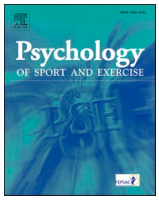
Psychology of Sport & Exercise 80 (2025) 102870

Contents lists available at ScienceDirect

Psychology of Sport & Exercise

journal homepage: www.elsevier.com/locate/psychsport



Athlete burnout and biomarkers: An exploratory, longitudinal *N-of-1* study

Hanna L. Glandorf *, Daniel J. Madigan, Owen Kavanagh, Sarah H. Mallinson-Howard

School of Science, Technology & Health, York St John University, Lord Mayor's Walk, York, YO31 7EX, UK

ARTICLE INFO

Keywords:
Allostatic load
Epigenetics
Exhaustion
Health
Stress
Wellbeing

ABSTRACT

Burnout is an increasingly common problem among athletes. In addition to negatively affecting mental health, burnout may also be related to changes in physiological functioning. Research outside of sport suggests that the hypothalamus-pituitary-adrenal (HPA) axis, immune, anabolic, and cardiovascular systems, in particular, may be affected. However, few studies have explored the relationship between burnout and biomarkers of these systems in athletes. Consequently, the aim of the present multidisciplinary study was to explore the relationship between athlete burnout and acute and chronic biomarkers using a longitudinal *N-of-1* design. Following a pre-registered protocol with open data, code, and materials, in two athletes, we examined burnout and acute salivary biomarkers (cortisol, testosterone, dehydroepiandrosterone-sulphate [DHEA-S], secretory Immunoglobulin A [sIgA], and C-reactive protein) in 12 samples over six months. In another two athletes, we examined burnout and chronic biomarkers from hair and blood (hair cortisol, glycated haemoglobin [HbA1c], triglycerides, total cholesterol, high-density lipoprotein cholesterol, and DNA methylation in the BDNF, SLC6A4, and NR3C1 genes) in six samples over 12 months. Dynamic regression modelling showed that burnout symptoms predicted decreased testosterone and developed simultaneously with decreases in DHEA-S and sIgA. Visual analyses suggested that burnout symptoms also developed in conjunction with increases in HbA1c and SLC6A4 methylation and preceded increases in hair cortisol and BDNF methylation. Our findings provide a preliminary "physiological fingerprint" that could help explain athlete burnout development and consequences which can be used to guide future theory and research in this area.

1. Introduction

Burnout appears to be an increasing concern among athletes (Madigan et al., 2022). Aside from affecting athletes' mental health, burnout may also be related to changes in physical and physiological functioning. Allostatic load theory may help to explain why this is the case (Juster et al., 2011; McEwen & Stellar, 1993). According to this theory, burnout may be related to a multisystem dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis and the immune, anabolic, and cardiovascular systems. The relationship between burnout and biomarkers of these systems has been examined outside of sport, however, few studies have examined this relationship in athletes. Consequently, the aim of the present study was to extend our understanding of this relationship in athletes by examining a variety of different biomarkers of the aforementioned systems and using a longitudinal *N-of-1* design to do so.

1.1. Burnout and biomarkers

Athlete burnout is a multidimensional psychosocial syndrome with three symptoms: emotional and physical exhaustion (physical and emotional aspects of exhaustion), sport devaluation (reductions in interest and development of a negative attitude towards one's sport), and a reduced sense of athletic accomplishment (reduced sense of athletic efficacy and accomplishment, Raedeke & Smith, 2001). Previous studies have shown that a significant minority of athletes can experience moderate-to-severe burnout symptoms during the athletic season (Gerber et al., 2018). More recent evidence, too, suggests that the prevalence of athlete burnout is increasing and has been doing so for the past two decades (Madigan et al., 2022). Worryingly for those involved in safeguarding the wellbeing of athletes, beyond its own symptoms, burnout has been associated with important outcomes such as worse quality motivation (Li et al., 2013) and physical and mental health (Glandorf et al., 2023). Biomarkers represent the medical state of an individual which can indicate normal or pathogenic processes, usually

* Corresponding author.
E-mail address: hanna.glandorf@yorksj.ac.uk (H.L. Glandorf).

https://doi.org/10.1016/j.psychsport.2025.102870
Received 5 August 2024; Received in revised form 6 May 2025; Accepted 8 May 2025
Available online 14 May 2025

related to one or more bodily systems (Bärtl et al., 2022). The assessment of biomarkers involves the measurement of blood pressure, heartrate, or determining levels of proteins or lipids from saliva and blood (Danhof-Pont et al., 2011). Some biomarkers show variation on an acute basis (e.g., minutes to weeks), while others are more chronic (e.g., weeks to months), depending on the processes they relate to. Biomarkers that vary on an acute basis often represent reactions to external stimuli (e.g., increased cortisol levels in response to acute stress; Bayes et al., 2021). Chronic biomarkers tend to represent either an accumulation of trends over time (e.g., long-term cortisol levels accumulated in hair; Brianda et al., 2020) or an adaptation of a system to environmental changes (e.g., consistently elevated blood glucose levels in response to chronic stress; Bayes et al., 2021). Certain biomarkers may show changes on both an acute and chronic basis as they respond to moment-to-moment stimuli and can stabilise over time (e.g., blood pressure; Danhof-Pont et al., 2011). It is for these reasons that biomarkers are useful proxies for health status and are routinely used in both the research and clinical contexts.

1.2. Theoretical propositions

Athlete burnout may be linked to biomarkers of specific systems. McEwen and Stellar's (1993) allostatic load theory provides a starting point for understanding the ways in which this may manifest. According to this theory, chronic stress exposure causes changes in a number of bodily systems. An individual will experience stress when environmental challenges exceed their ability to cope with those challenges. Long-term exposure to stress is then thought to have a negative impact and cause changes in bodily systems via allostatic overload. Allostatic overload is defined as a multisystem dysfunction in the hypothalamus-pituitary-adrenal (HPA) axis, the immune, anabolic, and cardiovascular systems. Because the accumulation of chronic stress is also thought to underpin burnout development, it has been suggested that burnout may be tied to biomarkers of these systems via allostatic overload (Juster et al., 2011).

The theoretical propositions from allostatic load theory have been further elaborated in relation to burnout. Melamed and colleagues (2006) suggested that stress causes the autonomic nervous system (ANS) and the HPA axis to be more active to provide the resources to manage the cause(s) of stress. Accordingly, biomarkers of the ANS and HPA axis may be upregulated on a short-term basis, which can help the individual to adapt (Danhof-Pont et al., 2011). However, over time, when stress becomes chronic, burnout will develop (Juster et al., 2011). Burnout symptoms are then expected to lower the resources available to the individual and thereby contribute to the chronic stress experience. Concurrent, chronic activation of the ANS and HPA axis due to chronic stress has been suggested to result in an exhaustion of these systems (Melamed et al., 2006). This exhaustion is thought to lead to insufficient signalling and thus a lack of regulation between the ANS and vital functions (e.g., blood pressure) as well as the HPA axis and immune system (e.g., inflammation) and metabolism (e.g., anabolic processes; Bayes et al., 2021). Changes in regulatory signalling may cause an overactivity of vital functions as well as damage to the metabolism and immune system, which can manifest as acute reactions and chronic accumulations or adaptations in related biomarker levels. Overactivity of vital functions and damage to these bodily systems may further link with cardiovascular diseases over time. Consequently, burnout may be related to changes in acute and chronic biomarkers by reducing an individual's resources and thus contributing to the chronic stress experience, which affects the ANS and HPA axis, causing a cascade of changes in other systems through the aforementioned signalling pathways.

Apart from affecting biomarkers through a physiological signalling cascade, burnout has been proposed to cause changes in bodily systems via epigenetic mechanisms (Bayes et al., 2021). Epigenetic mechanisms represent changes to DNA that do not affect the genetic code but determine whether specific genes are expressed (Bakusic et al., 2017). DNA methylation is an example of such a mechanism and occurs when a methyl group is attached to the base cytosine (Alasaari et al., 2012). Methylation can have inhibitory effects such that expression of a highly methylated gene is decreased. In this regard, studies have suggested that apart from demographic (e.g., age, biological sex) and environmental factors (e.g., early-life socioeconomic status; Lam et al., 2012), prolonged exposure to cortisol (a key stress hormone of the HPA axis) may cause alterations in DNA methylation (Bayes et al., 2021). Increased methylation, therefore, may represent an adaptation to the upregulation of the HPA axis. As burnout is expected to cause an upregulation of the HPA axis, DNA methylation of specific genes could develop at the same time or follow the development of burnout and thus be an additional relevant biomarker of burnout.

1.3. Empirical support outside of sport

There is empirical support for the links between burnout and certain biomarkers. Most of this evidence exists outside of sport. For example, previous reviews have shown that burnout is related to acute biomarkers of the HPA axis, immune system, and the ANS (see Bayes et al., 2021; Danhof-Pont et al., 2011). In particular, burnout has been associated with cortisol (e.g., Brianda et al., 2020), testosterone (e.g., Atik et al., 2020) and the precursor of these hormones, dehydroepiandrosterone-sulphate (DHEA-S; e.g., Lennartsson et al., 2016). With regards to the immune system and ANS, burnout has been linked to the acute inflammatory marker C-reactive protein (CRP; e.g., Metlaine et al., 2018) and to fluctuations in blood pressure (Bärtl et al., 2022). Along with associations between burnout and biomarkers, these studies have identified extraneous variables (e.g., diurnal rhythm of salivary markers, food consumption, heavy exercise, venipuncture) that can affect acute biomarkers and thus need to be controlled for through study design (Bosch, 2014; Pilger et al., 2018; Weckesser et al., 2014).

Chronic biomarkers that have been explored in relation to burnout relate to the HPA axis, metabolism, and cardiovascular system. With regards to the HPA axis, studies have started to measure cortisol from hair to estimate the accumulation of cortisol concentrations over time, which have been shown to be associated with burnout (e.g., Brianda et al., 2020). In connection to the metabolism, burnout has been associated with glycated haemoglobin – a reflection of two-to-three-month blood glucose concentrations (HbA1c; e.g., Metlaine et al., 2018) – and accumulated allostatic load indices that include blood lipids (e.g., triglycerides, total cholesterol, HDL cholesterol; e.g., Bärtl et al., 2022). Although, compared to acute biomarkers, chronic biomarkers are less sensitive to short-term changes, extraneous variables that can affect these biomarkers levels have also been highlighted (e.g., food consumption, medical conditions, hair treatment, Danhof-Pont et al., 2011; Parker & Bristow, 2020).

Studies exploring epigenetic mechanisms have predominantly focused on stress-associated genes that are part of neuronal processes or those related to the HPA-axis. This work has found burnout to be related to methylation changes in the SLC6A4 gene which encodes for the human serotonin transporter and plays a role in cognitive processes such as emotional regulation (Alasaari et al., 2012; Bakusic et al., 2017). Burnout has also been related to methylation of the BDNF gene which codes for brain-derived neurotrophic factor and is important for maintaining normal brain function (Bakusic et al., 2020). Finally, burnout has been related to methylation changes in the NR3C1 gene which codes for glucocorticoid receptors that are involved in HPA axis activity and gene regulation surrounding the metabolism and immune system (Bakusic et al., 2021). Since these genes have also been associated with stress and depression, previous research has highlighted the need to control for extraneous variables (e.g., depression diagnosis) through sample selection and thereby avoid confusion between burnout and depressive symptoms (Bakusic et al., 2017; Cresswell & Eklund, 2006).

1.4. Empirical support inside of sport

Research in sport has started to examine the relationship between athlete burnout and biomarkers. Glandorf and colleagues (2023) recently systematically reviewed this literature. Their review showed that research in sport has predominantly focused on biomarkers of the HPA axis (cortisol, DHEA-S, and testosterone), followed by biomarkers of the immune system (CRP and secretory immunoglobulin A [sIgA]). Some of the reviewed studies showed burnout to be associated with certain biomarkers. For example, burnout was associated with increases in salivary cortisol in Monfared and colleagues' (2020) path analysis and Becker and colleagues (2021) found some correlations between burnout symptoms and CRP. However, when considered as a whole, findings were mixed. Moreover, and perhaps most noteworthy, only a small number of studies have been conducted in this area ($n = 8$).

Beyond the limited number of studies in sport, there are limitations to those that have examined biomarkers of burnout. First, most of the work is cross-sectional in nature. Cross-sectional designs can inform about associations between variables of interest; however, they cannot provide any information regarding the directionality or causality of a relationship (Danhof-Pont et al., 2011). To determine whether burnout causes changes in biomarkers, develops simultaneously, or is preceded by change, longitudinal research is required. There are a few noteworthy examples that have employed such designs. Martin and colleagues (2021), for example, conducted a three-wave design that showed a significant relationship between burnout and testosterone-cortisol ratio change in path analyses. However, these longitudinal studies have predominantly focused on discrete aspects of particular systems (e.g., nutrition status, Hew-Butler et al., 2021; HPA axis, Martin et al., 2021).

A systematic approach of exploring biomarkers linked to a range of bodily systems would be useful to expand on the current evidence base (e.g., ANS, HPA axis, immune system, metabolism, cardiovascular system, epigenetic mechanisms; Melamed et al., 2006; Bakusic et al., 2017). In line with research outside of sport, exploring acute (e.g., salivary cortisol) and chronic biomarkers (e.g., HbA1c) would allow for the examination of both *reactions* and *adaptations* (Van der Horn et al., 2020). Such a separation may further our understanding of burnout development and how it relates to biomarkers over time.

As we currently know very little about the relationship between burnout and biomarkers in sport, we are still in an exploratory phase of building and testing theory. In the present study, we aim to employ an *N*-of-1 design to provide preliminary support for possible relationships. This approach focuses on one or a few individuals over a period of time and allows for the assessment of a range of variables (Kwasnicka et al., 2019). Such an approach would provide a detailed examination of potential biomarkers and relationships over time (McDonald et al., 2020). These examinations can then inform the design of future studies with larger samples, which is important as biomarker studies are resource heavy (e.g., require financially expensive equipment and consumables).

1.5. The present study

Against this background, the aim of the present study was to examine the relationship between athlete burnout and biomarkers. To do so, we used an *N*-of-1 design. Based on existing theory and research, we chose to focus on biomarkers of the ANS (blood pressure, heart-rate variability), HPA axis (salivary and hair cortisol, DHEA-S, testosterone), immune system (CRP, sIgA), and metabolism (HbA1c, triglycerides, total cholesterol, HDL cholesterol) as well as providing an initial exploration of epigenetics (DNA methylation of BDNF, SLC6A4, NR3C1 genes). To help further understand these processes, we also quantified psychological stress. In presenting our work, we separated acute (from saliva: cortisol, DHEA-S, testosterone, testosterone/cortisol ratio, CRP, IgA) and chronic biomarkers (from blood and hair: HbA1c, triglycerides, total cholesterol, HDL cholesterol, hair cortisol, BDNF, SLC6A4, NR3C1 methylation). Due to the exploratory nature of this research, we had no specific hypotheses, however, our broad expectation was that burnout would be related to changes in the biomarkers.

2. Methods

2.1. Design

The present study used an *N*-of-1 design and was pre-registered on PsychArchives prior to data collection (Glandorf, 2022). The data, code, and materials are available on PsychArchives (https://doi.org/10.23668/psycharchives.6531).

2.2. Participants

We recruited four athletes – two to examine acute biomarkers from saliva, and two to examine chronic biomarkers from blood and hair.

Athletes were eligible if they were over 18 years old and actively competed in their sport. They were not eligible if they had a chronic health condition that affects the endocrine system, the cardiovascular system or the immune system or took medication that affects any of these systems, or were bald or had dyed hair (specific to hair biomarkers). Due to the possible similarity of symptoms between burnout and clinical depression, we excluded individuals who had been diagnosed with Major Depressive Disorder (Bianchi et al., 2015). Athlete samples for acute markers and chronic markers were kept separate to avoid anticipatory stress effects of blood collection on the levels of salivary biomarkers (e.g., Weckesser et al., 2014).

Acute biomarkers (saliva). The two athletes (A1, A2) assessed for acute biomarkers were female (sex assigned at birth). Athlete A1 was 21 years old at the start of the study. She was competing in athletics at an international level and had 14 years of competition experience. Across the season, her training hours ranged from 4 to 19 h/week. Athlete A2 was 20 years old. She was competing both in netball and cycling, and the study followed her over the netball season where she competed at a regional level (her primary sport). She had 13 years of competition experience. Across her season, the training hours ranged from 3 to 5 h/week.

Chronic biomarkers (blood and hair). The two athletes (C1, C2) assessed for chronic biomarkers were male (sex assigned at birth). Athlete C1 was 23 years old and competed in athletics at a national level. He had nine years of competition experience. Across the season, his training hours ranged from 6 to 12 h/week. Athlete C2 was 24 years old and competed in athletics at a national level. He had 10 years of competition experience. Across the season, his training hours ranged from 4 to 8 h/week.

2.3. Procedure

Before study commencement, ethical approval was received from the researchers' institutional ethics board and athletes provided informed consent.

For acute biomarkers (saliva), there were twelve data collection points, each approximately two weeks apart, so the collection spanned approximately 24 weeks in total (see Supplementary Figure A1 for an overview). The time gaps between sampling waves were based on the expected time it would take to see changes in these biomarkers (days to weeks; Martin et al., 2021). Twelve timepoints were chosen to cover the competitive season of the athletes and to allow for the use of dynamic regression modelling (our planned analysis; see statistical analyses below). Electrocardiograms were carried out at the beginning of each sampling collection. Afterwards, athletes were asked to rinse their mouth with water. They then completed the questionnaires. Blood pressure readings were taken. The saliva sample collection was carried out last after 10 min had passed following the mouth rinsing.

For the chronic biomarkers (blood and hair), there were six data collection points each approximately two months apart, covering 12

months in total (see Supplemental Figure A2 for an overview). The time gaps between sampling waves were based on the expected time it would take to see changes in these biomarkers (weeks to months; Danhof-Pont et al., 2011). Six timepoints were chosen to cover the whole athletic season of the athletes and to provide sufficient data for visual analysis over time. Electrocardiograms were carried out at the beginning of each sampling collection. Athletes then completed the questionnaires. Blood pressure readings were taken afterwards. The blood sample was taken after these readings. The hair sample was taken last. To minimise athletes' emotional discomfort due to repeatedly sampling hair over a period of time, hair samples were only taken at the first, second, and third timepoint.

2.4. Measures

2.4.1. Questionnaires

Athlete Burnout. We measured athlete burnout using the 15-item Athlete Burnout Questionnaire (ABQ; Raedeke & Smith, 2001). The ABQ has three dimensions: emotional and physical exhaustion (EPE), devaluation (DEV), and reduced sense of accomplishment (RSA), with five items each (see supplemental Table A for example items). Participants indicated the extent to which they have experienced each symptom and respond on a 5-point Likert scale from 1 (almost never) to 5 (almost always). The ABQ has previously shown strong psychometric properties (e.g., Grugan et al., 2024). Participants who were tested for acute biomarkers were asked to report their burnout symptoms over the last week to align the measure with the sampling schedule of the biomarkers. Participants who were tested for chronic biomarkers were asked to report their burnout symptoms over the last month (as described in the original instructions for the ABQ) and aligned with research showing burnout changes over approximately two months (Gerber et al., 2018; Raedeke & Smith, 2001). Thus, burnout measurements for those tested for acute markers were concurrent, while burnout measurements for participants tested for chronic markers had specific gaps between measurements.

Perceived Stress. We measured perceived stress using the 10-item Perceived Stress Scale (PSS-10; Cohen et al., 1983). The PSS-10 measures perceived stress by asking participants about the frequency of specific feelings and thoughts in response to different situations (see supplementary Table A for example items). Participants answer on a 5-point Likert type scale from 0 (never) to 4 (very often). The PSS-10 has shown good reliability and validity in athletes (e.g., Chiu et al., 2016). All participants were asked to report their stress symptoms over the past week as stress is expected to show changes over this period (see Cohen et al., 1983). Thus, all stress measurements showed gaps between measurements, with larger gaps between samples for those tested for chronic biomarkers than for acute biomarkers.[1]

2.4.2. Autonomic nervous system functioning

Blood Pressure. Systolic (SBP) and diastolic blood pressure (DBP) were recorded with a blood pressure monitor that has been validated for clinical use (M3 HEM-7154-E, Omron Healthcare UK). At each timepoint, three recordings were taken, and an average calculated.

Heart-Rate Variability. Electrocardiogram (ECG) monitoring was used to assess heartrate variability (HRV) using a Custo Med Cardio 100 BT monitor. A 5-min recording was taken at each timepoint. To estimate heart rate variability (HRV), we used the Root Mean Square of Successive Differences (RMSSD) between each heartbeat. This is a time-domain method that has been shown to be a reliable measure of HRV (Tegegne et al., 2018).

2.4.3. Acute salivary biomarkers

Saliva Collection. We used the passive drool method to avoid parasympathetic stimulation of salivary glands (Bosch, 2014) into polypropylene 2 mL cryovials to collect saliva samples. To account for the circadian rhythm of the salivary biomarkers, athletes provided samples between 2pm and 4pm (Pilger et al., 2018). Athletes were asked to refrain from eating, smoking, or exercising within the 2 h before collection of the sample. After practising the technique, 4-min samples were collected in order to calculate flow rate. The volume of saliva was recorded, and samples stored in ice until they were transferred to the laboratory. The sample was then centrifuged for 6 min at 10,000 x G to remove bacteria and mucins. Supernatants were removed and the sample stored at −80 °C until analysis.

Salivary Cortisol, Testosterone, DHEA-S, CRP, and sIgA Analysis. All samples were analysed in duplicates using commercial enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics) validated for the determination of the chosen biomarker.

2.4.4. Chronic blood biomarkers

Blood Collection. Venous blood samples were collected in a serum tube with silica particles and an ethylenediaminetetraacetic acid (EDTA) tube. Blood samples were centrifuged for 10 min at 1000 x G to obtain blood serum. Serum was then stored at −80 °C until use for blood lipid analysis. Blood from the EDTA tube was used for immediate HbA1c analysis as well as for DNA purification and later methylation analysis.

HbA1c Analysis. All samples were analysed in duplicates. Blood from the EDTA tube was analysed for HbA1c levels with a hand-held point of care device (Syringa, Medigenix, UK) on the same day as collection that has been validated for clinical use.

Blood Lipid Analysis. All samples were analysed in duplicates. Blood serum was used to analyse for triglycerides, total cholesterol, and HDL on an ABX Pentra 400 (Horiba ABX Diagnostics) that has been validated for research and clinical use. Triglycerides and HDL were measured with an enzymatic colorimetric method, while total cholesterol was measured directly using selective inhibition colorimetric assays.

DNA Methylation Analysis. Blood from the EDTA tube was used to purify DNA first, which was later analysed for methylation levels. DNA purification was carried out according to the manufacturer's instructions with PureLink Genomic DNA kits (Invitrogen, UK). Genomic DNA was then stored in −20 °C until further analysis. The purity and quantity of DNA were determined by a NanoDrop spectrophotometer. DNA methylation in the regions of the BDNF, SLC6A4 and NR3C1 genes were analysed via bisulfide conversion and next-generation sequencing.[2] Primers were selected based on primers from previous burnout research (Bakusic et al., 2020 for BDNF; Bakusic et al., 2021 for SLC6A4 and NR3C1). These primers targeted exon 1b and 4 in the BDNF gene, part of the CpG island overlapping with the SLC6A4 promoter region, and the whole CpG island of NR3C1 1F region (see supplementary Table B and C for gene target and primer information). For an outline of the protocol and steps, outcomes of each step, kits, equipment and settings used see supplemental Table D and E.

2.4.5. Chronic hair biomarkers

Hair Collection. Hair was collected in line with recommended guidelines for sample collection (Parker & Bristow, 2020). A 2-cm strand of hair (25–40 mg) was cut from the back of the head, as close to the scalp as possible. Hair samples were stored at room temperature in a breathable container in the laboratories where temperatures and humidity were controlled to ensure samples remained stable.

[1] For the chronic biomarkers, stress measurements at a previous timepoint likely had little effect on any of the measurements at the next timepoint. As the study focuses on the relationship between burnout and biomarkers, the time gaps between measurement were matched to this relationship and stress deemed less important to study design.

[2] This sequencing approach differs from what was specified in the pre-registration as the originally proposed approach is now outdated.

Hair Analysis. Hair washing and grinding as well as the following cortisol extraction from the collected hair were carried out as per the validated protocol detailed by Parker and Bristow (2020). The extracted cortisol was then analysed via commercial solid-phase ELISA kits (State College). Each sample post extraction was analysed in duplicates. For quality control, 15 % of samples were extracted in duplicates and then analysed in duplicates. Each sample was analysed for the first (first cm from scalp) and the second segment (second cm from the scalp) to analyse for the cortisol concentration from the last two months individually.

2.5. Statistical analysis

All statistical analyses were carried out in R and R Studio (4.2.2; R Core Team, 2023).

2.5.1. Pre-processing

In line with associated guidelines for the questionnaires we used for the measurement of psychological variables, we first computed total and subscale scores for each of the questionnaire-based measures (i.e., Raedeke & Smith, 2001; Cohen et al., 1983). The testosterone-to-cortisol ratio was calculated by dividing the testosterone levels by cortisol levels. For DHEA-S and sIgA, concentrations were corrected for salivary flow rate by multiplying the absolute concentration by the saliva flow rate. This is commonly recommended practice as the secretion of these biomarkers heavily depends on the flow rate (Bosch, 2014). Hair cortisol concentrations were corrected for the individual sample hair weight of the sample, the amount of methanol used for extraction, and the reconstitution volume. For the methylation results, the number of methylated and unmethylated reads was determined with Bismark. Results were filtered for loci with more than 100X coverage, before the percentage of methylation per target was calculated. Further details of pre-processing can be found in the supplemental materials Table F.

2.5.2. Dynamic regression modelling (acute biomarker analysis)

For acute biomarkers, we used dynamic regression modelling to explore the relationships between stress, burnout, and the biomarkers (while the chronic biomarkers were analysed with visual analyses only). We chose this modelling approach because dynamic regression modelling allowed us to model autoregressive effects for the predictor variable (i.e., stability), while also estimating different lagged effects from the predictor onto the outcome variable (see Fig. 1 for overview). Based on theoretical conceptualisations of the variables and previous research (e.g., Danhof-Pont et al., 2011; Glandorf et al., 2024; Melamed et al., 2006), we expected all variables to have autoregressive effects and since we were interested in lagged effects between the psychological and physiological variables, this approach was deemed appropriate.

Following guidelines for longitudinal data modelling (e.g., Brown & Stenling, 2024), we considered the existence of stationary and non-stationary change in all variables. While dynamic regression models assume stationary change (e.g., stable mean with variation over time), we expected some level of non-stationary change in burnout and stress over six months. To turn potential non-stationary change into stationary change and thereby fit the model, we used differencing. This is an approach that has been used in previous longitudinal research on athlete burnout and biomarkers (e.g., Martin et al., 2021). Thus, the employed models estimated how much of a difference in the outcome variable could be expected from a one-unit difference in the predictor variable from one timepoint to the next. Due to the exploratory nature of this study, we did not have particular hypotheses with regards to how much of a lag would be appropriate for the relationship between the psychological and physiological variables. However, as we used dynamic regression modelling for the acute biomarkers only and these biomarkers are expected to change on a short-term basis from days to weeks, we used both a lag of 0 and a lag of 1 for all models to explore whether the predictor simultaneously develops with the outcome variable (0 lag) or is able to predict the outcome variable at the next timepoint (1 lag; McDonald et al., 2020).[3] We included both lags simultaneously in our models to allow us to differentiate between contemporaneous development of the outcome and predictor and a lagged response in the outcome variable. We ran predictive (psychological variables predicting biomarkers) and reciprocal models (psychological variables serving as the outcome) separately (90 models for each athlete). To determine statistical significance, we used an alpha value of $p < .05$.[4]

2.5.3. Visual analysis (acute and chronic biomarker analysis)

For each athlete, figures with each psychological variable (stress, total burnout, EPE, DEV, RSA) and biomarker (Acute: salivary cortisol, testosterone, DHEA-s, CRP, sIgA; Chronic: hair cortisol, HbA1c, triglycerides, total cholesterol, HDL, DNA methylation; Both: BP, HRV) were created to visualise their development over time. These were visually analysed by all authors for potential relations between stress and burnout with the biomarkers. In line with recommendations for visual analysis in single-case research, visual inspection focused on evaluating the overall trend over time with a focus on the shape of each development and to what extent the shapes of the psychological construct and biomarker matched (Ledford et al., 2018). Timepoint-to-timepoint change in direction (increase vs. decrease) was further assessed and compared while considering a simultaneous development and a lagged development of psychological variables to biomarkers.

3. Results

We now present the findings for acute and chronic biomarkers, and in doing so we present each participant's findings individually.

3.1. Acute biomarkers

The analyses for acute biomarkers were based on dynamic regression modelling. Where significant relationships were found, we followed this with visual analysis.

3.2. Descriptive statistics

Descriptive statistics in terms of mean, SDs, and ranges were calculated for each athlete for all variables and presented together with reference levels from previous research (see supplementary Table G). Compared to Athlete A2, Athlete A1 generally showed lower athlete burnout levels both on the total score (A1 = 2.01 ± .27; A2 = 2.17 ± .16) and the dimensional scores apart from RSA where Athlete A1 showed higher levels than Athlete A2 (A1 = 2.52 ± .26; A2 = 2.13 ± .23). On average, stress levels were also lower in athlete A1 (A1 range = 3–9; A2 range = 11–19). See supplementary Table H for a comparison of all variables to reference levels.

[3] This approach differs from the pre-registration where we only considered a lag of 1. Based on reviewers suggestions, we adopted a model that considers both a lag of 0 and lag of 1 as it represents a better exploratory approach.

[4] To determine whether the effects of the dynamic regression models were sufficiently powered, we ran a sensitivity analysis in R (4.2.2; R Core Team, 2023). The analysis was based on a data simulation matched to the study (total sample size and timepoints), fitting a dynamic regression model matched to the study (two lagged effects, one autoregressive effect), and a power simulation based on the simulated data and specified model (n = 1000 simulations). The sensitivity analysis found standardised effects (β) above (−).53 to be sufficiently powered (power = 80.3 %).

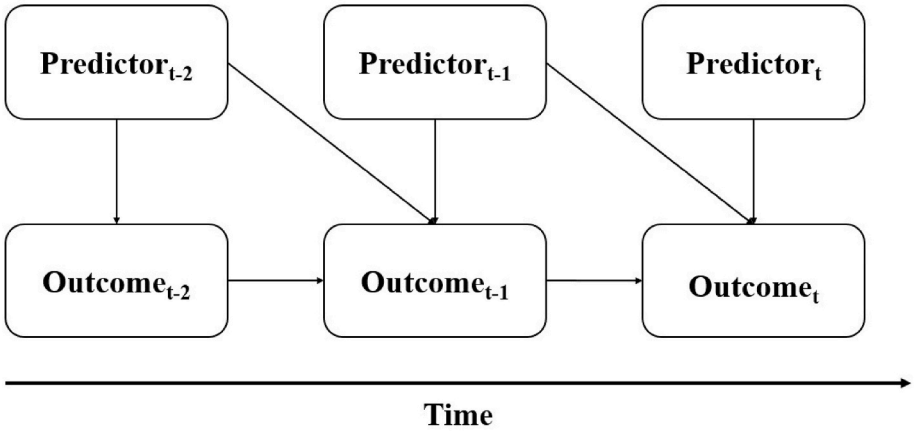


Fig. 1. Overview of the Theoretical Dynamic Regression Models. *Note.* Burnout and stress were used as predictor variables first, while biomarkers were used as outcome variables. Then, in separate models, burnout and stress were used as outcome variables, while biomarkers were used as predictor variables. The lag of 0 and lag of 1 were run in the same model together with the autoregressive effect of the outcome predicting itself at the next timepoint.

3.3. Dynamic regression modelling and visual analysis

Model overviews and regression results are shown in Tables 1 and 2. Dynamic regression models for stress, total burnout, and the burnout dimensions and biomarkers were calculated separately. To improve readability, statistics for significant results are reported in-text, while all other results can be found in Tables 1 and 2

Blood Pressure. For Athlete A1, stress was found to significantly predict decreases in DBP at the next timepoint ($F(3,6) = 6.05$, $p < .05$, $b = -1.53$, $\beta = -1.07$, $R^2 = .75$, see Table 2). This relationship was also evident in the visualisation where increases in stress occurred prior to decreases in DBP (see Fig. 2A). For Athlete A2, all models predicting DBP were found to be significant. However, this was driven by the autoregressive effects of DBP (see Table 1). This was the same for the significant model predicting EPE from SBP (see Table 1).

Heartrate Variability. Athlete A2 showed a significant model for total burnout and HRV and a significant reciprocal model for HRV and EPE. However, these were driven by autoregressive effects.

Salivary Cortisol. For the psychological variables predicting salivary cortisol, none of the models were significant. For Athlete A1, however, in a significant reciprocal model, salivary cortisol was found to significantly predict increases in stress at the next timepoint ($F(3,6) = 9.77$, $p < .05$, $b = 27.59$, $\beta = .69$, $R^2 = .83$, see Table 2). This relationship was also evident in the visualisation where increases in salivary cortisol occurred prior to increases in stress (see Fig. 2B). Athlete A2 also showed a reciprocal model for salivary cortisol and EPE, however, this was driven by autoregressive effects.

Testosterone. For Athlete A1, DEV was shown to significantly negatively predict testosterone at the next timepoint ($F(3,6) = 5.3$, $p < .05$, $b = -186.49$, $\beta = -.64$, $R^2 = .73$, see Table 2). This relationship was also evident in the visualisation where increases in DEV occurred prior to decreases in testosterone (see Fig. 2C). Athlete A2 further showed a significant model for RSA and testosterone, however, no significant relationships were found (see Tables 1 and 2).

For Athlete A2, in a significant reciprocal model, testosterone was shown to develop simultaneously with increases in stress ($F(3,6) = 5.65$, $p < .05$, $b = .02$, $\beta = .67$, $R^2 = .74$, see Tables 1 and 2). This relationship was also visible in the visualisation where increases in stress occurred at the same time as increases in testosterone (see Fig. 2D).

TC-Ratio. For Athlete A1, DEV was shown to significantly negatively predict T/C ratio at the next timepoint ($F(3,6) = 9.48$, $p < .05$, $b = -1585.31$, $\beta = -.51$, $R^2 = .83$, see Tables 1 and 2). This was visible in the visualisation where increases in DEV occurred prior to decreases in TC-Ratio (Fig. 3A). Athlete A2 also showed a significant model for RSA and T/C ratio but this model showed no significant relationships (see Table 1). The reciprocal models showed significant models for T/C ratio and EPE and T/C ratio and stress, however, this was driven by autoregressive effects.

DHEA-S Flow Rate. For Athlete A1, DEV developed simultaneously with decreases in DHEA-S flow rate with marginal significance ($F(3,6) = 4.85$, $p = .05$, $b = -429.52$, $\beta = -.61$, $R^2 = .71$, see Table 2). This relationship was also evident in the visualisation where increases in DHEA-S flow rate occurred simultaneously with decreases in DEV (Fig. 3B). In Athlete A2, significant models for DHEA-S flow rate and EPE and DHEA-S flow rate and RSA were shown, however, this was driven by autoregressive effects.

CRP. For the psychological variables predicting CRP, none of the models were significant. The reverse direction showed significant models for CRP predicting EPE in Athlete A2. However, this was driven by autoregressive effects.

sIgA Flow Rate. For Athlete A1, total burnout was shown to significantly positively predict sIgA flow rate at the next timepoint ($F(3,6) = 4.88$, $p < .05$, $b = 83.41$, $\beta = .56$, $R^2 = .71$, see Table 2). This relationship was also evident in the visualisation where increases in total burnout occurred prior to increases in sIgA flow rate (see Fig. 3C). In the reciprocal model, sIgA flow rate was also found to develop simultaneously with decreases in DEV ($F(3,6) = 4.95$, $p < .05$, $b = -.01$, $\beta = -.76$, $R^2 = .71$, see Table 2). This relationship was also evident in the visualisation where increases in DEV occurred simultaneously with decreases in sIgA flow rate (see Fig. 3D). For Athlete A2, the reciprocal models of sIgA flowrate and total burnout, EPE, and DEV were significant as well. However, in the models for total burnout and EPE, this was driven by autoregressive effects. In the model for DEV, sIgA flow rate showed to predict decreases in DEV at the next timepoint ($F(3,6) = 3.40$, $p < .05$, $b = -.00$, $\beta = -.62$, $R^2 = .63$, see Table 2). This relationship was also evident in the visualisation in the beginning of the study where increases in sIgA flow rate occurred prior to decreases in DEV (see Fig. 3D). This relationship, however, was not present for the later timepoints.

3.4. Chronic biomarkers

The analyses for chronic biomarkers were based on visual analysis only.

3.5. Descriptive statistics

Descriptive statistics in terms of mean, SDs, and ranges were calculated for each athlete over the course of the study for all variables and presented together with reference levels from previous research (see

Table 1
Overview of Dynamic Regression Models for Acute Biomarkers, Burnout Dimensions, and Stress for each Athlete.

		Athlete A1						Athlete A2					
		Psych → Biomarker			Biomarker → Psych			Psych → Biomarker			Biomarker → Psych		
BM	BO	F (3,6)	p-value	R^2	F (3,6)	p-value	R^2	F (3,6)	p-value	R^2	F (3,6)	p-value	R^2
DBP	TB	1.01	.45	.34	2.26	.18	.53	12.38	**<.01**	.86	.68	.52	.73
	EPE	1.99	.22	.50	.09	.97	.04	6.91	**<.05**	.78	7.12	**<.05**	.78
	DEV	.55	.67	.22	2.23	.19	.53	5.02	**<.05**	.72	.12	.95	.06
	RSA	.54	.67	.21	.50	.70	.20	8.66	**<.05**	.81	1.48	.31	.42
	Stress	6.05	**<.05**	.75	2.34	.17	.54	6.46	**<.05**	.76	3.05	.11	.60
SBP	TB	.82	.53	.29	.63	.62	.24	.98	.46	.33	4.13	.07	.67
	EPE	.90	.50	.31	1.40	.33	.41	.95	.47	.32	6.26	**<.05**	.76
	DEV	1.72	.26	.88	1.48	.31	.43	.96	.47	.33	.18	.90	.08
	RSA	.80	.54	.29	.35	.79	.15	1.06	.43	.35	3.10	.11	.61
	Stress	1.11	.42	.36	1.68	.27	.46	1.33	.35	.40	2.53	.15	.56
HRV	TB	3.00	.13	.64	2.09	.22	.56	1.02	**<.01**	.34	3.32	.10	.62
	EPE	1.21	.40	.42	.38	.77	.19	1.68	.27	.46	8.48	**<.05**	.81
	DEV	1.91	.25	.54	.52	.69	.24	1.64	.28	.45	1.09	.42	.35
	RSA	1.05	.45	.39	.59	.65	.26	.88	.50	.31	1.10	.42	.36
	Stress	.98	.47	.37	3.81	.09	.70	1.40	.33	.41	2.30	.18	.54
Cor	TB	.52	.69	.21	.83	.52	.29	.54	.67	.21	3.34	.10	.63
	EPE	.05	.98	.02	.43	.74	.18	.68	.60	.25	6.35	**<.05**	.76
	DEV	.88	.50	.31	1.21	.38	.38	3.58	.09	.64	.24	.87	.11
	RSA	.15	.93	.07	.60	.64	.23	.73	.57	.27	1.38	.34	.41
	Stress	.04	.99	.02	9.77	**<.05**	.83	.81	.53	.29	1.88	.23	.49
Tes	TB	1.67	.27	.46	.58	.65	.22	4.26	.06	.68	3.31	.10	.63
	EPE	1.45	.32	.42	1.19	.39	.37	1.57	.29	.44	6.58	**<.05**	.77
	DEV	5.30	**<.05**	.73	1.20	.39	.38	1.04	.44	.34	.21	.88	.10
	RSA	1.16	.40	.37	.47	.71	.19	6.7	**<.05**	.77	1.91	.23	.49
	Stress	1.33	.35	.40	1.72	.26	.46	4.09	.07	.67	5.65	**<.05**	.74
T/C	TB	3.10	.11	.61	.55	.67	.22	2.62	.15	.57	3.24	.10	.62
	EPE	1.42	.33	.42	.06	.98	.03	.95	.48	.32	6.94	**<.05**	.78
	DEV	9.48	**<.05**	.83	.76	.56	.28	.91	.49	.31	.17	.91	.08
	RSA	1.58	.29	.44	.50	.70	.20	5.24	**<.05**	.72	1.74	.26	.47
	Stress	.55	.32	.73	2.57	.15	.56	2.82	.13	.59	5.00	**<.05**	.71
DHE	TB	1.67	.27	.45	.97	.47	.33	.48	.71	.19	4.59	.05	.70
	EPE	.84	.52	.30	.01	.99	.01	.35	.79	.15	6.16	**<.05**	.76
	DEV	4.85	**<.05**	.71	2.73	.14	.58	.40	.76	.17	.29	.83	.13
	RSA	.92	.49	.32	1.22	.38	.38	2.08	.20	.51	8.13	**<.05**	.80
	Stress	1.19	.39	.37	1.48	.31	.43	.80	.54	.29	3.73	.08	.65
CRP	TB	.47	.71	.19	1.14	.41	.36	.82	.53	.29	4.12	.07	.67
	EPE	.96	.47	.32	.68	.59	.26	1.01	.45	.34	7.51	**<.05**	.79
	DEV	.22	.88	.10	.62	.63	.24	.78	.55	.28	3.12	.11	.61
	RSA	.18	.91	.08	.42	.75	.17	.44	.73	.18	2.14	.20	.52
	Stress	1.12	.41	.36	2.56	.15	.56	2.75	.14	.58	2.40	.17	.55
sIgA	TB	4.88	**<.05**	.71	1.33	.35	.40	2.62	.15	.57	6.79	**<.05**	.77
	EPE	.62	.63	.24	.07	.97	.03	1.45	.32	.42	6.39	**<.05**	.76
	DEV	4.31	.06	.68	4.95	**<.05**	.71	3.93	.07	.66	3.40	**<.01**	.63
	RSA	3.16	.11	.61	2.24	.19	.53	1.51	.30	.43	1.67	.27	.46
	Stress	.34	.80	.14	2.18	.19	.52	.91	.49	.31	1.79	.25	.47

Note. Each combination of biomarker and burnout (dimensions) constitutes a single model. BM = Biomarker, BO = Burnout, TB = Total Burnout, EPE = Emotional and Physical Exhaustion, DEV = Devaluation, RSA = Reduced Sense of Accomplishment, DBP = Diastolic Blood Pressure, SBP = Systolic Blood Pressure, HRV = Heartrate Variability, Cor = Cortisol, Tes = Testosterone, T/C = Testosterone/Cortisol Ratio, DHE = DHE Rate, CRP = C-reactive Protein, sIgA = secretory Immunoglobulin A Flow Rate.

supplemental Table G). Athlete C1 generally showed lower athlete burnout levels both on the total score and the dimensional scores compared to Athlete C2. On average, stress levels were similar for Athlete C1 and C2 (C1 = 11.17 ± 3.86; C2 = 11.20 ± 2.95). See supplemental Table H for a comparison of all variables to reference levels.

3.6. Visual analyses

Blood Pressure. Athlete C1 showed a negative, simultaneous relationship between RSA and DBP from timepoint 0 to 3, but not beyond this (see Figure B). Athlete C2 showed a negative, simultaneous relationship between EPE and DBP from timepoint 2 to 4, but not before this (see Figure B). Athlete C1 further showed a positive, simultaneous relationship between stress and DBP from timepoint 0 to 4, but not beyond this (see Figure B). There was no relationship between stress and DBP for Athlete C2.

Athlete C1 showed a negative, simultaneous relationship between total athlete burnout and SBP from timepoint 0 to 3, but not beyond this (see Figure C). Athlete C1 showed a positive, simultaneous relationship between stress and SBP from timepoint 0 to 2 only, but not beyond this (see Figure C). Athlete C2 did not show any relationships. For all figures, see supplemental Figure B and C.

Heartrate Variability. Athlete C1 and C2 showed a negative, simultaneous relationship between total athlete burnout and HRV from timepoint 0 to 3, but not beyond this (see Figure D). In Athlete C1, this appeared to be the same for the burnout dimensions, while Athlete C2 only showed this for RSA. Neither of the athletes showed a relationship between stress and HRV. For all figures, see supplementary Figure D.

Triglycerides. Neither Athlete C1 or C2 showed a relationship between total athlete burnout or the burnout dimensions and triglycerides. Athlete C1 showed a positive, simultaneous development of stress and triglycerides, with a decline at the beginning of the study and an increase at the end of the study (see Figure E). However, the variability in triglycerides was low and could represent variation around a constant mean. For all figures, see supplementary Figure E.

Total cholesterol. Neither Athlete C1 or C2 showed a relationship

Table 2
Overview of Regression Results for Models of Acute Biomarkers, Burnout Dimensions, and Stress for each Athlete.

		Athlete A1						Athlete A2					
		Psych → Biomarker			Biomarker → Psych			Psych → Biomarker			Biomarker → Psych		
BM	BO	Pred	*b (β)*	*p*-val	Pred	*b (β)*	*p*-val	Pred	*b (β)*	*p*-val	Pred	*b (β)*	*p*-val
DBP	TB	DBP	.01 (.02)	.98	TB	−.28 (−.29)	.44	DBP	−.31 (−.31)	.31	TB	−.29 (−.30)	.62
		TB L0	16.06 (.66)	.16	DBP L0	.02 (.47)	.16	TB L0	5.50 (.16)	.59	DBP L0	.01 (.36)	.59
		TB L1	7.50 (.31)	.48	DBP L1	−.01 (−.34)	.38	TB L1	−20.97 (−.58)	.10	DBP L1	−.01 (−.26)	.59
	EPE	DBP	−.29 (−.30)	.35	EPE	−.06 (−.06)	.93	DBP	−.57 (−.58)	.11	EPE	−.63 (−.64)	.09
		EPE L0	.97 (.09)	.77	DBP L0	.02 (.18)	.77	EPE L0	2.67 (.17)	.70	DBP L0	.01 (.17)	.70
		EPE L1	7.25 (.69)	.06	DBP L1	−.01 (−.11)	.82	EPE L1	−3.84 (−.25)	.57	DBP L1	−.01 (−.16)	.68
	DEV	DBP	.07 (.07)	.88	DEV	−.20 (−.21)	.52	DBP	−.87 (−.88)	**<.01**	DEV	−.13 (−.14)	.64
		DEV L0	4.05 (.28)	.61	DBP L0	.01 (.17)	.61	DEV L0	−4.94 (−.08)	.74	DBP L0	−.00 (−.18)	.74
		DEV L1	−4.49 (−.32)	.46	DBP L1	−.04 (−.58)	.09	DEV L1	−2.49 (−.06)	.80	DBP L1	−.00 (−.12)	.83
	RSA	DBP	−.01 (−.01)	.98	RSA	−.27 (−.28)	.53	DBP	−.55 (−.56)	.08	RSA	−.22 (−.23)	.68
		RSA L0	4.09 (.25)	.55	DBP L0	.02 (.24)	.55	RSA L0	4.37 (.13)	.61	DBP L0	.01 (.40)	.61
		RSA L1	−4.47 (−.30)	.52	DBP L1	.00 (.03)	.94	RSA L1	−12.96 (−.38)	.20	DBP L1	−.00 (−.11)	.87
	Stress	DBP	.12 (.03)	.89	Stress	−1.20 (−1.23)	.05	DBP	−.85 (−.85)	**<.05**	Stress	−.60 (−.62)	.12
		Stress L0	−.52 (−.37)	.21	DBP L0	−.48 (−.71)	.21	Stress L0	−.64 (−.34)	.31	DBP L0	−.27 (−.56)	.31
		Stress L1	−1.53 (−1.07)	**<.01**	DBP L1	.01 (.02)	.96	Stress L1	−.56 (−.29)	.38	DBP L1	−.11 (−.22)	.71
SBP	TB	SBP	−.54 (−.54)	.17	TB	−.45 (−.47)	.26	SBP	−.54 (−.55)	.24	TB	−.67 (−.70)	**<.05**
		TB L0	−2.24 (−.07)	.86	SBP L0	−.00 (−.08)	.86	TB L0	6.49 (.15)	.81	SBP L0	.00 (.07)	.81
		TB L1	−2.11 (−.07)	.86	SBP L1	−.01 (−.27)	.57	TB L1	4.29 (.10)	.87	SBP L1	−.01 (−.24)	.49
	EPE	SBP	−.46 (−.47)	.35	EPE	.24 (.25)	.50	SBP	−.58 (−.58)	.17	EPE	−.83 (−.85)	**<.01**
		EPE L0	1.90 (.14)	.75	SBP L0	.01 (.13)	.75	EPE L0	.99 (.05)	.94	SBP L0	.00 (.02)	.94
		EPE L1	1.02 (.08)	.84	SBP L1	−.05 (−.63)	.18	EPE L1	.66 (.04)	.96	SBP L1	−.01 (−.12)	.66
	DEV	SBP	−.44 (−.45)	.23	DEV	−.40 (−.42)	.26	SBP	−.57 (−.58)	.17	DEV	−.09 (−.10)	.75
		DEV L0	−7.73 (−.42)	.28	SBP L0	−.02 (−.45)	.28	DEV L0	1.97 (.03)	.95	SBP L0	.00 (.02)	.95
		DEV L1	−6.34 (−.36)	.32	SBP L1	.00 (.08)	.84	DEV L1	−2.11 (−.04)	.91	SBP L1	−.00 (−.15)	.68
	RSA	SBP	−.52 (−.52)	.96	RSA	−.36 (−.38)	.35	SBP	−.48 (−.49)	.38	RSA	−.29 (−.31)	.36
		RSA L0	.33 (.01)	.98	SBP L0	.00 (.01)	.98	RSA L0	9.95 (.24)	.67	SBP L0	.03 (.15)	.68
		RSA L1	.22 (.02)	.96	SBP L1	−.00 (−.06)	.89	RSA L1	5.54 (.14)	.76	SBP L1	−.01 (−.53)	.20
	Stress	SBP	−.47 (−.47)	.24	Stress	−.58 (−.59)	.14	SBP	−.62 (−.63)	.14	Stress	−.66 (−.67)	.07
		Stress L0	−.60 (−.33)	.45	SBP L0	−.17 (−.30)	.45	Stress L0	−.24 (−.10)	.84	SBP L0	−.03 (−.08)	.84
		Stress L1	−.27 (−.15)	.74	SBP L1	−.02 (−.05)	.91	Stress L1	−.84 (−.36)	.47	SBP L1	.11 (.26)	.51
HRV	TB	HRV	−.31 (−.32)	.47	TB	−.29 (−.30)	.57	HRV	−.48 (−.49)	.23	TB	−.76 (−.79)	**<.05**
		TB L0	48.78 (.58)	.12	HRV L0	.01 (.74)	.12	TB L0	−33.38 (−.20)	.73	HRV L0	−.00 (−.12)	.73
		TB L1	−10.38 (−.12)	.79	HRV L1	.00 (.38)	.46	TB L1	4.94 (.03)	.96	HRV L1	.00 (.00)	.99
	EPE	HRV	−.54 (−.57)	.21	EPE	−.02 (−.02)	.97	HRV	−.35 (−.36)	.36	EPE	−.75 (−.76)	**<.05**
		EPE L0	13.31 (.37)	.35	HRV L0	.01 (.49)	.35	EPE L0	−56.39 (−.78)	.27	HRV L0	−.00 (−.27)	.27
		EPE L1	4.37 (.12)	.76	HRV L1	.00 (.16)	.79	EPE L1	31.74 (−.44)	.50	HRV L1	.00 (.03)	.90
	DEV	HRV	−.38 (−.39)	.30	DEV	−.46 (−.49)	.40	HRV	−.41 (−.42)	.27	DEV	−.11 (−.12)	.65
		DEV L0	2.31 (.05)	.90	HRV L0	.00 (.07)	.90	DEV L0	122.53 (.42)	.27	HRV L0	.00 (.34)	.27
		DEV L1	−25.40 (−.53)	.23	HRV L1	.00 (.11)	.83	DEV L1	36.58 (.19)	.58	HRV L1	−.00 (−.09)	.76
	RSA	HRV	−.46 (−.49)	.30	RSA	−.34 (−.35)	.49	HRV	−.55 (−.57)	.16	RSA	−.59 (−.62)	.13
		RSA L0	17.78 (.32)	.44	HRV L0	.01 (.37)	.44	RSA L0	−19.48 (−.12)	.79	HRV L0	−.00 (−.12)	.79
		RSA L1	−1.01 (−.02)	.97	HRV L1	.00 (.17)	.75	RSA L1	−6.21 (−.04)	.93	HRV L1	−.00 (−.14)	.74
	Stress	HRV	−.51 (−.53)	.22	Stress	−.71 (−.73)	**<.05**	HRV	−.56 (−.58)	.14	Stress	−.75 (−.76)	**<.05**
		Stress L0	−2.74 (−.57)	.46	HRV L0	−.04 (−.21)	.46	Stress L0	−3.28 (−.37)	.44	HRV L0	−.03 (−.30)	.44
		Stress L1	−2.27 (−.46)	.48	HRV L1	−.01 (−.05)	.86	Stress L1	−4.45 (−.48)	.32	HRV L1	−.01 (−.06)	.88
Cor	TB	Cor	.52 (.04)	.89	TB	−.43 (−.44)	.34	Cor	−.48 (−.47)	.27	TB	−.80 (−.83)	**<.05**
		TB L0	−.08 (−.18)	.49	Cor L0	−1.07 (−.28)	.49	TB L0	−.10 (−.26)	.69	Cor L0	−.30 (−.12)	.69
		TB L1	.06 (.14)	.62	Cor L1	−.45 (−.20)	.64	TB L1	−.08 (−.21)	.75	Cor L1	−.23 (−.09)	.77
	EPE	Cor	−.02 (−.02)	.94	EPE	−.02 (−.03)	.94	Cor	−.42 (−.41)	.33	EPE	−.88 (−.90)	**<.01**
		EPE L0	.02 (−.01)	.96	Cor L0	−.17 (−.02)	.96	EPE L0	−.00 (−.00)	.99	Cor L0	−.00 (−.00)	.99
		EPE L1	−.00 (−.09)	.73	Cor L1	−2.36 (−.44)	.30	EPE L1	.04 (.27)	.74	Cor L1	−.85 (−.14)	.57
	DEV	Cor	.04 (.04)	.86	DEV	−.25 (−.27)	.48	Cor	−.45 (−.44)	.14	DEV	−.31 (−.32)	.44
		DEV L0	−.07 (−.26)	.31	Cor L0	−2.59 (−.39)	.31	DEV L0	−.11 (−.18)	.53	Cor L0	−.61 (−.29)	.53
		DEV L1	.03 (.13)	.59	Cor L1	1.00 (.25)	.47	DEV L1	−.31 (−.72)	**<.05**	Cor L1	−.17 (−.08)	.83
	RSA	Cor	−.01 (−.01)	.98	RSA	−.24 (−.25)	.58	Cor	−.48 (−.47)	.27	RSA	−.60 (−.63)	.12
		RSA L0	−.05 (−.17)	.54	Cor L0	−1.40 (−.22)	.54	RSA L0	.13 (.36)	.45	Cor L0	.75 (.30)	.45
		RSA L1	−.02 (−.07)	.82	Cor L1	.76 (.20)	.65	RSA L1	.07 (.19)	.70	Cor L1	.53 (.19)	.63
	Stress	Cor	.01 (.01)	.99	Stress	−.60 (−.62)	.01	Cor	−.45 (−.43)	.28	Stress	−.73 (−.74)	.06
		Stress L0	−.00 (−.05)	.93	Cor L0	−1.01 (−.01)	.93	Stress L0	−.00 (−.08)	.89	Cor L0	−2.50 (−.06)	.88
		Stress L1	.00 (.04)	.93	Cor L1	27.59 (.69)	**<.01**	Stress L1	−.01 (−.38)	.48	Cor L1	4.36 (.09)	.80
Tes	TB	Tes	−.53 (−.56)	.13	TB	−.49 (−.52)	.26	Tes	−.12 (−.12)	.69	TB	−.91 (−.94)	.09
		TB L0	−78.51 (−.15)	.67	Tes L0	−.00 (−.22)	.67	TB L0	28.04 (.05)	.90	Tes L0	.00 (.06)	.90
		TB L1	−204.34 (−.40)	.29	Tes L1	−.00 (−.22)	.66	TB L1	446.42 (.79)	.11	Tes L1	−.00 (−.12)	.74
	EPE	Tes	−.74 (−.78)	.08	EPE	.09 (.09)	.80	Tes	−.43 (−.44)	.25	EPE	−.83 (−.85)	**<.05**
		EPE L0	−77.87 (−.36)	.38	Tes L0	−.00 (−.40)	.38	EPE L0	27.80 (.12)	.86	Tes L0	.00 (.05)	.86
		EPE L1	11.78 (.05)	.87	Tes L1	−.00 (−.78)	.11	EPE L1	116.58 (.49)	.45	Tes L1	.00 (.18)	.48
	DEV	Tes	−.64 (−.67)	**<.05**	DEV	−.33 (−.35)	.52	Tes	−.51 (−.52)	.19	DEV	−.15 (−.16)	.61
		DEV L0	4.83 (.02)	.96	Tes L0	.00 (.04)	.96	DEV L0	−74.01 (−.08)	.83	Tes L0	−.00 (−.08)	.83
		DEV L1	−186.49 (−.64)	**<.05**	Tes L1	.00 (.47)	.39	DEV L1	117.49 (.18)	.62	Tes L1	−.00 (−.21)	.55
	RSA	Tes	−.59 (−.62)	.13	RSA	−.37 (−.39)	.33	Tes	−.07 (−.07)	.80	RSA	−.29 (−.31)	.65
		RSA L0	68.35 (.20)	.59	Tes L0	.00 (.24)	.59	RSA L0	−136.55 (−.26)	.34	Tes L0	−.00 (−.61)	.34
		RSA L1	39.11 (.13)	.75	Tes L1	.00 (.21)	.66	RSA L1	345.65 (.66)	.09	Tes L1	−.00 (−.33)	.45

(*continued on next page*)

Table 2 (*continued*)

		Athlete A1						Athlete A2					
		Psych → Biomarker			Biomarker → Psych			Psych → Biomarker			Biomarker → Psych		
	Stress	Tes	−.55 (−.58)	.15	Stress	−.61 (−.63)	.11	Tes	−.35 (−.36)	.35	Stress	−.55 (−.56)	.15
		Stress L0	−9.34 (−.32)	.48	Tes L0	−.01 (−.29)	.48	Stress L0	23.56 (.80)	**<.05**	Tes L0	.02 (.67)	**<.05**
		Stress L1	−8.86 (−.30)	.51	Tes L1	−.00 (−.02)	.97	Stress L1	8.47 (.28)	.54	Tes L1	.01 (.26)	.48
T/C	TB	T/C	−.38 (−.40)	.17	TB	−.32 (−.33)	.53	T/C	−.12 (−.12)	.71	TB	−.89 (−.92)	.07
		TB L0	610.31 (.11)	.67	T/C L0	.00 (.26)	.67	TB L0	346.90 (.10)	.82	T/C L0	.00 (.10)	.82
		TB L1	−2017.13 (−.37)	.22	T/C L1	.00 (.12)	.81	TB L1	2624.68 (.77)	.16	T/C L1	−.00 (−.04)	.91
	EPE	T/C	−.54 (−.56)	.10	EPE	.06 (.06)	.89	T/C	−.39 (−.41)	.32	EPE	−.82 (−.84)	**<.01**
		EPE L0	−2.22 (−.00)	.99	T/C L0	−.00 (−.00)	.99	EPE L0	203.46 (.14)	.85	T/C L0	.00 (.05)	.85
		EPE L1	−129.60 (−.06)	.85	T/C L1	−.00 (−.18)	.76	EPE L1	630.60 (.43)	.55	T/C L1	.00 (.21)	.39
	DEV	T/C	−.55 (−.58)	**<.05**	DEV	−.06 (−.07)	.92	T/C	−.40 (−.41)	.29	DEV	−.16 (−.16)	.60
		DEV L0	394.83 (.12)	.51	T/C L0	.00 (.57)	.51	DEV L0	23.07 (.00)	.99	T/C L0	.00 (.00)	.99
		DEV L1	−1585.31 (−.51)	**<.05**	T/C L1	.00 (.55)	.40	DEV L1	1199.14 (.31)	.43	T/C L1	−.00 (−.15)	.65
	RSA	T/C	−.55 (−.58)	.12	RSA	−.29 (−.30)	.49	T/C	.01 (.01)	.97	RSA	−.30 (−.32)	.64
		RSA L0	565.95 (.16)	.61	T/C L0	.00 (.24)	.61	RSA L0	−857.30 (−.27)	.37	T/C L0	−.00 (−.54)	.37
		RSA L1	350.31 (.11)	.76	T/C L1	.00 (.01)	.99	RSA L1	2170.00 (.68)	.10	T/C L1	−.00 (−.23)	.59
	Stress	T/C	−.65 (−.68)	**<.05**	Stress	−.72 (−.74)	**<.05**	T/C	−.37 (−.39)	.33	Stress	−.64 (−.65)	.09
		Stress L0	−133.06 (−.43)	.24	T/C L0	−.00 (−.49)	.24	Stress L0	148.00 (.82)	.07	T/C L0	.00 (.59)	.07
		Stress L1	−115.69 (−.37)	.31	T/C L1	−.00 (−.55)	.19	Stress L1	81.39 (.44)	.36	T/C L1	.00 (.23)	.52
DHE	TB	DHE	−.21 (−.19)	.69	TB	−.10 (−.11)	.84	DHE	−.19 (−.20)	.66	TB	−.74 (−.77)	**<.05**
		TB L0	−409.75 (−.35)	.37	DHE L0	−.00 (−.43)	.37	TB L0	−1527.94 (−.56)	.42	DHE L0	−.00 (−.22)	.42
		TB L1	374.31 (.32)	.51	DHE L1	.00 (.15)	.78	TB L1	−1206.41 (−.43)	.50	DHE L1	.00 (.17)	.54
	EPE	DHE	−.61 (−.57)	.17	EPE	.03 (.03)	.94	DHE	−.31 (−.32)	.45	EPE	−.87 (−.89)	**<.01**
		EPE L0	−28.76 (−.06)	.88	DHE L0	−.00 (−.08)	.88	EPE L0	330.32 (.28)	.73	DHE L0	.00 (.08)	.73
		EPE L1	19.86 (.04)	.92	DHE L1	−.00 (−.09)	.87	EPE L1	516.92 (.44)	.59	DHE L1	.00 (.11)	.65
	DEV	DHE	−.12 (−.11)	.76	DEV	.07 (.07)	.88	DHE	−.31 (−.33)	.47	DEV	−.10 (−.11)	.72
		DEV L0	−429.52 (−.61)	.05	DHE L0	−.00 (−.84)	.05	DEV L0	−1046.65 (−.22)	.60	DHE L0	−.00 (−.16)	.60
		DEV L1	231.86 (.35)	.38	DHE L1	−.00 (−.08)	.86	DEV L1	−697.10 (−.22)	.61	DHE L1	.00 (.10)	.74)
	RSA	DHE	−.69 64)	.19	RSA	−.20 (−.21)	.54	DHE	.13 (.14)	.78	RSA	−.49 (−.52)	.06
		RSA L0	145.22 (.19)	.69	DHE L0	.00 (.16)	.69	RSA L0	−2887.52 (−.96)	.12	DHE L0	−.00 (−.40)	.12
		RSA L1	65.84 (.09)	.82	DHE L1	.00 (.55)	.19	RSA L1	−1957.07 (−.76)	.08	DHE L1	.00 (.50)	.08
	Stress	DHE	−.58 (−.54)	.17	Stress	−.68 (−.70)	.08	DHE	−.15 (−.16)	.73	Stress	−.83 (−.84)	**<.05**
		Stress L0	18.16 (.27)	.56	DHE L0	.00 (.25)	.56	Stress L0	23.70 (.16)	.79	DHE L0	.00 (.08)	.79
		Stress L1	26.33 (.38)	.41	DHE L1	.00 (.13)	.74	Stress L1	86.77 (.58)	.36	DHE L1	−.00 (−.43)	.16
CRP	TB	CRP	−.24 (−.23)	.64	TB	−.54 (−.56)	.20	CRP	−.22 (−.23)	.36	TB	−.76 (−.79)	**<.05**
		TB L0	−127.34 (−.32)	.52	CRP L0	−.00 (−.25)	.52	TB L0	−202.87 (−.32)	.41	CRP L0	−.00 (−.24)	.41
		TB L1	49.72 (.13)	.82	CRP L1	−.00 (−.43)	.30	TB L1	−54.62 (−.09)	.83	CRP L1	−.00 (−.21)	.45
	EPE	CRP	−.02 (−.02)	.97	EPE	.25 (.26)	.60	CRP	−.15 (−.16)	.56	EPE	−.82 (−.84)	**<.05**
		EPE L0	−85.11 (−.50)	.21	CRP L0	−.00 (−.55)	.21	EPE L0	−130.87 (−.48)	.30	CRP L0	−.00 (−.24)	.30
		EPE L1	57.41 (.34)	.47	CRP L1	.00 (.09)	.86	EPE L1	−63.61 (−.23)	.62	CRP L1	−.00 (−.10)	.68
	DEV	CRP	−.27 (−.25)	.58	DEV	−.41 (−.43)	.29	CRP	−.61 (−.64)	.19	DEV	.32 (.34)	.21
		DEV L0	−23.36 (−.10)	.84	CRP L0	−.00 (−.08)	.84	DEV L0	−328.68 (−.30)	.40	CRP L0	−.00 (−.18)	.40
		DEV L1	37.57 (.17)	.73	CRP L1	−.00 (−.25)	.52	DEV L1	234.16 (.32)	.35	CRP L1	−.00 (−.76)	**<.05**
	RSA	CRP	−.25 (−.23)	.60	RSA	−.34 (−.35)	.37	CRP	−.29 (−.30)	.31	RSA	−.59 (−.62)	.09
		RSA L0	50.17 (.19)	.68	CRP L0	.00 (.16)	.68	RSA L0	−116.66 (−.20)	.58	CRP L0	−.00 (−.19)	.58
		RSA L1	13.09 (.05)	.91	CRP L1	−.00 (−.03)	.94	RSA L1	−35.59 (−.06)	.86	CRP L1	−.00 (−.47)	.19
	Stress	CRP	−.01 (−.01)	.99	Stress	−.45 (−.46)	.24	CRP	−.17 (−.18)	.34	Stress	−.96 (−.97)	**<.05**
		Stress L0	15.25 (.67)	.18	CRP L0	.02 (.45)	.18	Stress L0	−7.43 (−.22)	.39	CRP L0	−.02 (−.39)	.39
		Stress L1	1.53 (.07)	.90	CRP L1	−.00 (−.07)	.84	Stress L1	−19.38 (−.56)	.06	CRP L1	−.00 (−.02)	.95
sIgA	TB	sIgA	.05 (.05)	.80	TB	.16 (.17)	.78	sIgA	−.52 (−.53)	.16	TB	−.79 (−.82)	**<.05**
		TB L0	−36.44 (−.24)	.28	sIgA L0	−.01 (−.65)	.28	TB L0	−187.41 (−.92)	.09	sIgA L0	−.00 (−.49)	.09
		TB L1	83.41 (.56)	**<.05**	sIgA L1	.00 (.28)	.46	TB L1	−139.17 (−.67)	.22	sIgA L1	−.00 (−.28)	.35
	EPE	sIgA	−.21 (−.23)	.47	EPE	.08 (.09)	.86	sIgA	−.22 (−.22)	.60	EPE	−.75 (−.76)	**<.05**
		EPE L0	−4.87 (−.08)	.80	sIgA L0	−.00 (−.13)	.80	EPE L0	−34.22 (−.39)	.57	sIgA L0	−.00 (−.15)	.57
		EPE L1	19.27 (.30)	.34	sIgA L1	.00 (.12)	.80	EPE L1	12.46 (.14)	.84	sIgA L1	.00 (.04)	.88
	DEV	sIgA	−.23 (−.25)	.46	DEV	−.40 (−.42)	.30	sIgA	−.85 (−.87)	**<.05**	DEV	−.26 (−.28)	.20
		DEV L0	−60.20 (−.67)	**<.05**	sIgA L0	−.01 (−.76)	**<.05**	DEV L0	−240.24 (−.68)	.06	sIgA L0	−.00 (−.51)	.06
		DEV L1	−14.74 (−.17)	.62	sIgA L1	−.00 (−.36)	.35	DEV L1	−105.28 (−.44)	.13	sIgA L1	−.00 (−.62)	**<.05**
	RSA	sIgA	−.50 (−.53)	.07	RSA	−.78 (−.81)	.06	sIgA	−.56 (−.58)	.13	RSA	−.67 (−.70)	.08
		RSA L0	38.34 (.39)	.17	sIgA L0	.01 (.56)	.17	RSA L0	−85.43 (−.44)	.31	sIgA L0	−.00 (−.43)	.31
		RSA L1	62.12 (.69)	**<.05**	sIgA L1	.01 (.68)	.08	RSA L1	−95.58 (−.49)	.27	sIgA L1	−.00 (−.21)	.62
	Stress	sIgA	−.20 (−.21)	.56	Stress	−.69 (−.71)	.06	sIgA	−.50 (−.51)	.20	Stress	−.72 (−.73)	.07
		Stress L0	.02 (.00)	.99	sIgA L0	.00 (.00)	.99	Stress L0	−.45 (−.04)	.94	sIgA L0	−.00 (−.03)	.94
		Stress L1	1.65 (.19)	.68	sIgA L1	−.04 (−.39)	.26	Stress L1	−2.93 (−.26)	.61	sIgA L1	−.00 (−.02)	.96

Note. Each combination of biomarker and burnout (dimensions) constitutes a single model; the two lagged effects (0; 1) and the autoregressive effect are run in the same model. BM = Biomarker, BO = Burnout, Pred = Predictor, p-val = p-value, TB = Total Burnout, EPE = Emotional and Physical Exhaustion, DEV = Devaluation, RSA = Reduced Sense of Accomplishment, DBP = Diastolic Blood Pressure, SBP = Systolic Blood Pressure, HRV = Heartrate Variability, Cor = Cortisol, Tes = Testosterone, T/C = Testosterone/Cortisol Ratio, DHE = DHEA-S Flow Rate, CRP = C-reactive Protein, sIgA = secretory Immunoglobulin A Flow Rate.

between stress, total athlete burnout, or the burnout dimensions and total cholesterol (see supplemental Figure F).

HDL Cholesterol. Athlete C2 showed a negative, simultaneous development of total athlete burnout and HDL with a general increase in total burnout and decrease in HDL (see Figure G). However, the variability in HDL was low and could therefore represent variation around a constant mean. This was the similar in EPE and RSA. Athlete C1 showed no relationships. For all figures, see supplementary Figure G.

HbA1c. Both Athlete C1 and C2 showed a positive, simultaneous development of total burnout and HbA1c (see Fig. 4A). While Athlete C1

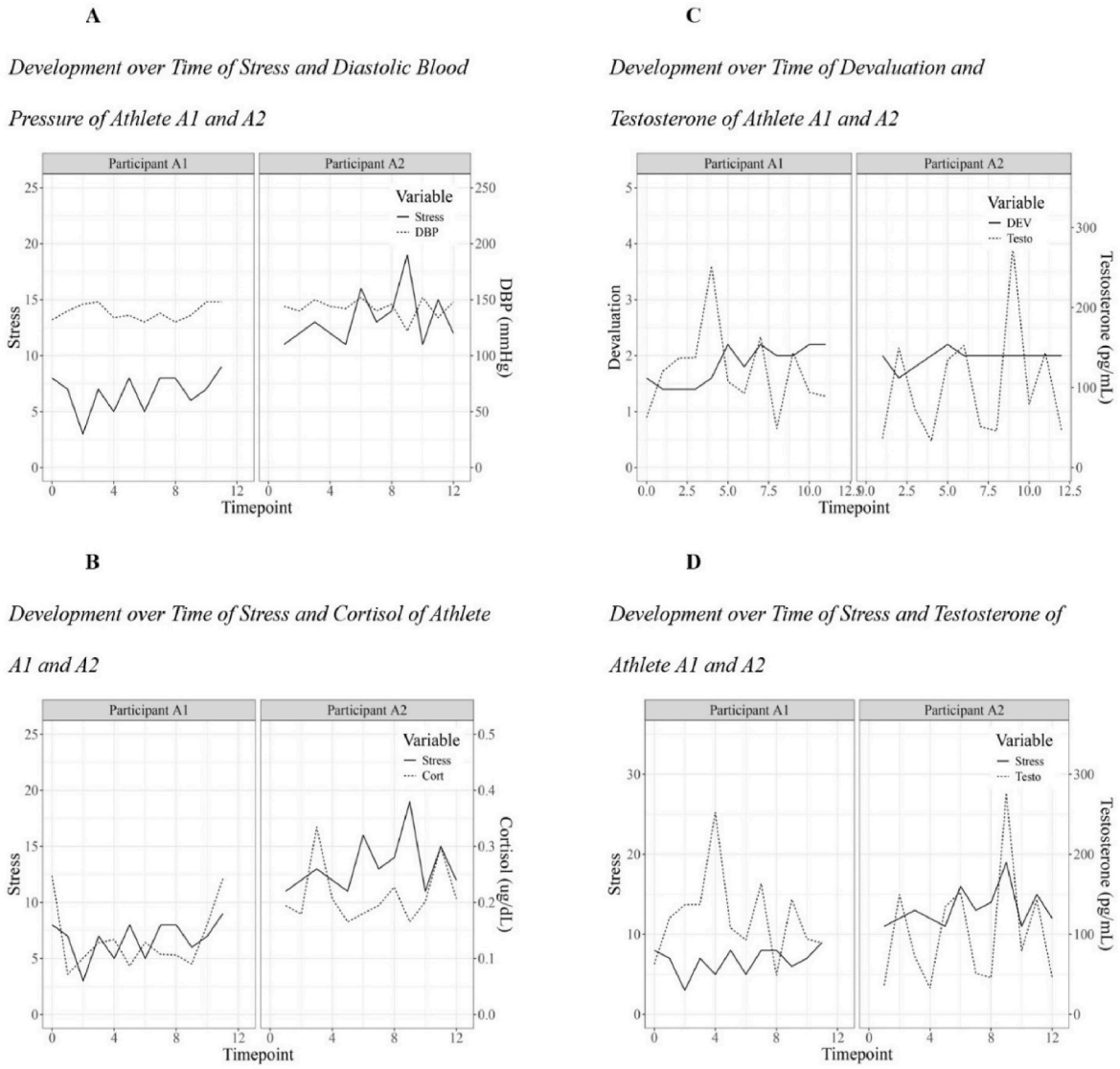


Fig. 2. Development over time of stress and devaluation with acute biomarkers.

showed a gradual decline until timepoint 4 and then an increase in both total burnout and HbA1c, Athlete C2 showed an overall increase and levelling off at the last two timepoints. This was similar for EPE and RSA (see Fig. 4B–C). DEV and stress did not seem to develop simultaneously to HbA1c. For all figures, see supplementary Figure H.

Hair Cortisol. To visualise the development of hair cortisol month-by-month both segment 1 hair cortisol and segment 2 hair cortisol were included in the same graph. As such, segment 2 was visualised on the midpoint between timepoints (.5, 1.5, 2.5), while segment 1 was visualised at each timepoint (1, 2, 3). Athlete C2 showed increases in total burnout to occur prior to increases in hair cortisol, demonstrating a positive, lagged relationship by one timepoint (see Fig. 4D). RSA showed a similar lagged development (see Fig. 4F), while EPE showed a similar gradual increase in both EPE and hair cortisol over the study (see Fig. 4E). DEV and stress, however, did not show a relationship with hair cortisol in Athlete C2. Athlete C1 did not show any relationships. For all figures, see supplemental Figure I.

BDNF Methylation. Neither of the athletes showed BDNF exon 1b methylation to have a relationship to burnout or the burnout dimensions. Athlete C1 showed a positive, simultaneous relationship between BDNF exon 1b methylation and stress, apart from the last timepoint (see Fig. 5A). This was similar for Athlete C2, however, increases in stress occurred prior to increases in BDNF exon 1b methylation, demonstrating a positive, lagged relationship by one timepoint (see Fig. 5A).

Athlete C1 showed a negative, simultaneous relationship between EPE and DEV with BDNF exon 4 methylation that disappeared on the last timepoint (see Fig. 5B–C). Athlete C2 showed a positive, simultaneous relationship between stress and BDNF exon 4 methylation which was the case for Athlete C1 as well, but only across the first three timepoints (see Fig. 5D). For all figures, see supplementary Figure J.

SLC6A4 Methylation. Athlete C2 showed a positive, simultaneous relationship between DEV and SLC6A4 methylation levels, but this was not the case for Athlete C1 (see Fig. 5E). Athlete C1 showed no relationships. For all figures, see supplementary Figure K.

NR3C1 Methylation. Overall, NR3C1 methylation levels showed little to no variation over time and thus did not match with the development of any of the psychological variables (see supplementary Figure L).

4. Discussion

The present study aimed to explore the relationship between athlete burnout and biomarkers using an *N*-of-1 design. We separated

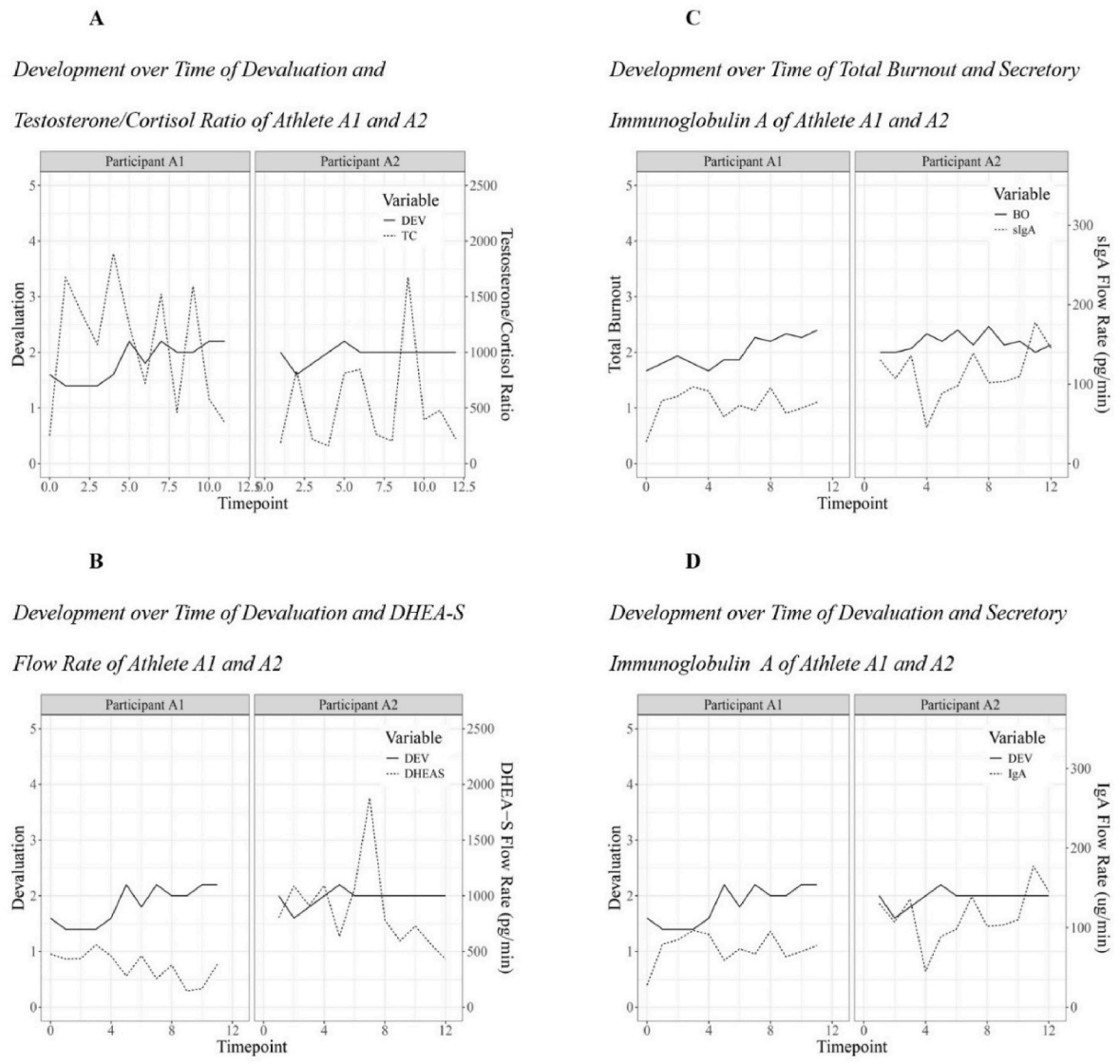


Fig. 3. Development over time of total burnout and devaluation with acute biomarkers.

biomarkers by system and based on their acute or chronic nature. In terms of main findings, among the acute biomarkers we examined, our dynamic regression modelling showed that devaluation predicted decreases in testosterone as well as testosterone/cortisol ratio and developed simultaneously with decreases in DHEA-S and sIgA. The findings for sIgA showed contrasting results as total burnout was shown to predict increases in sIgA, whereas sIgA predicted decreases in devaluation. Salivary cortisol was found to predict increases in stress. Among the chronic biomarkers we examined, our visual analysis showed that total burnout, exhaustion, and a reduced sense of accomplishment developed simultaneously with increases in HbA1c and preceded increases in hair cortisol. In relation to epigenetics, devaluation appeared to develop simultaneously with SLC6A4 methylation and preceded the development of BDNF exon 4 methylation. In addition, stress developed with increases in testosterone and BDNF exon 1b and 4 methylation and predicted decreases in DBP. We found little evidence, however, for relationships between burnout and systolic blood pressure, HRV, salivary cortisol, CRP, blood lipids, or NR3C1 methylation.

4.1. Key findings

4.1.1. Acute biomarkers

We found devaluation to predict decreases in testosterone and the testosterone/cortisol ratio, and stress to develop simultaneously with increases in testosterone. While stress has been associated with testosterone previously (Souza et al., 2018), the existing research regarding burnout is equivocal, with some research finding testosterone to be higher in a "burnout" group (Atik et al., 2020), and other work showing no differences (Souza et al., 2018). Testosterone plays a key role in metabolism, with notable effects on muscle mass and insulin sensitivity (Kelly & Jones, 2013). In the short-term, simultaneous increases in testosterone due to stress may reflect acute HPA axis activation and, thus, be advantageous in training and/or competition. For example, increased testosterone/cortisol ratios have been shown to be related to higher podium positions in indoor racing (Ficarra et al., 2023). Devaluation, however, predicted both decreases in testosterone and the testosterone/cortisol ratio, which indicates a dysregulation of the HPA axis. Since devaluation represents an emotional detachment from sport, such emotional suppression might affect the cognitive appraisal process by lowering the individual's coping resources. Reduced resources could then contribute to the chronic stress experience and thereby affect HPA

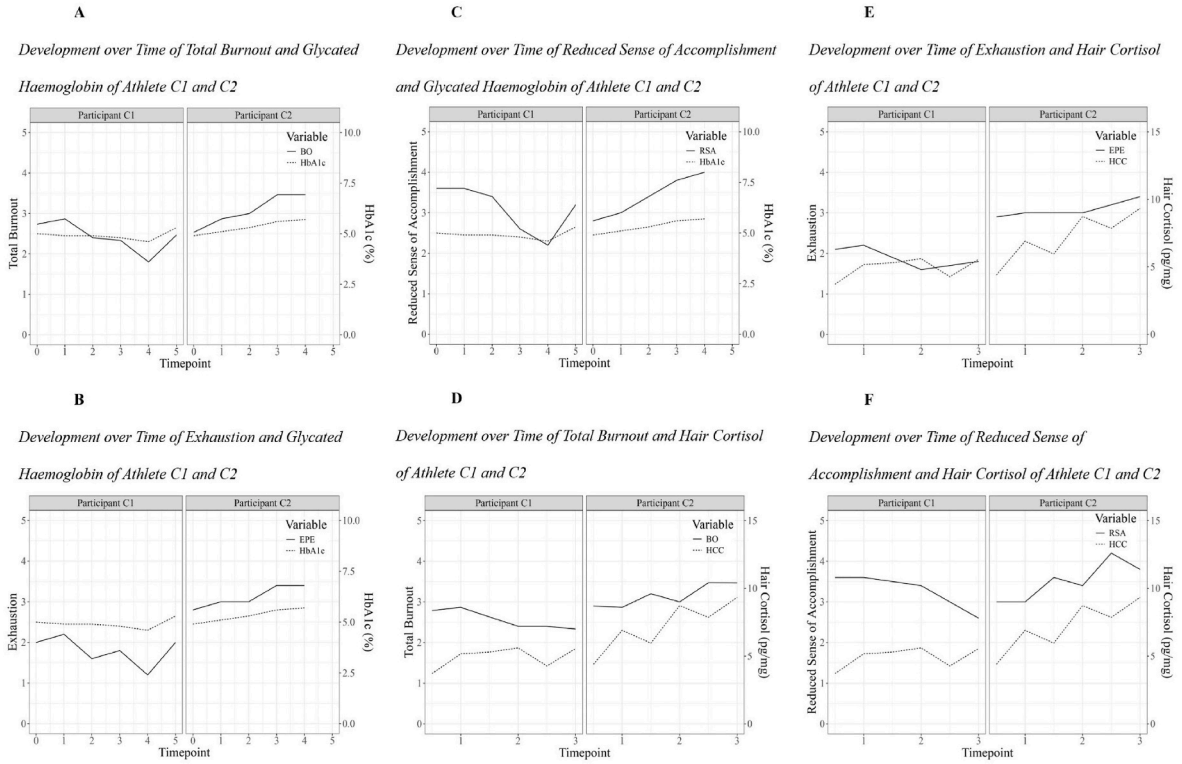


Fig. 4. Development over time of total burnout, exhaustion, and reduced sense of accomplishment with chronic biomarkers.

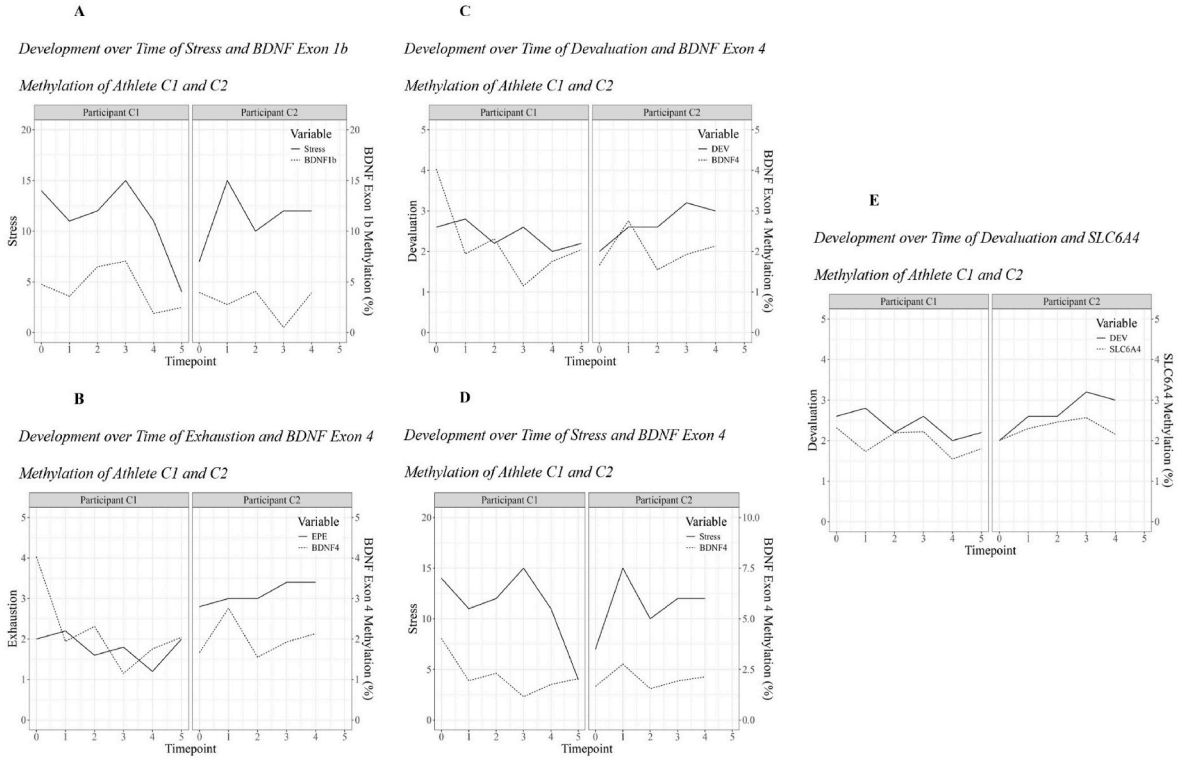


Fig. 5. Development over time of stress, exhaustion, and devaluation with chronic biomarkers.

axis activity. While previous research has found cortisol and testosterone to be upregulated under acute stress, cortisol has also been shown to predict reductions in testosterone over time (Crewther et al., 2023). As such, a sustained upregulation of cortisol due to burnout may reduce testosterone levels and thus cause a dysregulation of HPA axis activity. If this dysregulation continues over time, concomitant decreases in muscles mass and insulin sensitivity following the development of devaluation may negatively affect athletes' training and performance.

We found devaluation to develop simultaneously with decreases in DHEA-S. This pattern of relationships has been found previously in patients (Lennartsson et al., 2016) but, contrary to previous cross-sectional work (Souza et al., 2018), this is the first time these findings have emerged in sport. DHEA-S is a precursor to testosterone and cortisol, meaning it can be converted into both of these hormones. Similar to testosterone, chronic stress has been associated with decreases in DHEA-S levels and thus, devaluation may affect DHEA-S levels through a dysregulation of the HPA axis (Lennartson et al., 2013). As a precursor of these hormones, low levels of DHEA-S could translate into low levels of testosterone and thus may explain why devaluation predicts decreases in testosterone. Through its effects on testosterone, decreases in DHEA-S may also link with similar metabolic effects on muscle mass and insulin sensitivity. DHEA-S has also been shown to regulate immunocompetence such that low levels of DHEA-S may increase the risk for infection (Lennartsson et al., 2016). As a result, long-term decreases in DHEA-S in connection to devaluation and thus metabolic effects and more frequent infections may make it harder for athletes to achieve optimal performance.

Burnout showed a complex relationship with sIgA. While devaluation was found to develop simultaneously with decreases in sIgA in one athlete, sIgA predicted decreases in devaluation in another athlete. In addition, total burnout was found to predict increases in sIgA. This is the first-time burnout has shown relationships with sIgA in an athlete sample (see Glandorf et al., 2023). As devaluation showed the same direction for its relationship with sIgA, this suggests devaluation may develop simultaneously with or be predicted by decreases in this biomarker. In contrast, total burnout showed to predict increases in sIgA over time. Due to this contrast, the findings with regards to sIgA should be interpreted with caution, however, discrepancies in total versus dimension scores could explain why this is the case. As it is unlikely that burnout dimensions develop in tandem (Gerber et al., 2018), it is possible that unique relationships with sIgA are lost or false positives are created when dimensions are amalgamated. Alternatively, the difference in the direction of the relationship between devaluation and total burnout with sIgA could reflect the differential temporal development of the burnout symptoms. Devaluation may be the symptom that is predominantly linked to decreases in sIgA levels through its effects on the HPA axis and interactive signalling that affects the immune system. As sIgA is an antibody that plays a key part in adaptive and innate immunity (Macpherson et al., 2011), any decreases in sIgA that are linked to devaluation would likely make athletes more susceptible to infection. This increasing susceptibility to infection may coincide with increasing burnout symptoms over time. Thus, increases in total burnout may predict infection in which case sIgA level would be expected to rise (see Turner et al., 2021). If this is the case, these findings provide important information regarding athletes' illness susceptibility, especially if sIgA decreases linked to devaluation continue over a prolonged timeframe.

4.1.2. Chronic biomarkers

The relationship between burnout and cortisol was perhaps the most complicated that we observed. In this regard, while burnout was not associated with salivary cortisol, it did appear to precede increases in hair cortisol. Cortisol, as the main stress hormone, is the most widely examined biomarker in burnout research. Studies within sport have predominantly focused on salivary cortisol, and have been equivocal (e.g., Monfared et al., 2020). As highlighted by reviews inside and outside of sport, this inconsistency is likely due to the variability of methods used to measure salivary cortisol and the quick response of the biomarker to acute stress (Danhof-Pont et al., 2011; Glandorf et al., 2023). As we found salivary cortisol to predict stress, this suggests salivary cortisol may only be appropriate as a biomarker to acute stress. In contrast, salivary cortisol may not be suitable as a biomarker for burnout as burnout typically takes longer to show changes over time (~2 months; DeFreese & Smith, 2014). However, measuring cortisol from hair, which reflects an accumulation of cortisol over months (1–2 months) rather than moment-to-moment variation may be better suited when examining burnout development. In support of this idea, in-line with our findings, a growing body of work has started to link increases in burnout to increases in hair cortisol levels (e.g., Brianda et al., 2020). Since cortisol has immunosuppressive effects and facilitates glucose metabolism, consistently elevated cortisol levels may weaken the immune system and increase cardiovascular disease risk, both of which are certainly undesirable for athletes.

Our analyses suggested that exhaustion, reduced sense of accomplishment, and total burnout developed simultaneously with increases in HbA1c. These findings are similar to studies outside of sport (Metlaine et al., 2018). HbA1c reflects the average glucose concentration in blood over the last two to three months and is thus used to monitor diabetes as well as for an indicator of cardiovascular disease risk (Metlaine et al., 2018). We think changes in lifestyle factors may help explain this finding. For example, to deal with the experiences of exhaustion and a reduced sense of accomplishment, athletes may change their diets, for the worse. If this is the case, monitoring athletes and supporting healthy lifestyles may be important both for burnout identification and limiting potential health consequences that could develop should these lifestyles be maintained over long periods of time.

4.1.3. Epigenetic mechanisms

We provided a first exploration of epigenetics in context of athlete burnout. In doing so, our analyses showed that devaluation developed simultaneously with increases in SLC6A4 methylation. The SLC6A4 gene codes for the human serotonin transporter that is important for serotonin reuptake in the presynaptic neuron and as a consequence plays a role in a number of physiological and cognitive processes such as regulation of mood and emotions (see Bakusic et al., 2017). Increases in methylation levels in this gene and thus potential decreases in the expression of this gene may thus be related to changes in emotional regulation. Previous research outside of sport has consistently found a similar relationship between total burnout and specific dimensions with SLC6A4 methylation levels (Alasaari et al., 2012; Bakusic et al., 2017, 2021). Considering that devaluation reflects an emotional detachment from sport and SLC6A4 is likely important to emotional regulation, it might be that case that devaluation and simultaneous increases in SLC6A4 methylation represent the same symptom.

Our findings showed devaluation to precede increases of BDNF exon 4 methylation and stress to develop simultaneously with increases in BDNF methylation. In line with our findings, previous research has also shown BDNF methylation to be associated with both stress and burnout symptoms (Bakusic et al., 2017, 2020). The BDNF gene codes for brain-derived neurotrophic factor that is key to neuronal survival and growth and thus the development and maintenance of normal brain function. Increased BDNF methylation levels may suggest that the expression of BDNF in serum becomes downregulated as demonstrated in previous studies (Bakusic et al., 2020), which could cause disruptions to cognition, specifically learning and memory. Stress has previously been suggested to affect BDNF levels as increased cortisol secretion in response to stress might cause alterations in DNA methylation (Bayes et al., 2021). Similarly, devaluation might precede changes in DNA methylation due to an emotional detachment from the sport that then affects HPA axis activity. HPA axis activity might directly or indirectly through immune regulation affect DNA methylation levels (Ligthart et al., 2016). Although preliminary, these findings may open an additional useful avenue for understanding the biology of burnout.

4.1.4. Insufficient evidence

We found little evidence for relationships between burnout and blood pressure, HRV, CRP, any of the examined blood lipids, and NR3C1 methylation. The inclusion of these aspects was informed by work outside of sport that previously found burnout to be related to these factors (e.g., Bayes et al., 2021). The most notable difference then that may explain these discrepancies is that of the sample. The athletes in the

present study were all young, healthy individuals who exercise regularly, and doing so may counteract some of the physical health consequences of burnout found outside of sport, which means some of these biomarkers may be relevant in the work population but less so among athletes (see Danhof-Pont et al., 2011). This may also explain why we found stress to predict decreases in diastolic blood pressure, even though the bulk of the literature shows stress to increase blood pressure. Alternatively, because the time between measurements were focused on the temporality of burnout and the biomarkers, they may not have been the most appropriate to examine (changes in) stress and we thus may have missed aspects of the relationship between stress and the examined biomarkers. Overall, these findings suggest that these particular biomarkers may be of less interest to future research, which is important to consider due to the resource-heavy nature of biomedical research.

4.2. Theoretical implications

We are hopeful that the present findings can help inform athlete burnout theory. In this regard, such theory currently has little to say in relation to the biological or physiological processes underpinning burnout development or consequences. Firstly, then, based on the present findings, it seems that such an addition would be worthwhile. We borrowed most of our theorising from allostatic load theory (McEwen & Stellar, 1993), in addition to suggestions from occupational burnout theorists (e.g., Bayes et al., 2021; Melamed et al., 2006). We think that the most important revisions to sport theory would be a separation of an acute and chronic phase for the development of burnout and its physiological consequences. Based on the present findings, we have provided some suggestions for how this might be accomplished below.

In the acute phase, responses to stress and burnout development appear to occur through the upregulation of the HPA axis. Stress causes this initial upregulation and develops simultaneously with increases in cortisol and testosterone. Increases in cortisol may also develop simultaneously with increased methylation in genes associated with the nervous system, such as the BDNF gene. As stress becomes chronic, burnout symptoms will develop. Devaluation as an emotional detachment from sport may affect coping resources of the athlete and thereby cause changes in HPA axis activity as well as other systems through downstream signalling. Devaluation therefore may develop simultaneously with decreases in DHEA-S and sIgA. The dysregulation of HPA axis activity due to the development of devaluation may also predict decreases in testosterone and testosterone/cortisol ratios.

During the chronic phase, adaptations to burnout development likely occur. The emotional detachment of devaluation may result in simultaneous increases in gene methylation levels such as the SLC6A4 gene and predict increases in gene methylation in the BDNF gene. In turn, exhaustion and a reduced sense of accomplishment potentially develop with simultaneous increases in glycated haemoglobin due to dietary changes in response to these symptoms. Apart from the metabolism, exhaustion and a reduced sense of accomplishment will also precede increases in hair cortisol that could reflect the dysregulation of the HPA axis activity with burnout development. Over time, and ultimately, these physiological effects will likely make it more difficult to achieve optimal performance. These initial suggestions will hopefully provide a further means to build a more complete theory of athlete burnout that incorporates key biological and behavioural processes. We have summarised these theoretical implications in Fig. 6.

5. Limitations and future directions

The present study has several limitations. First, while our *N-of-1* design allowed us to explore a wide range of biomarkers, this design limits the generalisability of our findings. Future studies based on our preliminary findings should therefore be conducted. Second, sensitivity analyses showed that not all significant effects were sufficiently powered and thus, some true effects might have been missed. In the same vein, due to the small sample size, reciprocal effects could also not be tested in the same model. Nonetheless, the findings from this study may be used for power analyses in the future to determine appropriate sample sizes that also allow for examination of reciprocal effects in the same model. Third, as little longitudinal research has been conducted on the relationship between athlete burnout and biomarkers, the optimal time between measurement waves is still unclear. Time should be considered as a variable in future research to determine the intervals over which the relationship of burnout and biomarkers shows to be the strongest (Taris & Kompier, 2014). Fourth, biomarkers are part of a complex integrated system and will respond to other influences. For example, others sources of strain such as training load (e.g., Dobson et al., 2020) could be considered as variables in future work. Similarly, biological variables such as infections or injury could have affected

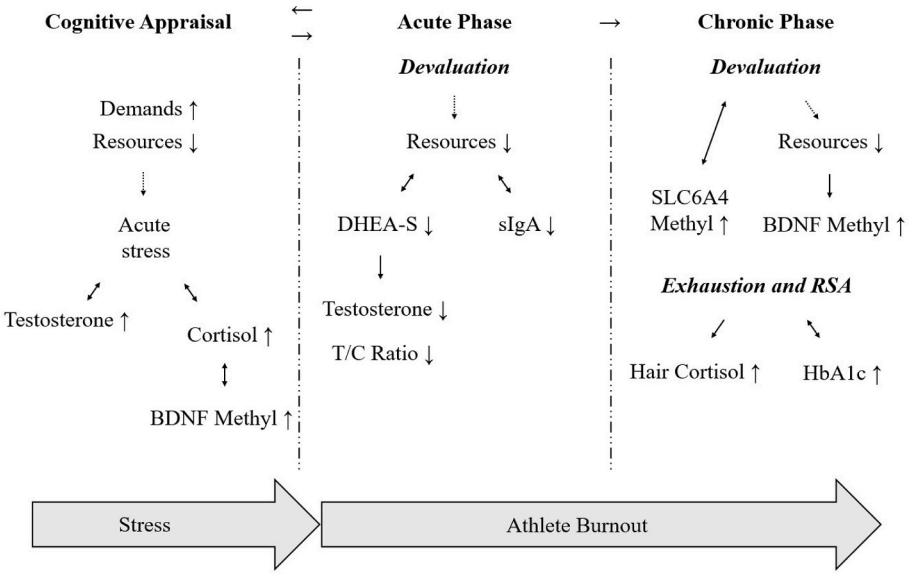


Fig. 6. Summary of the Theoretical Implications for Athlete Burnout Development and Physiological Consequences. *Note.* Methyl = Methylation. T/C = Testosterone/Cortisol. RSA = Reduced Sense of Accomplishment. Dashed, one-directional arrows ( ) indicate an assumed causation, bi-directional arrows ( ) indicate an assumed simultaneous development, solid, one-directional arrows ( ) indicate an assumed prediction over time.

biomarker levels. While participants only completed data collection if they were healthy, unknown infections/illness, sampling close to recovery from infection, or minor injuries may have affected biomarker levels. In the future, studies may collect more data about the participants' condition that could be included in analyses (e.g., injury and injury severity) and/or screen for infections prior to testing which may involve carrying out further biomedical tests (e.g., screening for SARS CoV-2 virus). Fifth, there may be biological sex differences in how burnout and biomarkers are related. Such differences could not be examined within our study. There are obvious hormonal markers that will be affected, such as testosterone, which is naturally higher in men (Kelly & Jones, 2013), and should therefore be examined in future work. Sixth, biomarker selection of this study was heavily based on work from organisational psychology due to the limited literature inside of sport, which ignores potential differences between athlete and non-athlete samples. Athlete samples have previously shown to have better biomarker profiles (e.g., Barbosa et al., 2021) and thus biomarkers of burnout may differ from those in the work population, which should be further explored in future research (Moore et al., 2025). Lastly, the study was likely affected by the healthy participant problem as the athletes showed predominantly low stress and burnout levels as well as "normal" biomarker levels (see Schaufeli & Enzmann, 1998). As such, relationships that only occur at higher burnout or stress levels might have been missed. It is worth considering pre-selection of participants for future research (e.g., screening for athletes with high levels of burnout).

6. Conclusion

We explored the relationship between athlete burnout and biomarkers. Our findings provide a preliminary "physiological fingerprint" that could help explain burnout development and its physiological effects. This "physiological fingerprint" can be separated into an acute phase which is primarily related to the effects of devaluation on the HPA axis and the immune system and a chronic phase which is related to the effects of (1) devaluation on the nervous system via gene methylation and (2) exhaustion and a reduced sense of accomplishment on the HPA axis and metabolism. The theoretical and empirical implications of this idea will hopefully help further our understanding of athlete burnout.

CRediT authorship contribution statement

Hanna L. Glandorf: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Daniel J. Madigan:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Owen Kavanagh:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Sarah H. Mallinson-Howard:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Further declarations

The submission fully follows the ethical publication standards and has received ethics approval by the researchers' institutional ethics board. This submission is our own original work, has not been published previously, and is not under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research received no external funding. Institutional support was provided by York St John University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.psychsport.2025.102870.

Data availability

Data is available on PsychArchives doi: https://doi.org/10.23668/psycharchives.15820.

References

Alasaari, J. S., Lagus, M., Ollila, H. M., Toivola, A., Kivimäki, M., Vahtera, J., Kronholm, E., Härmä, M., Puttonen, S., & Paunio, T. (2012). Environmental stress affects DNA methylation of a CPG rich promoter region of serotonin transporter gene in a nurse cohort. *PLoS One, 7*(9). https://doi.org/10.1371/journal.pone.0045813

Atik, D., Cander, B., Bulut, B., Kaya, H., Yazici, R., Guven, R., & Kazezoglu, C. (2020). Evaluation of the relationship between testosterone levels and burnout levels and job satisfaction in emergency department female employees; a prospective study. *Journal of the Pakistan Medical Association*, 1–14. https://doi.org/10.47391/jpma.775

Bakusic, J., Ghosh, M., Polli, A., Bekaert, B., Schaufeli, W., Claes, S., & Godderis, L. (2020). Epigenetic perspective on the role of brain-derived neurotrophic factor in burnout. *Translational Psychiatry, 10*(1), 1–8. https://doi.org/10.1038/s41398-020-01037-4

Bakusic, J., Ghosh, M., Polli, A., Bekaert, B., Schaufeli, W., Claes, S., & Godderis, L. (2021). Role of NR3C1 and SLC6A4 methylation in the HPA axis regulation in burnout. *Journal of Affective Disorders, 295*(2021), 505–512. https://doi.org/10.1016/j.jad.2021.08.081

Bakusic, J., Schaufeli, W., Claes, S., & Godderis, L. (2017). Stress, burnout and depression: A systematic review on DNA methylation mechanisms. *Journal of Psychosomatic Research, 92*, 34–44. https://doi.org/10.1016/j.jpsychores.2016.11.005

Barbosa, L. P., da Silva Aguiar, S., Santos, P. A., dos Santos Rosa, T., Maciel, L. A., de Deus, L. A., Neves, R. V., de Araújo Leite, P. L., Gutierrez, S. D., Sousa, C. V., Korhonen, M. T., Degens, H., & Simões, H. G. (2021). Relationship between inflammatory biomarkers and testosterone levels in male master athletes and non-athletes. *Experimental Gerontology, 151*, 1–7. https://doi.org/10.1016/j.exger.2021.111407

Bärtl, C., Henze, G.-I., Giglberger, M., Peter, H. L., Konzok, J., Wallner, S., Kreuzpointner, L., Wüst, S., & Kudielka, B. M. (2022). Higher allostatic load in work-related burnout: The Regensburg Burnout Project. *Psychoneuroendocrinology, 143*, 1–11. https://doi.org/10.1016/j.psyneuen.2022.105853

Bayes, A., Tavella, G., & Parker, G. (2021). The biology of burnout: Causes and consequences. *World Journal of Biological Psychiatry, 22*(9), 686–698. https://doi.org/10.1080/15622975.2021.1907713

Becker, L., Dupke, A., & Rohleder, N. (2021). Associations between C-reactive protein levels, exercise addiction, and athlete burnout in endurance athletes. *Frontiers in Psychology, 12*, 1–6. https://doi.org/10.3389/fpsyg.2021.615715.

Bianchi, R., Schonfeld, I. S., & Laurent, E. (2015). Burnout–Depression Overlap: A Review. *Clinical Psychology Review, 36*, 28–41. https://doi.org/10.1016/j.cpr.2015.01.004.

Bosch, J. (2014). The use of saliva markers in psychobiology: Mechanisms and methods. *Monographs in Oral Science*, 99–108. https://doi.org/10.1159/000358864

Brianda, M. E., Roskam, I., & Mikolajczak, M. (2020). Hair cortisol concentration as a biomarker of Parental Burnout. *Psychoneuroendocrinology, 117*, 1–5. https://doi.org/10.1016/j.psyneuen.2020.104681

Brown, D. J., & Stenling, A. (2024). Working with time: Navigating the temporal jungle to capture change processes. *Sport, Exercise, and Performance Psychology. Advance Online Publication.* https://doi.org/10.1037/spy0000365

Chiu, Y., Lu, F., Lin, J., Nien, C., Hsu, Y., & Liu, H. (2016). Psychometric properties of the perceived stress scale (PSS): Measurement invariance between athletes and non-athletes and construct validity. *PeerJ, 4*(2016), 1–20.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385–396.

Cresswell, S. L., & Eklund, R. C. (2006). The convergent and discriminant validity of burnout measures in sport: A multi-trait/multi-method analysis. *Journal of Sports Sciences, 24*(2), 209–220. https://doi.org/10.1080/02640410500131431

Crewther, B. T., Hecht, M., Grillot, R. L., Eisenbruch, A. B., Catena, T., Potts, N., Kilduff, L. P., Cook, C. J., Maestripieri, D., & Roney, J. R. (2023). Day-to-day coordination of the stress and reproductive axes: A continuous-time analysis of within-person testosterone and cortisol relationships in athletic and healthy men. *Physiology & Behavior, 263*, Article 114104. https://doi.org/10.1016/j.physbeh.2023.114104

Danhof-Pont, M., van Veen, T., & Zitman, F. (2011). Biomarkers in burnout: A systematic review. *Journal of Psychosomatic Research, 70*(6), 505–524. https://doi.org/10.1016/j.jpsychores.2010.10.012

DeFreese, J. D., & Smith, A. L. (2014). Athlete social support, negative social interactions, and psychological health across a competitive sport season. *Journal of Sport & Exercise Psychology, 36*(6), 619–630. https://doi.org/10.1123/jsep.2014-0040

Dobson, J., Harris, B., Claytor, A., Stroud, L., Berg, L., & Chrysosferidis, P. (2020). Selected cardiovascular and psychological changes throughout a competitive season in collegiate female swimmers. *The Journal of Strength & Conditioning Research, 34* (11), 3062–3069. https://doi.org/10.1519/JSC.0000000000003767

Ficarra, G., Caccamo, D., Rottura, M., Bitto, A., Trimarchi, F., & Di Mauro, D. (2023). Testosterone:cortisol ratio as a predictor of podium in adolescent rowing athletes. *Heliyon, 9*(11), Article e22315. https://doi.org/10.1016/j.heliyon.2023.e22315

Gerber, M., Gustafsson, H., Seelig, H., Kellmann, M., Ludyga, S., Colledge, F., Brand, S., Isoard-Gautheur, S., & Bianchi, R. (2018). Usefulness of the Athlete Burnout Questionnaire (ABQ) as a screening tool for the detection of clinically relevant burnout symptoms among young elite athletes. *Psychology of Sport and Exercise, 39*, 104–113. https://doi.org/10.1016/j.psychsport.2018.08.005

Glandorf, H.. *Exploring the Physiological Consequences of Athlete Burnout: A Longitudinal N-of-1 Study*. PsychArchives. https://doi.org/10.23668/psycharchives.6531.

Glandorf, H. L., Madigan, D. J., Kavanagh, O., & Mallinson-Howard, S. H. (2023). Mental and physical health outcomes of burnout in athletes: A systematic review and meta-analysis. *International Review of Sport and Exercise Psychology*. https://doi.org/10.1080/1750984X.2023.2225187

Glandorf, H. L., Madigan, D. J., Kavanagh, O., Mallinson-Howard, S. H., Donachie, T. C., Olsson, L. F., & Rumbold, J. L. (2024). Athlete burnout and mental and physical health: A three-wave longitudinal study of direct and reciprocal effects. *Sport, Exercise, and Performance Psychology*. https://doi.org/10.1037/spy0000355

Grugan, M. C., Olsson, L. F., Vaughan, R. S., Madigan, D. J., & Hill, A. P. (2024). Factorial validity and measurement invariance of the athlete burnout questionnaire (ABQ). *Psychology of Sport and Exercise, 73*. https://doi.org/10.1016/j.psychsport.2024.102638

Hew-Butler, T., Aprik, C., Byrd, B., Landis-Piwowar, K., Smith-Hale, V., VanSumeren, M., … Martin, J.. *Changes in nutrient biomarkers, body composition, and burnout symptoms in collegiate athletes across a season* [Preprint]. https://doi.org/10.20944/preprints202105.0602.v1.

Juster, R., Sindi, S., Marin, M., Perna, A., Hashemi, A., Pruessner, J., & Lupien, S. (2011). A clinical allostatic load index is associated with burnout symptoms and hypocortisolemic profiles in healthy workers. *Psychoneuroendocrinology, 36*(6), 797–805. https://doi.org/10.1016/j.psyneuen.2010.11.001.

Kelly, D. M., & Jones, T. H. (2013). Testosterone: A metabolic hormone in health and disease. *Journal of Endocrinology, 217*(3). https://doi.org/10.1530/joe-12-0455

Kwasnicka, D., Inauen, J., Nieuwenboom, W., Nurmi, J., Schneider, A., Short, C. E., … Naughton, F. (2019). Challenges and solutions for N-of-1 Design Studies in health psychology. *Health Psychology Review, 13*(2), 163–178. https://doi.org/10.1080/17437199.2018.1564627.

Lam, L. L., Emberly, E., Fraser, H. B., Neumann, S. M., Chen, E., Miller, G. E., & Kobor, M. S. (2012). Factors underlying variable DNA methylation in a human community cohort. *Proceedings of the National Academy of Sciences of the United States of America, 109*(Suppl 2), 17253–17260. https://doi.org/10.1073/pnas.1121249109

Ledford, J. R., Lane, J. D., & Severini, K. E. (2018). Systematic use of visual analysis for assessing outcomes in single case design studies. *Brain Impairment, 19*(1), 4–17. https://doi.org/10.1017/BrImp.2017.16

Lennartsson, A.-K., Theorell, T., Kushnir, M. M., & Jonsdottir, I. H. (2016). Changes in DHEA-S levels during the first year of treatment in patients with clinical burnout are related to health development. *Biological Psychology, 120*, 28–34. https://doi.org/10.1016/j.biopsycho.2016.08.003

Lennartsson, A.-K., Theorell, T., Rockwood, A. L., Kushnir, M. M., & Jonsdottir, I. H. (2013). Perceived stress at work is associated with lower levels of DHEA-S. *PLoS One, 8*(8). https://doi.org/10.1371/journal.pone.0072460

Li, C., Wang, C. K. J., Pyun, D. Y., & Kee, Y. H. (2013). Burnout and its relations with basic psychological needs and motivation among athletes: A systematic review and meta-analysis. *Psychology of Sport and Exercise, 14*(5), 692–700. https://doi.org/10.1016/j.psychsport.2013.04.009

Ligthart, S., Marzi, C., Aslibekyan, S., Mendelson, M. M., Conneely, K. N., Tanaka, T., Colicino, E., Waite, L. L., Joehanes, R., Guan, W., Brody, J. A., Elks, C., Marioni, R., Jhun, M. A., Agha, G., Bressler, J., Ward-Caviness, C. K., Chen, B. H., Huan, T., … Dehghan, A. (2016). DNA methylation signatures of chronic low-grade inflammation are associated with complex diseases. *Genome Biology, 17*(1). https://doi.org/10.1186/s13059-016-1119-5

Macpherson, A. J., Geuking, M. B., & McCoy, K. D. (2011). Immunoglobulin A: A bridge between innate and adaptive immunity. *Current Opinion in Gastroenterology, 27*(6), 529–533. https://doi.org/10.1097/mog.0b013e32834bb805

Madigan, D., Olsson, L., Hill, A., & Curran, T. (2022). Athlete burnout symptoms are increasing: A cross-temporal meta-analysis of average levels from 1997 to 2019. *Journal of Sport & Exercise Psychology, 44*(3), 153–168. https://doi.org/10.1123/jsep.2020-0291

Martin, J., Byrd, B., Hew-Butler, T., & Moore, E. W. G. (2021). A longitudinal study on the psychological and physiological predictors of burnout in NCAA collegiate swimmers. *Journal of Applied Sport Psychology*, 1–17. https://doi.org/10.1080/10413200.2021.1974603

McDonald, S., Vieira, R., & Johnston, D. W. (2020). Analysing N-of-1 observational data in health psychology and behavioural medicine: A 10-step SPSS tutorial for beginners. *Health Psychology and Behavioral Medicine, 8*(1), 32–54. https://doi.org/10.1080/21642850.2019.1711096

McEwen, B., & Stellar, E. (1993). Stress and the individual. *Archives of Internal Medicine, 153*(18), 20–93. https://doi.org/10.1001/archinte.1993.00410180039004

Melamed, S., Shirom, A., Toker, S., Berliner, S., & Shapira, I. (2006). Burnout and risk of cardiovascular disease: Evidence, possible causal paths, and promising research directions. *Psychological Bulletin, 132*(3), 327–353. https://doi.org/10.1037/0033-2909.132.3.327

Metlaine, A., Sauvet, F., Gomez-Merino, D., Boucher, T., Elbaz, M., Delafosse, J. Y., Leger, D., & Chennaoui, M. (2018). Sleep and biological parameters in professional burnout: A psychophysiological characterization. *PLoS One, 13*(1). https://doi.org/10.1371/journal.pone.0190607

Monfared, S., Lebeau, J., Mason, J., Cho, S., Basevitch, I., & Perry, I. (2020). A bio-physio-psychological investigation of athletes' burnout. *Research Quarterly for Exercise & Sport, 92*(1), 189–198.

Moore, L., Isoard-Gautheur, S., & Gustafsson, H. (2025). Psychophysiological markers of athlete burnout: A call to arms. *International Journal of Sports Medicine, 46*(2), 69–78. https://doi.org/10.1055/a-2433-3930

Parker, K., & Bristow, M. (2020). *Hair cortisol analysis protocol*. protocols.io. https://doi.org/10.17504/protocols.io.bqevmte6

Pilger, A., Haslacher, H., Meyer, B. M., Lackner, A., Nassan-Agha, S., Nistler, S., Stangelmaier, C., Endler, G., Mikulits, A., Priemer, I., Ratzinger, F., Ponocny-Seliger, E., Wohlschläger-Krenn, E., Teufelhart, M., Täuber, H., Scherzer, T. M., Perkmann, T., Jordakieva, G., Pezawas, L., & Winker, R. (2018). Midday and Nadir salivary cortisol appear superior to cortisol awakening response in Burnout Assessment and Monitoring. *Scientific Reports, 8*(1). https://doi.org/10.1038/s41598-018-27386-1

R Core Team. (2023). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Raedeke, T. D., & Smith, A. L. (2001). Development and preliminary validation of an athlete burnout measure. *Journal of Sport & Exercise Psychology, 23*, 281–306.

Schaufeli, W. B., & Enzmann, D. (1998). *The burnout companion to study and practice: A critical analysis*. Philadelphia: Taylor & Francis.

Souza, R. O., Alves, D. L., de Assis, F., Palumbo, D. de P., & Moiano, J. V. M. (2018). Analysis of dehydroepiandrosterone sulphate, cortisol and testosterone levels in performance athletes affected by burnout syndrome. *Journal of Exercise Physiology Online, 21*(2), 1–8.

Taris, T. W., & Kompier, M. A. (2014). Cause and effect: Optimizing the designs of longitudinal studies in occupational health psychology. *Work & Stress, 28*(1), 1–8.

Tegegne, B., Man, T., van Roon, A., Riese, H., & Snieder, H. (2018). Determinants of heart rate variability in the general population: The Lifelines Cohort Study. *Heart Rhythm, 15*(10), 1552–1558. https://doi.org/10.1016/j.hrthm.2018.05.006

Turner, S. E. G., Loosemore, M., Shah, A., Kelleher, P., & Hull, J. H. (2021). Salivary Iga as a potential biomarker in the evaluation of respiratory tract infection risk in athletes. *Journal of Allergy and Clinical Immunology: In Practice, 9*(1), 151–159. https://doi.org/10.1016/j.jaip.2020.07.049

van der Horn, H., Out, M., de Koning, M., Mayer, A., Spikman, J., Sommer, I., & van der Naalt, J. (2020). An integrated perspective linking physiological and psychological consequences of mild traumatic brain injury. *Journal of Neurology, 267*(9), 2497–2506. https://doi.org/10.1007/s00415-019-09335-8

Weckesser, L. J., Plessow, F., Pilhatsch, M., Muehlhan, M., Kirschbaum, C., & Miller, R. (2014). Do venepuncture procedures induce cortisol responses? A review, study, and synthesis for stress research. *Psychoneuroendocrinology, 46*, 88–99. https://doi.org/10.1016/j.psyneuen.2014.04.012