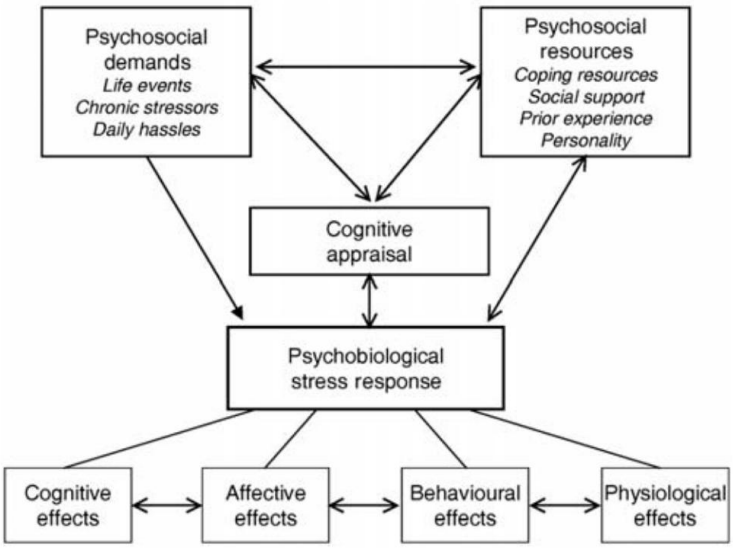


*Figure 2: The transactional model of stress, as adapted by Ayers and Steptoe (2007, p. 217). Stress is understood as the interplay between the external demands, personal resources, and the stress response, mediated by cognitive appraisal.*

The transactional theory of stress, put forth by Susan Folkman and Richard Lazarus, argues that stress cannot be understood as a single variable but rather as a multidimensional phenomenon. The bidirectional interaction between the stressor and the acute and long-term effects is directed by appraisal and coping strategies. In this sense, the appraisal is based on the interpretation of resources and demands (primary appraisal) and the interpretation of the coping strategies (secondary appraisal) (Folkman et al., 1986; Lazarus & Folkman, 1984, p. 315). Coping is the attempt to meet the demand through emotional regulation or problem-solving (Folkman et al., 1986). The final reaction to stress is then divided into four domains: physiological regulation, motor behavior, and changes in cognition and affect. The activity of the HPA axis, as part of the physiological response, is therefore just a way to facilitate energy availability under motivated challenges. However, without desired goals, there would also be no response to a threat (Lazarus, 2006). Figure 2 depicts a recent version of the transactional model of stress.

### 1.2.7. Allostasis – Beyond Homeostasis

In the 1980s, the epidemiologist Joseph Eyer and the neurobiologist Peter Sterling collaborated on the relationship between environmental and psychosocial stressors and health (Eyer & Sterling, 1977; Sterling & Eyer, 1981). Their work led them to realize that existing physiological theories could not adequately explain many epidemiological observations, such as the link between hypertension and socioeconomic status or the increase in blood pressure with age. In their view, previous physiological research focused on the body without acknowledging the central nervous system as the "pre-eminent regulatory influence on somatic physiology" (Sterling & Eyer, 1988, p. 630). Instead of adjusting the *Milieu Intérieur* to a "normal" range by local feedback mechanisms, Sterling and Eyer adopted a brain-centered model in which the internal environment is adjusted to both anticipatory and reactive needs. This also has the advantage of allowing for experience or learning processes. This dynamic regulation was called allostasis (Sterling & Eyer, 1988). Interestingly, Sterling and Eyer seemed to use the terms "arousal" or "psychological arousal" interchangeably with stress (Sterling & Eyer, 1988). According to Mason, whose work on hormonal regulation during stress was cited multiple times in their articles, arousal would count as the most nonspecific of all stress reactions (Mason, 1975).

Further updates of the theory of allostasis were made by Bruce McEwen. In his definition, homeostasis was kept as a very narrow term that applies only to life-essential parameters, such as blood pH levels. Allostasis was defined as the process supporting homeostasis (McEwen, 2000; McEwen & Stellar, 1993). The second, widely accepted concept introduced by McEwen is that of *allostatic load* (see Figure 3), which he metaphorically describes as "the price the body pays […] and it represents either the presence of either too much or inefficient operation of the allostasis response systems" (McEwen, 2000, p. 174). In further research, he shows how chronic exposure to glucocorticoids, cytokines, adrenal catecholamines, and other mediators of allostasis can have negative health effects. He particularly focused on the hippocampus, but also included other morphological and functional changes (McEwen, 2000, 2007, 2013; McEwen & Akil, 2020).

While allostasis and allostasis load are widely used constructs, the definitions put forth by the presented research groups differ slightly. In earlier articles, Peter Sterling rejects the concept of homeostasis entirely or superseded it with allostasis ('Allostasis', 2012; Sterling & Eyer, 1988). In later articles, homeostasis and allostasis were differentiated by peripheral feedback and brain-derived feedforward control mechanisms, respectively (Schulkin & Sterling, 2019). McEwen, however, used the term allostasis to describe the process that

maintains homeostasis (McEwen, 2000). However, there appears to be a demarcation problem in McEwen's definition of allostasis. For example, the circadian variation in body temperature of approximately 0.2–0.8 °C (Geneva, 2025) can be interpreted either as a homeostatic parameter essential for survival or as an allostatic adjustment that maintains other, more fundamental parameters.

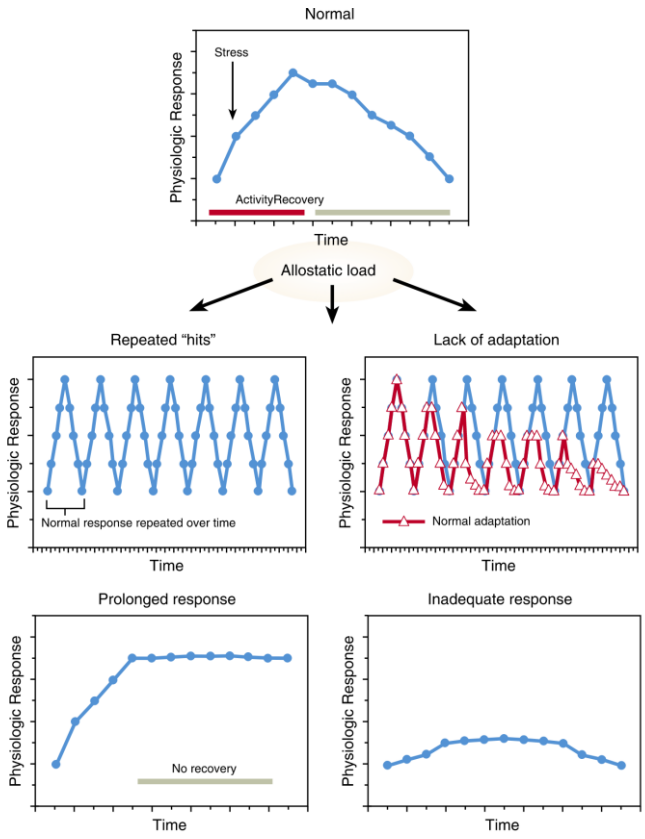


*Figure 3: The allostatic load model explains maladaptation due to adaptive demands, known as allostatic load. The top panel illustrates a normal response to stress. The middle panel shows the response to repeated stressors without (left) and with (right) reduction of adaptation over time. The lower panel illustrates prolonged responses without recovery (left) or inadequate response patterns (right) (McEwen, 2007, p. 880)*

Overall, the concept of allostasis is widely accepted and frequently referred to in the context of stress and health. Its original definition was adopted multiple times. But all definitions build on the idea of dynamic adjustments to support stability, and the inclusion of anticipatory regulations (Word et al., 2022). Allostasis does not replace homeostasis as one of the most fundamental physiological principles (J. Michael et al., 2009). The argument put forth by proponents of allostasis – namely, that homeostasis, as a concept, is too static and includes only peripheral feedback mechanisms (Schulkin & Sterling, 2019; Sterling & Eyer, 1988) – is too narrow by today's standards. Many authors integrated feedforward control

mechanisms and changing set points into the original definition of homeostasis or introduced alternative concepts (Davies, 2016; Moore-Ede, 1986; Mrosovsky, 1990; Romero et al., 2009; Woods & Ramsay, 2007). Nevertheless, allostasis is one of the most widely used theories and will be adhered to in the present dissertation. Allostasis allows for quantification of the transactional model of stress within a neuroendocrine paradigm. However, as noted by Birk, this shift remains a methodological challenge: „McEwen's work exemplifies a tendency of modern stress research, wherein the importance of the individual's perception of something as a stressor is explicitly recognized, yet not explored very thoroughly." (Birk, 2021, p. 260).

### 1.2.8. Summary of the Concept of Stress

History shows that data can only be interpreted within the framework of the prevailing *zeitgeist* and contemporary theories. Science stands on the shoulders of earlier science. However, this also implies that it is difficult to revise established concepts or to discard outdated models (Hinkle, 1974). To that point, Donald Hebb wrote about a theory of the 1930s that it "[…] was evidently like the gin that was being drunk about the same time; it was homemade and none too good, as Skinner pointed out, but it was also habit-forming; and the effort to escape has not really been successful." (Hebb, 1955, p. 243).

Early on, stress research emerged as an interdisciplinary field drawing on biology, psychology, medicine, and systems theory. This exchange between different fields expanded our understanding of how stress affects an organism. However, the debate on what stress is and how to quantify it is still ongoing.

This chapter should not be understood as a comprehensive review of the history and philosophy of stress research. Many theories from more distant fields were omitted, as were some that are directly relevant. However, it may be read as my current understanding of the topic and should help contextualize the research outlined in this thesis.

## 1.3. A Working Definition of Stress

As there is no consensus on what stress is, a working terminology is required for the present thesis. **Stress** is used as the overarching process connecting the (external) demand and (internal) processing, with biological, psychological, and behavioral responses. It is a state of heightened energetic and regulatory requirements relative to baseline conditions and can be inferred from coordinated psychophysiological adjustments. This usage follows common

practice in human experimental research, while acknowledging that narrower definitions have been proposed. More specifically, ***psychological*** *or* ***psychosocial stress*** is conceptualized as the subjective experience of demands appraised as threats or challenges and is operationalized through self-report measures (perceived stress, state anxiety). A more nuanced differentiation of psychological components, such as goal relevance, resources, or controllability, is beyond the scope of the presented studies.

The ***stress response*** will be considered the coordinated response of the HPA axis or the ANS to demands, as the primary neuroendocrine mediators of a system-wide regulation. In the presented studies, the stress response will be operationalized via glucocorticoids (saliva cortisol) and the cardiac regulation (heart rate and heart rate variability), while other proxies and mediators, for example, cytokines (Segerstrom & Miller, 2004), or alpha amylase (Nater & Rohleder, 2009), were only partly considered.

A ***stressor*** is an external or internal demand that can trigger the stress response, either anticipatorily or reactively. Beyond general responses, stressors may induce specific psychophysiological responses.

***Allostasis*** is understood as the brain-coordinated regulatory process that involves psychological (cognitive and affective), behavioural, and peripheral physiological regulation, including the stress response, to meet predicted demands. It involves feedforward mechanisms with nested feedback (Theriault et al., 2025). Accordingly, the **allostatic load** is the cumulative cost of these regulatory processes across time.

## 1.4. Physiology of the Stress Response

The physiological stress response is primarily mediated by two interconnected systems: The ANS, which can be considered the fast-response system, and the HPA-axis, which relies on humoral signaling and operates on slower timescales (Ulrich-Lai & Herman, 2009). While there are considerable stressor-specific patterns of regulation at the level of the central nervous system, the ANS and HPA axis are involved in most peripheral and central regulations (Pacák & Palkovits, 2001). Both systems are embedded in hierarchically structured reciprocal feedback loops and modulated centrally by predictive allostatic (feedforward) mechanisms (Friston, 2010; Theriault et al., 2025; Ulrich-Lai & Herman, 2009).

Briefly, a key purpose[4] of the brain is the reduction of surprise through the minimization of uncertainty (Shannon entropy). In computational frameworks, free energy represents the upper limit of surprise. Its minimization corresponds to the reduction of interoceptive prediction errors, thereby adjusting autonomic outflow to meet anticipated metabolic demands (Friston, 2010). In this view, allostasis can be described as the predictive regulation of the brain's energetic needs in concert with homeostasis. These allostatic needs are continuously updated by exteroceptive and interoceptive information, which are integrated via large-scale networks, including the salience network and default mode network (Barrett & Simmons, 2015; Kleckner et al., 2017). Importantly, within the embodied predictive interoceptive coding (EPIC) paradigm, interoception is considered more important compared to exteroception, as it bears direct consequences for allostatic load (Kleckner et al., 2017).

Top-down integration from prefrontal regions and the limbic system cannot initiate the stress response directly but act primarily through projections to hypothalamic structures, especially the paraventricular nucleus (PVN) and the bed nucleus of the stria terminalis (BNST). Bottom-up information converges in these integrative areas, but can also modulate ANS and HPA activity output through faster feedback mechanisms involving the brainstem and spinal cord (Gibbons, 2019; Ulrich-Lai & Herman, 2009).

### 1.4.1. Autonomic Nervous System

The ANS can be subdivided into the parasympathetic and sympathetic nervous systems (Gibbons, 2019). The enteric nervous system is often considered a third part of the ANS but is (semi-)autonomous (Gibbons, 2019; Sharkey & Mawe, 2023). The two branches of interest, the parasympathetic and sympathetic nervous systems, can act on a wide range of tissues simultaneously through their hierarchical structure. Feedback regulations are integrated at the level of the spinal cord (intermediolateral column), the medulla oblongata, midbrain, and pons, and the forebrain. In general, more rostral structures can modulate or override lower-level regulations (Friston, 2010; Karemaker, 2017). This way, while autonomic processes can operate with little higher-order effort, they can be recruited for goal-directed adjustments (see later).

The primary neurotransmitters of the ANS are acetylcholine and noradrenaline (norepinephrine). A multitude of other neurotransmitters (most notably, neuropeptide Y, nitric oxide, substance P, etc.) are released by the ANS and influence, for instance, the cardiovascular system. However, the conditions of release and the functional roles of these non-adrenergic

[4] Here "purpose" is the goal directed behavior of a system (Rosenblueth et al., 1943)

and non-cholinergic mediators remain incompletely characterized (Shanks et al., 2024). Adrenaline and noradrenaline are also released directly from neurons in the brainstem to not only modulate the peripheral ANS but also to project to other brain structures, most notably the PVN, where they modulate the HPA response (Pace & Myers, 2024; Ulrich-Lai & Herman, 2009) (see Chapter 1.4.2).

The sympathetic nervous system acts via direct innervation of the end organ, releasing the neurotransmitter noradrenaline. In addition, the sympathetic innervation of the adrenal medulla (and the sweat glands) acts via acetylcholine, which is the neurotransmitter used by the parasympathetic branch and the preganglionic synapses of the ANS (Karemaker, 2017). In response to the stimulation, chromaffin cells of the adrenal medulla synthesize adrenaline (epinephrine) and noradrenaline, which are released into the venous blood as hormones. Most noradrenaline, however, is released from sympathetic neurons and spills over to the bloodstream. Tissue-specific actions are enabled by nine subtypes of adrenoreceptors (AR-α1, AR-α2, and AR-β, with labelled subtypes) with distinct affinities for adrenaline and noradrenaline (Basarrate et al., 2024).

Functionally, the sympathetic branch is associated with the fight-or-flight response, while the parasympathetic branch triggers adaptations favoring a state of rest-and-digest (Gibbons, 2019; Karemaker, 2017). However, this distinction is now considered to be too simplistic. Parasympathetic efferences not only antagonize sympathetic function but also exhibit well-established synergistic effects (Berntson et al., 1991; Paton et al., 2005). Different psychophysiological states can require co-activation or co-inhibition of both branches. Figure 4 depicts this autonomic space model with orthogonal sympathetic and parasympathetic action (Berntson et al., 1991) and the expected interplay during exercise at increasing intensity (S. Michael et al., 2017; White & Raven, 2014).

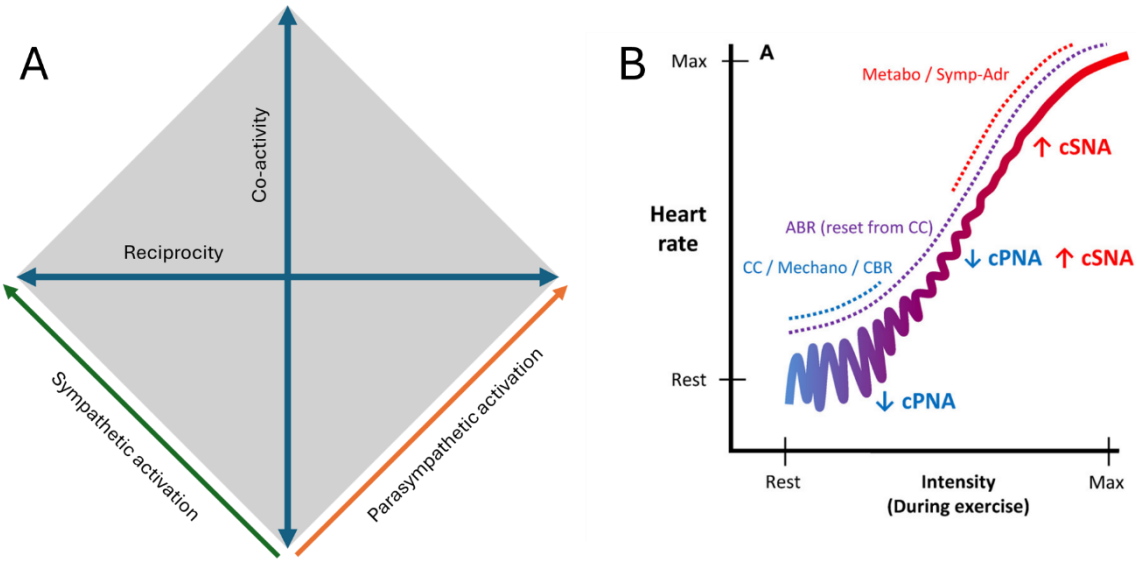


*Figure 4: Panel A shows the autonomic regulation as a 2-dimensional space. According to this model, different tasks need specific patterns of sympathetic and parasympathetic activity. The figure is adapted from Berntson et al. (1991). Panel B depicts the typical pattern of autonomic regulation during exercise as intensity increases. Heart rate adaptations from rest to about 100 beats per minute are primarily due to parasympathetic withdrawal, whereas higher intensities are mostly due to increased sympathetic activity (S. Michael et al., 2017, p. 301). Based on the autonomic space model (Panel A), this would resemble a leftward shift towards a reciprocal sympathetic activation. Other tasks, like mental challenges, elicit different patterns (Berntson et al., 1991; Weissman & Mendes, 2021). cPNA, cardiac parasympathetic neural activation; cSNA, cardiac sympathetic neural activity; CC, central command; Mechano, mechanoreflex; CBR, central baroreflexes; ABR, arterial baroreflex; Metabo, metaboreflex; Symp-Adr, sympatho-adrenal; Thermo, thermoregulatory influences.*

The autonomic space is especially important for tissues that show dual regulation, such as the heart, but not for single-innervation tissue, such as sweat glands (Karemaker, 2017; Weissman & Mendes, 2021). Skeletal muscle would be another example with no parasympathetic innervation. However, their functionality is governed by direct somatic and sympathetic innervation and is influenced by autonomic regulation, including vascular tone, temperature, and metabolism (Roatta & Passatore, 2008; Thomas & Segal, 2004), exemplifying the complex interplay of the systems.

Important peripheral effects of sympathetic activity include, but are not limited to, increases in cardiac output and heart rate, vasodilation and vasoconstriction dependent on receptor type, bronchodilation, increases in blood glucose and free fatty acids. The effects of catecholamines on the immune system are complex but are primarily anti-inflammatory (Strahler et al., 2015; Zouhal et al., 2008). Bone marrow, thymus, spleen, and lymph nodes all express adrenoreceptors and respond to catecholamines (Segerstrom & Miller, 2004).

The vagus nerve is the primary route for parasympathetic information. Importantly, the vagus nerve consists of approximately 80 - 90% afferent fibers (Berthoud & Neuhuber, 2000).

This dominance of afferent information, projecting to important brain structures that contribute to homeostatic and allostatic control, is a central pillar in multiple theories around emotion, cognition, and self-control.

One of the most important frameworks is the neurovisceral integration theory by Thayer and Lane (2000, 2009). Based on this theory, the vagus nerve is a major path of the central autonomic network, integrating interoceptive information to control goal-directed behavior. The central autonomic network includes the amygdala, hypothalamus, PAG, solitary nucleus, and the nucleus ambiguus. Importantly, the interaction between the rostral (prefrontal cortex) and the caudal (medulla oblongata) is bidirectional. Top-down, the prefrontal cortex can continuously inhibit the amygdala and other subcortical regions involved in threat responses. Increased prefrontal control correlates with executive function and emotional regulation, reduces sympathetic outflow, and increases vagal tone (Thayer & Lane, 2009). This is reflected in increased heart rate variability and reduced heart rate (Thayer et al., 2009).

Other theories, such as Damasio's somatic marker theory (Damasio & Carvalho, 2013) and Barrett's constructed emotions (Barrett, 2016), share similar arguments and neurophysiological underpinnings, focusing more on the emergence of feelings from interoceptive signals. In the context of the predictive processing framework (see Chapter 1.2.7), vagal afferents provide the primary "prediction error" signals that the brain must resolve – either by changing the body (allostasis) or updating the internal model (learning). The more recently outlined vagal tank theory (Laborde et al., 2018b) integrates the neurovisceral information theory with psychological theories on self-regulation. In the vagal tank theory, cardiac vagal activity serves as a barometer of freed cognitive resources in a metaphorical tank. A full tank supports the main functions of the vagal outflow: rest, reactivity, and recovery (Laborde et al., 2018b).

Beyond the psychological role, the mentioned neural connections of the vagus also exert systemic endocrine and immunological effects. Afferent fibers project to the solitary nucleus in the medulla oblongata and further activate the locus caeruleus and the HPA axis in response to inflammatory cytokines and endotoxins, nociception, or other homeostatic demands (Berthoud & Neuhuber, 2000; Tracey, 2002). In addition to system-wide responses, efferent vagal outflow can modulate inflammation locally. Cholinergic signals to exposed macrophages, which express high levels of acetylcholine receptors, reduce the release of TNF-α, IL-1, and IL-6. This helps keep inflammation local rather than spreading throughout the system (Borovikova et al., 2000; Tracey, 2002). Systemic reduction of TNF-α is likely the result of vagal innervation of the liver and spleen. The exact mechanism is debated, but

animal models and invasive studies support the effects (Martelli et al., 2014; Zila et al., 2017). Indirect evidence from correlating vagally mediated heart rate variability (HRV) with markers of inflammation is also in line with this connection (D. P. Williams et al., 2019).

Stimulation of the cardiac-vagal interaction can be achieved, for example, by cold water face immersion (Richer et al., 2022) or electrical (often transcutaneous) nerve stimulation (Austelle et al., 2024). Another intervention that has received more attention recently is breathing exercises (Laborde et al., 2022; Lehrer & Gevirtz, 2014), with potential upstream effects on arousal, emotion, immune function, pain, and overall recovery processes. Slow-paced breathing, an intervention implemented in the present thesis (see Chapter 2.3), specifically targets this pathway to modulate arousal and recovery.

Psychological stress, as well as physical exercise, reliably elevates adrenaline and noradrenaline (Athanasiou et al., 2022; Jezova et al., 2004; Zouhal et al., 2008). Direct measurement via blood plasma samples remains the gold standard. However, adrenaline and noradrenaline have short plasma half-lives and are rapidly cleared. Also, multiple blood draws within a short timeframe can induce additional stress (Rohleder, Nater, et al., 2004). Urinary assays integrate catecholamine output over extended intervals but lack temporal resolution for rapid changes (Grouzmann & Lamine, 2013). Microneurographic recordings of muscle sympathetic nerve activity MSNA (Katayama & Saito, 2019) are frequently implemented for local (mostly peroneal) real-time measures. A less invasive proxy of ANS activity, with high temporal resolution, is alpha-amylase (Nater & Rohleder, 2009; Rohleder, Nater, et al., 2004). Cardiac measures, such as HRV (H.-G. Kim et al., 2018; Laborde et al., 2018a; S. Michael et al., 2017) and pre-ejection period, typically estimated via impedance cardiography (S. Michael et al., 2017; Pilz et al., 2023), have become the standard for non-invasive, low-cost indicators of ANS activity with high-temporal resolution and broad applicability. All these measures have their advantages and limitations. In this thesis, HRV is used across all studies as an accessible and reliable method for assessing cardiac vagal activity (Bellenger et al., 2016; Laborde et al., 2017).

### 1.4.2. Hypothalamic-Pituitary-Adrenal Axis

The HPA axis is widely regarded as the main structure of the physiological stress response. Its actions start at the PVN of the hypothalamus. The PVN is regulated by homeostatic disruption and allostatic regulation and, consequently, communicates with many other brain centers. Important connections arise from the noradrenergic and neuropeptidergic neurons in the brainstem, which are involved in autonomic regulation and metabolic needs,

respectively. Further, connections to the amygdala and the hippocampus (via the BST) are especially relevant for anticipatory (feedforward) regulations (Herman et al., 2016). The PVN releases vasopressin (arginine-vasopressin, AVP) and corticotropin-releasing factor (CRF) into the hypophyseal port connected to the pituitary gland. Besides the pineal gland, the pituitary gland is a major hormonal gland located directly at the CNS. The anterior pituitary is primarily controlled by the hypothalamus and secretes five main hormones (Yeung et al., 2006). AVP and the more potent CRF stimulate the release of adrenocorticotropic hormone (ACTH) into the bloodstream (Ulrich-Lai & Herman, 2009). The other hormones from the anterior pituitary gland are growth hormone (GH), prolactin, luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and follicle-stimulating hormone (FSH). Importantly, there is no one-on-one regulation by the hypothalamus. Each pituitary hormone is predominantly regulated by one hypothalamic transmitter, but there is considerable overlap (Yeung et al., 2006). Besides ACTH, exercise demands or psychosocial stressors also drive other pituitary hormones (Ahtiainen et al., 2003; Kraemer & Ratamess, 2005; Petrowski & Kahaly, 2025). These transient changes could also counteract some of the immunosuppressing effects of the catecholamines and the HPA axis (Dorshkind & Horseman, 2001). Together, the anterior pituitary hormones regulate growth, reproduction, and metabolism. It is ACTH, in turn, that promotes the release of glucocorticoids when it reaches the adrenal cortex (J. S. Kim & Iremonger, 2019; Pace & Myers, 2024; Ulrich-Lai & Herman, 2009).

Cortisol accounts for approximately 95% of glucocorticoids in humans (Kraemer & Ratamess, 2005) and can target nearly every cell of peripheral tissues and the central nervous system through glucocorticoid receptors (GR) (Basarrate et al., 2024). GR are widespread in the brain, especially in the PVN of the hypothalamus and the hippocampus (Binder, 2009; Oyola & Handa, 2017). The affinity of cortisol for the second important receptor class, mineralocorticoid receptors (MR), is about 10 times higher than for GR, leaving MR receptors occupied during basic diurnal and ultradian fluctuations (Hartmann et al., 2021). This is reflected in their functional differences. MRs are involved in the upregulation of the HPA axis, while CRs are involved in the two primary feedback mechanisms downregulating cortisol release at the level of the pituitary and the hypothalamus. To allow for effective regulation, genomic feedback, which acts within tens of minutes to hours, is supported by two faster, non-genomic mechanisms that act within seconds to minutes. The fastest mechanism involves inhibition of pituitary ACTH secretion. The second fastest mechanism is coupled to endocannabinoid release in the PVN (Di et al., 2003; M. N. Hill & Tasker, 2012;

J. S. Kim & Iremonger, 2019). The non-genomic pathways are likely dominant in studies investigating acute stress responses (see Chapters 0 and 3).

Cortisol can be measured in plasma, serum, saliva, hair, or urine (El-Farhan et al., 2017; Iob & Steptoe, 2019; Strahler et al., 2017), and it is the most frequently reported physiological marker in stress research (Giacomello et al., 2020). Each of these measurement sights has strengths and limitations. Hair cortisol is mostly seen as a proxy for long-term stress (Iob & Steptoe, 2019). On the other hand, blood samples show the fastest response to stress. About 1–10% of circulating cortisol is free. The rest is bound to corticosteroid-binding globulin (CBG) and to a smaller extent to albumin. Only the free cortisol is considered bioactive. Blood samples measure total cortisol, whereas saliva measures only unbound cortisol (Hellhammer et al., 2009; Kajantie & Phillips, 2006a; Perogamvros et al., 2011).

Cortisol is secreted in a pulsatory fashion with strong ultradian and diurnal patterns (see Figure 5). The circadian rhythm is regulated by the suprachiasmatic nucleus of the hypothalamus (SCN), which is influenced by external *zeitgebers*, primarily light. The SCN communicates with the PVN and directly with the adrenal gland via sympathetic fibres. Local pacemaker cells in the adrenal glands, together with constant feedback loops, superimpose an ultradian cortisol pattern (Leliavski et al., 2015; Russell & Lightman, 2019).

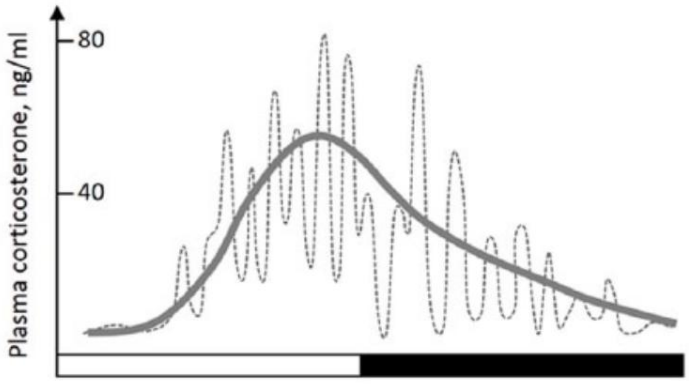


*Figure 5: Cortisol shows a strong circadian and ultradian pattern. The additional awaking cortisol response, 30-45 minutes after waking up, supports synchronizing peripheral body clocks (Leliavski et al., 2015, p. 21)*

In healthy humans, these interacting processes lead to a sharp peak about 30-45 minutes after awakening and the lowest levels of cortisol during early nighttime. This cortisol awakening response is influenced by the allostatic needs of the previous and upcoming days and prepares the body for anticipated demands. Its central role is to align the central with the different peripheral body clocks (Stalder et al., 2025). The cortisol awakening response was

proposed as a predictor of exercise-induced overreaching (Anderson & Wideman, 2017). Alterations of the cortisol awakening response, as well as diurnal regulations (cortisol slope), are also associated with stress-related psychiatric disorders and psychosocial function (Boggero et al., 2017). However, the causal structure remains unclear, with sex differences, comorbidities, and other confounders further complicating the picture (Joseph & Golden, 2017; Wang et al., 2024).

Besides the master pacemaker of the SCN, the adrenal rhythm constitutes an important regulatory output relevant for the sleep-wake cycle and energy availability (Leliavski et al., 2015; Stalder et al., 2025). Interestingly, contrary to previous understanding, the SCN of the adult brain has been shown to express GR. Nevertheless, despite this receptor expression, the master clock appears largely resistant to glucocorticoid signaling, which may contribute to the stability of circadian rhythms in the face of cortisol fluctuations (Sládek et al., 2026).

Depending on the time of day, the superimposed ultradian wave shows peaks approximately every 30 to 50 minutes (Faghih et al., 2015). Acute stress responses may vary in cortisol amplitude depending on baseline levels relative to the pulsation pattern.

The primary organ for cortisol clearance is the liver, where metabolism is mediated predominantly by A-ring reductase. A smaller proportion of cortisol is metabolized in the kidneys and subsequently excreted (Finken et al., 1999). In renal tissue, the first step of cortisol conversion involves enzymatic inactivation by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2. High levels of 11β-HSD are also present in saliva, leading to rapid conversion of cortisol to cortisone (Blair et al., 2017; Seckl, 2024). Salivary cortisone, although physiologically inert, has been shown to reflect plasma cortisol more accurately than salivary cortisol, probably due to the regulatory influence of CBG (Blair et al., 2017; Del Corral et al., 2016). 11β-HSD type 1 exhibits tissue-specific functions but predominantly acts as a reductase in the liver, brain, skeletal muscle, inflammatory cells, and adipose tissue. This reaction regenerates cortisol from cortisone and amplifies the effects of HPA activation, with wide-ranging clinical implications (S. Gregory et al., 2020; Kupczyk et al., 2022).

Cortisol has many physiological functions. Primarily, it can be considered a hormone for metabolic regulation. It exerts catabolic and anti-anabolic effects, mobilizing amino acids and elevating blood glucose levels through gluconeogenesis, and limiting glucose uptake in order to preserve substrate availability (Kraemer et al., 2020; Viru & Viru, 2004). Cortisol is released during exercise and higher-intensity physical activity. In particular, endurance exercise at intensities exceeding approximately 60% of maximal oxygen uptake (VO2max) is associated with considerable cortisol secretion (E. E. Hill et al., 2008). However, the

metabolic actions of cortisol are characterized by a notable delay, making it a less favorable candidate for immediate energy supply (Viru & Viru, 2004). Instead, cortisol's role is often interpreted as exerting a rapid permissive effect on adrenaline action at the target cell (Hackney & Walz, 2013; Viru & Viru, 2004).

As already mentioned, baseline cortisol fluctuations synchronize the central clock with other tissues (Gnocchi & Bruscalupi, 2017). Cortisol is also integral to the function of the immune system (see Figure 6). Like adrenal catecholamines (see Chapter 1.4.1), cortisol and ACTH act primarily anti-inflammatorily by inhibiting NF-κB pathways and reducing cytokine expression. This effect is time- and dose-dependent and has been shown to follow a biphasic pattern (Yeager et al., 2011; Zefferino et al., 2020). Acute psychological stress (Steptoe et al., 2007; Strahler et al., 2015) or physical challenges (Izquierdo et al., 2009) lead to a short-term increase in pro-inflammatory markers and, in the case of demanding physical exercise, may temporarily reduce immune function (Bernecker et al., 2013; Souza et al., 2021)[5]. In that case, the combined stress response from HPA and ANS activity counteracts immunological responses. From an evolutionary perspective, Nesse and colleagues (2016) proposed that the stress response evolved not primarily to protect against pathogens themselves, but rather against the potential costs of excessive immune responses.

Under chronic stress or allostatic overload, elevated concentrations of inflammatory mediators are thought to reflect dysregulation of the stress system and reduced sensitivity to cortisol and catecholamines (Rohleder, Joksimovic, et al., 2004). Most adverse stress-related effects on metabolism and the cardiovascular system are believed to arise from these chronic inflammatory processes. (Brotman et al., 2007; Gianotti et al., 2021; Hackney & Walz, 2013; Kivimäki et al., 2023; Kivimäki & Steptoe, 2018).

[5] Exercise does not invariably lead to immune suppression. In addition to exercise intensity, duration, and mode, biological factors may modulate the immune response (Gonçalves et al., 2020). In addition, observations traditionally interpreted as evidence of post-exercise immune suppression call for a more nuanced interpretation (J. P. Campbell & Turner, 2018).

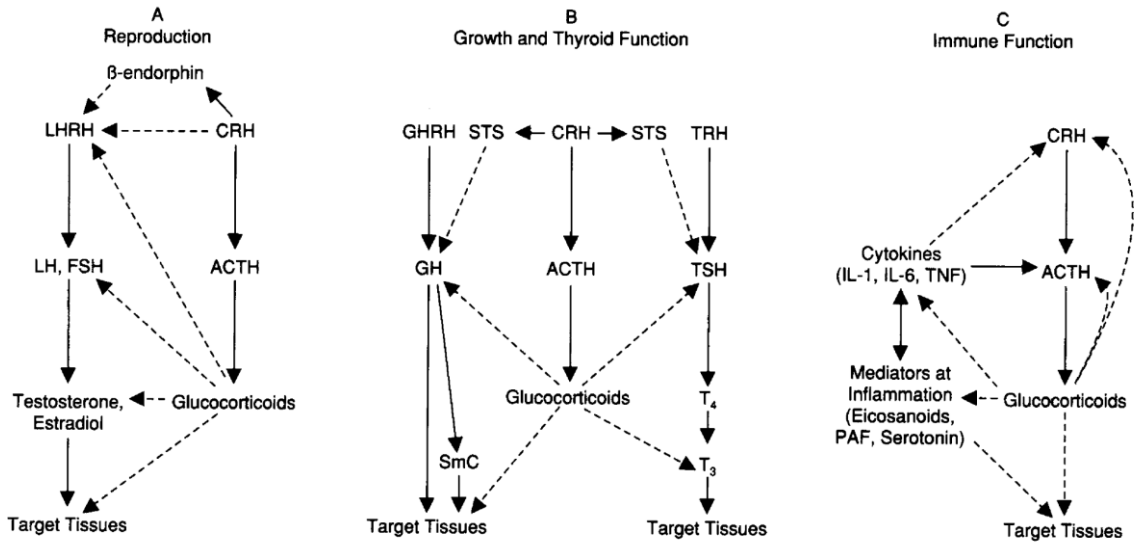


*Figure 6: Simplified interaction of stress-associated molecules with (A) the reproductive system, (B) growth and thyroid function, and (C) the immune system. LHRH, luteinizing hormone releasing hormone; CRH, corticotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; ACTH, corticotropin; GHRH, growth hormone releasing hormone; STS, somatostatin; TRH, thyrotropin-releasing hormone; GH, growth hormone; TSH, thyroid-stimulating hormone; $T_4$, thyroxine; SmC, somatomedin C; $T_3$, triiodothyronine; IL-1, interleukin 1; IL-6, interleukin 6; TNF, tumor necrosis factor; and PAF, platelet-activating factor (Chrousos & Gold, 1992, p. 1249)*

Many brain structures, including the prefrontal cortex, hippocampus, and amygdala, show high expression of MR and GR (McEwen, 2000). The locus caeruleus is also affected by glucocorticoids, which, in turn, release noradrenaline (Pace & Myers, 2024). Through these pathways, the acute and chronic effects of cortisol extend to cognitive and emotional processes. Short-term elevations in cortisol have been shown to improve memory formation (Sherman et al., 2023) and to promote negative affect, with neural correlates resembling state anxiety (Daviu et al., 2019). Negative emotions during challenges and threats are an evolutionary advantage. The metaphorical smoke-detector function of this phenomenon would predict a high rate of false-positive “alarms” for avoiding unnecessary danger (Nesse et al., 2016). Importantly, the subjective experience of stress and cortisol responses are not always tightly coupled (Dalile et al., 2022; Het et al., 2012) and may be elicited by different types of stressors (Dickerson & Kemeny, 2004) or moderated by factors such as menstrual cycle or health status (J. Campbell & Ehlert, 2012; Duchesne & Pruessner, 2013). These findings pose challenges for theories of constructed emotions and embodied cognition (Barrett, 2016; Barrett & Simmons, 2015; Damasio & Carvalho, 2013).

Unlike acute stress, long-term allostatic (over-)load, through exposure to cortisol and related neurotransmitters, damages neural structures, such as the hippocampus, amygdala, and

the PFC. This exerts extensive negative effects on health and cognition (Lupien et al., 2018; McEwen, 2000). Stress-associated changes of the hippocampus and the amygdala, as well as alterations in HPA-feedback, are found in psychopathologies, especially depression, anxiety disorder, and post-traumatic stress disorder (PTSD), indicating a causal relationship between chronic exposure to cortisol and psychopathologies (Bangasser & Valentino, 2014; Lupien et al., 2018; McEwen & Akil, 2020).

### 1.4.3. Summary: Physiology of the Stress Response

In summary, the two major mediators of the stress response, the ANS and the HPA, are tightly coupled and hierarchically organized to affect all organ systems of the human body (see Figure 7).

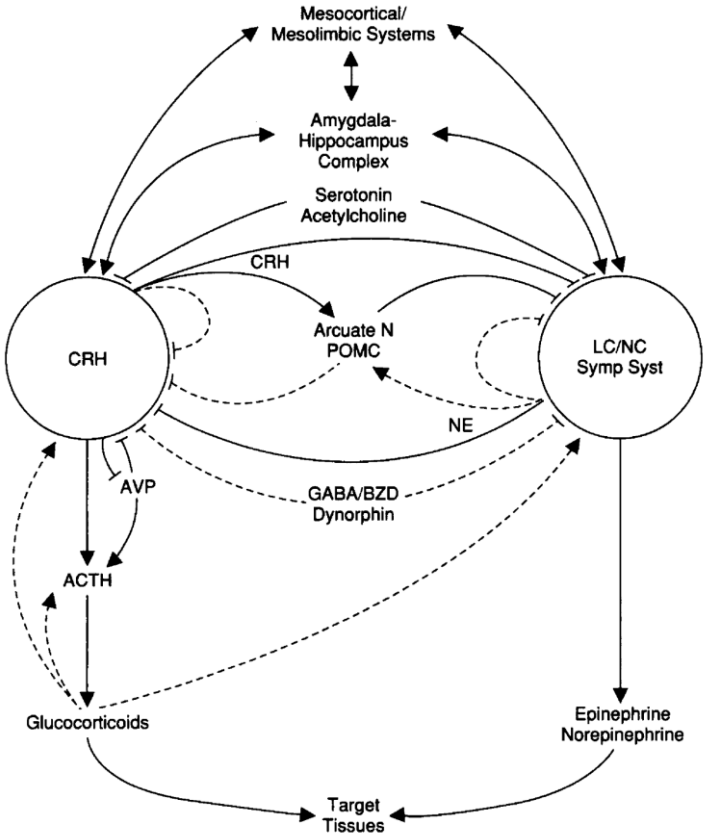


*Figure 7: A simplified representation of the HPA and the ANS stress response systems. ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; BZD, benzodiazepine; CRH: corticotropin-releasing hormone; GABA, δ-aminobutyric acid; LC/NE, locus ceruleus/noradrenaline system; POMC, proopiomelanocortin; (Chrousos & Gold, 1992, p. 1247)*

Their baseline activity follows a circadian rhythm, influenced by external *zeitgebers*. Continuous adjustments of the ANS and HPA are based on multiple integrated feedback

mechanisms on the one hand, and top-down feedforward regulation on the other hand. By comparing predictions with exteroceptive and, with higher priority, interoceptive information, the brain adjusts energetic flow and updates internal models to keep uncertainty minimal. The integrative process, sometimes referred to as “sense-making”, is psychologically manifested in context-dependent feelings or states. For instance, under certain conditions, unexplained deviations in heart rate from the predicted trajectory, combined with nociceptive input, may be constructed as pain or anxiety. However, during a training session, an experienced athlete may interpret the same signals differently. In the face of perceived challenges or threats, resources can be mobilized through coordinated activation of stress response systems. Accordingly, the ANS and HPA operate in concert with metabolic, immune, and cardiovascular processes to support optimal function under imposed demands. Consequently, stress responses and allostatic regulation can only be adequately understood through the assessment of multiple endocrine and autonomic markers over time. Adverse consequences of allostatic overload are partly attributed to “neurotoxic effects” (Lupien et al., 2018) of stress mediators, primarily cortisol, as well as increased pro-inflammatory and reduced anti-inflammatory processes. However, it is important to remember that changes in health behavior can also have indirect effects (D. Hill et al., 2022; Stults-Kolehmainen & Sinha, 2014).

## 1.5. Stress Models in Exercise Science

The prominent strength and conditioning coach Cal Dietz starts his principles of training with: “If the athlete isn’t physically stressed, you are wasting your time.” (Peterson & Dietz, 2012, p. 3). This reflects the widely accepted contention that training must impose necessary stress to drive positive adaptation. At the same time, this view is largely rooted in an outdated conceptualization of stress that treats it as a (metaphorical) driver of physiological adaptation to purely mechanical and metabolic stimuli (Kiely, 2018).

Most classical theories of stress focus on negative adaptations rather than positive ones. However, Selye coined the term *eustress* and made clear that stress itself does not necessarily entail negative consequences, but is essential to life and development (Selye, 1975b). Crucially, Selye’s eustress-distress dichotomy is largely a philosophical argument and not empirically grounded in his research. Other theories of positive biological adaptations were described early on but have been under scrutiny for oversimplification and excessive generalization (Bondy, 2023). One biological foundation for the beneficial effects of low-level stressors comes from the theory of hormesis. In 1888, Hugo Schulz reported that low doses of toxins could stimulate yeast growth, whereas higher doses had detrimental effects

(1888). Schulz's linkage to homeopathy sidelined his observations for a time[6]. However, these and similar findings gained attention in toxicology and medicine as the biphasic response to stressors, termed *hormesis* (Calabrese, 2018). According to the principle of hormesis, low-dose stressors can have positive effects on the system, whereas higher doses lead to negative adaptations (Mattson, 2008).

Training science made ideas about homeostasis, hormesis (which is seldom explicitly mentioned), and stress more concrete: adaptation arises from repeated, structured, and planned mechanical and metabolic challenges. Anecdotal connections have been made to the ancient Greeks and to *Milo of Croton*, who is said to have carried a calf from birth until he could do so with a grown bull (Stojiljković et al., 2013). In the twentieth century, the physiological underpinnings of such accounts were attributed to the so-called *supercompensation* or *adaptive reconstruction* phenomenon. Originally, supercompensation referred to the specific finding of increased muscle glycogen storage after depletion, which has been attributed to either Yampolskaya in the 1950s (Yakovlev, 1975) or Folbrot in the 1940s (Verkhoshansky & Siff, 2009). Rigorous documentation of the effect, however, stems from the *Scandinavian Biopsy Studies* (Bergström & Hultman, 1966; Hawley et al., 2015). This very specific finding was generalized early on and applied to different adaptations of exercise training by Soviet exercise scientists in the 1950s (Verkhoshansky & Siff, 2009).

At that time, there was no connection to the GAS theory of stress. In the Soviet Union, science was ideologically monitored, and Selye's work was suppressed at least until the 1960s, according to Viru (2002). However, the parallels were drawn later, and GAS became a conceptual backdrop for training periodization (Verkhoshansky & Siff, 2009). One of the first to directly apply the GAS theory to training was the Austrian exercise physician Ludwig Prokop (1959). Today, GAS is frequently invoked in contemporary models of training adaptation. However, these links are largely analogical: GAS summarized endocrine and morphological responses to noxious agents and did not predict "supercompensation", whereas training adaptations result from repeated, specific mechanical and metabolic stimuli.

A quantitative shift came with the *Fitness-Fatigue model*, introduced by Calvert et al. (1976). It was advanced by Eric W. Banister (1991) and is often referred to as a revision of the GAS theory in discussions of training periodization (Chiu & Barnes, 2003; H. Gregory G. & Travis, 2015; Kiely, 2015; Zatsiorsky et al., 2020). However, no explicit connections to stress were

[6] Hormesis is based on low-dose toxins with clear physiological mechanisms. Homeopathy builds on the effects of hyper-dilutions with no clear physiological underpinnings and very limited experimental evidence (Ernst, 2010).

provided in the original articles (Banister, 1991; Calvert et al., 1976). The GAS itself represented a conceptualization of Selye's observations, with a primary interest in the underlying biological mechanisms. In contrast, the Fitness-Fatigue model was developed from a phenomenological, systems-theory perspective and was formulated without making strong assumptions about specific physiological processes. It effectively treated the organism as a black box. The discrepancy between GAS and training adaptation was also summarized by Kiely (2015, 2018), who advocates incorporating a more holistic view of training. A comparison of GAS and an updated depiction of the Fitness-Fatigue model is shown in Figure 8.

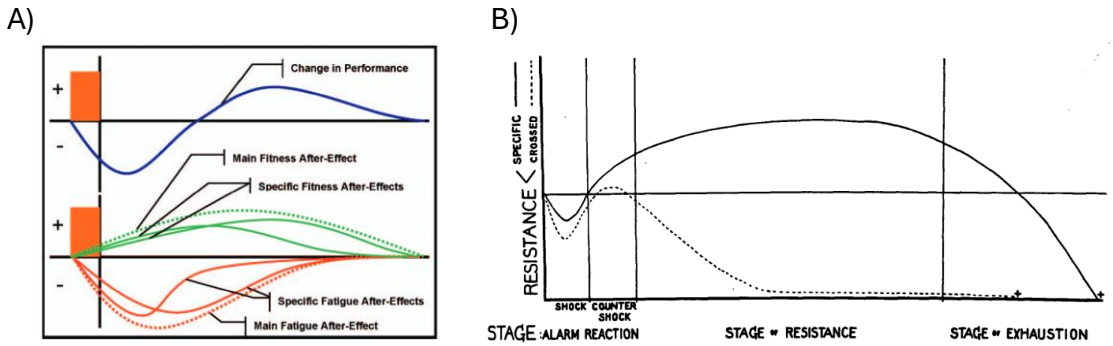


*Figure 8: Comparison of (A) the Fitness-Fatigue Model (Chiu & Barnes, 2003, p. 44) and (B) the General Adaptation Syndrome (GAS; Selye, 1946, p. 123). In (A), the overall positive effects on performance are a result of a fast-dissolving fatigue component superimposed by a slower effect on fitness. The model predicts an overall improvement of function, which serves as the basis for structured training adaptations. In (B), the GAS represents the negative response to stress. After an initial shock phase, the second stage marks higher resistance (i.e., lower response) against the specific stressor and reduced resistance to other stressors. This is fundamentally different from the positive, performance-related changes in the Fitness-Fatigue model. The last phase in the GAS model describes exhaustion of the organism's adaptive competencies, maladaptation, and, finally, death.*

A key innovation of the Fitness-Fatigue model is its differentiation between two outcomes of an exercise bout: fatigue and fitness. Predicted performance is the sum of the two constructed components. Fitness and fatigue show independent exponential decay over time, with fatigue dissipating more rapidly. This allows long-term improvements through systematic training inputs while avoiding accumulated fatigue that would lead to overreaching and overtraining (Banister, 1991). This represents a further distinction from stress models. The window of opportunity for long-term change through structured training inputs does not rely on the reestablishment of physiological stability. Rather, it is the masking effects of fatigue that diminish in their influence on outcomes of interest (i.e., performance). Only the superimposed performance function resembles the response pattern depicted in the original GAS model.

Later adaptations of the Fitness-Fatigue model acknowledge that different physiological systems exhibit distinct trajectories of both fitness and fatigue (Chiu & Barnes, 2003). Framed in this way, the adaptation profile to exercise may still be directly related to more recent theories of stress. Exercise demands can be viewed as deliberate allostatic challenges to multiple systems, leading to beneficial remodeling consistent with hormetic processes. Examples of biphasic ("U-shaped") exercise-induced regulations include the effects of reactive oxygen species (Powers et al., 2020; Shadel & Horvath, 2015) or the reduction of serum cortisol during low-intensity exercise, whereas higher intensities provoke robust cortisol secretion (E. E. Hill et al., 2008). Furthermore, positive exercise adaptations can be described as allostatic regulation through modification of perception (e.g., changes in pain threshold (Tesarz et al., 2012)), active interference via internal or external action (e.g., elevated muscle protein synthesis (Damas et al., 2015)), and perceptual weighing (e.g., desensitization to anabolic stimuli (Ogasawara et al., 2013)).

Adaptations are specific to the demands but also introduce effects distributed throughout the organism via neural, endocrinological, and immunological mediators. When multiple demands – from training as well as other life stressors – accumulate beyond recovery capacity, allostatic overload can occur in two principal forms. Type 1 allostatic overload occurs when energy demands can exceed the supply to tissues or organs (McEwen & Wingfield, 2003). This corresponds to low energy availability, a hallmark of Relative Energy Deficiency in sports (Mountjoy et al., 2023). Type 2 allostatic overload occurs when sufficient energy is available, but its distribution remains ineffective. The cost of allostasis is reflected in elevated glucocorticoid levels, increased inflammatory activity, and low vagal tone (McEwen & Wingfield, 2003). Overtraining can then be described as a combination of low energy availability and high repair costs, compounded by psychosocial distress.

This reframing allows a more stringent integration of exercise training with other biological adaptations, including psychosocial stress. It also improves the explanatory power of empirical and anecdotal knowledge regarding the complexity of training responses to additional biopsychosocial demands and recovery strategies (Kiely, 2015, 2018). It aligns with conceptualizations of overtraining syndrome, in which factors such as psychological demands, nutrition, sleep, infectious diseases, and recovery must be considered (Bell et al., 2020; Meeusen et al., 2013; Radak et al., 2008).

In summary, while training adaptations recruit mediators traditionally associated with the stress response, equating exercise periodization with GAS is conceptually misleading. To explain the interaction between training and other life demands and the multifaceted

adaptations that arise, a modern model of training must include multilevel, psychophysiological regulation through predictive feedforward and feedback mechanisms. Such a model can also better explain experimental findings on the interactions of recovery, stress, and training, including overreaching and overtraining syndrome.

## 1.6. Interaction between Stress and Physical Exercise

The interaction between stress and exercise is twofold. Most studies focus on exercise as a means to alleviate psychosocial stress (Gosain et al., 2020; Park & Jang, 2019; Traylor et al., 2020) or on the association between perceived stress and exercise participation (Stults-Kolehmainen & Sinha, 2014). Although considered an acute stressor, exercise can exert beneficial effects on stress-related outcomes. Conversely, high allostatic load resulting from psychosocial demands can impair training and adaptive responses to exercise, potentially increasing the risk of overtraining. Both directions will be briefly explored in the following subchapters, given their relevance to the presented studies.

### 1.6.1. Effects of Exercise on Stress Regulation:

Acute physical activity can increase allostatic demands. This is evident in the metabolic energy demands during and after physical exercise (Børsheim & Bahr, 2003), as well as in cardiac-autonomic regulations (Leal-Menezes et al., 2025), which are supported by elevated adrenal catecholamines and cortisol levels (Athanasiou et al., 2022). Consequently, under a response-centered theory of stress, acute exercise can be viewed as a stressor. The chronic effects are more complicated. On the one hand, high training demands may involve increased cortisol exposure. For example, endurance athletes show higher hair cortisol levels than controls (Skoluda et al., 2012). Another study found a correlation ($r = 0.34$) between vigorous physical activity and hair cortisol in university students (Gerber et al., 2013). On the other hand, long-term exercise functions as a health-promoting factor by improving allostatic function through different pathways. This discrepancy is sometimes referred to as the *exercise glucocorticoid paradox* (Chen et al., 2017). Several possible explanations should be considered for these (seemingly) paradoxical findings. First, the intensity and volume of training play an important role. Both cited studies support a relationship between training volume or intensity and chronic cortisol exposure (Gerber et al., 2013; Skoluda et al., 2012).

Second, the effects of exercise on stress regulation are not limited to cortisol exposure. Fuchs and Klaperski (2018) describe the positive effects of physical activity on stress regulation from a psychophysiological perspective and identify four levels (see Figure 9): (1)

Physical activity can act on the level of the stressor. For example, a person may meet psychosocial requirements by engaging in physical activity. (2) The increase in personal resources may be reflected in social support from a regular exercise group. (3) Physical activity may reduce the psychophysiological stress response to other stressors beyond the exercise context. (4) Regular physical activity has a compensatory effect on health, counteracting the negative effects of high exposure to stress mediators. For example, while chronic exposure to elevated cortisol levels promotes hypertension and cardiovascular events, regular physical activity has the opposite effect. In this context, it is noteworthy that the mechanisms of action overlap. Chronic stress favors inflammation (see Chapter 1.4), with elevated concentrations of pro-inflammatory cytokines such as TNF-α and IL-6. These mediators contribute to the development of atherosclerosis, a predominant risk factor for cardiovascular events (Brotman et al., 2007; Wirtz & von Känel, 2017). In contrast, chronic exercise increases cardiac vagal tone, reduces sympathetic activity (El-Malahi et al., 2024; Joyner & Green, 2009), and reduces exposure to pro-inflammatory cytokines (Abd El-Kader & Al-Shreef, 2018; Khosravi et al., 2019).

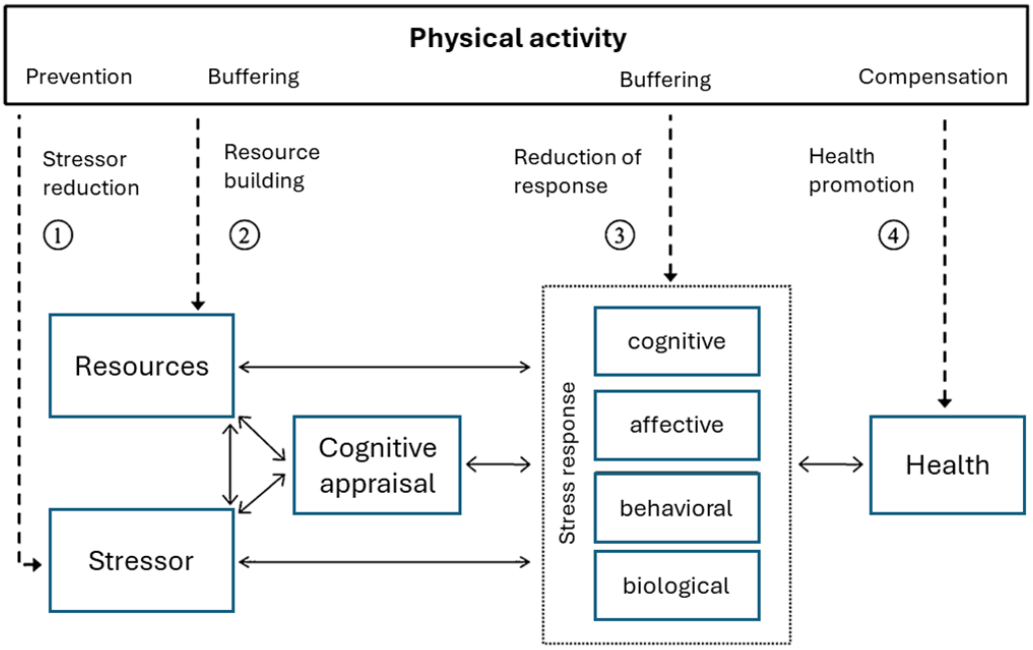


*Figure 9: Model of stress regulation, adopted from Fuchs and Klaperski (2018, p. 209). The model is based on a transactional model of stress (Ayers & Steptoe, 2007; Lazarus & Folkman, 1984). Physical activity can potentially influence all four levels of interaction.*

The buffering effect in the model by Fuchs and Klaperski should also be briefly elaborated on, as it directly relates to the presented studies. While this effect cannot be isolated from

the other conceptual pathways, the reduction of the stress response through exercise and training is frequently referred to as the cross-stressor adaptation hypothesis (CSAH; Sothmann, 2006; Sothmann et al., 1996). Sothmann and colleagues proposed that dealing with stressors would induce learning through habituation to the known stressor (i.e., reduced neuroendocrine response) and sensitization (i.e., increased response) to novel stressors. As already mentioned, adaptations to exercise training involve the stress response systems, as one of their primary functions is energy management. According to Sothmann and colleagues (1996), exercise training would also improve the specificity and regulatory capacity of the response, as improved management of energy distribution between different response systems would be an evolutionary advantage. This also fits within the allostasis and predictive processing paradigm. Efficient regulation would free resources by reducing prediction error. Multiple studies have addressed the CSAH. The early meta-analysis by Jackson and Dishman (2006) did not find evidence for the CSAH. Forcier and colleagues (2006), on the other hand, investigated only cardiovascular responses and found that fitter individuals showed lower reactivity to psychological stressors. However, both literature syntheses suffered from high heterogeneity, underrepresentation of females, and inconsistent measurement of fitness and stress responses. In particular, the heterogeneity of stress-induction protocols is of importance. Forcier et al. (2006) included studies that appeared to induce only very mild stress, as indicated by heart rate responses of less than 10 beats per minute. Later studies addressed this limitation by implementing the TSST as a more robust stress-induction procedure. More recently, Mücke et al. (2018) summarized cross-sectional and longitudinal studies on the effect of fitness on heart rate and cortisol during the TSST. Although they did not quantitatively estimate effects due to the high heterogeneity of the studies, this review supports a reduced cortisol response in fitter individuals. Approximately 60% of the included participants were male. Some recently published studies warrant individual consideration due to their rigorous methodology, longitudinal design, and sample sizes.

Klaperski et al. (2014) randomly allocated healthy men to a 12-week aerobic exercise training group, a relaxation group, or a wait-list control group. In the final sample of n = 96 participants, stress responses to the TSST were assessed before and after the intervention. Following training, the exercise group showed reduced cortisol, heart rate, and HRV metrics during TSST. In contrast, the relaxation group demonstrated only a reduction in cortisol activity, while cardiovascular indices remained unchanged. An important limitation of this otherwise rigorous study is that HRV was derived from the internal algorithm of the heart rate monitor rather than from raw inter-beat interval data (Klaperski et al., 2014).

Gerber and colleagues (2020) investigated the effects of aerobic endurance training compared to an active control condition in a clinical sample of inpatients with major depression. The intervention lasted six weeks, while the control group engaged in light stretching and ball juggling. After considerable attrition, the final sample comprised n = 25 participants. Overall, cortisol responses to the TSST were low, and no significant differences between groups were detected. Meta-analytic evidence indicates that individuals with depression often show blunted cortisol reactivity; however, findings are highly heterogeneous (Burke et al., 2005). Although the small final sample size limits statistical power, the study by Gerber et al. (2020) remains informative. In a secondary analysis, the authors reported no between-group differences in symptom reduction or subsequent physical activity. Nevertheless, the aerobic exercise group demonstrated clinically meaningful improvements in working memory (Imboden et al., 2020), supporting the beneficial effects of exercise on psychological functioning.

Arvidson and colleagues (2020) reported another randomized controlled trial in healthy participants, comparing six months of aerobic endurance exercise with a control group that maintained habitual physical activity. This study did not support the CSAH for any of the outcomes examined. Interestingly, Arvidsen et al. (2020) measured plasma ACTH and total serum cortisol, whereas a majority of other studies relied on saliva samples to assess free cortisol (Mücke et al., 2018). Most circulating cortisol in serum is bound to CBG and considered biologically inactive. Furthermore, serum and salivary cortisol do not exhibit a linear relationship (Hellhammer et al., 2009). This limits the study's comparability with the broader CSAH literature.

While standardizing the stress-induction is desirable, no studies to date have compared different laboratory-based stress-induction protocols. Hence, it remains unclear whether cross-stressor adaptation depends on the type of stressor.

### 1.6.2. Combined Effects of Exercise and Psychosocial Stressors

Psychological stressors and mental health influence training and performance. The influential consensus statement on *Recovery and Performance in Sport* (Kellmann et al., 2018) accepts recovery as a biopsychosocial process. Consistent with the training-as-stress model (see Chapter 1.5), it describes the balance between total stress (induced by training, performance, competition, daily life, etc.) and recovery as essential in sport (Kellmann et al., 2018). Discourses on overtraining have also introduced psychological factors, particularly stress, as both a cause and a diagnostic feature (Meeusen et al., 2013). Some have

even proposed considerable overlap between stress-related psychiatric disorders and overtraining (Angeli et al., 2004; Armstrong & VanHeest, 2002).

Some smaller studies suggest a synergistic effect of psychological stressors and exercise demands on the physiological response. Huang et al. (2010) examined catecholamine, 8-isoprostane (a marker of oxidative stress), and IL-2 responses to cycling at 60% of VO2max compared with the same exercise demand combined with a Stroop test and mental arithmetic. Their study indicates higher levels of adrenaline and 8-isoprostane under the combined stressor condition. Using a similar design, the same research group examined the combined stress of endurance exercise and a firefighter-specific decision task in n = 9 firefighters. The results were comparable to those of the first study and again suggested a synergistic effect of exercise and mental demand (Huang, Webb, Garten, et al., 2010). Unfortunately, both studies are limited by small sample sizes (n = 7 and 9), the absence of a pure mental challenge condition, and insufficient statistical analyses. It therefore remains unclear whether the mental demand can be identified as a stressor. These findings should be interpreted with caution.

Wasmund et al. (2002) used MSNA, blood pressure, and heart rate to evaluate the combined autonomic response to static handgrip exercise and an arithmetic task. This investigation did not find a synergistic effect and suffered from the same limitations as the previously mentioned studies. Taken together, the direct evidence for synergistic physiological effects of exercise and psychosocial stressors remains very limited.

### 1.6.3. Effects of Stress on Training

Direct experimental evidence on the effects of stress on training adaptations is also sparse. Stults-Kolehmainen and Bartholomew (2012) reported slower recovery of isometric force production after an intensive resistance exercise protocol in participants who reported higher levels of perceived stress. Two years later, they presented a longer follow-up of 96 hours, further strengthening their findings. They also observed greater soreness and perceived fatigue at the end of the observation period (Stults-Kolehmainen & Sinha, 2014). Ruuska et al. (2012) reported differences in the adaptations after only 2 weeks of aerobic endurance training, depending on self-reported baseline stress. One limitation of this study is that training intensity was controlled by heart rate, but data on the mechanical work performed during the sessions were not reported. The negative association between training adaptation and perceived stress may be partly mediated by performance during training. In addition, adverse effects of stress on exercise adaptations could involve reduced self-

confidence and self-efficacy (Tossici et al., 2024). In contrast, relaxation methods have been shown to improve performance in some studies. For example, Pinto and McGill (2020) reported faster recovery during repeated countermovement jumps when participants performed a muscle-relaxation technique compared to standing still. While interesting, this study does not provide mechanistic explanations nor control metrics on the propagated relaxation. Pelka et al. (2017) used different relaxation techniques (progressive muscle relaxation, systematic breathing[7], power naps, yoga, and a control condition) between two consecutive bouts of repeated sprint exercise separated by 25 minutes. In their within-subject design with n = 27 students, higher running speed was observed during the second bout after breathing and a power nap. No effects were found for mean power or heart rate.

Some other studies reported negative health outcomes (Simms et al., 2021) or increased injury risk associated with psychological factors and perceived stress (Ivarsson et al., 2017; Ivarsson & Johnson, 2010; Mann et al., 2016; J. M. Williams & Andersen, 1998). However, these findings are not robust, and the underlying mechanisms remain debated. In addition, psychological traits may act as mediators (Podlog & Ivarsson, 2025; Tranaeus et al., 2024).

Taken together, while the general consensus is that stress influences performance, recovery, and adaptation (Soligard et al., 2016), high-quality and consistent evidence remains lacking. Experimental evidence on the effects of stress on exercise adaptations faces several methodological challenges. Prolonged experimental stress induction cannot be justified for ethical reasons. An alternative strategy is to use relaxation and stress-reduction techniques as potentially positive interventions. Breathing exercises, in particular, have shown some promising results. The use of slow-paced breathing as a mechanistically and experimentally supported means to increase parasympathetic and reduce sympathetic activation will be further explored in Chapter 2.3.

## 1.7. Aim of the Project

The previous chapters laid out the theoretical and practical background of this work. In this dissertation project, I address multiple distinct scientific topics related to the interaction between stress and exercise. Three studies are presented in the following chapter that address the stress response in relation to training status and fitness, as well as the role of a physiological “stress-management” strategy during a strenuous training regimen.

[7] The description of „systemic breathing“ (Pelka et al., 2017) is consistent with slow-paced breathing as described in this project and by others (Laborde et al., 2024).

First, we tested responses to two different laboratory stress-induction protocols in male and female athletes. We report a broad range of psychophysiological outcomes elicited by a psychosocial stressor (TSST) and a psychophysical stressor (Maastricht Acute Stress Test; MAST). Although this paper represents a pilot study with a limited sample size, its methodology laid the foundation for further experiments.

The second paper utilized the methods and insights from the first study and elaborated on the role of aerobic fitness as a mediator of the stress response to the MAST and TSST. While similar experiments have been conducted previously, this is the first study to differentiate the effect of fitness across two different stressor paradigms, strengthening our understanding of the transferability of stress-buffering between domains.

The third paper expanded the topic from a slightly different angle, addressing the role of a recovery strategy intended to reduce allostatic load through increased vagal activity. The intervention group practiced slow-paced breathing for four weeks, while the control group did not. All participants underwent a demanding sprint interval training. Based on the model of training adaptations outlined above, we hypothesized greater improvements in performance and better cardiac-autonomic recovery in the slow-paced breathing group.

### 1.7.1. Thesis-wide methodological principles

All three studies were guided by common methodological principles derived from the theoretical background as well as methodological and social considerations:

**Objective 1:** Stress cannot be operationalized by a single physiological outcome measure. For example, similar cortisol responses do not imply equal overall stressor impact. Therefore, the first objective of the PhD project was to clearly distinguish between response patterns by implementing multiple subjective and objective outcomes.

**Objective 2:** Second, exercise science remains male-biased, with approximately 63% of participants included in respective studies being men (Cowley et al., 2021). A similar pattern can be found in related fields such as psychology, medicine, and physiology (Beery & Zucker, 2011). The influence of sex-related biology (including sex hormones) on physical performance (Hunter et al., 2023), disease risk (Bangasser & Valentino, 2014; Prabhavathi et al., 2014) as well as autonomic and HPA function (Gu et al., 2022; Kajantie & Phillips, 2006b; Kudielka & Kirschbaum, 2005) is well established at mechanistic and experimental

levels. Therefore, the project's second objective was to include participants of both biological sexes and model sex-specific effects where appropriate.

**Objective 3:** All presented studies followed the open science paradigm and provide openly accessible, structured, and managed data, in accordance with the FAIR principles (Wilkinson et al., 2016). Rapid dissemination of acquired knowledge was established by publishing preprints, not merely to increase visibility and exposure, but also to foster further transparency by enabling pre-peer-review comparison of manuscripts. Studies 2 and 3 were also preregistered on Open Science Framework.

# 2. Materials and Methods

This chapter gives an overview of the central methods used across the three studies. Because each study includes an extensive methods section detailing study design, participant recruitment, inclusion criteria, allocation, measurement, and statistical analysis, the present chapter summarizes the methodological framework common to the project. Particular emphasis is placed on the stress-induction protocols, training intervention, and the slow-paced breathing intervention, which constitute central methodological pillars across the dissertation.

## 2.1. Stress-induction protocols

Stress-induction protocols are used to study the psychophysiological stress response in humans under controlled laboratory settings. Other approaches to inducing stress responses include pharmacological administration (See & Waters, 2011), carbon dioxide inhalation (Wetherell et al., 2006), hypoglycemia (Haas et al., 2022), or cold exposure (Schwabe & Schächinger, 2018). However, with the transition from one-dimensional response-centred theories of stress to transactional theories (see Chapter 1.2), the sole increase in cortisol and adrenaline may be interpreted as homeostatic regulation rather than stress. Nevertheless, the responses of the HPA axis and ANS are still used to evaluate the effectiveness of the stress response. In addition, the mentioned protocols were either invasive, insufficiently effective, or unreliable in producing psychophysiological response patterns. Therefore, Kirschbaum and colleagues developed the TSST as a more robust method to induce HPA and ANS responses (C. Kirschbaum et al., 1993). The cortisol response to the TSST is robust, with meta-analytically estimated effect sizes of SMD = 0.93 (95% CI [0.84, 1.01]) (Goodman et al., 2017). Sex differences were found, with females typically

showing lower cortisol levels. At the same time, some studies report higher subjective stress in female participants. Both findings are related to menstrual cycle, hormonal contraception and sex hormone levels (Gervasio et al., 2022; Gu et al., 2022; Kudielka & Kirschbaum, 2005; Liu et al., 2017).

The TSST is one of the most frequently used laboratory stress-induction protocols. It includes public speaking in front of an audience and a mental arithmetic exercise. Some variations of the TSST, including a group version (von Dawans et al., 2011), a version for children (Yim et al., 2010), virtual versions (Zimmer et al., 2019), and a sham version (Het et al., 2009; Wiemers et al., 2013), have been validated. In the presented research, the standard TSST was implemented and followed the guidelines published by Labuschagne et al. (2019).

The effectiveness of the TSST is based on psychological theories of stress. Uncontrollability, uncertainty, and novelty were already discussed by Mason as being the main drivers of the stress response (1968). Goal-directedness and risk were also identified early as potential drivers of the stress (Lazarus & Folkman, 1984). Based on this research, Dickerson and Kemeny (2004) identified the components of laboratory-based stress-induction protocols for inducing a stress response and defined preservation of the *social self* as a major goal during the TSST, which is challenged by social evaluation under uncontrollability, uncertainty, and novelty.

The second stress-induction protocol used in the present research is the Maastricht Acute Stress Test (MAST). This test was introduced by Smeets et al. (2012) as an extended version of a socially evaluated cold-pressor test (Schwabe & Schächinger, 2018), with elements similar to the TSST. During the classical cold-pressor test, the tested person submerges one hand in ice water. This leads to vasoconstriction and a subsequent increase in blood pressure. The test was originally proposed as a vasomotor function test, and the results show some predictive power for the development of hypertension and cardiovascular diseases (Kasagi et al., 1995). Another application is the study of pain perception (Tesarz et al., 2012). However, except for blood pressure, other markers of stress (e.g., subjective, heart rate, cortisol) do not show robust response patterns during the classic cold-pressor test. It should be noted that rapid elevation of blood pressure activates the baroreflex, which counteracts the effect on heart rate (Schwabe & Schächinger, 2018). The cold-pressure test lacks psychological components of social evaluation, uncertainty, uncontrollability, and novelty (Dickerson & Kemeny, 2004). It is a passive coping test in which the tested person

endures the pain. Active and passive coping may induce different autonomic and immunological responses to the stressor (Bandler et al., 2000; Bosch et al., 2001).

The MAST addresses these issues by adding social evaluation and the active component of a mental arithmetic test. Repeated exposure to ice water with an unknown duration adds components of uncontrollability and uncertainty. Unlike the socially evaluated cold pressure test, the MAST has a similar duration to the TSST, making it a suitable candidate for comparison. Based on a sample of n = 20 male undergraduate students, Smeets (2012) compared the MAST to the TSST and found no significant differences in cortisol, alpha-amylase, and affect. However, other direct comparisons have not yet been published. While older studies tested the hypothesized stress-buffering effect of exercise (Sothmann et al., 1996) using different physiological or psychological challenges (Forcier et al., 2006; Jackson & Dishman, 2006), more recent studies primarily used the TSST (Arvidson et al., 2020; Caplin et al., 2021; Gerber et al., 2020; Klaperski et al., 2013; Mücke et al., 2020; Wunsch et al., 2019). The MAST has not yet been employed in the context of stress, exercise, and physical fitness. Addressing this gap is essential to understanding whether the potential stress-buffering effects of fitness and exercise depend on the nature of the stressor. For example, beyond physiological adaptations, athletes and regular exercisers have been shown to exhibit greater pain tolerance (Tesarz et al., 2012), which may be particularly relevant for stressors that include a nociceptive component, such as the MAST. The presented research addresses this gap.

## 2.2. Sprint interval training

Sprint interval training (SIT) is a form of high-intensity interval training (HIIT). It consists of multiple relatively long sprints (30 – 45 seconds) at an intensity above the maximal aerobic speed and close to maximal sprinting speed. The sprints are interspersed with prolonged recovery phases (typically > 2 minutes) at very low intensity or at complete rest (Buchheit & Laursen, 2013). Compared to other forms of HIIT, it is considered more closely related to the neuromuscular and anaerobic energy systems. However, the repeated character under the accumulation of fatigue leads to increased dependence on the aerobic system with each set performed (Buchheit & Laursen, 2013; MacInnis & Gibala, 2017). Tucker et al. (2016) showed that, despite lower overall energy expenditure during the SIT session, there is a considerable excess post-exercise oxygen consumption (EPOC) after SIT compared with lower-intensity training. Combined with findings of high local oxygen uptake (Kriel et al., 2019; Manchado-Gobatto et al., 2020), this may explain improved fitness through local working-muscle adaptations in oxygen transport and utilization as well as increases in

muscle glycogen (Hall et al., 2023; Smith et al., 2023), especially when controlled for training volume or net training time (Mølmen et al., 2025). This results in improvements in aerobic and anaerobic capacity following SIT (Boullosa et al., 2022).

Because SIT is typically performed with maximal intent (i.e., maximal voluntary sprinting speed under accumulating fatigue), formal VO2max or performance testing is not required for prescription, which is an ecological advantage, though baseline testing can aid screening and monitoring. (Buchheit & Laursen, 2013). This ecological advantage also controls for test validity and reliability during program design.

Some argued that SIT is more effective than other forms of endurance training. But this is not supported by meta-analyses comparing SIT to other forms of HIIT or to moderate-intensity continuous training methods (Boullosa et al., 2022; Gist et al., 2014; Rosenblat et al., 2020). However, its effects are generally deemed to be comparable or slightly higher for anaerobic power. It must be noted that almost all studies are conducted on healthy individuals (mostly men), and prior experience with SIT relative to other forms of endurance training is not always reported, but would be expected to be lower. While some have recommended SIT for other populations, including clinical use (e.g., Minghetti et al., 2018; Sjöros et al., 2018), others exercise caution. The applicability of SIT is limited by the high complexity and physical demands of the training, required self-control or motivation, and high negative affect during the sessions (Biddle & Batterham, 2015; Hardcastle et al., 2014). In a small (n = 9) within-subject experiment, relatively fit (Vo2max = 48.9 ± 5.3 ml/kg/min) men performed one session of three different HIIT protocols and analyzed affect, preference, and self-efficacy for regular self-organized training. SIT showed high levels of unpleasantness and the lowest outcomes among other forms of HIIT (Townsend et al., 2017). In addition, a SIT exercise bout leads to reductions in vagal-associated HRV for up to two hours after the end of the session (Stuckey et al., 2012) and induces higher short-term fatigue than other HIIT forms (Benítez-Flores et al., 2023). Four times 30-second maximal intent sprint with 7.5 minutes of rest increased serum cortisol significantly more than a TSST in n = 32 healthy men (Hermann et al., 2019). According to Hall et al. (2023), only 2 weeks of SIT are sufficient to induce performance improvements. Most studies implement SIT for 4 weeks, with an average of 3 sessions per week. A typical work/rest ratio is 1:8. A shift towards higher work/rest ratios leads to pronounced aerobic stimulus, whereas reducing the work (i.e., the duration of a single sprint) relative to the resting time induces stronger stimulation of anaerobic and neuromuscular components.

In summary, SIT is a very demanding exercise protocol eliciting strong cardiovascular, autonomic, endocrine, and subjective responses. Short training periods of 2–6 weeks have been shown to produce effects similar to those of moderate-intensity continuous training or other forms of HIIT.

## 2.3. Slow-paced breathing

Under resting conditions, healthy adults typically breathe at a rate of 12 – 20 cycles per minute. Breathing depth and frequency dynamically change according to homeostatic and allostatic needs. It has long been reasoned that consciously altering breathing patterns influences physiological and psychological states (Russo et al., 2017). Breathing interventions are increasingly used because they provide a simple means of modulating autonomic activity through cardiorespiratory reflex mechanisms. In addition, correct execution can be observed reliably based on simple physiological measures (Nicolò et al., 2020).

One approach to inducing relaxation is the deliberate reduction of respiration rate. Slow-paced breathing (SPB) was defined as a conscious control of the respiratory cycle by following an explicit pacemaker, such as a sound or a visual clock. The cadence of respiration is generally lower than ten breaths per minute (Zaccaro et al., 2018). Most interventions use a frequency of six breaths per minute – a frequency close to the *resonance frequency (i.e., eigenfrequency)* of the physiological feedback loops between respiration and heart rate (Vaschillo et al., 2006). Two connected physiological effects underlie the effectiveness of SPB: the first is the elevation of respiratory sinus arrhythmia (RSA), and the second is increased baroreflex engagement (Grossman & Taylor, 2007; Russo et al., 2017).

The baroreflex is a closed-loop feedback mechanism controlling blood pressure. Baroreceptors, primarily in the aortic arch and the carotid artery, respond to stretch from blood-pressure fluctuations. This triggers a reduction in heart rate to control overall blood flow. Both physiological (receptor- and signaling-based) and physical (e.g., viscosity, blood volume) factors are responsible for a short delay between changes in heart rate and the resulting effects on blood pressure, which can cause the feedback system to oscillate, resulting in rhythmic changes in heart rate and blood pressure. SPB deliberately exploits these oscillations by pacing respiration at a frequency that maximizes their amplitude, a phenomenon referred to as resonance (Lehrer & Gevirtz, 2014; Vaschillo et al., 2006).

While the Baroreflex drives the blood pressure response, RSA describes the coupled heart rate oscillation. Figure 10 shows an example of the resulting heart rate oscillation, in phase with the specified breathing cadence. During SPB at a rate mirroring the delay time of the

baroreflex feedback loop, RSA and the baroreflex become more synchronized (coherent), creating sinusoidal fluctuations in heart rate during breathing (Lehrer & Gevirtz, 2014; Vaschillo et al., 2006).

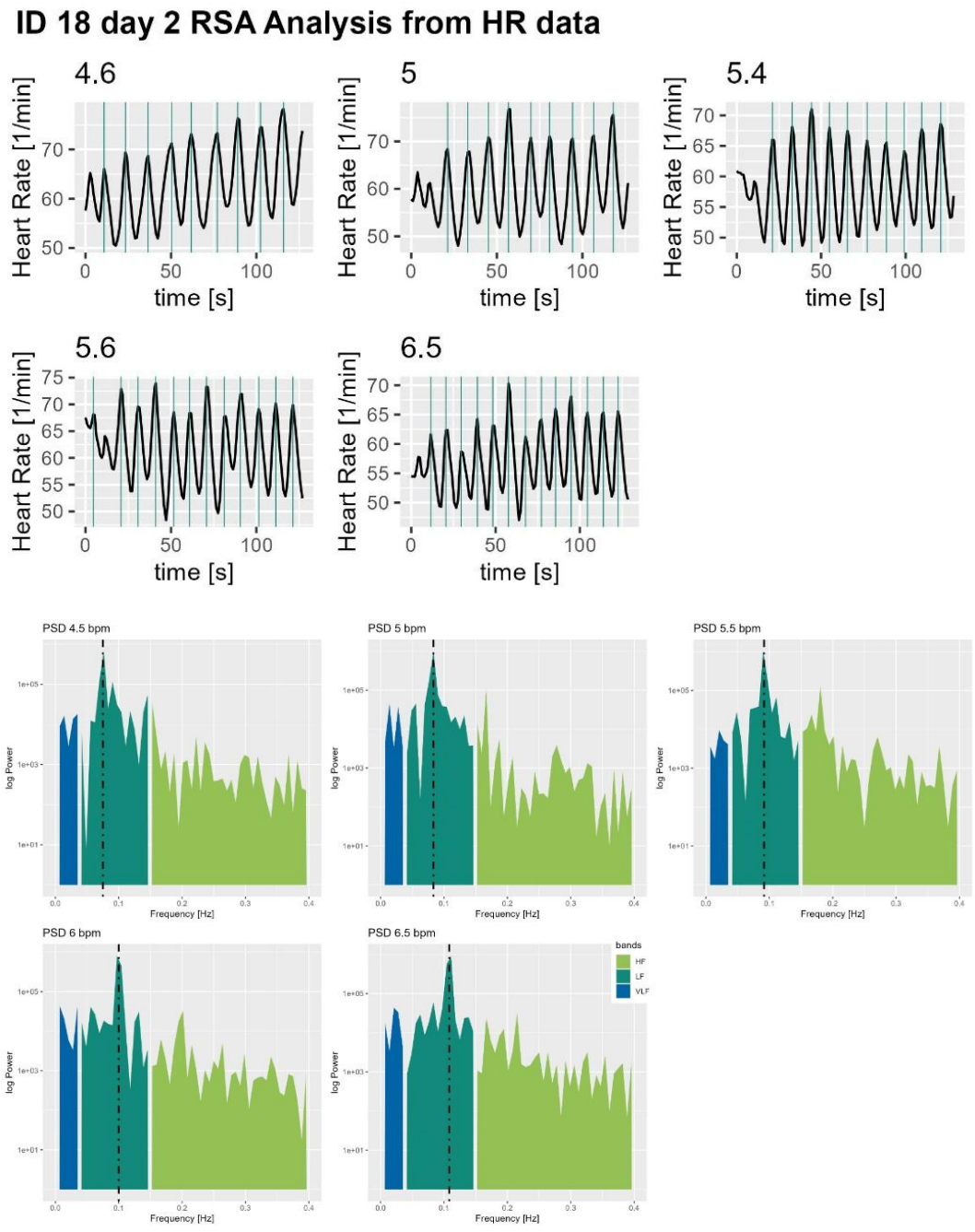


*Figure 10: Demonstrative data from two-minutes of slow-paced breathing at five different breathing rates. The test procedure and analysis followed the guidelines by Shaffer and Meehan (2020). The upper panel shows heart rate oscillations synchronized with breathing. The prescribed breathing frequencies were 4.5, 5.0, 5.5, 6.0, and 6.5 breaths per minute. The number at the top of each plot indicates the breathing frequency achieved by the participant. The lower panel shows the power spectral density of normal-to-normal heart periods from the same recording. Colors represent predefined HRV power bands. The vertical black line indicates the prescribed breathing frequency. Close alignment of the achieved peak with the prescribed breathing frequency is visible.*

The RSA primarily serves as a regulatory function to support optimal gas exchange, arterial CO2 levels, and the efficiency of the cardiac system. For example, a higher heart rate during inspiration improves lung perfusion and oxygen extraction and reduces energy demand during expiration (Ben-Tal et al., 2014; Elstad et al., 2018; Grossman & Taylor, 2007; Hayano & Yasuma, 2003). Some researchers propose that RSA is related to cardiac vagal regulation and may serve as an indicator of broader parasympathetic activity. A bidirectional relationship between cardiac vagal tone and overall parasympathetic activity would not only provide a valuable proxy for parasympathetic function but also create opportunities for breathing interventions with upstream effects on other psychophysiological states. These ideas have been formalized within several theoretical frameworks (Laborde et al., 2018a; Lehrer & Gevirtz, 2014; Porges, 1995; Thayer & Lane, 2000).

SPB has been shown to acutely and chronically increase vagally mediated HRV metrics (You et al., 2023). This has implications not only for mental and physical health (Lehrer et al., 2020) but also for sports performance and training (Laborde et al., 2024). However, most studies have either focused on acute changes in coordinative and technical skill learning rather than long-term physiological adaptations and changes in physical performance, or have not been designed as randomized controlled trials (Jiménez Morgan & Molina Mora, 2017; Laborde et al., 2024; Pagaduan et al., 2020, 2022).

# 3. Publications

The dissertation includes three publications that represent central empirical components of the project. While additional studies and data collections were conducted during the doctoral period, the following papers constitute the primary, completed contributions available for cumulative presentation. The first study characterizes psychophysiological response patterns to two established laboratory stress paradigms and evaluates potential sex differences in athletes. Building on this foundation, the second investigation explores aerobic fitness as an individual factor that may shape the magnitude and coordination of stress responses across stressor modalities. The third study moves from description and moderation toward intervention and tests whether deliberate modulation of autonomic regulation through slow-paced breathing can influence recovery and subsequent training outcomes. Together, these works address both determinants and modifiability of stress responses and thereby contribute to a multilevel understanding of how stress regulation interacts with exercise, performance, and recovery.

## Study 1: Cross-Sectional Investigation of Acute Stress Responses to Two Different Laboratory Stress Tests in Male and Female Athletes

**Authors:** Peter Raidl, Barbara Wessner, Michael Methlagl, and Robert Csapo

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- Validation, P.R.
- Formal analysis, P.R.
- Investigation, P.R.
- Resources, R.C., B.W.
- Data curation, P.R.
- Writing—original draft preparation, P.R.
- Writing—review and editing, P.R., B.W., M.M., R.C.
- Visualization, P.R.
- Supervision, R.C.
- Project administration, P.R., R.C

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*Article*

# Cross-Sectional Investigation of Acute Stress Responses to Two Different Laboratory Stress Tests in Male and Female Athletes

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**Abstract**

Background: Regular exercise was previously shown to reduce glucocorticoid and cardiac-autonomic responses to psychosocial stressors. Specifically, laboratory-based stress induction procedures are recognized as valid experimental manipulations of the physiological stress response. Nevertheless, comparative research between different types of stressors is limited. This study was designed to examine the multi-system psychophysiological response to two stress-induction procedures—psychosocial (Trier Social Stress Test; TSST) and psychophysical (Maastricht Acute Stress Test; MAST)—in male and female athletes. Methods: In a crossover pilot study, 12 athletes (6 female) underwent a TSST and a MAST, one month apart. Saliva hormones and cardiac-autonomic response (heart rate and HRV) were analyzed, besides an untargeted proteomics analysis. Results: The MAST revealed a lower elevation of heart rate (SMD = −1.47 [−2.51, −0.43]) and reduction in RMSSD (SMD = 0.98 [0.01, 1.95]) compared to the TSST. No statistically significant differences were found for hormones or subjective stress (all $p < 0.05$). Sex comparisons of the area under the curve exposed overall lower responses in women for aldosterone (SMD = −1.50, [−2.45, −0.51]), cortisol (SMD = −1.35, [−2.28, −0.39]), cortisone (SMD = −1.43, [−2.38, −0.46]), overall glucocorticoids (SMD = −1.44, [−2.38, −0.46]), and stronger reduction in testosterone-to-cortisone (SMD = 1.41, [0.44, 2.35]). Interestingly, sex differences were more evident in response to the TSST. Conclusions: Found sex differences underscore the importance of sex sensitive research in stress and exercise science. Our data support the presented methodological approach and encourage properly powered research on stressor comparison in relation to sports and physical fitness.

**Keywords:** stress; exercise; hormone; cortisol; HRV; proteomics



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## 1. Introduction

The physiological stress response is essential for development, learning, and survival [1]. Predictions of and reactions to threat are met by acute, systemic regulations involving the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis, which support coping strategies across psychological, behavioral, and metabolic domains [2–4]. However, scientific research dating back to the 20th century has also established the detrimental effects of excessive or prolonged (i.e., chronic) stress on human health [5]. Chronic stress, along with episodes of acute extreme stress, has been linked to the

development of immunological [6], cardiovascular [7–9], metabolic [10–12], neurological, and psychological disorders [13], highlighting its systemic impact and clinical relevance.

While the long-term health consequences of stress are well established, a growing body of research has investigated factors that may buffer or mitigate these effects. Among these, physical activity and exercise are widely recognized for their potential psychological benefits, including improved mood [14], reduced anxiety [15], and enhanced regulation of the physiological stress response [16]. Yet, the underlying mechanisms remain incompletely understood [17,18]. Multiple pathways have been proposed by which physical exercise might interact with stress, including providing personal resources, compensating for the negative health consequences of stress, and promoting physiological adaptations in the systems mediating the stress response (i.e., ANS and HPA) [19].

In fact, exercise itself can act as a potential physical stressor, activating many of the same physiological pathways as psychological stress. Herein, a clear non-linear dose–response relationship can be described, with exercise at higher intensities and longer durations leading to a more pronounced glucocorticoid and catecholamine response along with vagal withdrawal and sympathetic activation [20,21]. The mentioned biomarkers also serve as standard variables for operationalizing psychosocial stress. Individual differences in the glucocorticoid response to the same absolute volume-load of physical activity are primarily explained by physical fitness [22]. The relationship between external demands and the physiological response is more complex in the context of psychosocial stressors [23]. In addition, psychosocial stressors are less scalable than exercise stressors. So dose–response patterns are harder to study in the earlier case.

One possible explanation for the psychological benefits of regular physical activity is provided by the cross-stressor adaptation hypothesis [24,25]. According to this hypothesis, repeated exposure to exercise-induced stress responses may foster resilience to other stressors, especially psychosocial. This is particularly relevant given that humans exhibit pronounced stress responses to social and psychological threats [26,27]. To date, only a limited number of studies have directly tested the cross-stressor adaptation hypothesis under controlled experimental conditions [28–30]. While these studies are methodologically sound and well-designed, overall, they found limited evidence for the effect of exercise training on the physiological stress response. However, several methodological challenges remain unsolved: the populations currently studied are heterogeneous, with an overall high percentage of men. In addition, there is no consensus on a surrogate metric for stress. While cortisol is the most frequently used biomarker representing HPA activity, it has been argued that other glucocorticoids, although lower in concentration, exhibit higher sensitivity to stress [31,32]. Additionally, the stress response is characterized by a vast array of interdependent hormonal adaptations, including the gonadal axis and sex hormones [33,34]. At the same time, the autonomic response is a significant component of stress, but robust measures are underrepresented in the current literature. Finally, a key issue concerns the selection and standardization of stress-induction protocols. It is currently unclear whether different acute stressors elicit distinct response patterns, particularly in athletic populations.

Laboratory-based paradigms are frequently used to elicit a measurable stress response, with the Trier Social Stress Test (TSST) representing one of the most widely established methods. During the TSST, participants are required to deliver a mock job interview and perform mental arithmetic tasks in front of an evaluative committee, under the impression that they are being recorded and assessed [35]. This procedure reliably elicits robust physiological and psychological stress responses [36] through a combination of social evaluation and uncontrollability, paired with elements of novelty, unpredictability [37]. The Maastricht Acute Stress Test (MAST) was later developed as a complementary paradigm that combines elements of the TSST with the Cold Pressor Test [38]. In this protocol,



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the dominant hand is immersed in ice water to provoke autonomic and cardiovascular activation, while participants engage in cognitive tasks. So far, only one study directly compared the MAST and the TSST in terms of autonomic, endocrine, and subjective response [38]. While this comparison is a valuable initial step, it was conducted with male participants and a narrow set of examined biological markers, restricting a more comprehensive evaluation of the stress response.

Importantly, no study to date has directly compared the response to these two paradigms in habitual exercisers or athletes. A more differentiated understanding of stress reactivity in this population is essential to facilitate further research on the stress-buffering effect of exercise. If true cross-stressor adaptation occurs, no difference in overall response patterns between the TSST and MAST may be expected in athletes and regular exercisers. Conversely, if stress adaptation is domain-specific, regular exercisers may show reduced reactivity primarily to the MAST, which places greater emphasis on physiological stress. Such differential reactivity would have important theoretical and practical implications for future research on exercise-based stress resilience.

In addition to exploring the role of different stress paradigms, the present study also addresses a persistent sex gap in the literature. Despite well-established differences between men and women, endocrinological research in exercise science remains heavily male-dominated, and sex differences in stress reactivity among athletes and habitual exercisers remain poorly understood [39,40].

Finally, an important limitation of previous research is the narrow scope of biological markers typically assessed in response to acute stress. Cortisol is the principal glucocorticoid in humans and is widely used as a central indicator of stress-related endocrine reactivity. However, other glucocorticoids display slightly different time kinetics, activation patterns, and may show greater sensitivity to stressors [32]. Further, sex hormones are of particular interest in exercise science, and their role in acute and chronic adaptations is well studied [41,42]. Observational studies indicate that sex hormones are influenced not only by the physical, but also the psychological components of sports and competition [43,44]. But the role of experimentally induced psychosocial stress on sex hormones in athletes is currently not established.

Against this background, the objective of this study was to compare two established laboratory stress test protocols—the TSST and the MAST—using a crossover design in a mixed-sex sample of self-identified athletes. To enable a comprehensive evaluation of the stress responses, multiple indicators, including glucocorticoids, heart rate variability, subjective stress, and the saliva proteome, were assessed. Thus, the present study addresses three central limitations of prior research: the lack of stressor comparison in trained populations, the underrepresentation of mixed-sex studies, and the narrow focus on a few biological markers. With this broad approach, we aimed to explore novel candidate biomarkers, providing a basis for further research on stress reactivity and resilience.

We hypothesized (i) that the MAST would elicit a lower glucocorticoid response than the TSST, as physically active individuals may be more accustomed to physical rather than psychosocial stressors; (ii) differences in cardiac autonomic response between MAST and TSST, due to the cold-pressor effects of the MAST [45]; and (iii) that while women would report higher subjective stress, they would show overall lower cortisol responses, reflected in a higher cortisone-to-cortisol-ratio (Cn:C) due to sex-specific hormonal regulation, e.g., via 11 $\beta$-Hydroxysteroid Dehydrogenases type 2 (11-$\beta$HSD2; [33,46]).

## 2. Results

All 12 recruited participants (6 female, 6 male) were deemed eligible and completed both experimental sessions. Due to technical issues during salivary analysis, complete biological data are reported for 10 (5 female) participants. These were considered to be



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missing at random. Full datasets of all other outcome measures were retained for all participants. Baseline characteristics are shown in Table 1.

**Table 1.** Participants' characteristics.

| Sex | N | Age (Years) | BMI (kg/m$^2$) | DASS21-Depression | DASS21-Anxiety | DASS21-Stress |
|---|---|---|---|---|---|---|
| Female | 6 | 30.5 ± 5.0 | 22.8 ± 1.5 | 1.8 ± 2.1 | 0.8 ± 0.7 | 3.5 ± 2.1 |
| Male | 6 | 27.3 ± 4.87 | 25.5 ± 2.4 | 0.7 ± 1.2 | 1.5 ± 1.7 | 1.0 ± 1.3 |

Values are represented as mean ± standard deviation. BMI: Body mass index, DASS21: Depression, anxiety, and stress scale with 21 items.

None of the participants fell below the predefined cut-off levels for the DASS-21 subscales. They reported a training age of 2 to 20 years, consistently conducted 3–6 sessions per week for at least the last 3 months, and were competitively active in their sports. Five participants were enrolled in university-level education, and ten reported holding regular employment. None were shift workers or reported irregular working schedules.

### *2.1. Psychobiological Measures*

#### 2.1.1. Quantified Hormones

Figure 1 provides an overview of the time course of salivary endocrine markers during the MAST and TSST for male and female participants. Descriptively, cortisol concentrations peaked 15 to 20 min after stress cessation, with cortisone peaking 5 min later.

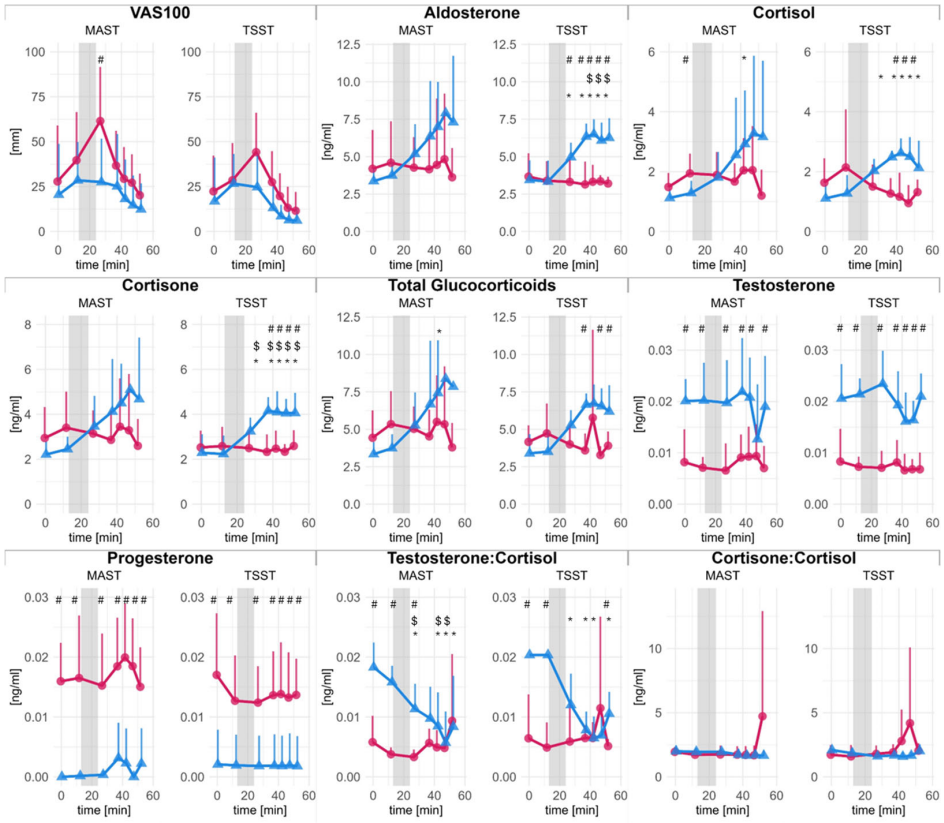


**Figure 1.** Saliva hormone concentrations and subjective stress over time. Error bars represent 95% confidence intervals (CI) Females (red dots) and males (blue triangles) are shown separately. The *x*-axis (abscissa) indicates the time in minutes, where the grey area corresponds to the stress-induction procedure. Statistically significant differences in post hoc comparisons are denoted with symbols: $ significant difference to baseline; # significant difference between male and female; * significant difference to baseline in men.



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A complete statistical analysis and results for time kinetics are disclosed in the supplements. Figure 1 presents changes in saliva hormone levels and VAS100 scores for men and women, separated by stress-induction protocol. Briefly, changes over time were found in men but not in women for aldosterone, cortisol, cortisone, total glucocorticoids, and testosterone-to-cortisol-ratio (T:C). Statistically significant differences between men and women were found for glucocorticoids and aldosterone, particularly during TSST. Overall, changes were more pronounced and robust during TSST than during MAST. Unsurprisingly, for testosterone and progesterone, significant main effects of sex were found. Absolute levels of testosterone at baseline were $20.33 \times 10^{-3} \pm 0.33 \times 10^{-3}$ ng/mL in men and $8.24 \times 10^{-3} \pm 0.08 \times 10^{-3}$ ng/mL in female participants. Progesterone concentrations in women ($16.49 \times 10^{-3} \pm 0.75 \times 10^{-3}$ ng/mL) were within the physiological range and borderline detectable in men.

AUCi comparisons for each quantified hormone are depicted in Figure 2. The mixed ANOVA revealed significant main effects of sex in case of aldosterone ($F(1, 8) = 12.9$, $p = 0.007$), cortisol ($F(1, 8) = 8.63$, $p = 0.019$), cortisone ($F(1, 8) = 10.32$, $p = 0.012$, overall glucocorticoids ($F(1, 8) = 10.09$, $p = 0.013$) and testosterone-to-cortisol ratio ($F(1, 8) = 5.64$, $p = 0.045$). No other effects reached statistical significance. The direct comparison indicated lower response in women for aldosterone (SMD = −1.50, [−2.45, −0.51]), cortisol (SMD = −1.35, [−2.28, −0.39]), cortisone (SMD = −1.43, [−2.38, −0.46]), overall glucocorticoids (SMD = −1.44, [−2.38, −0.46]), and a more pronounced reduction in testosterone-to-cortisone (SMD = 1.41, [0.44, 2.35]).

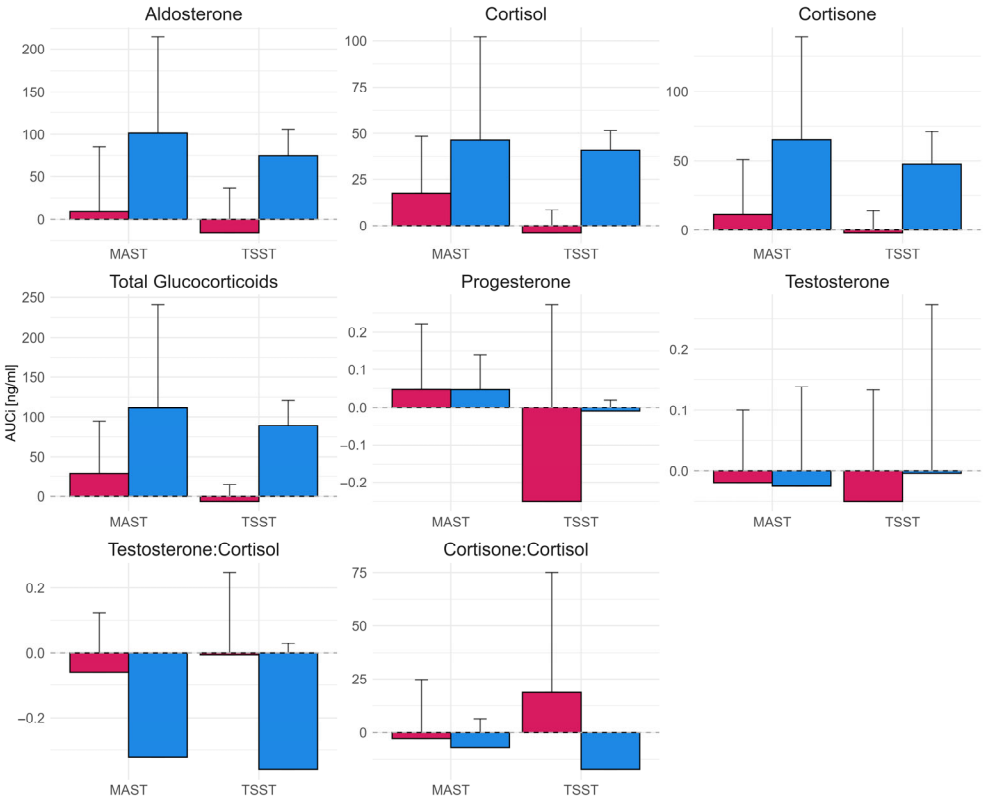


**Figure 2.** Area under the curve with respect to the baseline (AUCi) of saliva hormone concentrations during TSST and MAST for male (blue bars; right) and female (red bars; left) participants. Error bars represent 95% confidence intervals.

Direct comparison between the TSST and MAST revealed no statistically significant effects. However, the sex differences were pronounced during the TSST, with substantially lower responses in women for aldosterone (SMD = −2.33, 95%CI [−3.89, −0.71]),



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cortisol (SMD = −4.35, 95%CI [−6.69, −1.95]), cortisone (SMD = −2.77, 95%CI [−4.47, −0.99]), and overall glucocorticoids (SMD = −3.91, 95%CI [−6.07, −1.69]). In contrast, the corresponding differences during the MAST were smaller and not statistically significant (aldosterone: SMD = −1.07, 95%CI [−2.27, 0.19]; cortisol: SMD = −0.72, 95%CI [−1.87, 0.47]; cortisone: SMD = −1.02, 95%CI [−2.21, 0.23]; overall glucocorticoids: SMD = −0.90, 95%CI [−2.08, 0.32]).

#### 2.1.2. Autonomic Response

Figure 3 presents the autonomic response during the TSST and MAST for male and female participants. The results of the time-kinetic analysis are available in the supplements.

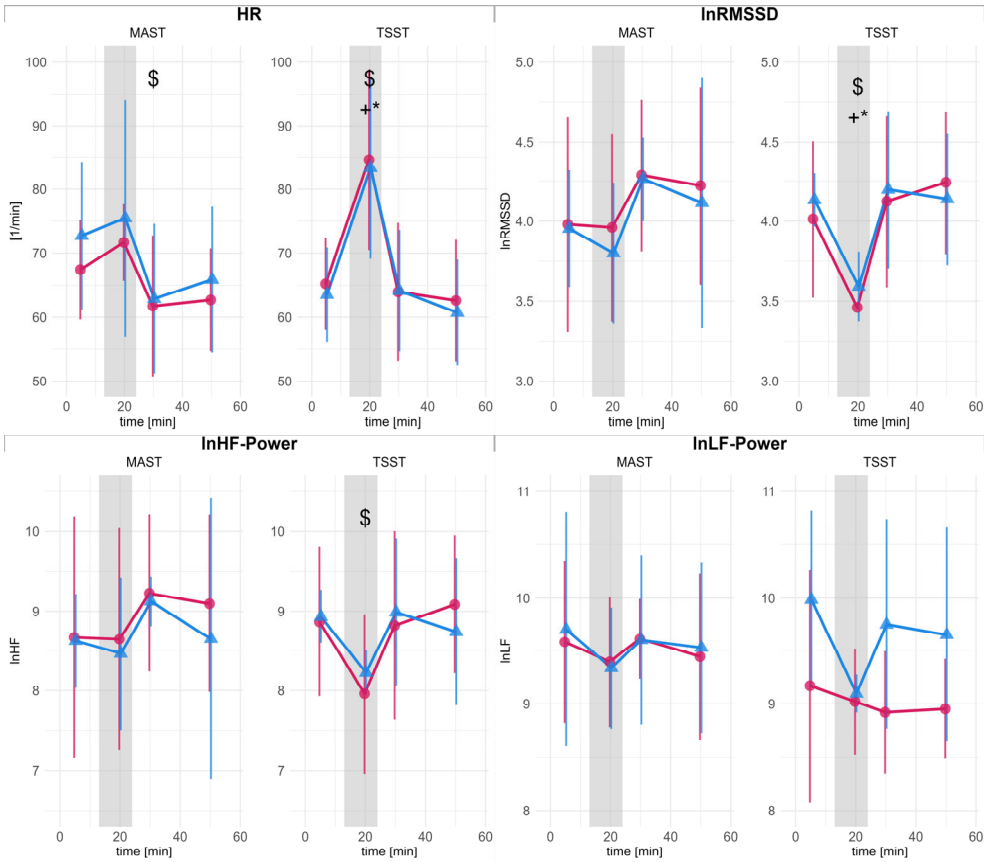


**Figure 3.** Heart rate (HR) and heart rate variability (HRV) metrics for male (blue triangles) and female (red dots) participants. Error bars represent 95% confidence intervals (CI). The abscissa indicates time in minutes, with the gray area indicating the stress-induction procedure. HR: mean heart rate; RMSSD: root mean square of successive differences (ln-scaled); LF: low frequency power (ln-scaled), HF: high frequency power (ln-scaled). Statistically significant differences in post hoc comparisons are denoted with symbols: $ significant difference to baseline; * significant difference to baseline in men; + significant difference to baseline in women.

The quality of the raw heart period data was rated as good. Excluded datapoints were less than 5%, and most outliers occurred outside the periods of analysis.

The mixed-ANOVA on the differences between baseline and during stress test indicated a significant main effect of test condition for HR ($F(1, 10) = 16.07$, $p = 0.002$), with lower HR during MAST compared to TSST (SMD = −1.47 [−2.51, −0.43]). Similarly, a significant test condition effect was found for RMSSD ($F(1, 10) = 5.57$, $p = 0.040$). The TSST led to a stronger reduction in RMSSD (SMD = 0.98 [0.01, 1.95]).



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2.1.3. Proteomics

The untargeted approach identified 1416 different proteins, of which 422 were eligible for further analysis. The mixed ANOVA (time × sex) and estimated marginal means contrasts revealed 38 statistically significant effects for TSST, all corresponding to the main effect of sex. During the MAST condition, 28 significant effects were identified, of which 21 corresponded to sex differences, three to time, and four to time effects within female sex only. Nevertheless, the already liberal limit of 20% false discovery rate (FDR) identified none of those significant effects as interesting (i.e., $p \leq$ critical value). All significant changes are listed in the supplements.

Comparison of the maximal fold change between male and female participants for each stress test (see Figure 4) revealed that during MAST, three proteins showed a stronger change for males compared to females (*Nicotinamide phosphoribosyltransferase*, *Thioredoxin domain-containing protein 17* and *Rho GDP-dissociation inhibitor 1*) and two showed a more pronounced change for females (*Mucin-7* and *Calumenin*), which were all statistically significant. During TSST, three proteins showed stronger responses in men (*Actin-related protein 2/3 complex subunit 5*, *Neutrophil cytosol factor 4*, and *Lysosome-associated membrane glycoprotein 2*), whereas no significantly higher responses were found for females. There was no overlap in the sex dependent effect on the protein between MAST and TSST. This is evident in Figure 4, which shows proteins with significant sex differences during the MAST as black triangles in the depiction of the TSST and vice versa. None of the effects passed the accepted, predefined FDR threshold.

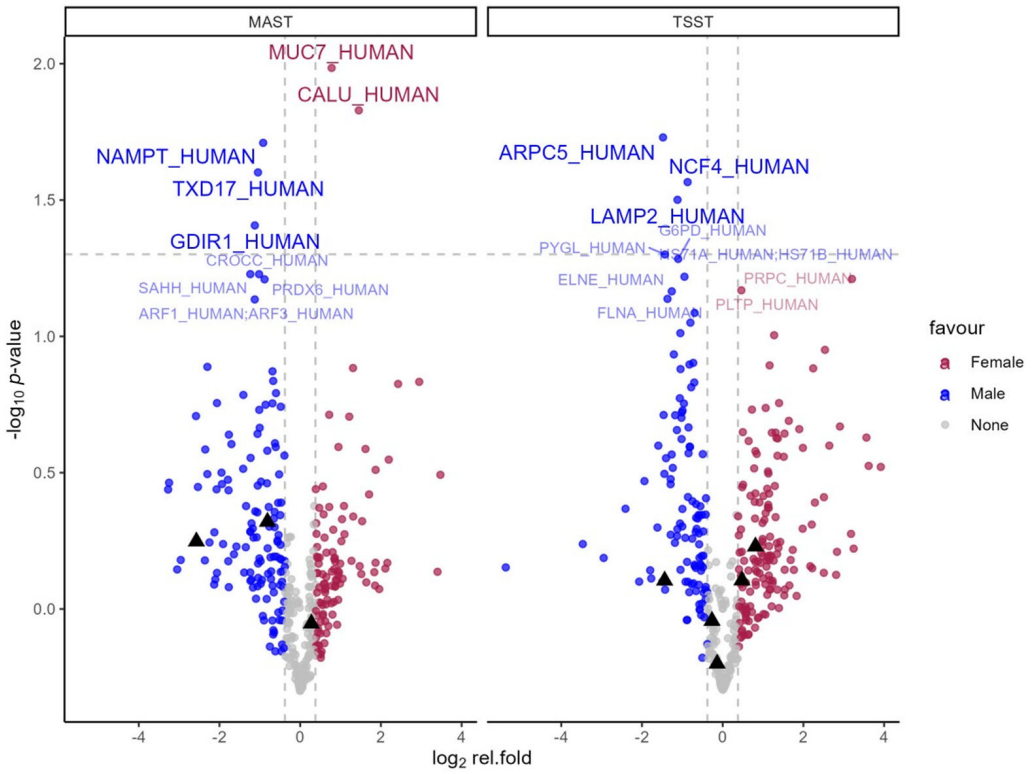


**Figure 4.** Volcano plots of relative fold change comparing men and women during each stress test. Blue dots represent >30% higher fold change in men. Red dots represent >30% higher fold change in females. The gray horizontal line represents 5% alpha level. Significant effects ($p < 0.05$) over 30% are labeled with UniProtKB entry names for brevity. For additional information, proteins that showed a significant difference in one stress test condition are highlighted with a black triangle in the other condition.



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### *2.2. Self-Reported Measures*

#### 2.2.1. STAI-10

The subjective anxiety levels were 30.7 ± 8.1 points at baseline and 31.3 ± 9.2 during the follow-up. The (condition × sex) ANOVA on the STAI-10 change score revealed no statistically significant main or interaction effects. Following this, no changes in STAI-10 were found in either condition.

#### 2.2.2. VAS100

Descriptively, women displayed an increase in reported stress from baseline to directly after the stressor of ΔVAS100 = 33.7 mm (95%CI [−0.1, 67.5]) during MAST and ΔVAS100 = 21.8 mm (95%CI [−6.3, 49.9]) during TSST. The change in men was estimated as ΔVAS100 = 7.0 mm (95%CI [−26.8, 40.8]) and ΔVAS100 = 7.8 mm (95%CI [−20.3, 35.9]), respectively (Figure 2). The ANOVA (time × sex) for the separate analysis of each stress condition revealed a statistically significant main effect of time for the MAST ($F(1, 10) = 4.79$, $p_{adj} = 0.032$) and the TSST ($F(1, 10) = 6.65$, $p_{adj} = 0.005$), while the main effect for sex and interaction did not reach statistical significance ($p_{adj} > 0.05$). Estimated means establish no change from baseline to directly after the stressor (MAST: ΔVAS = 20.3 mm, 95%CI [−3.6, 44.2]; TSST: ΔVAS = 14.8 mm, 95%CI [−5.06, 34.7]). Nevertheless, a significant decrease in VAS100 from the maximal displacement following the stressor was found for all later time points. VAS100 continuously dropped by −28.3 mm (95%CI [−41.6, −14.9]) during MAST and −25.6 mm (95%CI [−37.1, −14.1]) during the TSST till the last follow-up 25 min after the stress cessation. However, the ANOVA model for the maximal change scores (condition × sex) could not identify any statistically significant main effects or interactions, which was confirmed by hypothesis-derived contrasts (all $p > 0.05$).

## 3. Discussion

This study examined the acute stress response to two established laboratory stress-induction protocols—the TSST and the MAST—in a mixed-sex sample of self-identified athletes. Both protocols elicited measurable stress responses, while systematic differences were observed only in autonomic regulation. However, distinct patterns for each sex emerged in several hormonal parameters, particularly during the TSST.

### *3.1. Comparison of TSST and MAST*

We hypothesized that regular exercisers and athletes would show a reduced response to the MAST, which incorporates a strong physical component. Contrary to this hypothesis, we found no significant differences between the MAST and the TSST in hormonal response or subjective stress. While the absence of statistical differences should not be interpreted as evidence of equivalence, the largely similar endocrine responses suggest that both protocols are appropriate tools to study the stress response in athletes. Notably, descriptive data indicate greater inter-individual variability in hormonal responses during the MAST (see Figures 1 and 2). Even under standardized conditions, individual responsiveness to psychological stress [47] as well as exercise stressors [48] shows substantial variability. In athletes, this variability may be further amplified by differences in training status [49] or altered pain tolerance [50]. In contrast to the hormonal findings, autonomic responses differed between the two stress tests. HR was higher and RMSSD lower during the TSST, indicating stronger vagal withdrawal. The cold stimulus during MAST typically provokes vasoconstriction in the exposed limb, accompanied by sympathetic activation. The baroreflex then counteracts excessive elevation of blood pressure and ANS activity [45]. Our data suggest that regular exercisers may exhibit altered autonomic responses to cold



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stress, potentially mediated by an increased baroreflex sensitivity. However, this hypothesis is not supported by earlier studies (e.g., [51]).

Research on the cold pressor test highlights considerable individual variability in autonomic reactivity, with some individuals classified as hyperreactors [52]. Such heightened responses are observed in both trained and untrained individuals [53], but the difference in prevalence appears to be unclear. Although we did not formally assess pain perception, participants' informal feedback indicated varying levels of physical discomfort. Since pain tolerance is believed to be linked to sympathetic nervous system activity [54,55], this variability likely contributed to the broader spread in endocrine and autonomic responses. Furthermore, while cold tolerance is typically not closely linked to fitness, the specifics of training environments (i.e., regular training in the cold) may partially account for individual differences [56].

Taken together, these findings suggest that although overall HPA activation was similar between the stressor paradigms, the autonomic response was more pronounced during the TSST. This pattern is only partly consistent with the assumption of stressor-specific adaptation in physically active individuals. The stronger autonomic activation during the psychosocially loaded TSST suggests that cross-stressor adaptation may be limited or more complex than previously assumed. During exercise, the cardiac autonomic response is a direct function of exercise intensity. Depending on the metric, the response can be described as linear or even exponential [21]. On the other hand, the activation of the HPA axis depends on surpassing a threshold of intensity or volume. Systemic glucocorticoid release is only observed during more strenuous exercise bouts lasting approximately 90 min or more [20,22]. Furthermore, the endocrine response to exercise appears to be influenced by motivational and situational factors, as evident in the comparison of endocrine levels after competition and training [44]. This may suggest that exercisers are more frequently exposed to adaptive regulatory functions of the ANS, while the HPA axis and gonadal regulations are less pronounced and therefore less trained. We further want to highlight that the relationship between stress, as a broader construct, and the different measured metrics is complex. This is evident by the limited evidence for a correlation between subjective stress and cortisol [57,58]. Furthermore, original work on the cross-stressor adaptation hypothesis focused on the presumed lower reactivity of HPA or ANS-related metrics in physically fitter individuals [25]. Later work, however, also investigated a faster recovery from stress [30,59,60]. However, while reactivity and recovery of the stress response relate to distinct physiological processes [61], their role in health-related outcomes is currently not fully understood [62].

### *3.2. Sex Specific Responses*

Our findings are consistent with reported sex differences [36,63], as women exhibited significantly lower glucocorticoid responses following stress exposure. This partially supports our hypothesis that increased enzymatic conversion of cortisol to cortisone via 11β-HSD2 [33,46] may blunt active cortisol levels in women. However, we observed no significant sex difference in the cortisone-to-cortisol ratio, and 11β-HSD2 could not be reliably quantified in our proteomic data. The follow-up time of 25 min after stress cessation may have been too short to reveal differences, as the cortisone-to-cortisol ratio showed slight deviations in the female sample during the final measurement time points (see Figure 1).

Sex differences were also apparent in aldosterone responses, which paralleled those seen in glucocorticoids. Aldosterone plays an essential role in short-term cardiovascular regulation through sodium retention and vasoconstriction and is a known modulator of sympathetic tone and baroreflex sensitivity [64]. Given its responsiveness to acute stress



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and its pathophysiological implications [65], aldosterone may warrant further investigation in sex-specific stress and exercise research.

Finally, a direct comparison of the two stress test protocols revealed that sex differences were significant only during the TSST, but not the MAST. While exploratory, this finding indicates that the physiological stress responses to the MAST may be less sex-dependent in physically active individuals. However, the VAS100 indicated a different picture. The elevation of self-reported stress was more pronounced in women, specifically during TSST. But confirmatory studies are needed to evaluate this trend.

The untargeted proteomics approach revealed no changes that passed the FDR threshold. Upon further inspection, some proteins exhibited significant sex differences during both MAST and TSST, suggesting that overall sex differences may be more pronounced than the response to either stress test. A direct comparison of the maximal fold change between men and women revealed different patterns during the MAST and TSST. No protein that surpassed the threshold of 30% difference in fold change in one test condition reached a similar effect in the other test condition. This would indicate that MAST and TSST lead to different sex specific responses.

### *3.3. Summary*

The present study implemented a broad-spectrum, multimodal approach to compare psychophysiological responses across two conceptually distinct laboratory stress protocols. A major strength of the study is the use of LC-MS for hormone quantification, the inclusion of both sexes, and a comprehensive outcome panel, providing a multidimensional view of the acute stress response.

However, several limitations must be acknowledged. Most notably, the small sample size ($n$ = 12; with missing saliva samples for one woman and one man) limits the statistical power and the generalizability of the findings. While the observed effect sizes are broadly consistent with recent meta-analyses [36,63], replication in larger cohorts is warranted. Attempts were made to minimize the potential influence of the menstrual cycle and contraceptives based on self-reporting. However, more rigorous methods should be implemented in future studies [66]. Psychological confounders were taken into account by implementing a screening questionnaire for depression, anxiety, and stress pre-enrollment. However, other confounders, like physical fitness, related health status [67], and psychological traits [47], should also be controlled for in future studies. Caution is also necessary when comparing absolute hormone concentrations to those in other studies. Although our values align with physiologically plausible ranges, differences in sampling methods and assay techniques may affect comparability [68–70].

We found higher HR and lower RMSSD during the TSST compared to the MAST. However, this difference may be partly attributable to posture and physical activity during the respective protocols. During the MAST, participants are seated, but stand during the TSST. The resulting reduction in RMSSD may therefore reflect orthostatic and mathematical effects rather than physiological differences per se. A feasible methodological solution for future studies may involve mathematical correction of RR intervals, as described by Sacha [71].

## 4. Materials and Methods

To examine the acute stress response in a mixed-sex sample of regular exercisers, we employed a randomized, counterbalanced crossover design. Each participant completed two experimental sessions, undergoing both the TSST and MAST. The study was approved by the ethics committee of the University of Vienna (REC ID: 00880, 2023).



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### *4.1. Participants*

Twelve young, healthy, non-smoking individuals (6 females, 6 males; age = 20–40 years) who self-identified as athletes and well-trained were recruited. All participated in a regular, structured training regime to improve athletic performance and competed in their primary sports discipline. Female participants were excluded if they used hormonal contraceptives, were pregnant or lactating, or reported menstrual cycles shorter than 21 days or longer than 35 days. All participants were naïve to the study's specific aims and were informed that the research examined hormonal responses to stress and physical demands. They arrived prepared for physical exercise but were not given further procedural details to minimize expectancy effects.

### *4.2. Procedure*

Experimental sessions were scheduled in the afternoon (between 2:00 p.m. and 3:30 p.m.) to control for diurnal variation in hormone levels [72]. Participants were required to be well-rested and abstain from food or drink (except water) for at least one hour, from caffeine for 18 h, and from intense physical training, nicotine, and alcohol for 24 h prior to testing. The female menstrual cycle status was shown to modulate the effect of psychological stressors. To minimize variation, female participants were tested 20 to 22 days after the first day of their last menstruation. This phase typically elicits the most pronounced cortisol response [73]. Each experimental session followed a standardized timeline (see Figure 5). During the sessions, participants were not permitted to use their mobile phones or wear watches. All sessions took place during fall 2024 at a laboratory at the University of Vienna (Austria), which was kept at 22 °C throughout all sessions [71].

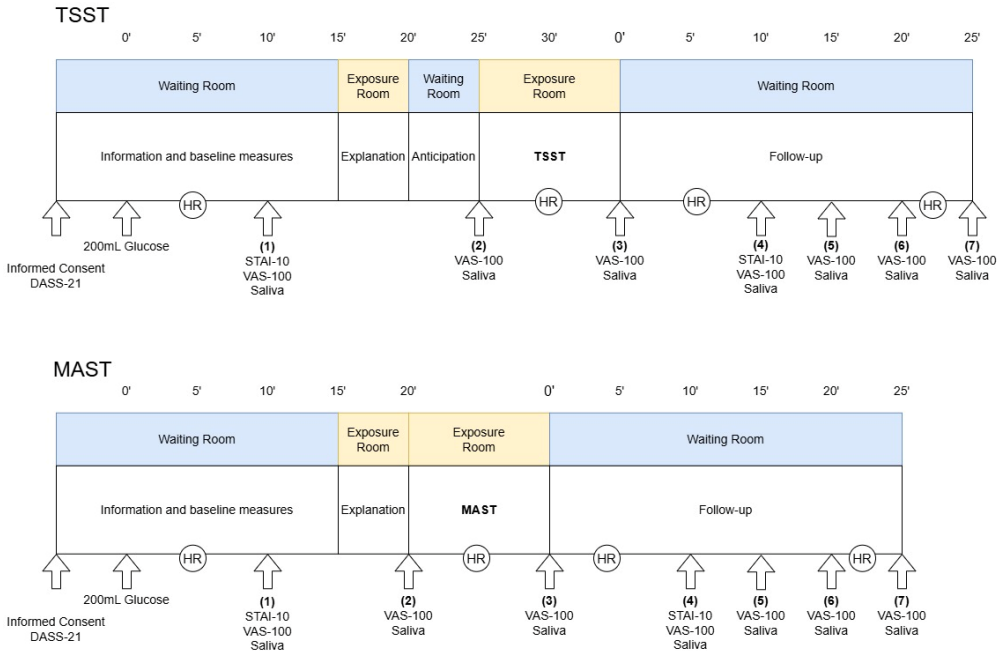


**Figure 5.** Study design. The **upper** panel illustrates the experimental procedure for the Trier Social Stress Test (TSST), while the **lower** panel shows the equivalent for the Maastricht Acute Stress Test (MAST). All measurements were timed based on the administration of glucose and the end of the stress exposure. HR: heart rate measurement—participants were advised to sit quietly for robust measurement; VAS-100: Visual analog scale for subjective stress; DASS-21: Depression, anxiety and stress scale with 21 items; STAI-10: State anxiety inventory with 10 items.

On the first experimental day, participants provided written informed consent and completed the Depression, Anxiety and Stress Scale (DASS-21). Individuals scoring above



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the established cutoffs for depression (>10), anxiety (>6), or stress (>10) were excluded to control for the influence of clinically relevant psychological symptoms [74]. Since the stress response is closely linked to energy availability and metabolism, it was recommended to control for variations in blood glucose levels to enhance the reliability of the stress-induction [75,76]. Therefore, participants consumed 30 g of dextrose dissolved in 200 mL of water and rinsed their mouths with an additional 100 mL of water at the start of each session. This was followed by a 15 min seated resting period. Participants then underwent either the TSST or the MAST, as described in detail below. Throughout the session, they wore a heart rate monitor (Polar H10, Polar Electro Oy, Kempele, Finland) to continuously gather heart rate (HR) and HRV. Subjective ratings of stress and saliva samples were collected seven times throughout the experiment. The second experimental day followed the same structure, including the remaining stress-induction procedure.

#### 4.2.1. Trier Social Stress Test

The TSST was conducted according to the guidelines by Labuschagne et al. [75]. Participants were escorted to the exposure room and instructed to stand on a marked spot 1.5 m in front of a desk. Two experimenters dressed in white lab coats, pants, and dark shoes were seated behind the desk, facing the participant. The assigned sex of the experimenters was shown to influence cortisol secretion [77]. Therefore, the two experimenters represented two different genders (male and female). The experimenter, representing the opposite gender to the participant, was designated as the main interviewer and would initiate and conduct the session. A video camera and microphone were positioned to enhance the perceived evaluative context.

The lead investigator read out the instructions, informing the participants that they would have five minutes in the waiting room to prepare for a mock job interview of high personal relevance. During the task, they would be required to speak about their qualifications and personality traits in front of the two evaluators, who were introduced as experts in behavioral psychology and body language. Following the ten-minute speech (which would be interrupted after five minutes), they would complete a mental arithmetic task, the details of which would be described later.

After these instructions, participants were returned to the waiting room. A saliva sample and VAS100 were collected. This time point will further be described as the anticipatory phase, as participants were now aware of the upcoming challenge. According to theoretical underpinnings regarding the predictive function of allostatic regulation [71,72], anticipatory stress was found to be associated with peak cortisol secretion [78]. The anticipatory phase lasted 5 min. However, the length of the anticipatory phase does not appear to affect the overall cortisol response [77]. Subsequently, participants re-entered the test room, and the lead investigator exited. After five minutes, the arithmetic task was introduced, requiring participants to repeatedly subtract 13 from the starting number 1009 as quickly and accurately as possible. The interviewer would ask the participant to start again if there was a mistake. Also, the interviewer would instruct them to increase speed, look into the camera, or speak louder if needed. Both interviewers were trained to give as little feedback as possible and keep a neutral expression. Upon completion of the task, the lead investigator returned and guided the participant to the follow-up phase in the waiting room.

#### 4.2.2. Maastricht Acute Stress Test

The MAST was conducted using the original material provided by Smeets et al. [38]. Participants were brought into the test room by the lead investigator and seated in front of a computer, with a camera and microphone facing them. To keep the condition similar to



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the TSST, the evaluator representing the opposite gender, and dressed identically to the TSST interviewers, administered the test.

Instructions were read aloud from the computer screen. Following this, a saliva sample and VAS100 were collected to assess anticipatory stress. The lead investigator then exited the room, and the evaluator initiated the test.

During the task, participants were repeatedly instructed to immerse their dominant hand and wrist in a container of ice water (1–4 °C) for unspecified periods, described as lasting 45 to 90 s (the actual durations were 90 s, 60 s, 60 s, 90 s, and 60 s, respectively). Between the cold exposure, they performed a mental arithmetic task, subtracting 13 from a four-digit starting number (2043, 1584, 1229, and 1431) as rapidly and accurately as possible. The evaluator asked them to start again if there was a mistake, but did not provide any other information or feedback. Upon completion of the task, the lead investigator re-entered the room and escorted the participant to the waiting room for the follow-up period.

### *4.3. Measures*

A total of six hormones were quantified at six different timepoints, and two additional ratios—cortisone-to-cortisol and testosterone-to-cortisol—and one sum-score (total glucocorticoids) were calculated. Heart periods were continuously recorded and analyzed across four specific timeframes. Four metrics of cardiac regulation were determined. Two subjective metrics were collected at six and two timepoints, respectively. Additional untargeted proteomics analyses were performed at six time points. All mentioned metrics were recorded on both experimental days under the conditions TSST and MAST for each participant. A detailed description of each metric follows.

#### 4.3.1. Psychobiological Measures

Stimulated salivary samples (Salivettes, Sarstedt, Germany) were administered at seven time points: (1) at baseline, (2) anticipation (after explanation of the upcoming test), (3) immediately after the stress test as well as (4) 5 min, (5) 10 min, (6) 15 min, and (7) 25 min post-stress (Figure 5). The synthetic swab was kept in the mouth for two minutes at each time point. The samples were stored at 5 °C until the end of the session, centrifuged (2000 RCF, 4 °C) for two minutes, and frozen at −20 °C for later analysis.

The frozen saliva samples were sent to an external laboratory (BIOLYZ Inc., Tulln, Austria) for analysis. Hormones were extracted from samples using solid-phase extraction (SPE) and analyzed by targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analysis was performed on the ZentoTOF 7600 coupled to a Shimadzu Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan). Data were acquired in multiple reaction monitoring (MRM) mode using optimized transitions for each analyte. All analyses were quantified using heavy-isotope labeled internal standards to ensure accuracy, reproducibility, and compensation for matrix effects. Data processing and quality control were performed using Analyst software (v1.7, SCIEX, Framingham, MA, USA).

Proteins were processed using the single-pot, solid-phase-enhanced sample preparation (SP3) protocol. Samples were reduced, alkylated, and proteins were captured on carboxylate-coated paramagnetic beads in the presence of organic solvent, followed by bead-based cleanup and on-bead digestion with sequencing-grade trypsin and Lys-C (Promega). Resulting peptides were desalted and analyzed by targeted liquid chromatography-mass spectrometry (LC-MS) using a ZenoTOF 7600 (SCIEX, Framingham, MA, USA) and an ACQUITY M-CLASS LC (Waters, Milford, MA, USA). Data were acquired in data-independent acquisition (DIA) mode using the Zeno-SWATH method. DIA data were processed using DIA-NN (v1.8) with a UniProt reference proteome database (Homo sapiens UP000005640) and common contaminants, applying a 1% lab-level false discovery rate at the peptide and



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protein levels. Quantitative data were normalized using DIA-NN's built-in normalization and interference correction.

Heart period data (RR intervals) were recorded continuously using a chest strap ECG device (Polar H10, sampling rate 1000 Hz). Raw RR intervals were extracted and analyzed in R using the RHRV package (v5.0.0; [79]). Time- and frequency-domain metrics were derived from 3 min periods at each analysis time point. During the stress exposure phase of each session, three consecutive 3 min segments were extracted. Since no time-related differences were observed within this period, values were averaged across the three windows.

Artifact correction was performed using an adaptive threshold filter [80], based on a 50 bpm running mean and a physiological RR range of 20 to 220 bpm. The filtered RR time series was used to calculate mean heart rate (HR) and the root mean square of successive differences (RMSSD). Frequency domain analysis was performed via Fast Fourier Transformation on the interpolated (spline, 4 Hz) data. All 3 min analysis windows were detrended and tapered (10%), without additional smoothing. Absolute power was computed for the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.5 Hz) bands. All HRV metrics were natural log-transformed to improve interpretability.

#### 4.3.2. Self-Reported Measures

The German version of the DASS-21 was administered at the first test day to verify participant inclusion and inform sample characteristics. The DASS-21 is a widely used questionnaire consisting of 21 items on a 4-point Likert scale. All items are loaded positively and ask for experienced symptoms corresponding to the three distinct subscales (depression, anxiety, and stress) within the last week (example item: "I felt that my mouth was dry"). Response options range from 0 = "did not apply at all" to 3 = "does apply most of the time or very strongly". Validity and reliability were tested for multiple cultures and languages, including German, and are considered good [81]. We estimated internal consistency for our sample using the R package *psych* (v2.5.6) and report Cronbach's alpha with bootstrapped ($n$ = 1000 iterations) 95%CI. In our sample, internal consistency was found to be acceptable ($\alpha$ = 0.66 [0.34, 0.77], anxiety: $\alpha$ = 0.65 [0.36, 0.95], stress: $\alpha$ = 0.52 [0.15, 0.73]).

To ensure comparability with the existing body of literature, we measured self-reported stress experience by implementing two of the most frequently used assessments in laboratory stress settings [57]: the State Anxiety Inventory (STAI-10; [82]) and a visual analog scale. The STAI-10 has been shown to capture experiential discomfort during stress protocols without interfering with the physiological stress response [77]. We administered the German version of the STAI-10 at baseline and during the early recovery phase (time point 4). The questionnaire consists of ten items measured on an 8-point Likert scale. Participants rated how well the statements apply to them at the moment of administration. Response options range from 1 = "not at all" to 8 = "completely". An example item is: "I feel relaxed". Four items are inversely loaded and were reversed before being summed. In our study, the STAI-10 shows acceptable to good internal consistency ($\alpha$ = 0.84 [0.57, 0.92]).

A horizontal 100 mm visual analog scale (VAS100) was used simultaneously with saliva samples (timepoints 1–7). Verbal anchors but no tick marks were offered at each end of the scale (0 = "not stressed", 100 = "highly stressed"). The values were manually measured and digitized at the end of each experiment. Compared to the STAI-10, the VAS100 offers faster administration and was implemented for higher temporal resolution. It has been successfully used in similar settings [83–85], despite limited evidence for its psychometric validity.



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### *4.4. Statistical Analysis*

Endocrine, subjective, and HRV metrics were analyzed over the time course of each experimental session. Estimates are reported with standardized mean differences and 95% confidence intervals (CIs), calculated with the non-centrality parametric method [86]. Standardized effect sizes were adjusted for small samples (Hedges' g) and for repeated measures (Cohen's $d_{rm}$), as appropriate [87]. For variables bounded at zero (e.g., hormones), 95%CIs were truncated accordingly [88]. Parametric inference tests were accompanied by visual inspection of distribution properties (qq-plots), which were deemed unproblematic except for HRV metrics. The latter were natural-log transformed before the analyses. Sphericity was assumed to be violated by default because of the small sample. Greenhouse-Geisser correction was used as an appropriate solution.

To better understand the time kinetics of all outcomes, separate mixed ANOVA models (sex × time) were fitted for each stress-induction protocol. The STAI-10 operates on a scale from 10 to 80, and we opted not to treat it as interval-scaled. Therefore, a robust ANOVA model with trimmed means (20% trim level) was used, incorporating the R package WRS2 (v1.1.7). The analysis followed the same logic as for the previously mentioned outcomes.

To test the presented hypotheses, the overall hormonal change during each experiment was summarized as the area under the curve with respect to increase ($AUC_i$) [89]. For HR, HRV metrics, STAI-10, and VAS100, the maximal change scores relative to the baseline were calculated. A mixed ANOVA (condition × sex) was performed on each outcome of interest, followed by pairwise comparison tests based on estimated marginal means.

In the proteomics analysis, proteins not identified in more than 20% of the samples were excluded. The remaining missing values were imputed using 50% of the lowest detected value for each protein. For each stress test, mixed ANOVAs (timepoint × sex) followed by contrasts against baseline were applied. To correct for type II errors, a threshold false discovery rate (FDR) of 20% was accepted as *statistically interesting* [90] using the Benjamini–Hochberg procedure. Between-sex comparisons of maximum fold changes were conducted using additional ANOVAs (sex × condition). Volcano plots visualize $log_2$-transformed fold ratios between men and women. A minimally relevant effect was defined as a 30% difference in fold change.

All inferential tests were performed against an alpha level of $\alpha = 5\%$. As a pilot study, focusing on broad-spectrum analysis including proteomics, the sample size was justified by available resources. The statistical power for induced stress and sex differences was estimated using G*Power (v3.1.9.7) and ranged from 50% to 90% for medium (f = 0.25) to large (f = 0.4) effect sizes (r = 0.7).

## 5. Conclusions

This study is the first to directly compare the TSST and MAST in a mixed-sex sample of self-identified athletes. While most outcome measures did not differ significantly between the stress protocols, the autonomic response did. An untargeted proteomic analysis also revealed indications of potential mechanistic differences. We further observed pronounced sex differences in glucocorticoid and aldosterone responses, particularly during the TSST, underscoring the importance of including both sexes in stress and exercise research. Our data also indicate greater between-subject variability in response to the MAST. Future research should investigate how regular exercise influences stress responses across sexes and stressor types. To advance our understanding of cross-stressor adaptation, studies should not only consider the characteristics of the exercise stimulus but also systematically compare the specific demands of different stress-inducing protocols, including the typical response to exercise training.



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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Vienna (Reference number: 00880, approval date: 4 May 2023).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The original data presented in the study are openly available in OSF at https://doi.org/10.17605/OSF.IO/F9ENX.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| ANS | Autonomic nervous system |
| AUCi | Area under the curve with respect to increase |
| Cn:C | Cortisone-to-cortisol-ratio |
| DASS-21 | Depression Anxiety Stress Scale (21-itmes) |
| FDR | False discovery rate |
| HF | High Frequency HRV |
| HPA | Hypothalamic–pituitary–adrenal axis |
| HR | Heart rate |
| HRV | Heart rate variability |
| LF | Low-frequency HRV |
| MAST | Maastricht Acute Stress Test |
| RMSSD | Root mean square of successive differences |
| STAI-10 | State Anxiety Inventory (10-items) |
| T:C | Testosterone-to-cortisol-ratio |
| TSST | Trier Social Stress Test |
| VAS100 | Visual analogue scale (100 mm) |

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## Study 2: Does aerobic capacity protect against stress? Insights from two stress-induction paradigms

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- Methodology, P.R., A.D., B.W., U.N., R.C.
- Validation, P.R.
- Formal analysis, P.R.
- Investigation, P.R., A.V.
- Resources, R.C. & B.W.
- Data curation, P.R., A.V.
- Writing—original draft preparation, P.R.
- Writing—review and editing, P.R., A.V., A.D., B.W., R.C.
- Visualization, P.R.
- Supervision, R.C.
- Project administration, P.R. and R.C

# Does aerobic capacity protect against stress? Insights from two stress-induction paradigms

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All data and analysis code are made available on OSF:

https://osf.io/ex2t4/overview?view_only=6a67433550424110b82d24dc0613b5d9

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The authors declare no conflicts of interest.

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**Participants Consent Statement**

All participants provided informed consent in accordance with the terms of the ethics application.

**Keywords:** stress, cortisol, HRV, cardiac capacity

## Abstract

Introduction: Prior evidence suggests that individuals with higher fitness exhibit attenuated endocrine and autonomic responses to acute stress. However, it remains unclear whether this stress-buffering effect depends on the type of stressor. This study examined whether aerobic capacity modulates psychophysiological responses to two stress induction paradigms (Trier Social Stress Tests, TSST; Maastricht Acute Stress Test, MAST). We hypothesized that fitter participants show lower stress reactivity, with stronger attenuation expected for the MAST, which includes a physical stress component.

Methods: Healthy participants (n = 59; 28 women; age: 27.1 ± 5.5 yrs) completed the TSST and MAST on separate days under standardized conditions. We assessed self-reported stress, salivary hormones (cortisol, cortisone, DHEAS), and autonomic cardiac regulation (heart rate, RMSSD). Aerobic fitness was determined via graded exercise testing ($VO_2$peak: 50.4 ± 8.6 ml/kg/min). We used multilevel regression models to examine the associations between VO2peak and stress responses.

Results: Higher $VO_2$peak was significantly associated with lower heart rate across both stress induction paradigms (b = 0.99 bpm, p = 0.002). For other outcomes, no significant association with $VO_2$peak was found. The associations did not differ between TSST and MAST.

Discussion: Aerobic fitness was linked to lower heart rate during the experimental sessions, indicating a modest cardiovascular advantage under acute stress. Contrary to our hypothesis, this effect did not differ between the two stress paradigms, suggesting that the influence of fitness was not specific to the type of stressor. Aerobic capacity, therefore, seems to reduce cardiovascular load during stressful situations in a general manner.

# 1. Introduction

The stress response is a crucial physiological function for maintaining and re-establishing dynamic homeostatic control [1]. The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) are systemic regulators, and their effectors are frequently used as measures of the stress response [1].

While activation of the HPA axis and the ANS is necessary for optimal function and survival, chronic or inadequate stress is detrimental to health [2–4]. Given its psycho-physiological nature, the impact of stress on health can be addressed on multiple levels. One potential avenue is to strengthen the systems responsible for coordinating the stress response. Acute physical activity is known to stimulate both the HPA axis and the ANS, and repeated training exposure challenges these systems [5] in ways that may promote long-term adaptations. Physical fitness can therefore be viewed as an accumulated marker of such training-induced adjustments. It has been suggested that these adaptations could enhance the capacity to cope with acute stressors, potentially conferring a stress-buffering effect [6]. However, the nature and extent of this proposed effect are still not well understood.

One challenge in studying the potential protective effects of physical fitness is the difficulty of inducing acute stress in a standardized, reproducible manner. One of the most frequently applied laboratory stress induction protocols in humans is the Trier Social Stress Test (TSST) [7]. It consists of a mock interview and a mental arithmetic task performed under social pressure, providing a valid and reliable laboratory stressor [7,8]. Through its standardization, the TSST allows the interaction of physical fitness and stress to be studied under consistent test conditions, marking a major improvement over earlier studies [9,10]. However, the TSST involves only psychosocial stress and does not include any physical stress component. It is,

nevertheless, conceivable that physical fitness may be especially relevant for coping with physically demanding stressors.

To test the potential specificity of the stress-buffering effect of physical fitness, we therefore implemented a crossover design, comparing the stress response to the TSST with that to the Maastricht Acute Stress Test (MAST) [11]. In contrast to the TSST, the MAST combines psychosocial stress with the physical challenge of cold exposure, allowing us to test whether physical fitness provides greater protection against stressors that include a physical component. Aerobic fitness was assessed using a graded exercise test (GXT), and its association with a broad spectrum of stress metrics – including endocrine, cardio-regulatory, and subjective measures – was analyzed.

We hypothesized that higher aerobic capacity ($VO_2$peak) would be associated with attenuated psychophysiological stress responses, reflected in (a) reduced glucocorticoid release, (b) lower heart rate (HR) and higher heart rate variability (HRV) indicating more favorable autonomic regulation, and (c) lower subjective stress ratings. Given the differing sensory and psychosocial demands of the two stress paradigms, we further hypothesized that the magnitude of this fitness-related stress-buffering would differ between the MAST and TSST, with a stronger protective influence of fitness expected for the MAST.

Additionally, we conducted two exploratory analyses. First, as sex differences in stress and exercise research remain insufficiently understood [9,12], we examined whether the associations between physical fitness and stress responses differed between males and females. Second, we considered ventilatory thresholds as alternative indicators of aerobic fitness, as they provide physiologically distinct, motivation-independent markers of cardiorespiratory fitness.

# 2. Materials and Methods

## 2.1. Study Design

The study was approved by the Ethics Committee of the University of Vienna (Reference Number: 00960). Data processing and all hypotheses were preregistered on OSF (https://osf.io/txsc5). Data collection took place between May 2024 and June 2025 at the Department of Sport and Human Movement Science, University of Vienna. The study was designed as a crossover experiment with a balanced sample of male and female subjects. Each participant completed both a TSST and MAST on two separate days, spaced one month apart. The order of the tests was randomly allocated and counterbalanced, stratified by sex. To reduce participant enrollment time while minimizing interference between test days, the assessment of aerobic capacity via GXT was scheduled two weeks after the first experimental day.

We used the *mlmpower* package (v. 1.0.10) to perform a Monte Carlo simulation of the presented multilevel model (see data processing and statistical analysis). We estimated medium to small main effects of condition and fitness ($R^2$ = 0.06 – 0.12) and high variance explained by cluster structure (ICC = 0.2 – 0.3). A cross-level interaction was expected, with a small effect size (0.02). Typical variance for z-transformed $VO_2$peak was based on internal data sets (σ = 8 ml/kg/min)**.** We used 2000 simulations under an accepted 5 % type 1 error rate. A sample of n = 60 participants was estimated to achieve a power of 90%, 65%, and 30% for the fixed effects of $VO_2$peak, condition, and their interaction, respectively.

The study was announced on the institute's website, and potential volunteers were contacted directly. Eligibility was screened via phone or email, after which participants were invited to the laboratory to provide informed consent. No financial compensation was offered. However, participants received their individual CPET results, which were discussed with an experienced

sport scientist. We did not inform participants about the specifics of the experiments. The aim of the study was described as understanding the hormonal response to “challenges” (instead of stress). Participants were therefore partially blinded to the study's aim and structure. It was not possible to blind the experimenters.

### 2.2. Participants

We aimed to recruit a balanced sample of male and female participants aged 18 to 40 years. Exclusion criteria were acute injuries or health concerns, use of medications or performance-enhancing drugs regulated by the *World Anti-Doping Agency*, regular intake of ashwagandha or rhodiola rosea within the last month [13], diagnosed chronic diseases, and conditions affecting hormonal balance, including the use of hormonal contraception, pregnancy, lactation, or irregular menstrual cycles. Participants were also required to maintain a regular sleep schedule for the time of the experiment, avoid traveling across multiple time zones in the weeks of testing, and refrain from intense exercise, supplement intake, or alcohol and other drugs for at least 24 hours before each experimental day. In addition, they were asked to abstain from caffeine and nicotine for 18 hours, and from eating, drinking caloric beverages, chewing gum, or brushing their teeth for one hour prior to testing.

### 2.3. Study Protocol

An overview of the experimental design is shown in Figure 1. Appointments were kept consistent within each participant and scheduled between 2 pm and 4 pm to reduce bias related to the circadian variability of hormonal markers [14]. It has previously been demonstrated that the stress response is influenced by the menstrual cycle [15]. Therefore, female participants attended the stress tests on day 21 (±1) of their cycle.

Written informed consent was obtained after participants received detailed information at the beginning of the first visit. They were then equipped with a chest strap heart rate monitor and

seated in a quiet waiting room. To minimize personal interactions, the investigator leading the experiment left the room between measurements. The waiting room contained only neutral reading material, a pen, and paper. To control for individual variations in blood glucose, which are directly linked to cortisol regulation, participants consumed 30 g of dextrose dissolved in 200 ml of water [16] and subsequently rinsed their mouth with 100 ml of water to prevent contamination of saliva samples. Fifteen minutes after ingesting the glucose drink, participants were guided into the test room and given instructions for the upcoming stress test. After the stress test, they were returned to the waiting room for follow-up measurements of the stress response.

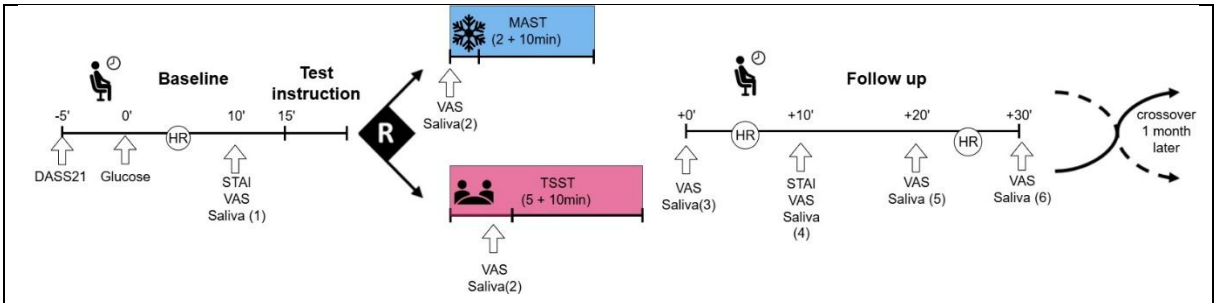


*Figure 1. Study design. Two experimental days were separated by a one-month washout period. The order of the experiments was counterbalanced within each sex. After a baseline assessment, participants completed either the Trier Social Stress Test (TSST) or the Maastricht Acute Stress Test (MAST), followed by a 30-minute recovery period. Vertical arrows indicate measurement time points for State Anxiety Inventory (STAI), the visual analog scale for perceived stress (VAS), and saliva sampling. Heart rate (HR) was recorded continuously throughout the experiment, with explicit time windows for standardized analyses.*

## Stress Induction Protocols

**TSST:** The TSST consists of a five-minute mock job interview, followed by a five-minute mental arithmetic task performed in front of a committee. The present study followed the guidelines provided by Labuschagne et al. [16]. The committee comprised one male and one female

interviewer, both dressed formally and instructed to maintain neutral facial expressions and body language to increase uncertainty. Interviews with female participants were conducted by a male interviewer, and those with male participants by a female interviewer. After receiving task instructions, participants were returned to the waiting room for a five-minute preparation phase. A saliva sample was collected at the end of this phase to capture anticipatory changes in stress (see *Physiological measurements*).

**MAST:** The MAST combines elements of the TSST with repeated cold exposure of the dominant hand as a physical stressor. The MAST was performed under the same conditions as the TSST, following the protocol described by Smeets et al. [11]. Briefly, each test was administered by a single tester — male for female participants and female for male participants. Participants received task instructions while seated at a laptop computer. They were then repeatedly asked to immerse their dominant hand in ice water. The cold exposure lasted between 60 and 90 seconds and alternated with arithmetic tasks similar to those in the TSST.

## 2.4. Measures

### Physiological Measures

Saliva samples were collected at six time points: at baseline, during stress anticipation, and after each stress test protocol (see *Figure 1*). Synthetic swabs (Salivettes, Sarstedt, Germany) were placed in the mouth for two minutes and stored at 5°C until the end of the experiment. Subsequently, they were centrifuged at 2000 RCF (4°C) and frozen at -20°C until analysis. Analyses were performed by an external laboratory (*BIOLYZ* Inc., Tulln, Austria) using targeted liquid chromatography-mass spectroscopy (LC-MS) with a ZenoTOF 7600 mass spectrometer (SCIEX, USA) and an ACQUITY M-CLASS LC system (Waters, USA). Concentrations of free cortisol, cortisone, and DHEAS were quantified against heavy isotope-labeled internal standards.

Heart periods were continuously recorded at 1000 Hz using a chest-strap ECG sensor (Polar H10, Finland) on each experimental day. Three resting time frames were analyzed: after glucose administration, and after collection of the third and fifth saliva samples (see Figure 1). During these periods, participants were instructed to sit still, rest their backs against the backrest of a chair, and breathe normally. Raw data were filtered for artifacts and ectopic beats using an adaptive threshold filter with a 50-beat sliding window and thresholds between 1333 to 300 ms, corresponding to heart rates of 45 to 200 bpm. HRV metrics were calculated in five-minute segments across the entire experiment using one-minute moving windows. Mean HR was chosen as an overall metric of cardiac autonomic load. The root mean square of successive differences (RMSSD) served as the primary outcome for vagally-mediated cardiac control [17].

### Self-reported Measures

Participants completed the *Depression Anxiety Stress Scale* (DASS-21) at the beginning of each experimental day. The DASS-21 assesses symptoms experienced during the preceding week across three subscales – depression, anxiety, and stress – using a 4-point response format (0 = "did not apply to me at all" to 3 = "applied to me very much or most of the time"). Scale scores were computed according to the official scoring guidelines, with higher scores indicating greater symptom severity.

Participants further completed the 10-item *State Anxiety Inventory* (STAI10) [18] before and after each stress test (see Figure 1). This questionnaire is widely used to assess transient anxiety in laboratory stress paradigms. Previous meta-analytic evidence indicates that administering the STAI questionnaire before or after stress induction does not affect the stress response [14]. The German version [18] applied in this study employs an 8-point Likert scale (1 = "not at all" to 8 = "completely") with four items reverse-scored.

A 100-mm visual analog scale (VAS100) was administered simultaneously with saliva sampling. The horizontal scale, which was labeled at the extremes with “relaxed” (minimum) and “stressed” (maximum) in German but did not contain intermediate ticks, was digitized after the session.

The long version of the International Physical Activity Questionnaire (IPAQ) [19] was administered on the day of the exercise test. It assesses physical activity during the preceding seven days, and metabolic equivalents (METs) for moderate and vigorous activity were calculated according to the official scoring guidelines.

### Fitness

A GXT was conducted on a separate occasion using a stationary cycle ergometer (Ergoline GmbH, Ergoselect 200, Germany) in a climatized laboratory. Each stage lasted 2 minutes, with individualized increments in load determined from the expected maximal work rate [20]. Participants were instructed to arrive well-rested and refrain from intense physical activity for 24 hours preceding the test.

Respiratory parameters were measured using a metabolic gas analysis system (Cortex, METALYZER 3B, Germany). Heart rate was continuously recorded using a chest strap ECG (Polar H10, Polar, Finland).

Maximal oxygen uptake ($VO_2$peak) during the final phase of the test was defined as the primary parameter of aerobic capacity. In addition, oxygen uptake at the first and second ventilatory thresholds (VT1 and VT2) served as motivation-independent measures reflecting distinct metabolic transitions and associated autonomic responses. To control for differences in body dimensions, $VO_2$peak and oxygen uptake at VT1 and VT2 were adjusted for body mass.

## 2.5. Data Processing and Statistical Analyses

Overall hormonal responses during each experiment were quantified by calculating the area under the curve with respect to baseline ($AUC_i$) [21]. Similarly, overall reported subjective stress was quantified as the area under the curve with respect to zero ($AUC_g$), which is more appropriate given its fixed lower limit.

For hormonal and VAS100 data, single missing time points were linearly interpolated; datasets with more than one missing value were excluded. Both hormonal and HRV metrics were natural log-transformed to approximate normale distributions. Except for RMSSD, the presented estimators were transformed to improve interpretability. Data points exceeding three times the interquartile range were classified as extreme outliers.

Statistical analyses were performed in R (v.4.5.1). The significance level was set at $p < 0.05$. Hypothesis testing employed multilevel linear models with random intercepts, specifying a repeated-measures structure for the stress test condition (level 1 variable: MAST vs. TSST) nested within participants (level 2 variables: sex and fitness). Models were fitted using the *lme4* package (v.1.1.37). Metric predictors for fitness were grand-mean centered. Model comparisons were based on changes in Akaike Information Criterion (AIC) and likelihood ratio ($\chi^2$) tests under maximum likelihood estimation. Parameter estimates were obtained using restricted maximum likelihood, and effects were considered statistically significant when the bootstrapped 95% confidence intervals (95% CIs) did not include zero.

The time kinetics of the various stress markers were examined in a similar framework, with time points treated as the lowest level. Estimated marginal means were used for contrast testing following significant fixed effects. For multiple pairwise comparisons, *p*-values were Bonferroni-adjusted and reported alongside 95% CIs.

# 3. Results

## 3.1. Sample Characteristics

Of the 63 participants initially deemed eligible, four discontinued participation due to acute illness (n = 2), pregnancy (n = 1), or excessive discomfort during the protocol (n = 1). The final sample comprised 31 men and 28 women, whose $VO_2$peak values were 55.1 ± 8.1 ml/kg/min and 44.2 ± 5.7 ml/kg/min, respectively. Overall, 58% of participants self-identified as competitive athletes — defined as those who participated in at least one official competition within the current year — and 37% trained or competed in a team setting. Sample characteristics are presented in Table 1.

*Table 1 Participant characteristics*

| | Female (n = 28) | Male (n = 31) | Total (n = 59) |
|---|---|---|---|
| Age (yrs) | 26.3 ± 5.7 | 27.7 ± 5.3 | 27.1 ± 5.5 |
| Body mass (kg) | 61.8 ± 6.9 | 78.6 ± 7.8 | 70.65 ± 11.2 |
| Height (cm) | 166.9 ± 5.50 | 180.6 ± 5.9 | 174.1 ± 8.9 |
| BMI (kg/m²) | 22.1 ± 1.9 | 24.1 ± 2.1 | 23.2 ± 2.2 |
| $Vo_2$peak (ml/kg/min) | 44.2 ± 5.7 | 55.1 ± 8.1 | 50.4 ± 8.6 |
| Team athletes (n) | 10 (36%) | 12 (39%) | 22 (37%) |
| Competitive athletes (n) | 13 (46%) | 21 (68%) | 34 (58%) |
| DASS21 Depression | 1 [0, 4] | 1 [0, 4] | 1 [0, 4] |
| DASS21 Anxiety | 1 [0, 8] | 1 [0, 10] | 1 [0, 10] |
| DASS21 Stress | 4 [0, 15] | 1 [0, 7] | 2 [0, 15] |

*Note: Values are presented as mean ± standard deviation, median [minimum, maximum], or number (%).*

## 3.2. Data Quality and Measurement Reliability

Twelve of the 720 saliva samples contained insufficient volume for analysis. Missing data points were interpolated in six cases, leaving six missing data points coming from three different cases. The quality of the heart rate period data was acceptable, with a mean rate of outlier intervals of 0.4 ± 0.9 %. Three datasets with >5% outlier heart periods due to a defective heart rate monitor were excluded. One CPET test had to be repeated because the participant was insufficiently accustomed to the cycle ergometer during the initial trial, which led to implausible results.

To evaluate the sensitivity of the salivary hormone measures, signal-to-noise ratios (SNRs) were calculated separately for each analyte's concentration-time curve. The SNR was defined as the difference between the mean maximal and baseline concentrations divided by the standard error of the mean, providing an index of how strongly the observed hormonal responses exceeded assay variability. The highest signal-to-noise ratio was observed for cortisol (SNR = 25.2), followed by VAS100 (SNR = 22.2), total glucocorticoids (SNR = 21.7), and cortisone (SNR = 19.3). Lower SNRs were found for the cortisone-to-cortisol ratio (cn2c; SNR = 11.2), and DHEAs (SNR = 5.2).

Internal consistency of the psychometric self-report measures was evaluated using Cronbach's α. For the DASS-21, internal consistency was good for the depression (α = 0.80, 95% CI [0.62, 0.86]) and stress subscales (α = 0.87, 95% CI [0.77, 0.92]), but low for anxiety (α = 0.39, 95% CI [0.10, 0.56]). Internal consistency of the STAI10 was acceptable (α = 0.76, 95% CI [0.65, 0.84]).

Two independent raters (PR and AV) determined the ventilatory thresholds. If the difference in relative oxygen uptake exceeded 5 ml/kg, raters discussed the case to reach a consensus. This was necessary in six cases. After this procedure intraclass correlation coefficients of ICC(2, 3) = 0.988 (95% CI[0.98, 0.993]) and ICC(2, 3) = 0.97 (95% CI[0.927, 0.985]) with standard errors

of measurement of SEM = 0.26 ml/kg/min (95% CI [0.19, 0.41]) and SEM = 0.16 ml/kg/min (95% CI[0.13, 0.21]) were reached for VT1 and VT2, respectively. The mean of the two ratings was used as a predictor for the following analyses.

### 3.3. Verification of Stress Induction

Before testing the hypothesized influence of aerobic fitness and stressor type on stress response, we first verified that both stress paradigms elicited robust endocrine, autonomic, and subjective responses. Because of multicollinearity, the anticipation phase (time point 2) was excluded from all glucocorticoid models. The final models for time kinetics included *cross-level interactions* and revealed statistically significant main effects of *time* and *time* × *condition* interaction for all key stress markers – cortisol, cortisone, total glucocorticoids, DHEAs, VAS100, STAI10, HR, and lnRMSSD – indicating effective stress induction across both tasks.

Except for the cortisone-to-cortisol ratio (Cn:C) and HR, the factor *sex* did not improve model fit for any outcomes. In the case of HR, the main effect of *sex* reached statistical significance, indicating overall higher HR in women than in men (ΔHR = 6.6 bpm, 95% CI [0.5, 12.8]). Figure 2 shows statistically significant effects between baseline and all other timepoints, as well as between the stress test conditions or sexes at each timepoint. Full parameter estimates for all time-course models are presented in the supplementary materials.

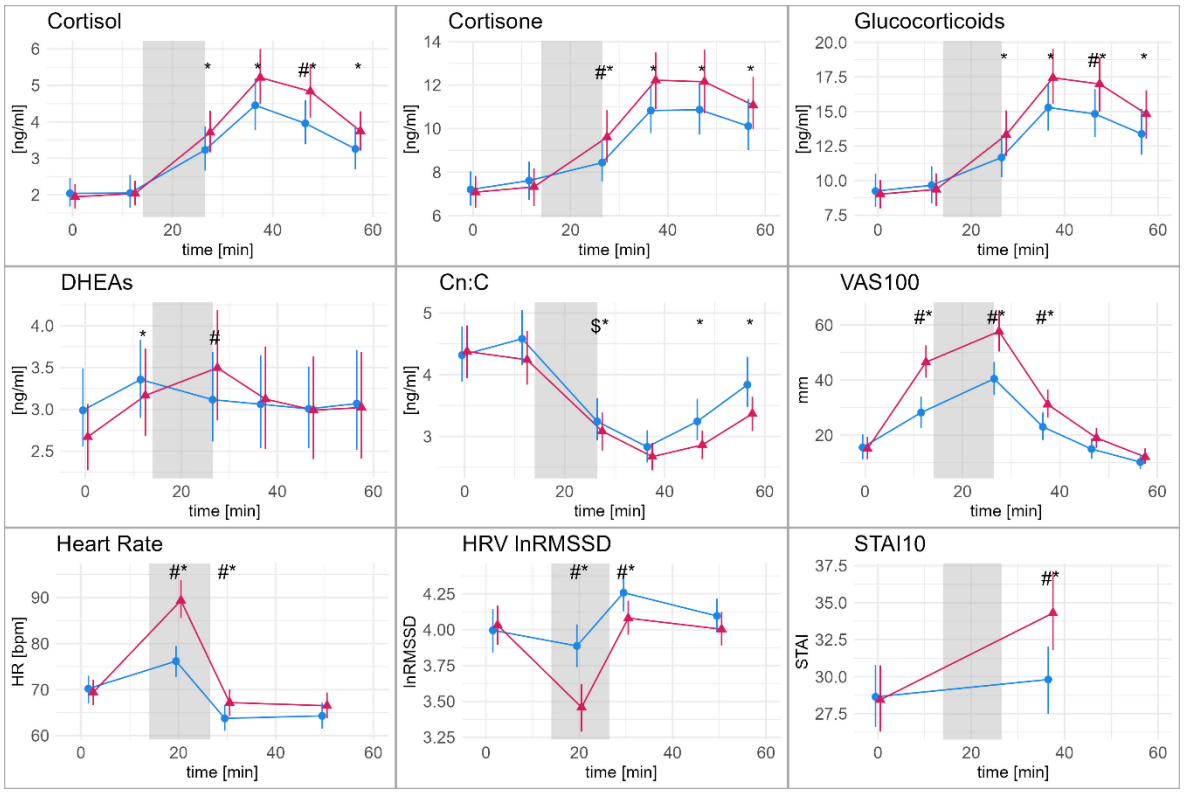


*Figure 2: Time course of salivary hormones, heart rate, HRV, and subjective stress responses. Error bars indicate 95% confidence intervals (CIs). The Maastricht Acute Stress Test (MAST) and the Trier Social Stress Test (TSST) are presented separately in blue triangles and red dots, respectively. *: Statistically significant differences compared to baseline; #: Statistically significant differences between MAST and TSST; $: differences between men and women; Cn:C: cortisone-to-cortisol ratio; VAS100: 100 mm horizontal visual analogue scale for subjective stress; lnRMSSD: natural log-transformed root mean square of successive differences; The x-axes (abscissa) indicate the time in minutes, with the grey area corresponding to the stress induction phase.*

## 3.4. Influence of Aerobic Fitness and Stressor Type

Mean cortisol response ($AUC_i$) was 76.1 ± 83.7 ng/ml·min. In the intercept-only model, 23.5 % of the variance in cortisol $AUC_i$ was attributable to person-level variability. The model fit improved with the addition of the fixed-effect predictor condition (ΔAIC = -3.16, $\chi^2(1) = 5.2$, p = 0.023). No further improvements were observed when aerobic fitness indicators (*$VO_2peak$*: ΔAIC = 3.5, $\chi^2(2) = 0.501$, p = 0.778; *VT1*: ΔAIC = 3.8, $\chi^2(2) = 0.2$, p = 0.905; *VT2*: ΔAIC = 2.81,

$\chi^2(2) = 1.188$, $p = 0.552$) were included. The final model revealed a variance attribution of ICC = 0.27 and a model fit of AIC = -251.7. Predicted geometric mean cortisol $AUC_i$ were 57.8 ng/ml·min (95% CI [25.1, 91.7]) and 87.8 ng/ml·min (95% CI [54.3, 122.0]) for MAST and TSST, respectively.

For subjective stress ratings (VAS100 $AUC_g$), the intercept-only model showed moderate between-person variability (ICC = 0.275). Adding *condition* improved the model fit (ΔAIC = -19.25, $\chi^2(1) = 21.253$, $p < 0.001$), with higher perceived stress during the TSST (predicted mean difference of $AUC_g$: 278.2 mm·min; 95% CI [278.2, 734.9]). Aerobic fitness did not significantly account for additional variance (*$VO_2$peak*: ΔAIC = 0.94, $\chi^2(2) = 3.065$, $p = 0.216$; *VT1*: ΔAIC = 0.79, $\chi^2(2) = 3.208$, $p = 0.201$; *VT2*: ΔAIC = 1.78, $\chi^2(2) = 2.222$, $p = 0.329$). In the final model (AIC = 219.6), 43.1 % of the variation was explained by the clustering structure of the data.

In the HR model, between-subject variability was relatively largest (ICC = 0.357). Including *condition* improved model fit (ΔAIC = -1060.33, $Chi^2(1) = 1062.328$, $p < 0.001$), and further improvements occurred with *$VO_2$peak* and the *$VO_2$peak* × *condition* interaction (ΔAIC = -8.19, $\chi^2(2) = 12.189$, $p = 0.002$). The mediating role of sex did not further improve the model (ΔAIC = 2.94, $\chi^2(4) = 5.061$, $p = 0.281$). In the final model (AIC = -75, $R^2 = 0.71$, ICC = 0.588), the predicted geometric mean HR at the 25$^{th}$ percentile of VO2peak (42.6 ml/kg/min) was 78.7 bpm (95% CI [74.6, 83.1] during the MAST and 91.8 bpm (95% CI [87.0, 96.9]) during the TSST. At the 75$^{th}$ percentile (55.1 ml/kg/min), the corresponding values were 71.6 bpm (95% CI [68.2, 75.1]) and 84.1 bpm (95% CI [80.1, 88.3]), respectively. This corresponded reduced HR of 0.99 bpm (95% CI [0.98, 1.00], $p = 0.002$) for each ml/kg/min change in VO2peak.

To evaluate whether ventilatory thresholds provided a better fitness proxy for HR during stress, we replaced $VO_2$peak with VT1 or VT2 in otherwise identical models. These substitutions did not improve explanatory power: marginal $R^2$ values were 0.718 for the VT1 model and 0.710

for the VT2 model, compared with 0.710 for the $VO_2$peak model. Accordingly, the model fits were not significantly different (VT1: ΔAIC = 5.65, $\chi^2(0) = 0$, $p > 0.999$, VT2: ΔAIC = 8.85, $\chi^2(0) = 0$, $p > 0.999$).

When HR reactivity was modeled as the change from baseline to the stress phase, only *condition* improved the fit (ΔAIC = -57.96, $Chi^2(1) = 59.962$, $p < 0.001$), whereas aerobic fitness did not ($VO_2$peak: ΔAIC = 3.58, $Chi^2(2) = 0.419$, $p = 0.811$).

Similarly, the fit for lnRMSSD (ICC = 0.294) was improved by the inclusion of *condition* (ΔAIC = -22.22, $\chi^2(1) = 24.2$, $p < 0.001$), whereas *$VO_2$peak* did not add further explanatory power (ΔAIC = 3.93, $\chi^2(2) = 0.1$, $p = 0.967$).

Figure 3 illustrates the observed data for cortisol, VAS100, absolute HR, and lnRMSSD, together with the predicted model including interaction between stressor condition and $VO_2$peak.

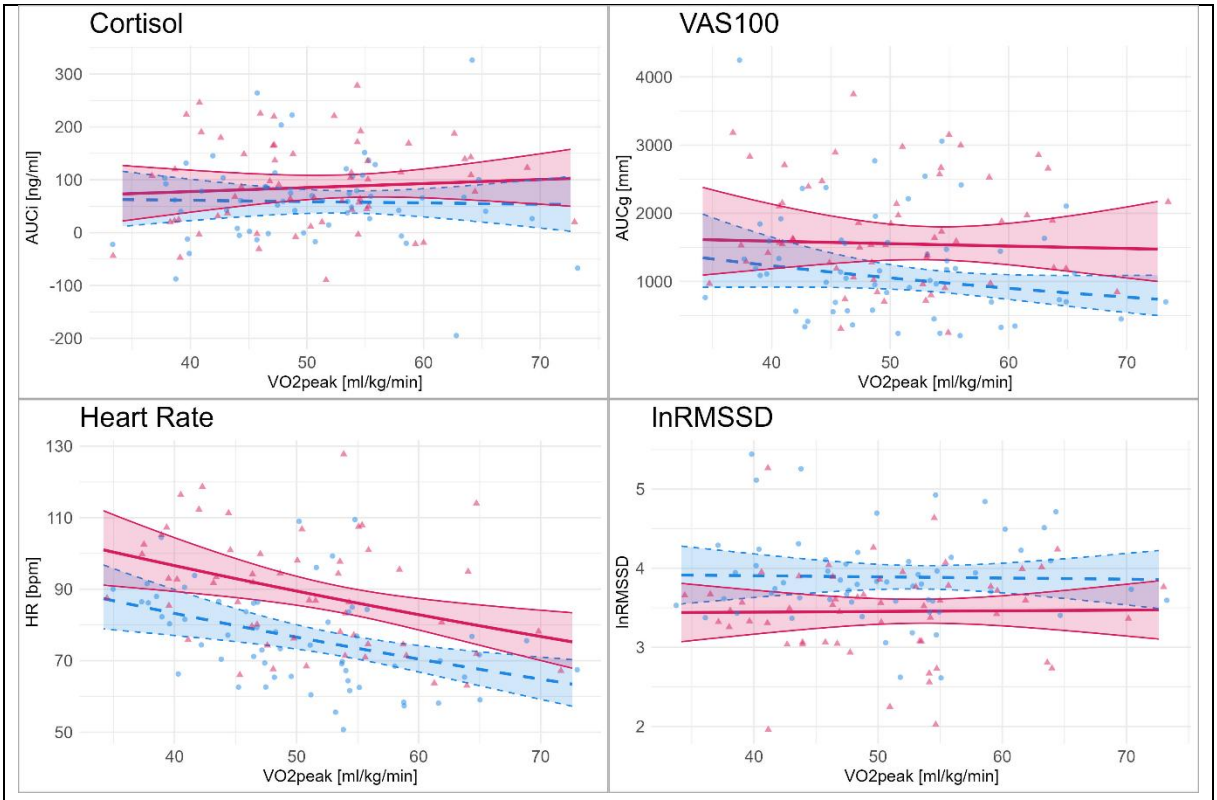


*Figure 3: Predicted area under the curve for cortisol, visual analogue scale (VAS100), heart rate (HR) during stressor condition, and natural log-transformed root mean square of successive difference (lnRMSSD) during stressor condition, by maximal oxygen uptake relative to body mass ($VO_2$peak). Error bars represent 95%*

*confidence intervals (CI) of the fixed effect estimator. The blue dashed lines represent the models for the Maastricht Acute Stress Test (MAST), while the blue circles indicate the sample data. Conversely, the models for the Tier Social Stress Test (TSST) are depicted as red solid lines, with the sample data shown as red triangles.*

# 4. Discussion

## 4.1. Overall Stress Response

This study examined the physiological and subjective stress responses to two established laboratory-based stress induction protocols, the TSST and MAST, and evaluated the modulating effect of aerobic fitness in a mixed-sex sample. Across the glucocorticoids, autonomic-cardiac, and subjective domains, both tests elicited pronounced stress reactions, confirming the validity of the paradigms. The TSST induced stronger activation of the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system, reflected by higher glucocorticoid and DHEAs concentrations, elevated heart rate, reduced vagal cardiac modulation, and greater subjective stress compared with the MAST.

Differences in task posture and engagement likely contributed to these effects. The TSST requires participants to stand while they actively engage in public speaking. This may introduce mild orthostatic and motor demands, which can amplify autonomic-associated cardiac activity [22]. The MAST, in contrast, is performed while seated and involves cold exposure, which may provoke localized vasoconstriction and transient autonomic adjustments. However, evidence is inconclusive as to whether partial body cold exposure has a measurable effect on HRV [23]. Taken together, these results reaffirm the TSST as a strong, reliable inducer of psychophysiological stress, while the MAST represents a less socially loaded, more physically tinged stressor.

It is noteworthy that our finding of divergent responses to the two stress paradigms updates results reported by Smeets et. al [11], who compared the TSST and MAST in a sample of 20 male undergraduate students in a crossover design and found no differences in salivary cortisol, alpha-amylase (often used as a marker of sympathetic activity [24]), or subjective stress. Several methodological and analytical factors likely explain this divergence. First, constructs and instruments differed: Smeets et al. used the *Positive and Negative Affect Schedule* (PANAS) at two time points, whereas we assessed state anxiety (STAI10) and perceived stress (VAS100). The PANAS [25] measures affect on two orthogonal dimensions. In contrast, the STAI10 captures anxiety, which is neurophysiologically and phenomenologically highly related to stress [26]. The VAS100 was used to report the level of overall stress. Compared to affective measures, stress and anxiety may be regarded as higher-order constructs that integrate affect with other components like arousal [27]. Second, the higher temporal resolution of our sampling (six vs. two time points) enabled time-course modeling and AUC-based summaries, increasing sensitivity to between-task differences.

Further, the autonomic indices comprised different physiological mechanisms. Smeets et al. measured blood pressure as an indicator of sympathetic activation [11]. In contrast, we assessed HR as a measure of overall cardiac autonomic load and RMSSD, which is interpreted as a measure of cardiac vagal modulation [17]. The stronger effects of the TSST in our study may therefore reflect the different autonomic pathways probed. Finally, sample size and composition may have played a role. Our sample was larger (n = 59 vs. n = 20) and included both sexes. The greater statistical power and biological heterogeneity may have revealed effects that were undetectable in the smaller, all-male cohort of Smeets et al. [11].

The inclusion of male and female participants represents a key strength of the present study. Research on physical activity and stress has traditionally relied predominantly on male samples

[6,10], despite accumulating evidence that biological sex and sex hormones modulate HPA-axis and autonomic regulation [9,12]. Meta-analytic evidence indicates that men typically show higher cortisol responses than women [8,28]. However, during the luteal phase – marked by elevated levels of estrogen and progesterone – women's cortisol responses to the TSST have been shown to approximate those of men [28]. In our exploratory analysis, sex-related differences were generally small. Across both stress induction protocols, the only significant difference in endocrine responses emerged for Cn:C shortly after the stressor.

Besides the direct effects of estrogen on the HPA axis, it also increases corticoid-binding globulin, which binds free, bioactive cortisol, measured in saliva [29]. The faster time kinetics, combined with the lower overall cortisol response in men (even if not significant in our study), may have led to a brief drop in Cn:C, before excess cortisol would be enzymatically converted to cortisone. This conversion could be further delayed by the higher saliva flow in men [30], reducing the contact time with 11beta-HSD, the main enzyme in this process. Regarding autonomic regulation, women exhibited higher mean heart rates than men, independent of time point or stressor. This finding aligns with well-established sex differences in cardiac structure and function, including smaller heart size and stroke volume in women [31]. No further sex-related differences were observed for endocrine, autonomic, or subjective outcomes, supporting the view that, under controlled hormonal conditions, acute stress responses are broadly comparable between sexes.

### 4.2. Stress Mitigation through Aerobic Fitness

With a mean $VO_2$peak of 50.4 ± 8.6 ml/kg/min, our participants demonstrated above-average aerobic fitness. Across all measured domains, stress responses to both tasks were largely independent of aerobic capacity. Cortisol, RMSSD, and subjective stress (VAS100) did not vary as a function of fitness. The only consistent association emerged for absolute HR during stress

induction, which was inversely related to $VO_2$peak. This pattern aligns with established cardiovascular adaptations to endurance training – such as increased cardiac dimensions and stroke volume [32] – that enable trained individuals to maintain lower heart rates at a given submaximal load. HR reactivity – the change of HR from baseline to during the stressor – did not vary significantly with fitness. Thus, while fitter participants exhibited a comparable relative stress response, their absolute cardiac demand was lower.

The expected interaction between fitness and stressor type – a stronger stress-buffering effect during the MAST – did not reach statistical significance, although descriptive trends in HR and VAS100 were in the hypothesized direction (Figure 3). It is conceivable that the physiological specificity of training adaptations limits their transferability to psychosocial stressors such as the TSST, whereas stressors with a physical component, like the MAST, may share greater overlap with the stimuli encountered during exercise training. Overall, however, the small and non-significant effects suggest that, under acute laboratory conditions, aerobic fitness may exert only a modest influence on HPA-axis or autonomic activation.

In an exploratory extension, we examined ventilatory thresholds (VT1 and VT2) as alternative fitness indices. These did not improve model fit for any stress parameter. Nevertheless, the use of submaximal thresholds may be advantageous in future work because they can be determined without requiring maximal effort and are, therefore, more readily available also in less-trained or clinical populations.

In summary, aerobic capacity was only associated with lower absolute HR during stress exposure, but did not attenuate endocrine, autonomic, or subjective stress responses. These findings suggest that under controlled laboratory conditions, the stress-buffering effect of fitness is modest. Endurance training elicits the strongest adaptive effects on cardiovascular function. Our data suggests that this system-specific training effect also shows the most

promising protective effect against psychosocial demands. Under this assumption, future studies may investigate the effect of other physical capacities and physiological adaptations to exercise training on psychological stress.

### 4.3. Strengths and Limitations

This study was the first to examine the relationship between fitness and stress responses across two different stressor paradigms based on a within-subject design. The high internal validity of our results is the result of collecting and analyzing multiple markers of stress and the application of state-of-the-art methods for hormone quantification and cardiac autonomic analysis. Both stress induction protocols were standardized and implemented according to best-practice guidelines, and physical fitness was determined by an incremental GXT, the current gold standard.

Furthermore, both biological sexes were included, and hormonal influences were partially controlled by scheduling women during the luteal phase and excluding those using hormonal contraception. However, the menstrual phase was self-reported only. To better account for intraindividual hormone variation, future studies should incorporate repeated ovulation testing [33,34] and statistically control for menstrual phase and contraceptive use – an approach that enhances ecological validity but requires larger samples.

To reduce the complexity of the statistical models and control for variation in time-kinetics, we used the area und the curve with respect to the baseline ($AUC_i$) for glucocorticoids. Alternative approaches – such as the AUC with respect to zero ($AUC_g$) or change scores – capture slightly different constructs. While $AUC_i$ is more related to changes from baseline and the sensitivity of the system, $AUC_g$ represents total hormone levels [21].

Baseline cortisol appears unrelated to exercise volume [35], although regular endurance training is related to higher chronic cortisol exposure [36], complicating the interpretation of absolute

concentrations. For these reasons, we decided to report $AUC_i$ but acknowledge the value of $AUC_g$ for estimating total allostatic load and mitigating baseline measurement error [37].

Finally, the follow-up period after stress cessation was limited to 30 minutes, and full hormonal recovery was not reached within this timeframe. Extending the recovery window, as recommended for the TSST [16], would allow a more precise characterization of post-stress regulation. It remains plausible that higher aerobic fitness accelerates HPA axis feedback and recovery kinetics even in the absence of lower peak responses [12].

## 5. Perspective

The positive effect of exercise on stress-related outcomes surpasses the acute response investigated in this study. The potentially negative health consequences of chronic stress include tissue catabolism, for example, in the hippocampus and other brain areas with a high density of glucocorticoid receptors [4], and skeletal muscle [38]. Also, stress modulates inflammatory processes and reduces cardiac vagal regulation, contributing to cardiovascular health [3]. Indirectly, these effects can be exacerbated by behavioral influences of stress, such as changes in diet and increased food intake [39]. Physical activity and exercise, on the other hand, have repeatedly been shown to positively influence all these outcomes via pathways that are unrelated to stress [40,41].

Taken together, this study demonstrated a robust stress response to the MAST and TSST spanning multiple interconnected psychophysiological systems. We did not find evidence for stress buffering effects of aerobic fitness on measures of HPA axis activity, cardiac vagal control, and subjective stress. Nevertheless, as with physical demands, people with higher aerobic capacity require a lower heart rate to adequately respond to the same stressor. This sparing

effect on the cardiovascular system under stress may be a key protective factor against everyday life stress.

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# Appendices

All supplementary files are available on OSF:

https://osf.io/ex2t4/overview?view_only=6a67433550424110b82d24dc0613b5d9

## Study 3: No Evidence for the Efficacy of Slow-Paced Breathing as a Recovery Strategy After Sprint Interval Training

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**Author's contribution:**

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- Validation, P.R.
- Formal analysis, P.R.
- Investigation, P.R., S.L
- Resources, R.C., B.W.
- Data curation, P.R.
- Writing—original draft preparation, P.R.
- Writing—review and editing, P.R., B.W., S.L., R.C.
- Visualization, P.R.
- Supervision, R.C.
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Human Kinetics
ORIGINAL INVESTIGATION

# No Evidence for the Efficacy of Slow-Paced Breathing as a Recovery Strategy After Sprint Interval Training

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***Purpose:*** Breathing practices are increasingly adopted by athletes due to the potential benefits for recovery, performance, and well-being. Slow-paced breathing is believed to increase cardiac vagal activity and relaxation, potentially supporting adaptive processes. Sprint interval training, by contrast, elicits a strong stress response. Therefore, we hypothesized that adding slow-paced breathing to highly demanding sprint interval training would enhance recovery, as indicated by cardiac autonomic control and sleep metrics, ultimately contributing to greater performance gains. ***Methods:*** Thirty-four participants were randomized into an intervention or control group. While both groups completed 4 weeks of supervised sprint interval training, only the intervention group practiced slow-paced breathing after each training and every evening before bedtime. Running performance, vagally mediated heart-rate variability (root mean square of successive differences), and sleep metrics were assessed throughout the intervention to evaluate the potential benefits of the breathing exercise. ***Results:*** Sprint interval training led to significant improvements in maximal power decrements (–8.26%, 95% CI, –11.48 to –5.04, $P < .001$) and overall work (8.84 kJ/kg, 95% CI, 4.58 to 13.1, $P < .001$), but performance gains did not differ between groups ($P > .05$). Unexpectedly, nocturnal heart rate showed a slight increase over time in the intervention group (+3.1 beats·min$^{-1}$, 95% CI, 0.47 to 5.72, $P < .05$) compared with the control group. ***Conclusion:*** Despite positive effects reported in other training and performance contexts, 4 weeks of daily slow-paced breathing did not confer additional performance improvements or physiological benefits when combined with sprint interval training. These findings suggest that 10-minute sessions of slow-paced breathing following highly demanding sprint interval training offer limited value for enhancing performance or supporting recovery.

***Keywords:*** breathing exercise, HRV, HIIT

Physical training at high volumes and intensities drives shifts in autonomic activity, alongside hormonal changes reflecting physiological stress.[1–3] In particular, high-intensity interval training (HIIT) is characterized by substantial physiological demands, including prolonged changes in heart rate (HR) and HR variability (HRV) as part of autonomic regulation, reflecting a delayed onset of recovery.[4–7]

One promising intervention to enhance relaxation and parasympathetic reactivation, measured as an increase in vagal-mediated HRV, is slow-paced breathing (SPB).[8–10] It is typically defined as breathing at a constant rate of under 10 cycles per minute.[9] The physiological effects of SPB have been framed through various theoretical models, including the polyvagal,[11] vagal tank,[12] neurovisceral integration,[13] resonance,[14] and biobehavioral integration theory.[15] A hallmark acute response to SPB is increased respiratory sinus arrhythmia (RSA), characterized by HR acceleration during inhalation and deceleration during exhalation. This cardiorespiratory coupling is mediated by central, neural, and mechanical feedback systems and reflects vagal efferent activity on the sinoatrial node.[9,14,15] The baroreflex, a key neural mechanism linking blood pressure regulation and HR control via stretch receptors in the carotid sinuses and the aortic arch, plays a central role in this process. Through the coordination of respiratory and cardiac function, SPB is thought to optimize gas exchange while reducing cardiac workload.[9,15,16] Although individual responses vary, the most pronounced effects on RSA and baroreflex sensitivity are observed at breathing rates near 6 breaths per minute.[14,17]

Beyond its peripheral effects, enhanced cardiac vagal regulation—via afferent signaling—also contributes to psychological well-being and emotional regulation.[13] Moreover, vagal efferent activity modulates inflammatory processes.[18,19] Accordingly, experimental studies on SPB have reported improvements in psychological well-being, cognitive function, and physical health.[20,21] These findings have sparked growing interest within sports science and practice.[22] While the psychological and behavioral benefits of SPB in athletic populations are well established,[23] evidence regarding its direct impact on physical performance and training adaptations remains scarce. Most studies synthesized in recent reviews focus on technical skill acquisition or lack structured training protocols and robust physical performance outcomes.[22,24]

Building on the proposed benefits of SPB, the present study investigated its role in the context of structured HIIT. Participants completed a 4-week sprint interval training (SIT)[25] on a nonmotorized treadmill. We hypothesized that (1) independent of the SPB intervention, 4 weeks of SIT would lead to improvements in anaerobic capacity, as indicated by increased absolute work output ($W_{abs}$) and reduced power decrements ($P_{dec}$); (2) participants in the intervention group, who performed SPB for 10 minutes following each training session and each evening, would show improved recovery, reflected by higher HR recovery (HRR), increased vagal-mediated HRV, and subjective measures of exertion; and (3) consistent with previous findings,[26,27] the SPB group would report improved sleep and exhibit lower nocturnal HR, potentially further supporting training adaptations.

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## Methods

The present study was designed as a randomized controlled trial. All data were collected between May 2023 and December 2024 at the Department of Sport and Human Movement Science of the University of Vienna. Participants underwent 4 weeks of repeated maximal effort sprints on a nonmotorized treadmill (Woodway Force3.0). Outcomes of interest were assessed at 3 time points—before (pretraining), midway through (midtraining), and after (posttraining) the training period—and included physical performance metrics recorded by the treadmill; physiological markers derived from continuous measurements of heart periods; and subjective assessments of exertion, recovery, and stress. In addition, participants tracked sleep duration and quality throughout the intervention. The study protocol, including all hypotheses and analysis steps, was approved by the ethics committee of the University of Vienna (reference number: 00971) and preregistered on the Open Science Framework (https://osf.io/xnp73).

### Participants

Thirty-four (female: n = 19) participants completed the intervention. They were 26.3 (SD = 3.2) years old, on average, healthy, and free of injuries. The inclusion criteria required regular participation in exercise involving running and no routine use of relaxation techniques or meditation within the past year. Individuals with a BMI ≥ 30 kg/m$^2$, any known medical condition, medication use, or a history of performance-enhancing drug intake were excluded. Participants' characteristics are summarized in Table 1.

All participants were screened for physical activity risk factors using the German version of the Physical Activity Readiness Questionnaire (PAR-Q)[28] and for potential clinically relevant symptoms of high-prevalence mental disorders using the 21-item Depression Anxiety Stress Scale.[29] Scoring above the cutoffs of 14, 7, and 9 on the subscales of stress, anxiety, and depression, respectively, resulted in exclusion from the study.

### Procedure

Participants were instructed to arrive well-rested for all sessions, abstain from caffeine and nicotine on the days of testing, and refrain from physical exercise for 24 hours and alcohol for 48 hours prior to each test session; on the familiarization day, only a 1-hour caffeine restriction was required.

At the initial visit, participants provided written informed consent and completed a medical history assessment. They were then equipped with a fitness tracker (Fitbit Inspire 3). Furthermore, each participant received information on the training structure, a training and sleep diary for daily use, and 3 copies each of the German version of the Acute Recovery and Stress Scale[30] and the Perceived Stress Scale,[31] to be completed on the morning of the 3 test days (see Figure 1). Participants then underwent an assessment to determine their individual resonance frequency—the breathing rate that produces the strongest effect on HRV and autonomic regulation. Individualized resonance frequencies were used to optimize cardiorespiratory coupling and maximize potential vagal effects, as outlined by Lehrer and Gevirtz.[14] The assessment followed the protocol outlined by Shaffer and Meehan.[17] A detailed description of the assessment, including analysis protocol and results, is available online through the Open Science Framework at https://doi.org/10.17605/OSF.IO/A87E5. As learning effects were expected, the resonance frequency test was repeated at the beginning of the second test day, at least 3 days later. If an individual's results deviated between the 2 tests, the second test result was considered.

The second day of the experiment was dedicated to familiarization with the training protocol. After a standardized warm-up, participants completed three 30-second runs at progressively higher effort levels (see Figure 1). To reduce running speed and mitigate high-speed running-related muscle injuries, the belt resistance was set to 5% of body mass during all runs. At the pretraining test, body mass and height were measured on a stadiometer and digital scale, and body composition was estimated via bioelectrical impedance analysis (Nutrigard-MS Data-Input). After the warm-up, participants completed four 30-second all-out sprints, each separated by 4 minutes of walking recovery. They were verbally encouraged by a research assistant, and standardized phrases were used to guide them through each run. Following the final sprint, they walked for another 4 minutes before sitting quietly for 10 minutes. Participants reported their subjective exertion using a 100-mm visual analog scale (VAS) at 5 time points: (1) before warm-up, (2) after warm-up, (3) after the last sprint, (4) after walking recovery, and (5) after passive recovery. The horizontal VAS was unmarked except for anchors at each end, ranging from "fully recovered" to "maximal exertion." The same test procedure was followed on all test days, as outlined in Figure 1.

Group allocation was performed after the pretraining test, using a biased coin minimization, stratified by sex.[32] To ensure balance between groups, we considered maximum running speed and total running distance during the pretraining test. Allocation was performed using a custom R script (available online at https://doi.org/10.17605/OSF.IO/A87E5). After allocation, the intervention group was taught SPB at their individual resonance frequency, leveraging a free mobile application (Breathe Havabee, India). Daily practices of 10 minutes were to be performed both before bedtime and after the active cool-down phase of each SIT session. The daily evening sessions were recorded in the participants' training diaries. The control group did not receive instruction on SPB. During recovery, they were asked to rest quietly and had the option of reading or listening to music.

All subsequent training sessions followed the same structure and were interspersed with at least 1 rest day. Aiming to increase flexibility and improve overall compliance, albeit at the cost of reduced control over the training sessions, up to 3 of the 10 training sessions could be completed unsupervised.

**Table 1 Participant Characteristics**

| Group | Participants (F/M) | Body mass, kg | Height, m | BMI, kg/m$^2$ | Body fat, % | DASS21 |
|---|---|---|---|---|---|---|
| Control | 9/7 | 69.4 (9.3) | 1.73 (0.08) | 23.35 (3.30) | 23.2 (7.9) | 6 (0–24) |
| Intervention | 10/8 | 70.3 (11.7) | 1.74 (0.10) | 23.22 (2.66) | 21.8 (7.0) | 4 (1–25) |

Abbreviations: BMI, body mass index; DASS, Depression Anxiety Stress Scale; F, female sex; M, male sex. Values are represented as mean ± SD or mean (range), as applicable.

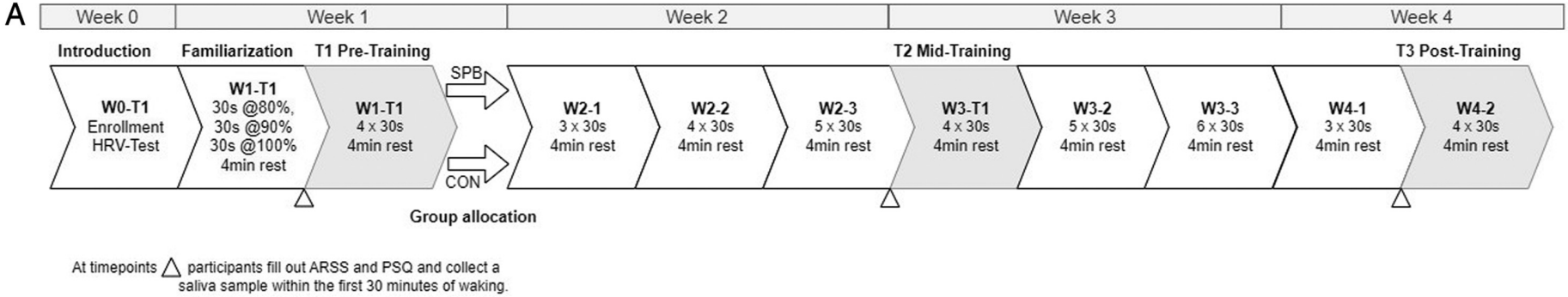


**Figure 1** — (A) Study protocol over 5 weeks. Group allocation was performed after the first performance test (pretraining). Testing sessions are highlighted in gray and labeled T1 to T3. (B) Description of a single session with group differences concerning the passive recovery phase. ARSS indicates Acute Recovery and Stress Scale; PSQ, Perceived Stress Scale; VAS, visual analog scale.

## Outcomes

Physical performance metrics were collected via the treadmill's software (Pacer Performance System, v2013.1.1). The treadmill was equipped with a strain gauge and force plate to collect horizontal and vertical forces at 200 Hz. The main outcomes of interest were defined as body mass-adjusted power (in watts per kilogram) and work (in kilojoules per kilogram). Power was estimated based on horizontal forces and belt speed. Work was calculated as the product of horizontal forces and distance covered. Power decrement ($P_{dec}$) was defined as the relative decline in maximum power across sets and was calculated as follows: $P_{dec} = 1 - \frac{\sum_{i=1}^{n} P_{max|i}}{n \times P_{max|best}}$, where $P_{max|i}$ is the maximum power achieved in a given set, $P_{max|best}$ is the highest power recorded across all sets, and $n$ is the number of sets. Total work ($W_{abs}$) was defined as the sum of work performed during all sets.

Physiological outcomes derived from heart periods were recorded with a Polar H10 sensor. Raw RR intervals were extracted and processed using a custom R script. Heart periods were filtered for ectopic beats and artifacts using an adaptive threshold filter, with an average window of 50 beats and a physiologically plausible HR range of 20 to 230 beats·min$^{-1}$.[33] HR was analyzed over the entire test session. HRR was calculated as the decrease in HR from its peak after the last set to values recorded after 1 minute of active recovery and the end of passive recovery. HRR within the first 2 minutes is a well-established procedure with acceptable reliability.[34] By contrast, HRR over the entire 14-minute recovery period is a less established approach; therefore, it is supplemented with a comparison of raw HR data. The root mean square of successive differences (RMSSD) was calculated as a robust time-domain vagal-mediated HRV metric that is minimally affected by respiratory rate.[13,35,36] To meet normality assumptions and facilitate interpretation, RMSSD values were ln-transformed prior to analysis (lnRMSSD). Frequency analysis using Fast-Fourier Transformation was performed on spline-interpolated heart period data (4 Hz) from the last minute of both active and passive recovery. Typical frequency bands for high-frequency (HF) and low-frequency (LF) spectral power were applied.[35] HF HRV primarily reflects cardiac vagal activity at rest but is also influenced by respiratory mechanics.[35–37] LF power reflects both sympathetic and parasympathetic input; however, its interpretative value remains controversial.[35,36] Consequently, HF and LF power were included only as complementary indices.

Nightly resting HR was extracted from the fitness tracker, which relies on photoplethysmography—an optical method shown to be reliable and valid at rest.[38,39] In addition, participants used a training and sleep diary to record all exercise and breathing sessions. This diary was also used to report their sleep quality (Likert scale: 1–10) and the time that they devoted to sleep.

## Statistical Analysis

An a priori power analysis was conducted with G*Power (v3.1.9.7).[40] We defined an alpha level of $\alpha = 5\%$ and a target power of $1 - \beta = 90\%$. It was based on effect sizes reported in a previous study investigating the effects of SPB on sleep in a comparable population and time frame ($\eta^2 = .14$).[26] To adopt a more conservative estimate, we set $\eta^2 = .06$ as the smallest effect size of interest. This corresponded to a required sample size of $n = 36$. To account for potential data loss, we increased the target sample by 15% ($n = 42$). However, the actual dropout rate exceeded expectations, and only $n = 34$ participants completed all training sessions, resulting in a statistical power of 80%. The full G*Power protocol is provided online at https://doi.org/10.17605/OSF.IO/A87E5.

The effects of SPB on performance ($P_{dec}$ and $W_{abs}$) and cardiac control (RMSSD and HR) were analyzed using analysis of covariance models with posttraining scores as dependent variables and the grand mean-centered baseline data as covariates. In addition, a $2 \times 3$ mixed-factorial analysis of variance (group × session) was conducted to report unadjusted data. $p$ values were Bonferroni-corrected for each hypothesis, based on the number of related outcomes. Contrast tests with marginal means were used for group- and time-based comparisons. Effects were reported along with 95% CI. Sleep quality, duration, and nocturnal HR data were analyzed using mixed linear models with restricted maximum likelihood estimations. This approach is well suited for the longitudinal data structure and can handle missing values. A 2-level structure with a random slope for time, nested within individuals as a random intercept, was implemented. The null model was initiated, and time, group, and their interaction were incorporated in a stepwise manner. The model fit was gauged through changes in the Akaike Information Criterion (Δ AIC). VAS for subjective exertion was analyzed with continuous ordinal regression. This approach was preferred over distribution-based methods and less powerful nonparametric tests as these scales are bounded and not purely interval-scaled.[41] To reduce model complexity in the presence of a limited sample size, only the last time point (5), corresponding to the end of the recovery phase, was analyzed. Effects were described as medians and median absolute deviation (MAD) between groups and over time. For the analysis of the ARSS, we modeled the sum of the subscales for recovery and load, respectively. Similarly, the 4 subscales of the PSQ (worry, tension, joy, and demand) were summarized to an overall score of stress, which is reported in the materials available online through the Open Science Framework at https://doi.org/10.17605/OSF.IO/A87E5.

Analyses were performed in R (version 4.4.2). All packages and detailed statistical processes are described in the materials available online through the Open Science Framework at https://doi.org/10.17605/OSF.IO/A87E5.

# Results

Of the 45 participants deemed eligible, 41 were allocated to the groups. Eighteen in the intervention group and 16 in the control group completed all required training and testing sessions and were included in the per-protocol analysis. Five participants dropped out due to illness (CON: 2 and INT: 3), 1 person due to a nonstudy-related injury, and 1 in for time-related reasons. A participants flowchart is available in the materials available online through the Open Science Framework at https://doi.org/10.17605/OSF.IO/A87E5. Diary logs indicated 100% and 95% adherence to the unsupervised training sessions and SPB, respectively. One participant in the control group failed to fully document all training sessions.

## Performance

Two participants emerged as extreme outliers (>3 × interquartile range) in the performance analysis, exhibiting an inverse pattern in peak power with increases rather than the expected decline across the 4 sprint sets, and were excluded from the analysis. Based on the remaining data, posttraining performance metrics were not significantly explained by group allocation when controlling for

pretraining performance. Consequently, contrast tests revealed no significant group differences for Δ $P_{dec}$ (0%, 95% CI, –4.08 to 3.88, $P_{adj} > .999$) or Δ $W_{abs}$ (–9.36 kJ/kg, 95% CI, –23.53 to 4.81, $P_{adj} = .376$). In the uncorrected analysis of variance, significant main effects of time were observed, indicating improvements in $P_{dec}$ by –8.26% (95% CI, –11.48 to –5.04, $P_{adj} \leq .001$) and in $W_{abs}$ by 8.84 kJ/kg (95% CI, 4.58 to 13.1, $P_{adj} \leq .001$). Notably, $P_{dec}$ decreased from pre- to midtraining (6.57%, 95% CI, 3.95 to 9.19, $P_{adj} \leq .001$), with no further change at posttraining ($P_{adj} = .9$). Figure 2 presents individual data along with estimated means and 95% CI.

## Cardiac Control

Results for cardiac control are summarized in Figure 3. Analysis of covariance showed no significant group differences in HRR during the first minute (HRR1min = 1.75%, 95% CI, –0.49 to 4, $P_{adj} > .999$) or over the full recovery period (HRR14min = –0.39%, 95% CI, –2.9 to 2.12, $P_{adj} > .999$). Session-to-session comparisons revealed no statistically significant changes in HRR1min (–0.05%, 95% CI, –1.72 to 1.62, $P_{adj} > .999$), but a small improvement in HRR14min (2.86%, 95% CI, 0.62 to 5.11, $P_{adj} = .018$). However, this training adaptation was not supported by changes in mean HR (Δ HR = –2.29 beats·min$^{-1}$, 95% CI, –5.21 to 0.63, $P_{adj} > .999$). When accounting for baseline data, lnRMSSD did not differ between groups (–0.49 ms, 95% CI, –1.04 to 0.06, $P_{adj} = .165$). Pre- to posttraining changes did not reach statistical significance (0.17 ms, 95% CI, –0.2 to 0.54, $P_{adj} = .756$). HRV frequency domain results are provided in the materials available online through the Open Science Framework at https://doi.org/10.17605/OSF.IO/A87E5. Briefly, HF spectral power showed no statistically significant effects, while LF power was higher in the SPB group (–1.12 ms$^2$, 95% CI, –2.08 to –0.15).

## Perceived Exertion

The analysis of VAS at the end of the cool-down phase showed the best model fit when session and group were included as predictors (Δ $AIC = 14$). At pretraining, VAS had a median of 49.5 mm (MAD = 28.2), which decreased over time (median = 24.0 mm, MAD = 22.2, $\beta_1 = 0.23$, $P = .019$). VAS values were slightly higher in the intervention group compared with the control group, but the difference failed to reach statistical significance (median = 48.0, MAD = 28.2, $\beta_2 = -1.48$, $P = .067$). Similarly, the group × time interaction was not significant ($P = .954$). The time course of the VAS during each testing session is illustrated in Figure 4.

## Sleep

The ICC of the null models indicated that 21% of the variance in sleep quality and 24% of sleep duration were attributable to individual differences. Neither the inclusion of a random slope nor the addition of fixed effects for group or time improved the model fit over the intercept-only model (sleep quality: Δ AIC = 1.4, $\chi^2(1) = 7.38$, $P = .061$; sleep duration: Δ AIC = 0.32, $\chi^2(1) = 5.68$, $P = .128$).

Nocturnal resting HR (Figure 5) suggested high variability between participants compared with the within-person variance (ICC = .93). Stepwise inclusion of fixed effects for time, group, and group × time interaction improved the model fit (Δ AIC = 5.9,

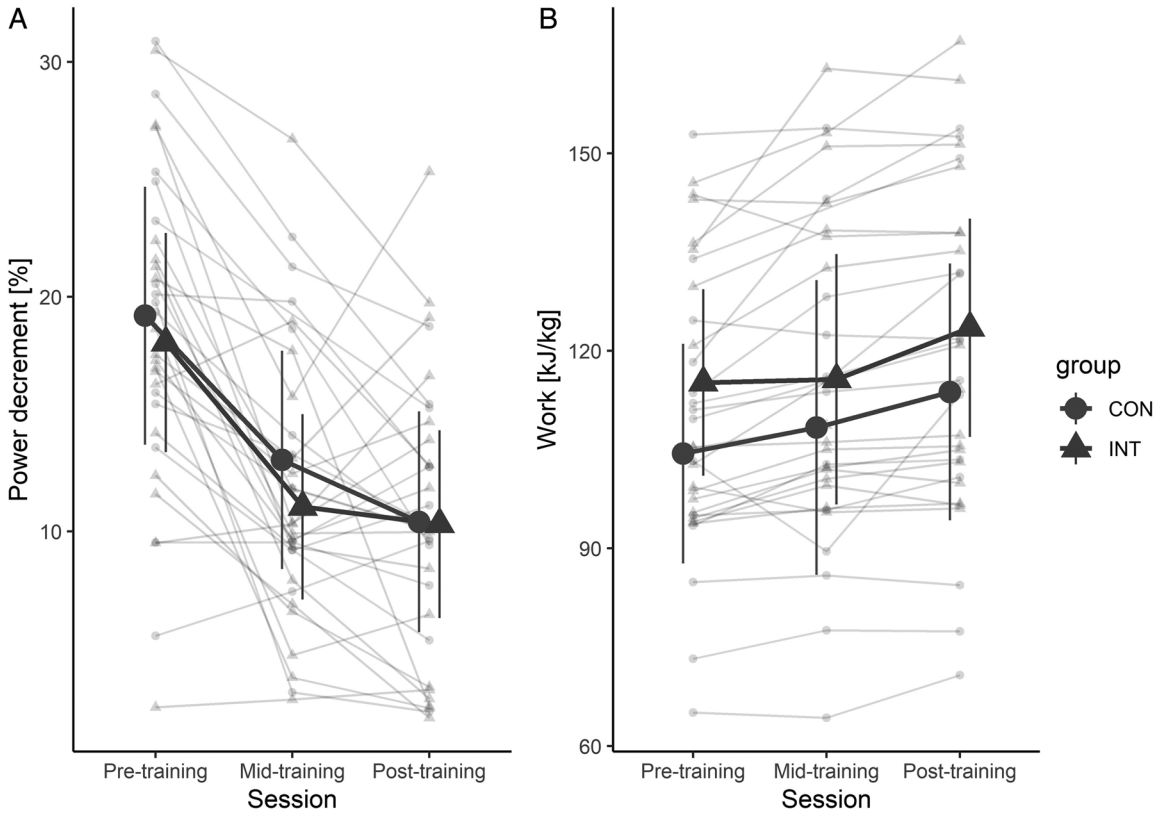


**Figure 2** — Performance metrics across the training period for each group. Thin lines represent individual participants. Group means and 95% CIs are presented for each group. (A) Percentage change in power decrement. (B) Work in kilojoules per kilogram body mass. CON indicates control group; INT, intervention group.

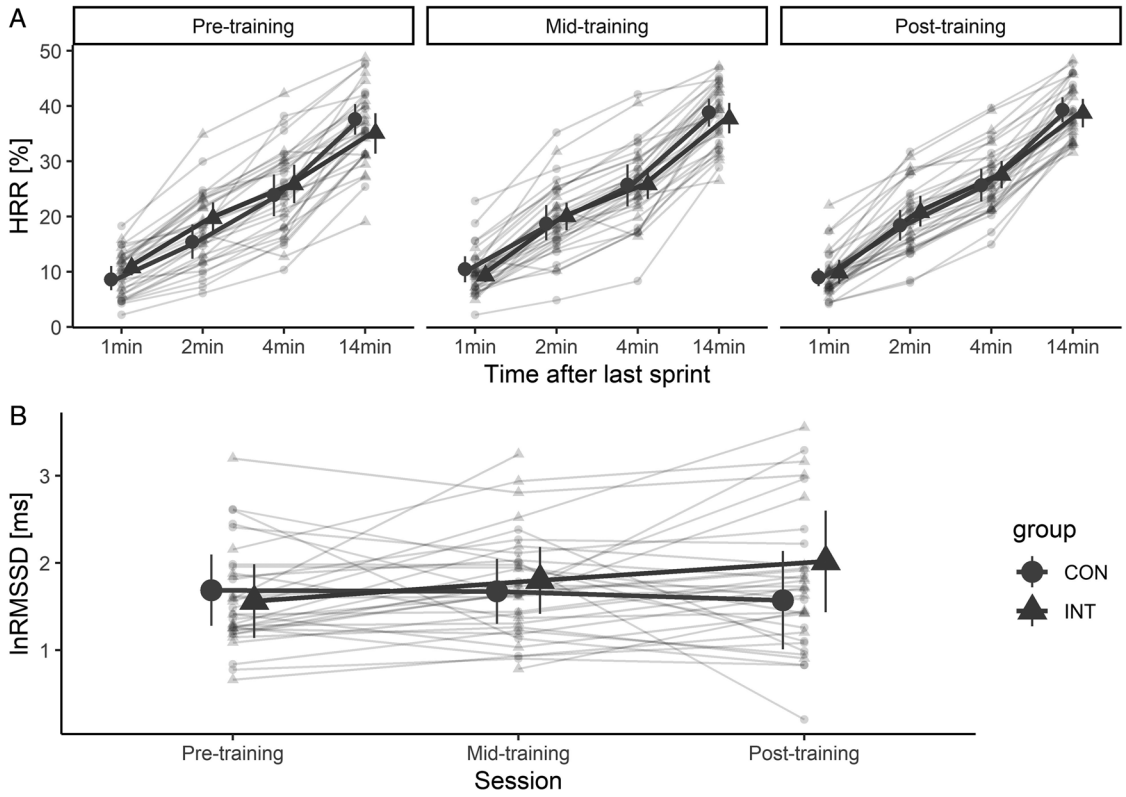


**Figure 3** — Metrics reflecting cardiac control during the training period for each group. Thin lines represent individual participants. Estimated marginal means and 95% CIs are presented for each group. (A) One-minute HRR over time for each testing session. (B) ln-transformed RMSSD post recovery. HRR indicates heart-rate recovery; CON, control group, INT, intervention group; RMSSD, root mean square of successive differences.

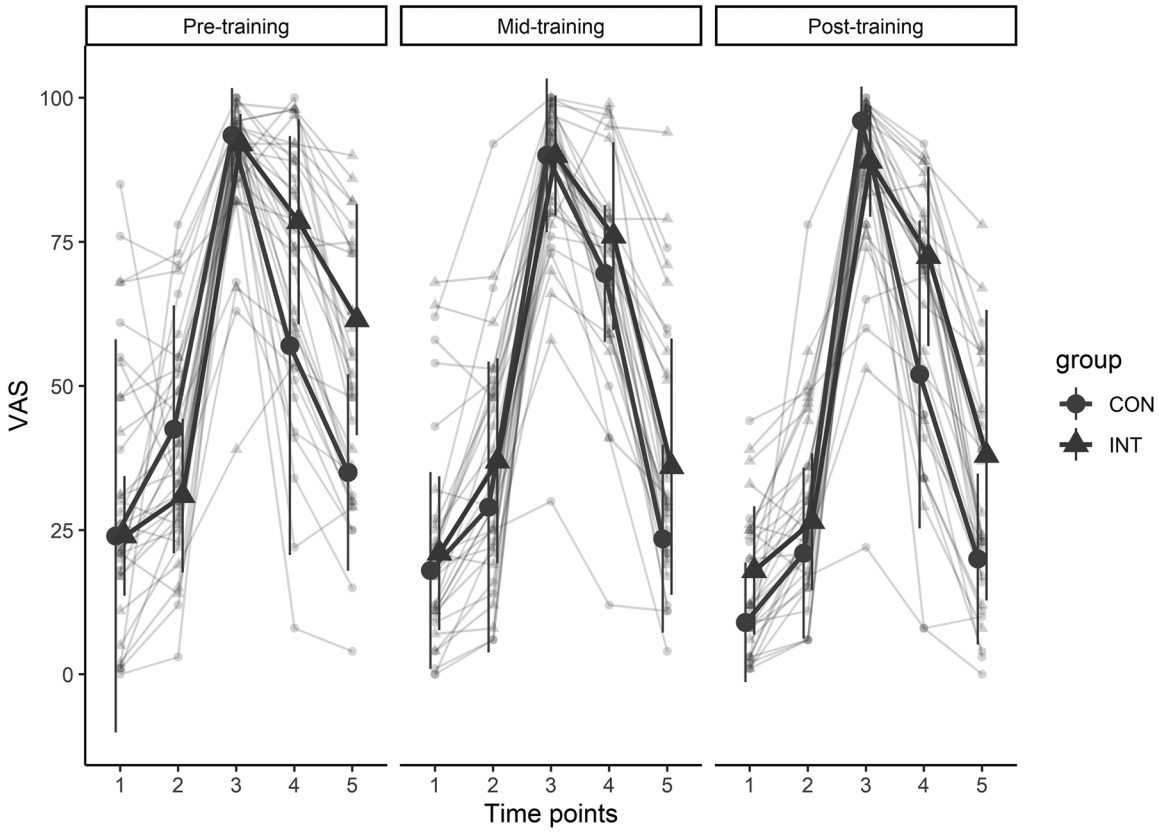


**Figure 4** — Time course of VAS scores during each testing session. Thin lines represent individual participant responses, with the 5 time points denoting (1) before warm-up, (2) after warm-up, (3) after the last sprint, (4) after 4 minutes of walking recovery, and (5) after 10 minutes of passive recovery, which incorporated slow-paced breathing for the intervention group and quiet sitting for the control group. Medians and median absolute deviations are presented for each time point and group. CON indicates control group, INT, intervention group; VAS, visual analog scale.

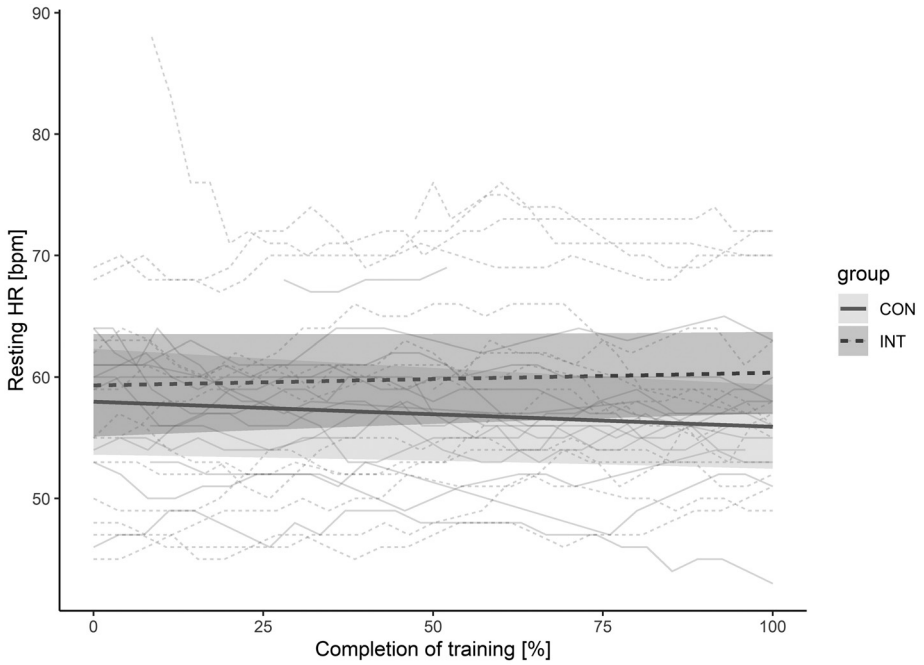


**Figure 5** — Continuous measures of nocturnal HR. Thin lines represent individual participants. Thick lines represent the estimated linear model with 95% CIs. Bpm indicates beats per minute; CON, control group; HR, heart rate; INT, intervention group.

$\chi^2(2) = 9.9$, $P = .007$). The estimated mean HR at the beginning of the training period was 58 beats·min$^{-1}$ (95% CI, 53.6 to 62.3). In the control group, resting HR decreased by −2.1 beats·min$^{-1}$ (95% CI, −4 to −0.2), while in the intervention group, a relative increase of 3.1 beats·min$^{-1}$ (95% CI, 0.47 to 5.72) was observed. No significant main effect for group differences was identified (1.35 beats·min$^{-1}$, 95% CI, −4.71 to 7.37). The standard deviations for the random intercept and slope were 8.3 and 3.3 beats·min$^{-1}$, respectively.

## Discussion

The overarching goal of the present study was to investigate the impact of SPB on the adaptations invoked by 4 weeks of SIT. The intervention group practiced SPB at individual resonance frequencies after each session and every evening before bedtime. Contrary to our hypothesis that SPB would enhance recovery, resulting in superior performance gains, reduced subjective exertion, and improved autonomic cardiac control and sleep, no such benefits were observed.

In accordance with previous findings,[25] SIT resulted in improvements in anaerobic power in a young, healthy population. The reduction in $P_{dec}$ (−8.26%) and concomitant increase in $W_{abs}$ (+8.84 kJ/kg) underscore its effectiveness. These improvements, however, were independent of the breathing intervention, indicating that SPB did not contribute to superior performance outcomes under the present conditions.

Contrary to a meta-analysis,[10] SPB also failed to produce meaningful changes in RMSSD or HRR over 1- and 14-minute intervals. Several physiological factors may explain this. One key consideration is the timing of SPB relative to the training stimulus. SIT provokes a prolonged autonomic response,[7,42] during which the autonomic system may be unresponsive to vagal stimulation. Consequently, SPB may have had little immediate influence. In addition, the cumulative physiological stress induced by SIT—including elevated catecholamines, fluid shifts, and metabolic and hormonal disturbances—may have diminished the potential effects of SPB.[43] It is possible that these competing factors obscured the effects of cardiac vagal reactivation.

Independent of group allocation, SIT induced changes in autonomic markers. HRR over 1 minute remained unchanged throughout the training, indicating limited influence on early-phase autonomic recovery, which is predominantly governed by sympathetic withdrawal.[4] By contrast, 14-minute HRR increased substantially after training, potentially indicating enhanced parasympathetic reactivation, cardiovascular efficiency, or faster metabolic clearance. However, this interpretation is not supported by concurrent changes in raw HR or RMSSD, which remained unchanged. Given the nonspecific nature of HRR and its susceptibility to confounding factors such as training load, overreaching, and overtraining,[44] the physiological relevance of this isolated finding remains uncertain.

Sleep-related outcomes also showed no benefit from SPB. Despite informal participant reports of a calming effect in the evening and very high adherence, sleep varied considerably between and within individuals. Notably, nocturnal HR increased slightly over time in the intervention group, whereas a minor reduction was observed in the control group. This unexpected trend may indicate reduced nocturnal parasympathetic tone in the SPB group and warrants further investigation. Elevated nocturnal HR is frequently reported in nonfunctional overreaching and overtraining.[44] One possible explanation is that SPB delayed the onset of autonomic recovery by interfering with early recovery processes, which rely on transient elevations in glucocorticoids, catecholamines, and blood pressure. This could have prolonged allostatic responses throughout the night. Alternatively, participants may have unintentionally hyperventilated during unsupervised evening SPB, leading to mild hypocapnia and increased sympathetic activity through reduced baroreflex and increased chemoreflex sensitivity.

Participants perceived the training as very strenuous. A high dropout rate of 20%, primarily due to infectious diseases, may

indicate elevated physiological strain or immune suppression related to the training load. While subjective exertion ratings during recovery differed between groups, individual differences in perception limit the interpretability of this finding.

## Practical Applications

Recent meta-analytic evidence indicates that certain postexercise recovery techniques—such as cold-water immersion or massage—can enhance vagally mediated HRV, potentially supporting recovery and adaptation.[45] Despite its potential as a safe, accessible, and cost-effective recovery strategy,[14,22,46] regular SPB did not improve performance or cardiac autonomic recovery when applied after SIT. Practitioners should be cautious when implementing SPB immediately postexercise, as delayed parasympathetic reactivation[3] and elevated ventilatory demands[47] may reduce its effectiveness. While SPB may offer psychological and cognitive benefits,[8,23,24] its role in enhancing adaptations to high-intensity endurance training remains uncertain.

While the study followed a rigorous protocol and achieved high adherence, several limitations should be noted. The final sample size was slightly below target due to higher-than-anticipated dropouts. Although common confounders of HRV (eg, caffeine, physical activity) were controlled where feasible, others (eg, menstrual cycle) could not be fully accounted for. Sleep assessments relied on self-report and wearable data. To increase ecological validity and participant compliance, up to 3 SIT sessions were completed unsupervised, which limited experimental control despite structured protocols and diary-based documentation. Moreover, the absence of systematic participant feedback on the subjective experience and feasibility of the SPB intervention limits the contextualization of its applicability. Finally, it is unclear whether the timing of SPB or the minimum duration of 10 minutes per application limited its potential effects. Future studies might consider a longer delay between exercise and SPB and extended sessions, as well as increasing the potential effectiveness of SPB by incorporating rhythmic muscle contractions.[48,49]

## Conclusion

In summary, SPB did not yield the expected benefits for recovery or performance during 4 weeks of SIT. Contrary to previous findings,[26,27] it also failed to improve sleep and was associated with a slight increase in nocturnal HR, suggesting possible interference with overnight parasympathetic recovery. These findings highlight the importance of further investigating the timing and implementation of SPB in high-intensity training contexts.

A strength of this study lies in its well-controlled training protocol and combined assessment of physical performance, cardiac autonomic control, and perceived exertion. Future research should explore longer interventions, extended cool-down periods before SPB, and robust methods for assessing sleep and nocturnal HRV. Sleep plays a pivotal role in maintaining optimal health and peak physical performance.[50] Cardiac vagal activity is typically suppressed for 10 to 60 minutes post-HIIT,[7] while oxygen uptake and ventilatory compensation for metabolic acidosis remain elevated for up to 45 minutes.[47] These physiological factors may have interfered with the intended effects of SPB.

The considerable interindividual variability observed suggests that within-subject designs—despite being challenging in applied settings—may offer greater sensitivity to detect effects. Finally, while faster recovery is often taken as a sign of improved fitness, accelerating recovery through postexercise interventions does not necessarily promote better long-term adaptations. For example, cold-water immersion may improve short-term recovery but attenuate strength gains in resistance training.[51] Future work should clarify whether SPB, particularly when practiced in the evening, can enhance performance through sleep-mediated mechanisms.

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**Supplementary Materials**: The data, description of the resonance frequency test, R code, and participant flowchart can be found on OSF: https://doi.org/10.17605/OSF.IO/A87E5

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# 4. Discussion

This cumulative dissertation aimed to advance our understanding of how stress and physical exercise interact at the psychophysiological level. The three presented papers discuss different aspects: Studies 1 (Raidl, Wessner, et al., 2025) and 2 (Raidl, Vogl, et al., 2025) both adopted similar study designs and methodologies. Study 3 (Raidl et al., 2026) provided insights into SPB as a practical and accessible strategy during a training intervention. Considered jointly, the findings enhance the interpretation of commonly applied laboratory paradigms, refine expectations derived from the CSAH, and identify constraints that shape the effectiveness of SPB as a recovery practice in demanding training protocols.

Study 1 revealed psychophysiological differences between the two stress paradigms, with lower cardiac-autonomic responses during the physically loaded MAST. Females showed overall lower physiological responses, consistent with meta-analytic findings on the TSST specifically (Gu et al., 2022; Liu et al., 2017). Importantly, the precision of the estimates presented is limited by the study's small sample size. Nevertheless, it informed subsequent planning and emphasized the need to consider both sex and stressor characteristics in experimental designs.

In study 2, we found stress response patterns similar to those in study 1. Yet, we did not find strong support for the CSAH. The only significant effect concerned the absolute heart rate during the TSST/MAST. This is not surprising given the lower resting heart rate and higher heart rate reserve typically found in aerobically fitter individuals (Emaus et al., 2010; Grant et al., 2013). Instead, the findings argue for domain-specific influences and caution against generalized interpretations of the CSAH. Several strengths and weaknesses of studies 1 and 2 are outlined in the manuscripts, some of which deserve revisiting here to guide future research:

We speculated that a lower heart rate during TSST/MAST indicates lower cardiovascular demand in fitter individuals during acute stress. However, we did not measure stroke volume, so we could not calculate cardiac output as a better estimate of cardiovascular demands (cardiac output = stroke volume × heart rate). In future research, transthoracic electrical bioimpedance (Harford et al., 2019) or modeling the arterial pulse waveform (Bogert & van Lieshout, 2005; Sugawara et al., 2003) can be valid and feasible methods during stress-induction and standardized exercise.

Another critical issue concerns the characterization of recovery. The follow-up periods in studies 1 and 2 were 25 minutes and 30 minutes after stress cessation, respectively. This was likely too short to evaluate the response pattern, including maximal values (i.e., reactivity) and return to baseline (i.e., recovery). This is evident in the figures on endocrine response over time presented in the manuscripts. However, recovery after stress-induction was considered to be at least as important as reactivity (Linden et al., 1997; Sapolsky, 1995). Impaired recovery was also proposed as a sign of allostatic overload (McEwen, 2007). Early meta-analyses provide evidence for faster cardiac recovery in fitter individuals (Forcier et al., 2006; Jackson & Dishman, 2006). Later studies that included endocrine measures could not support this (Klaperski et al., 2013; Rimmele et al., 2007). Unfortunately, many similar studies measured cortisol only up to 15 minutes after the end of the stressor (Mücke et al., 2018), which hampers the evaluation of recovery.

Peak magnitude and recovery kinetics are intrinsically linked. To robustly characterize recovery, future studies should extend the follow-up period to ≥ 60 minutes (Dickerson & Kemeny, 2004; Labuschagne et al., 2019). Coupling between the peak value and recovery should be reduced by (1) including maximal values as covariates in the statistical model, (2) expressing recovery as a percentage value in relation to baseline, (3) modelling individual decay curves (typically mono-exponential, log-transformed, or time-constant/linear) and comparing time-related parameters (e.g., half-life). (4) Summary metrics like area under the curve add information on total exposure, but should not be presented without the individual measures to capture the time-kinetics. Comparable principles apply to other outcomes, including heart rate recovery, which occurs on a shorter time scale but is governed by similar constraints (Bosquet et al., 2008; Mongin et al., 2023).

Another limitation concerns the control of the menstrual cycle and hormonal contraceptives. Menstrual cycle and contraceptives have been shown to influence the stress response to laboratory stressors (Gervasio et al., 2022). We decided to exclude participants with irregular menstrual cycles or using hormonal contraceptives. Further, we tested participants during the mid-luteal phase (self-reported; see discussion in the manuscript of study 2) to minimize variation within and between participants (Kajantie & Phillips, 2006b). While statistical control for menstrual phase or contraceptive use would be possible, we decided against it due to resource constraints. Including these factors in our statistical model would substantially increase the required sample size. At the same time, our approach probably introduced sampling bias and limits generalizability. A high percentage of female athletes have menstrual cycle dysfunctions. The estimated percentages range from under 10% to 70%,

depending on the sport, level of competition, and defined menstrual dysfunction (Carmichael et al., 2021; Gimunová et al., 2022; E. M. Kirschbaum et al., 2025). A retrospective analysis of training data found a relationship between intensity and volume with menstrual cycle-related dysfunction (Baranauskas et al., 2023). Furthermore, around 26% of females in the reproductive age use hormonal contraceptives (oral, injectable, or implant; United Nations, 2019). The use of hormonal contraceptives may even be slightly higher in athletes (E. M. Kirschbaum et al., 2025; Martin et al., 2018; Osborne et al., 2025). Taken together, our criteria for study participation exclude a high proportion of the population. Including users of contraceptives further complicates matters, as not every type of contraceptive may have the same effects on the stress response (Hampson, 2020; Langan-Evans et al., 2024). Even within the group of oral contraceptives, which is fairly well understood, current studies give little information on the type or generation of contraceptive pills taken (Barel et al., 2018; Gervasio et al., 2022; C. Kirschbaum et al., 1999; Rohleder et al., 2003). In summary, this is a significant research gap in the field as a whole. This is particularly important given accumulating evidence on potential health concerns from long-term oral contraceptives, which are partly cortisol-related (Brønnick et al., 2020; Hertel et al., 2017). Future studies must address this gap by improving sampling (e.g., stratification of typical hormonal contraceptives) and adding preregistered subgroup analyses.

In study 3, an interventional perspective was introduced by implementing slow-paced breathing during a demanding sprint interval training regimen. Although training itself produced clear performance improvements, the additional breathing practice did not enhance performance or cardiac autonomic regulation. While this study has several strengths and offers novel insights into the effects of SPB under high training demands, I want to highlight potential directions for future research: Introducing subjective and physiological monitoring with higher temporal resolution to evaluate the training demands and recovery would strengthen the argument or alternative explanations for the findings. Furthermore, the SPB intervention might have been too short or required a prolonged learning phase with biofeedback or coaching, even if the current literature does not support the need for biofeedback (Laborde et al., 2022; Lehrer et al., 2020). More importantly, the timing of the sprint exercise might have been a limiting factor in the intervention's effectiveness. While no formal analysis can be provided, some participants reported slight dyspnea, probably due to metabolic acidosis and increasing hypercapnia. This could create the opposite of the desired effect. Multiple studies have investigated breath-hold exercises during shorter repeated sprints, with potential positive adaptations (Précart et al., 2025). However, these changes would result from higher metabolic demands, further increasing the exerciser's allostatic needs. Future

studies should increase the time between the training and the breathing exercise and control blood lactate and blood oxygen saturation to better understand the underlying mechanisms.

Finally, I want to highlight the contention between the outlined theories around stress and the operationalization in our studies. *Stress* is not merely the elevation of cortisol or any other physiological metric, but a regulatory process (see Chapter 1.3). However, in the studies presented, we measured the psychophysiological *stress response* as a correlate of stress in a manner similar to how neural activities can be viewed as a correlate of mental processes. Cortisol was defined as the primary outcome as it was shown to be the most sensitive and widely accepted marker of acute responses to psychosocial stress-induction (Man et al., 2023). This rationale is not a trick to escape the *proxy problem*. The psychophysiological measures of the stress response are inherently important for health and human functioning (see Chapter 1.4). Where we used *stress* as a shorthand, we referred to the observed psychophysiological pattern rather than the construct.

To conclude, after outlining theoretical considerations on stress, the stress response, and its interaction with exercise, three studies were presented. Together, they offer new insights into the nature of the stress response in athletes and exercisers of both biological sexes. In particular, the direct comparison of two widely accepted stress-induction protocols can inform future research and help propose appropriate study designs. Study 3 also provides practical guidance on implementing slow-paced breathing during high-intensity training.

I hope this contribution enhances our scientific understanding of stress and physical exercise, guides future research, and supports practitioners in making informed decisions across psychological, physical health, and performance areas.

# Figures

# Abbreviations

ACTH
adrenocorticotropic hormone ..... 20
ANS
autonomic nervous system ..... 6
AVP
arginine-vasopressin ..... 20
BNST
bed nucleus of the stria terminalis ..... 15
CBG
corticosteroid-binding globulin ..... 21
CRF
corticotropin-releasing factor ..... 20
EPIC
embodied predictive interoceptive coding ..... 15
FSH
follicle-stimulating hormone ..... 20
*GAS*
General Adaptation Syndrome ..... 7
GH
growth hormone ..... 20
GR
glucocorticoid receptors ..... 20
HPA
hypothalamic-pituitary-adrenal ..... 7
HRV
heart rate variability ..... 19
LH
luteinizing hormone ..... 20
MAST
Maastricht Acute Stress Test ..... 36
MR
mineralocorticoid receptors ..... 20
PVN
paraventricular nucleus of the hypothalamus . 15
RSI
repeated sprint interval ..... 39
SCN
suprachiasmatic nucleus ..... 21
TSH
thyroid-stimulating hormone ..... 20
TSST
Trier Social Stress Test ..... 36
VO2max
maximal oxygen uptake ..... 22

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