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Predictive biomarkers of performance under stress: a two-phase study protocol to develop a wearable monitoring system

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ABSTRACT

Understanding and predicting individual responses to common stressors is essential for optimising performance in high-stress environments. This article outlines a protocol for a study to identify biomarkers that predict performance under heat, musculoskeletal, psychosocial and sleep stress, for future integration into a wearable sensor system. In Phase I, healthy adults aged between 18 and 45 years (n=104) will be recruited for an intervention trial that involves exposure to one of the four stressors: heat, musculoskeletal, psychosocial or sleep deprivation. Biomarkers will be identified from molecular markers in biological samples (eg, blood, saliva, sweat and stool), physiological measures and psychological assessments to predict cognitive and physical performance under stress. A within-subjects design will determine changes in molecular and non-molecular markers before and after stress exposure. In Phase II, we will use the biomarkers identified in Phase I to develop a wearable sensor to predict and monitor human performance under stress.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Individuals operating in high-stress environments, such as those exposed to intense physical, thermal and psychological demands, often experience compromised cognitive and physical performance.
⇒ Biomarkers, such as cortisol and heart rate, have been explored as stress indicators, but their combined use with cognitive and physiological measures for predicting performance remains underdeveloped.

WHAT THIS STUDY ADDS

⇒ This study adopts a multidisciplinary approach, integrating molecular, physiological and cognitive biomarkers to predict human performance under distinct stressors.
⇒ It establishes a foundation for using biomarkers in real-time monitoring, culminating in the development of wearable technology to enhance performance and stress management.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings could transform stress monitoring by providing validated biomarker panels that support the development of advanced wearable technologies.
⇒ This research may inform the design of tailored strategies for optimising stress resilience, physical and cognitive performance in highly demanding professions.

INTRODUCTION

In high-performance settings, stressors challenging physical and cognitive performance are common and can significantly impact operational readiness.[1] Stress is broadly defined in the scientific literature as any challenge to an individual's homeostasis, requiring an adaptive response.[2] While stress encompasses a spectrum from mild-to-severe challenges, stress can represent any situation where environmental demands exceed a person's natural regulatory capacity.[3] How distinct physical and psychological stressors lead to individual performance decrements is complex; however, leveraging this understanding is essential for developing effective strategies that enhance resilience and optimise performance in military personnel.

The impact of physical stressors on performance

The relationship between exercise tolerance and ambient temperature and humidity is well established, with the ability to perform sustained exercise impaired in extreme heat.[4] Performance decrements are also exacerbated by protective clothing, carried loads and poor fitness.[5] Heat strain causes a range of physiological responses, including dehydration, increased cardiovascular strain and impaired thermoregulation.[6] These harmful effects can result in heat-related illnesses such as heat exhaustion and heat stroke, which pose serious health risks and death if unmanaged.[6] Additionally, heat strain impairs cognitive function, leading to decreased cognitive performance, especially when the task demands higher cognitive load.[7]

In addition to the challenges posed by heat strain, several meta-analysis studies support the widespread prevalence of musculoskeletal injuries among military personnel.[8] These injuries can result from a variety of factors, including repetitive strain, overuse and acute trauma during training and operations.[9] Physical fitness is also a key factor, as soldiers with lower levels of physical fitness are more likely to injure themselves during training and deployment.[10] Moreover, inadequate sleep and rest periods can impair recovery, increasing the risk of injury.[11] Environmental factors also play a critical role in musculoskeletal stress, as adverse weather conditions, uneven terrain and the requirement to perform in various operational environments can exacerbate the risk of injuries.[12] For example, cold environments can increase the risk of muscle strains,[13] while hot environments can accelerate fatigue and decrease endurance.[14]

The impact of psychological stressors on performance

The types of stressors military personnel face constantly evolve, but combat stress, boredom, excessive workload and isolation remain persistent factors impacting physical and cognitive performance.[15] Maintenance of cognitive performance in military contexts is imperative, as suboptimal performance can be fatal. In any given scenario, defence personnel must rely on executive functions for cognitive flexibility, planning, initiating, inhibiting responses and error correction.[16] Researchers have shown that stressed participants make riskier decisions in psychosocial stress paradigms such as the Trier Social Stress Test (TSST), especially if they cannot divide their attention between tasks.[17] Acute stress even interferes with an individual's ability to accurately recall memories.[18] The sustained nature of the stressors and the combined impact of successive tasks exacts a cost on cognitive performance, resulting in decreased reaction times, lower accuracy and impaired decision-making.[19]

The impacts of sleep deprivation most clearly exemplify the profound effects of sustained stress on physical and cognitive performance. Fatigue and lack of sleep are pervasive in military environments, where demanding schedules, irregular sleep patterns and the need for sustained vigilance contribute to sleep deficits.[20] These sleep disturbances can decrease the ability to perform high-intensity physical activity,[21] increase susceptibility to inflammation-related illnesses,[22] and increase the likelihood of developing chronic conditions such as cardiovascular disease.[23] Cognitively, insufficient sleep impairs attention, working memory and executive function, which are critical for effective decision-making.[24] Physiologically, sleep deprivation is associated with significant hormonal disruptions, including changes in cortisol and other anabolic and catabolic hormones[25] that may impede cognitive performance in real time[26] and prolong recovery.[27] One of the ways to address this is to identify predictive factors that differentiate individuals who experience performance decrements from those who are well suited or can adapt more following sleep restriction, enabling the development of personalised strategies to enhance performance, resilience and recovery.

Optimising performance with biomarkers and wearable technology

Biomarkers are measurable indicators of biological states or conditions.[28] While usage of the term biomarker can vary in the literature, this protocol uses the term to encompass molecular, physiological and cognitive indicators of stress. In military and athlete settings, such biomarkers can be used to monitor and predict decrements in physical and psychological performance due to environmental stressors.[29 30]

Biomarkers can be measured in various biofluids such as blood, saliva, sweat and urine, providing insights into the body's response to stress.[31] Blood-based biomarkers such as the steroid hormone cortisol can be a time-sensitive marker of stress and recovery.[32] Lactate, commonly used to measure fatigue, is released by skeletal muscles and can reflect their metabolic state.[33] Non-invasive pathways to sample biofluids have also shown utility in monitoring hydration status, such as with sodium and potassium levels in sweat.[34] Non-invasive physical biomarkers on the skin, such as skin temperature, impedance and heart rate, are also effective in monitoring thermal and psychological stress.[35] Skin conductance can indicate hydration status and psychological stress, while heart and sweat rate are physical and psychological stress indicators.[36]

Integrating biomarkers into wearable technology to monitor and predict human performance under stress requires a multidisciplinary approach, combining advancements in biotechnology, data analytics and materials science.[37] The increased availability of multiomics technologies has further enabled the discovery of a wide range of stress markers by leveraging proteomics, transcriptomics, metabolomics and metagenomics.[38] This wealth of biological data has enhanced our ability to measure the effect of stress on multiple biological systems and create panels of biomarkers that indicate stress response.[38] A crucial step in leveraging predictive biomarkers involves assessing their stability in biofluids such as sweat or saliva, their sensitivity to changes

following stress exposure and ensuring they are unaffected by confounding factors such as diet or diurnal rhythm.[37]

Discovering reliable biomarkers that predict performance under physical and psychological stress is key to optimising and tailoring military personnel's capabilities and stress tolerance. Therefore, we have created a two-phase project to identify novel markers of stress that predict human performance under stressors common to military settings. In Phase I, stress markers will be isolated from blood (plasma and serum), saliva and sweat within four distinct stress trials. These stress trials include heat stress, musculoskeletal stress, psychosocial stress and sleep deprivation. The findings of this project will inform the development of a wearable sensor in Phase II for use in measuring and predicting performance in military personnel. Furthermore, this research may have wider applications for other high-stress occupations where optimal performance is crucial.

OBJECTIVES

The primary aim of Phase I is to identify predictive biomarkers of human performance under stress. These biomarkers will be derived from biological samples (blood, sweat, saliva and stool) and physiological and cognitive measures collected before, during and after each stress intervention. The predictive capability will be assessed based on how each biomarker is associated with primary physiological and cognitive measures during each stress trial. The secondary aim of this study is to examine reliable markers of stress occurring within and between each stress type.

The primary aim of Phase II is to develop and test a wearable sensor capable of detecting and monitoring the predictive biomarkers identified in Phase I. This Phase will involve trialling the sensor to evaluate its effectiveness in real-time monitoring and predicting human performance measures under stress.

PARTICIPANTS AND RECRUITMENT

Healthy individuals will be selected for Phase 1, with participants primarily recruited from university campuses, gyms, sports clubs and through online platforms such as social media and websites. We will aim to recruit 104 participants (n=26 for each trial, aiming for equal numbers of men and women and accounting for an attrition rate of 10%–15%). This sample size was selected as it balances the need for using an adequate sample size to detect novel biomarkers with the project's practical, time and budgetary restraints. The chosen sample size also aligns with findings from a review of 33 studies on circulating biomarkers associated with performance and resilience during military operational stress. The sample sizes ranged from a minimum of 7 to 800 participants, with an average of approximately 67 participants.[39] During the recruitment process, participants will choose which trial to participate in. Then, based on recruitment status (ie, the number of women and men already recruited), they will be sorted by their preference and eligibility for each stress trial based on the inclusion criteria. Due to the nature of inclusion for each stress trial, randomisation will not be used to allocate participants into groups. To promote participant retention, we will ensure flexible scheduling, frequent communication and providing gift cards on study completion. If participants choose to discontinue or deviate from the intervention protocols, we will collect all available data up to the point of withdrawal.

Inclusion criteria:

- 18–45 years of age.
- Men, women, non-binary and undisclosed.
- Willing and able to provide biological samples.
- Normal or corrected-to-normal sensory function (eg, vision; must bring glasses if required; hearing; must bring aids if required).

Exclusion criteria:

- Current or previous medical conditions (including psychiatric, neurological or sleep disorders, such as insomnia).
- Currently unwell, even if not diagnosed (eg, cold or influenza symptoms).
- Regular nap taker (ie, take naps 2 or more days per week).
- Night shift worker.
- Pregnant, planning to become pregnant or breastfeeding.
- Participation in other trials that could interfere with the current stress trial outcomes.
- Taking medication that affects exercise ability or thermoregulation (heat and musculoskeletal stress trial only).
- Presence of musculoskeletal injuries or cardiovascular issues that may be exacerbated by exercise (heat and musculoskeletal stress trial only).
- Taking medication such as anti-inflammatory or antioxidant supplements (heat and musculoskeletal stress trial only).
- Maximal oxygen uptake ($\dot{V}O_2$max) test results of <38.1 mL/kg/min (heat stress trial only).
- Less than 1 year of experience with resistance training (musculoskeletal stress trial only).

EARLY TERMINATION

During the stress trials, if a participant meets one of the following criteria, their participation will be discontinued:

- Withdrawal of consent.
- Adverse reaction to any aspect of the protocol.
- No visible or palpable veins as determined in the baseline session.
- Illness.

STRESS TRIAL DESIGN AND SETTING

Phase I of this project is a within-subjects intervention study. This study will be conducted at the Queensland University of Technology and encompass four stress trials (heat, musculoskeletal, psychosocial and sleep).

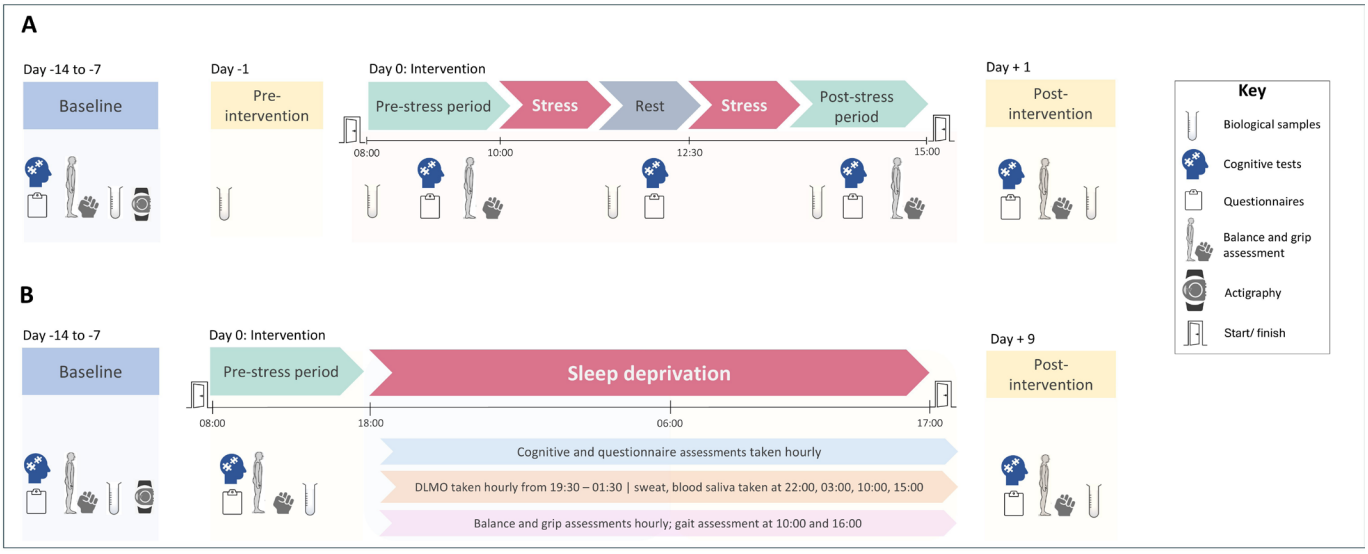

Figure 1 Trial timelines. (A) The study timeline for the heat, musculoskeletal and psychosocial stress trials. Baseline sessions will be scheduled 7–14 days before the intervention, with the pre-intervention session occurring 1 day before and post-intervention session occurring 1 day after the intervention session. (B) The study timeline for the sleep stress trial, where the post-intervention occurs 9 days after the stress intervention to monitor the effects of sleep disturbance. Actigraphy will be continuously monitored from baseline until the end of the post-intervention session for all stress trials.

Participants will select the specific trial they wish to join. They will be contacted with trial information and a consent form, provided they do not meet any exclusion criteria for their chosen stress trial. Sessions within each trial will be organised as follows: baseline session (7–14 days before the intervention session); pre-intervention session (1 day before the intervention session for the heat, musculoskeletal and psychosocial stress trials; day of intervention for the sleep trial); intervention session; post-intervention session (1 day post intervention for the heat, musculoskeletal and psychosocial stress trials; 9 days post intervention for the sleep trial) (figure 1).

Baseline session

The baseline laboratory sessions will involve confirming the screening information and obtaining informed consent from each participant. After a weight measurement, participants will complete questionnaires (including measures of psychological states and traits, eg, stress, anxiety, general sleep and chronotype), a cognitive battery (table 1), a balance test, a grip test, speech recording and biological sampling. The order of this data acquisition will be standardised for all participants. During this session, each participant will also be equipped with an accelerometer (GENEActiv, Cambridgeshire, UK) to record actigraphy for the duration of the trial. During the baseline session of the musculoskeletal stress trial, a five-repetition maximum test of each exercise will be performed to estimate each participant's one-repetition maximum. This estimated one-repetition maximum will then be used to prescribe a 50% load for each exercise during the intervention session. During the heat trial baseline, $\dot{V}O_2$max testing will be conducted to ensure participants meet the minimum fitness requirement.

Pre-intervention and post-intervention

The pre-intervention session will occur 1 day before the intervention session for the musculoskeletal, heat and psychosocial stress trials and on the day of the intervention for the sleep stress trial. Participants will attend the laboratory to provide biological samples (blood, saliva and sweat). Finally, participants will be asked to collect a stool sample at home using a Microba Insight sampling kit (Microba, Brisbane, Australia). The participants will provide the sample to the research team at the start of the intervention session. Participants in the heat trial will also have their body composition assessed through air displacement plethysmography (BodPod, Cosmed, Italy). The post-intervention session will mirror the baseline session, encompassing biological specimen collection, psychological questionnaires, cognitive tasks, and balance and grip strength assessment.

Intervention session

Heat stress trial

Biological samples will be collected after the participants arrive between 08:00 and 10:00 (blood via cannula, saliva and sweat) (figure 1A). Participants will receive a standardised snack before completing cognitive tests, followed by a brief questionnaire about their activities over the past 24 hours (table 1). Cognitive tests at the beginning (entry) and end (exit) of the intervention session will consist of the Balloon Analogue Risk Task, Parametric Go/No-Go (PGNG) task, Emotional Recognition Task (ERT) and Psychomotor Vigilance Task (PVT), while the spot test conducted after the first bout of stress will consist of the PVT, PGNG and ERT. Following these initial assessments, a blood sample will be collected and participants will be equipped with heart rate and skin

Table 1 Summary of cognitive tests, psychological questionnaires and visual analogue scales (VAS)

		Measure	D −14 to −7	D −1	D −0	D +1 and D +9§
Cognitive tests	Balloon Analogue Risk Task	Risk-taking	x		x	x
	Parametric Go/No-Go	Response inhibition	x		x	x
	Penn Emotion Recognition Task	Emotion recognition	x		x	x
	Psychomotor Vigilance Test	Attentional load	x		x	x
	Paced Audio Serial Addition Test	Sustained attention	x			x
	Verbal Paired Associates Learning	Associative/episodic memory	x			x
Psychological questionnaires	Karolinska Sleepiness Scale	Sleepiness	x			x
	PROMIS Sleep Disturbance Scale	Sleep quality	x			x
	PROMIS Sleep-Related Impairment Scale	Sleep quality	x			x
	Micro Munich ChronoType Questionnaire	Sleep regularity	x			x
	Perceived Stress Scale	Perceived stress	x			x
	Duckworth Grit Scale	Resilience	x		x	x
	Conor-Davidson Resilience Scale	Resilience	x			x
	Intolerance of Uncertainty Scale	Uncertainty intolerance	x			x
	Risk Propensity Scale	Risk propensity	x		x	x
	General Risk Propensity Scale	Risk propensity	x			x
	Positive and Negative Affect Scale	Mood	x		x	x
	Invincibility Belief Index	Invincibility perception	x			x
	Mini-Social Phobia Inventory	Social anxiety	x			x
	Depression Anxiety Worry Scale	Distress	x			x
	Health habits during last 24 hours	Health and lifestyle	x		x	x
VAS	Thermal Comfort Scale*	Perceived comfort			x	
	Thermal Sensation Scale*	Perceived sensation			x	
	Borg CR10 rate of perceived exertion*	Perceived effort			x	
	Repetitions in Reserve Scale†	Perceived intensity			x	
	Muscle Soreness Scale†	Perceived soreness			x	x
	Perceived Stress Perception Scale‡	Perceived stress			x	
	Reflexive scale and mood	Preparedness and mood			x	x
	Sleep quality and stress perception	Sleep quality and perceived stress	x	x	x	x

Cognitive tests will assess changes in decision-making, while psychological questionnaires will evaluate personality, psychopathology, risk propensity, resilience and health-related habits. VAS will be used to measure stress levels and perceived exertion. Assessments will occur during: D −14 to −7 (baseline), D −1 (pre-intervention), D −0 (intervention) and D +1 and D +9 (post-intervention).
*Assessments specific to the heat stress trial.
†Assessments specific to the musculoskeletal stress trial.
‡Assessments specific to the psychosocial stress trial.
§Assessments specific to the sleep stress trial.
PROMIS, Patient-Reported Outcomes Measurement Information System.

temperature monitors. Participants will then provide a nude weight and urine sample to measure their hydration status. Participants will then self-insert a rectal thermistor to a depth of 12 cm beyond the anal sphincter.

During both stress bouts, participants will exercise on a treadmill inside an environmental chamber maintained at 32°C Wet-Bulb Globe Temperature (37°C ambient temperature and 60% relative humidity). The exercise will consist of 60 min of walking at a lower intensity (4.6 km/hour, 1% incline), followed by 30 min at a higher exercise intensity (5.6 km/hour, 2% incline, wearing a 10 kg vest) inside the heat chamber. At the end of the first exercise bout, the participants will have a 60 min rest period, where they will briefly exit the chamber to provide a nude weight before returning. Participants will repeat the lower-intensity and higher-intensity exercise sequence, totalling approximately 4 hours inside the heat chamber.

During the rest periods, participants will be provided a nude weight measurement outside the heat chamber and will be provided with food. Participants will have access to water inside the heat chamber, but fluid consumption will be restricted to 30% of their sweat losses to achieve a 3%–4% body mass loss target throughout the trial. Biological samples (blood, sweat and saliva) will be collected, and participants will perform a shortened cognitive battery (PVT, PGNG and ERT) and complete visual analogue scales (VAS) to rate their stress response inside the heat chamber. During the rest period, they will complete self-report scales to assess perceived stress levels.

At the end of the session, physiological measurement devices will be removed, and final biological samples (blood, sweat and saliva) will be collected alongside recorded speech. Participants will complete the exit cognitive battery and receive a second Microba Insight sampling kit to collect an overnight stool sample at home. This stool sample will be handed to the research team at the post-intervention session.

Musculoskeletal stress trial

Participants in this trial will undergo the same arrival procedure described in the heat stress trial and arrive from 08:00 to 10:00; however, participants in this stress trial will have blood samples collected via venepuncture instead, ensuring unrestricted arm movement during exercise (figure 1A). Additionally, mid-upper arm circumference will be measured at the midpoint between the acromion process and the olecranon process. Mid-thigh circumference will be measured at the midpoint between the greater trochanter and the lateral epicondyle of the femur in a seated position. These measurements will be used as a proxy measure of muscle swelling. Participants will also use a visual analogue scale to rate their perceptions of muscle soreness.

Participants will then have the option to perform a light warm-up, including stretches and exercises, using an unloaded bar for up to 10 min. Following this, participants will perform a bout of three sets of 10 repetitions for each of 10 resistance exercises: back squat, bench press, deadlift, military press, front squat, bent-over row, incline bench press, bicep curl, close-grip bench press and upright row. After completing three sets of each resistance exercise, the participants will be asked to report their rating of perceived exertion and how many more repetitions they believe they can complete (ie, repetitions in reserve). This exercise bout will take around 45 min to complete. The load for each exercise will be set to 50% of each participant's one-repetition maximum, as estimated from the five-repetition maximum calculated during the baseline session. Following a 60 min rest period, the participants will repeat the same bout of resistance exercises.

During the rest period, participants will be allowed to consume water ad libitum and provided a protein bar. Mid-upper arm and mid-thigh circumference will be measured again. During the first recovery period, participants will also complete a baseline muscle soreness VAS and a shortened cognitive battery (PVT, PGNG and ERT). At the end of the second exercise bout, physiological measurement devices will be removed, and final biological samples (blood, sweat and saliva) will be collected alongside recorded speech. A final limb circumference and muscle soreness measurement will also be taken after the second bout. Finally, participants will complete the exit cognitive battery and be asked to collect an overnight stool sample to be handed in at the post-intervention session.

Psychosocial stress trial

Participants will undergo the same arrival procedure as outlined in the heat stress trial (including cannulation) and arrive from 08:00 to 10:00. Before stress induction, participants will have physiological measurement devices attached to record electrodermal responses and heart rate (Biopac Systems, Goleta, California, USA). The psychosocial stress trial consists of two stress periods separated by a 30 min rest period (figure 1A).

To induce social-evaluative stress, participants will complete the TSST, conducted by one evaluator and adapted from established protocols.[40] The TSST will consist of five phases: (1) introduction to the procedure; (2) preparation of a speech for a job; (3) speech delivery; (4) reciting mental arithmetic (subtracting backwards from 1000 in steps of 17); and (5) recovery (sit quietly and relax for 5 min). Participants will be informed that their speech and arithmetic calculations are recorded by video for later analysis of behavioural and speech-related markers. A self-report stress perception questionnaire will precede each phase of the TSST. The TSST will take ~90 min. During the second component of the protocol, participants will complete the Sing-a-Song-Stress Test (SSST) and tone avoidance task (TAT). In the SSST, participants will work through three reading tasks, one speaking and one singing task.[41] In the tone avoidance task, participants will press a button

in response to a visual stimulus as quickly as possible to avoid an unpleasant loud tone (1000 Hz, 1 s, 90 dBA). Each task will be preceded by a subjective self-report stress questionnaire, and physiological responses are recorded continuously. During each task, the evaluator will remain behind the participant, visible in a mirror on the wall in front of the participant. This test will take approximately 35 min to complete. Between the TAT and SSST, participants will also complete a subjective self-report stress questionnaire.

At the end of the trial, physiological measurement devices will be removed, and final biological samples (blood, sweat and saliva) will be collected alongside recorded speech. Participants will complete the exit cognitive battery and be asked to collect an overnight stool sample to be handed in at the post-intervention session.

Sleep stress trial

To ensure standardised sleep conditions, participants will be instructed to allocate a 9-hour window for sleep in the evening before and after the sleep intervention session, aligning with recommended sleep duration guidelines.[42] The sleep intervention protocol is outlined as follows (see figure 1B). On day 1, the preintervention session will begin in the laboratory at 08:00 to 10:00. Blood, sweat and saliva samples will be taken, and participants will complete a cognitive battery and psychological questionnaires as outlined in the heat intervention session. They will undergo a gait assessment, walking at a natural speed twice on a Zeno Walkway (ProtoKinetics, Havertown, Pennsylvania, USA) and undergoing a dual balance task. Participants will also have their speech recorded at the end of the arrival procedure. Participants will proceed with their usual daily activities outside the lab but will be asked to abstain from caffeine and alcohol. They will also be instructed to eat a meal before arriving at the laboratory at 18:00. On arrival, participants will be equipped with a polysomnography device (SOMNOmedics, GmbH, Germany) to measure electroencephalography (EEG) and electrocardiography (ECG). Participants will also have a cannula inserted. From 19:00, participants will undertake hourly cognitive or physical tests (randomised order for each participant; tests as per table 1). Following standard dim-lighting conditions starting at 19:00,[43] participants will also provide hourly saliva samples from 19:30 to 01:30 to capture the onset of melatonin secretion. Three biological samples (blood, sweat and saliva) will be collected at four set intervals (22:00 on day 1; 03:00, 10:00 and 15:00 on day 2).

After completing the exit cognitive battery, participants will undergo a gait assessment. Afterwards, their speech will be recorded, and the EEG/ECG devices will be removed. They will then be sent home with a stool sampling kit at 17:00. The stool sample will be returned by return mail according to the manufacturer's instructions.

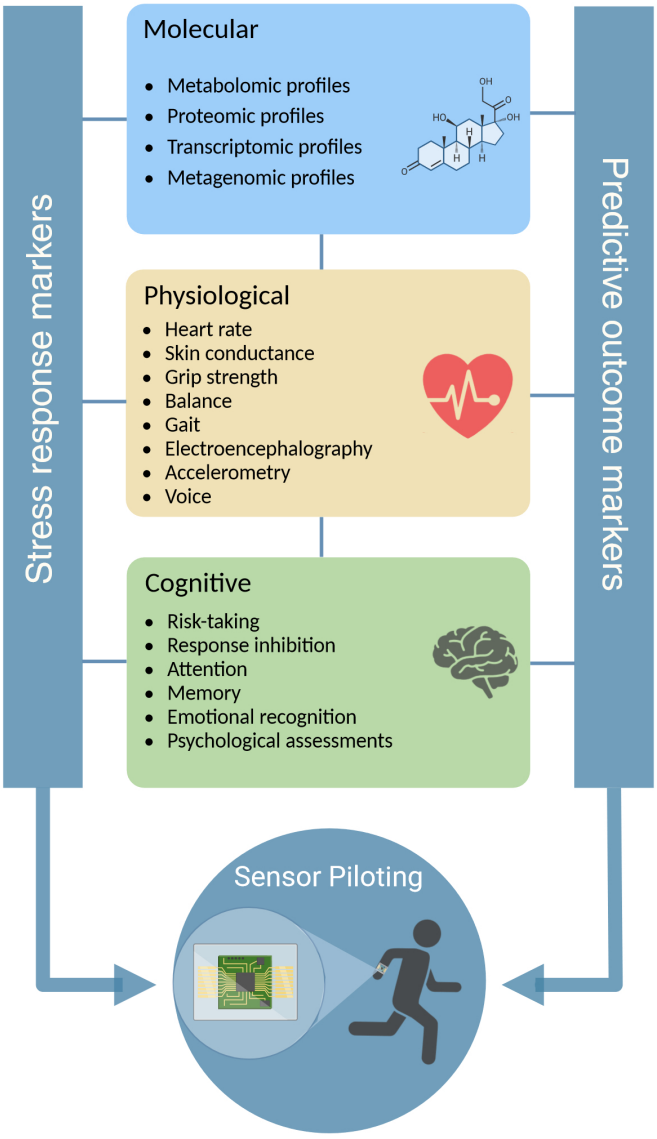

Figure 2 Overview of stress response domains and measures. This depicts the three domains of stress response markers: molecular, physiological and cognitive. Measures within each of these domains will be evaluated to assess their capability to monitor physical and psychological performance under stress. Markers correlated with stress response will then be integrated into a wearable sensor for piloting. Created by JF in 2024 in BioRender (https://BioRender.com/e62o964).

Biomarker analysis

Biomarkers within the molecular, physiological and cognitive domains will be analysed to identify predictors of human performance under stress (figure 2; see online supplemental appendix 1 material for collection procedure). The proteomic analysis will be conducted on the serum samples using liquid chromatography–tandem mass spectrometry employing data-independent acquisition. This will enable analysis of the relative quantification of proteins and identification of the molecular functions and biological processes represented by the proteins. Metabolomic profiling will be performed on the plasma

samples using gas chromatography–mass spectrometry to identify dynamic metabolic changes and link them to biological pathways related to stress exposure. miRNA from saliva samples will be analysed using miRNA sequencing (Illumina NovaSeq6000 platform) and verified through qRT-PCR assays, emphasising integrating these findings with proteomic and metabolomic data to identify predictive biomarker panels.

DNA from stool samples will be isolated and prepared before sequencing to explore the potential acute effects of induced stress on the gut microbiome (Illumina NovaSeq6000 platform). Metagenomic raw data will be processed using Microba's profiling pipeline.[44] Quantification of taxonomic abundances and metabolic pathway analysis will be conducted using the Microba Gene and Pathway Profiler software and ANCOM package in R. This will identify differentially abundant microbial features prestress and poststress exposure.

Sensor design and testing

The development of the wearable sensor will proceed through several stages, guided by the biomarker candidates identified in Phase I. Biofluids such as sweat and saliva will be evaluated to determine if our biomarkers of interest can be successfully detected, are stable and are measurable within biologically relevant ranges. Once detected, these analytes (electrolytes, metabolites, peptides and/or proteins) will be linked to biorecognition elements such as proteins, nucleic acids and artificial receptors, which are integral to the sensor's function. The wearable sensor, which could be integrated into devices such as contact lenses, mouthguards or skin patches, will employ colourimetric or electrochemical methods for data collection. Ideally, the final wearable sensor will be able to monitor several key biomarker targets continuously. Moreover, the wearable sensor will integrate measures such as heart rate and skin conductance to comprehensively measure physiological stress response.

In Phase II, we will pilot a wearable device/sensor to detect biomarkers identified in Phase I. 46 participants (n=23 per group) will be used to compare predictive biomarkers in stressed and unstressed individuals. This proposed sample size was estimated by referencing salivary cortisol, a well-documented biomarker with high specificity to stress, which can be monitored non-invasively.[45] A prior meta-analysis identified an effect size of cortisol response to stress as d=0.93.[46] Using G*Power, with a target statistical power of 0.80, this analysis suggests that a total sample size of 46 participants is needed, accounting for an expected attrition rate of 10%–15%. However, we recognise that the final sample size may need adjustment based on the number and variability of candidate biomarkers identified in Phase I. To refine our estimation after Phase I, we will consider using the prospective specimen collection, retrospective-blinded evaluation framework, which allows for consideration of biomarker performance metrics, the power to detect a sufficient proportion of useful biomarkers and the control of false leads.

Statistical analysis

The raw data will be cleaned to standardise the format for statistical analysis using R.[47] Exploratory data analysis will be conducted, including boxplots, histograms and tables, and estimates of each dataset's mean, median and standard deviation within each trial. Relationships between variables will be examined using multivariate linear regression and logistic regression. Mixed and random effects will be included to account for variability across individuals and experimental conditions. Principal components analysis and network analysis, classification, regression trees, neural network models and Bayesian networks will be used to integrate data across trials and assess predictive validity of biomarker panels.

DISCUSSION

The current study aims to identify biomarkers that predict human performance under conditions of heat, musculoskeletal, psychosocial and sleep stress, with the ultimate goal of developing a of wearable sensor. This investigation is driven by the growing need for evidence-based strategies to optimise performance in physically demanding environments while addressing the practical challenges associated with monitoring physiological responses in real time.

Achieving an adequate sample size is a recognised challenge due to stringent inclusion criteria, time constraints and financial limitations. To mitigate this, the study incorporates several strategies, including maximising the number of biological samples collected, ensuring a minimum of six collections per participant and employing a within-subjects design to minimise between-subject variability. Additionally, advanced statistical approaches such as generalised linear mixed models will account for individual differences and improve the reliability of detecting biomarker changes over time.

While this study seeks to identify a broad range of biomolecules as potential biomarkers, it is important to acknowledge the technical limitations of translating these findings into wearable sensor technology. Certain biomolecules may pose challenges due to their physical and chemical properties, such as instability, low detection sensitivity or incompatibility with existing sensor platforms. These limitations highlight the necessity of further research in subsequent phases to evaluate the feasibility of integrating specific biomarkers into a wearable device.

Trial status

The study has currently finished recruiting for Phase I. The project began development and recruitment for phase I in 2023, and Phase II will conclude in 2025 after sensor piloting.

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Competing interests None declared.

Patient and public involvement This study focuses on monitoring performance in healthy individuals, without involving a patient cohort or clinical interventions. Consequently, patients and the public were not directly involved in the research's development. The research questions and study design were informed by existing literature and the needs of military and high-stress occupations. Participants will have access to the study's results and the option to have their biological samples returned upon request, ensuring their rights and preferences are respected.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants. Phase I of this study involving human participants has been reviewed and approved by the University Human Research Ethics Committee (UHREC) at Queensland University of Technology (approval number 4824). As Phase II is contingent on the outcomes of Phase I and continued funding, a separate ethics approval will be sought for Phase II. In the current project, we have prioritised participant safety and have plans to manage participation risks. Stress trial safety risks will be managed through thorough screening and eligibility criteria, continuous monitoring of physiological strain and termination of sessions if strain exceeds predefined thresholds. In the event of a medical issue, we will follow recommended procedures, and any adverse event will be reported to the University Research Ethics Committee. The participants' time commitment will be acknowledged with gift cards on completion of their sessions. Personal information will be stored in de-identified format to minimise the risk of disclosure, with strict access controls and secure storage practices in place. We will accommodate participants' dietary preferences. COVID-19-safe practices will be adhered to with vaccination requirements, and rapid antigen testing will be made available to participants. The findings of the study will be disseminated through national and international conferences, as well as peer-reviewed journals. In addition, we will provide a collective summary of the trial findings to the participants, ensuring transparency and accountability to those involved in the study. Access to interim data will be limited to the trial lead and authorised study team members responsible for preparing progress reports. Significant protocol modifications, such as changes to eligibility criteria, outcomes or analysis methods, will be promptly communicated to UHREC, defence sponsors, investigators and participants as appropriate. Participants gave informed consent to participate in the study before taking part.

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Data availability statement No data are available. We plan to make the data available once they have been analysed, validated and published. Updates on data sharing will be provided as the study progresses, ensuring compliance with ethical and legal standards while respecting participant privacy. The authors can be contacted for any enquiries related to obtaining data.

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