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Environmental associations with heart-rate responses during field-based submaximal fitness testing in female football players: an exploratory multi-centre study

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ABSTRACT

This exploratory multi-centre study examined associations between wet-bulb temperature (WBT) and exercise heart-rate responses (HRe_x) obtained during routine field-based submaximal fitness testing (SMFT) in female football players. Data were collected from 84 players across three clubs competing in the Australian National Premier League, yielding 971 observations (12 ± 6 observations per player). Associations between WBT and HRe_x were examined using linear mixed-effects models accounting for repeated observations within players and testing sessions. A positive association was observed between WBT and HRe_x, with a 10 °C increase in WBT corresponding to an approximately 1.0 percentage point higher HRe_x (90% CIs: 0.1 to 1.9% points). This association remained relatively consistent across multiple model specifications. Allowing WBT slopes to vary between players improved model fit. Variation in individual WBT slopes (SD = 2.2% points) and residual within-athlete variability (SD = 2.0% points) exceeded the magnitude of the population-average association, although uncertainty surrounding these individual estimates was substantial. These findings should be interpreted as hypothesis-generating rather than evidence of causal relationships. Future confirmatory and experimental studies are required to determine whether higher environmental temperatures cause higher SMFT HRe_x regardless of changes in physiological state in female football players.

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KEYWORDS

Exercise physiology; heart rate; temperature; women's football; soccer

Introduction

Heart-rate-derived indices quantified during standardised submaximal fitness testing (SMFT) are widely used in team sports to monitor physiological (cardiovascular and metabolic) state and training adaptation (Shushan et al. 2022; Buchheit et al. 2025). These assessments are valued because they impose a relatively low physiological and mental cost, can be implemented frequently, and provide repeatable indicators of cardiovascular responses during standardised exercise tasks (Schneider et al. 2018; Shushan et al. 2023b). In applied settings, SMFT protocols are embedded within routine training sessions, often early within the session warm-up, helping to minimise confounding influences such as accumulated fatigue and cardiovascular drift (Arguedas-Soley et al. 2024). As a result, heart-rate (HR) responses during these protocols are increasingly used by practitioners to track physiological responses and monitor

longitudinal changes across the competitive season (Shushan et al. 2024b).

Despite their widespread application, HR responses obtained during SMFT may be influenced by several contextual factors beyond the underlying physiological state. For example, variation in recent training load, recovery status, and environmental conditions may all contribute to fluctuations in exercise HR responses. Accordingly, accurate interpretation of SMFT-derived HR metrics requires consideration of the context in which testing occurs (Schneider et al. 2018). This may influence the interpretation of longitudinal monitoring data, particularly when practitioners use subtle changes in HR-derived indices to infer alterations in physiological state or training adaptation.

Environmental conditions are of particular interest to the interpretation of HR-derived indices from SMFT because they could vary substantially throughout

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a team-sport season and have a strong mechanistic link to cardiovascular responses during exercise (Sawka et al. 2011; Racinais et al. 2015). Previous observations from team-sport settings have reported small positive associations between warmer environmental conditions and higher exercise heart-rate responses (H_{Rex}) during standardised submaximal exercise, with H_{Rex} increasing by approximately one percentage point for every 10 °C increase in environmental temperature (Lacome et al. 2018; Buchheit et al. 2025). However, the environmental index underlying these estimates has not been consistently specified, and the evidence has generally been derived from small cohorts or case studies with short observational periods, and has not typically accounted for the nested structure of observations within testing sessions. Thermoregulatory strain during exercise is influenced by both ambient temperature and atmospheric moisture, which together determine the effectiveness of evaporative heat loss and heat dissipation mechanisms (Sawka et al. 2011; Racinais et al. 2014).

Wet-bulb temperature (WBT) integrates these environmental characteristics into a single index that can be estimated from routinely available meteorological data (Stull 2011), making it a pragmatic indicator for longitudinal field-based monitoring. It does not, however, account for radiant heat or air movement, both of which influence thermal load during exercise and are captured by composite indices such as wet-bulb globe temperature (WBGT) (Racinais et al. 2015). WBT is therefore best regarded as a partial representation of the thermal conditions experienced during exercise, rather than a comprehensive measure of heat stress or individual heat strain. While environmental influences on HR responses during SMFT have received increasing attention, limited evidence has examined the association between WBT and H_{Rex} responses collected repeatedly during routine field-based monitoring (Lacome et al. 2018; Buchheit et al. 2025).

This knowledge gap may be particularly relevant in women's football. Despite the increasing professionalisation of the game and growing use of routine physiological monitoring practices, female athletes remain under-represented in much of the applied monitoring literature (Okholm Kryger et al. 2022; Lovell and Okholm Kryger 2025). Several physiological characteristics have been proposed to influence thermoregulatory responses during exercise in women, including differences in sweat rate, cutaneous blood flow responses, body composition, and body surface area-to-mass ratio (Notley et al. 2019). In addition, fluctuations in ovarian hormones throughout the menstrual cycle may alter thermoregulatory set points and resting body temperature (Oosthuysen and Bosch 2010). Collectively, these factors

suggest that associations between environmental conditions and cardiovascular responses observed in predominantly male cohorts may not necessarily generalise directly to female football players.

Although the magnitude of association between WBT and H_{Rex} appears modest, the existing evidence alone is not yet sufficient to develop a formal hypothesis about the effect of WBT on H_{Rex} during SMFT for examination in subsequent experimental studies under causal inference methodology. Therefore, the purpose of this exploratory multi-centre study was to examine associations between WBT and H_{Rex} responses during routine field-based SMFT in a large cohort of female football players.

Methods

Design

Repeated measures prospective cohort study.

Participants

Data were obtained from 84 female football players using an existing monitoring dataset collected during routine training across the 2024 competitive in-season. Players were drawn from three clubs competing in the Australian National Premier League (NPL) system, including two first-tier clubs (Club 1: $n = 26$; Club 2: $n = 24$) and one second-tier club (Club 3: $n = 34$), with players representing both U20 and senior squads. Based on competition level, participants were classified as Tier 2 and Tier 3 athletes (Compton et al. 2025). Self-reported menstrual health and hormonal-contraceptive status were available for 76 of the 84 players (90%), collected as part of routine club screening. Of these, 52 (68%) were naturally menstruating and not using hormonal contraception, including 2 who used a non-hormonal (copper) intrauterine device. The remaining 24 players (32%) reported using hormonal contraception: 15 (20%) a combined oral contraceptive pill, 6 (8%) a hormonal intrauterine device, 2 (3%) a progestin-only pill, and 1 (1%) a subdermal implant. Players were not excluded on the basis of menstrual or hormonal-contraceptive status, as the study aimed to characterise the WBT–H_{Rex} association within a representative cohort of female football players rather than to attribute responses to a specific hormonal profile.

All teams followed a typical competitive microcycle, including 3 training sessions of approximately 90 min, combining technical, tactical and conditioning content, and 1–2 competitive matches per week. This weekly structure remained reasonably consistent across the in-

season period, which spanned February to September. Ethical approval was obtained from the Human Research Ethics Committee of the University of Wollongong (approval number: 2023/334).

Submaximal fitness testing

The SMFT protocol consisted of a standardised 4-min continuous run performed at a fixed average speed of $11 \text{ km} \cdot \text{h}^{-1}$ on a 50-m shuttle course. We selected this protocol in line with recommendations favouring continuous exercise at a fixed external intensity for monitoring HR-derived measures (Shushan et al. 2023a). The duration was set at 4 min to allow HR to stabilise before the outcome measure was obtained (Shushan et al. 2024a). We determined the running speed in consultation with coaching staff, informed by practices reported in a survey of SMFT use in team sports (Shushan et al. 2023b). Running pace was controlled using an audio cue every 25 m (every 8.2 s) to ensure consistent speed (Shushan et al. 2023a). The SMFT was embedded within the routine warm-up and scheduled at least 48 h after the most recent match. This timing was intended to minimise residual match-related fatigue and maintain consistency with preceding training loads within the weekly microcycle.

Monitoring environmental conditions

All SMFT procedures occurred during evening times (~6:30–8:00 PM). Ambient air temperature ($^{\circ}\text{C}$) and relative humidity (%) were obtained for each session from a publicly available meteorological database (<https://www.timeanddate.com/weather/>). Values corresponded to the training location and time of testing (± 15 min). Public meteorological databases provide measurements obtained from nearby weather stations and are commonly used in sport and exercise research and field settings (Racinais et al. 2015; Schwarz et al. 2025) when direct on-field environmental measurements are not available. Although such data may not fully capture local microclimate conditions at the training surface (e.g., solar radiation, surface temperature), they provide a pragmatic estimate of ambient environmental conditions experienced during field-based training sessions. Wet-bulb temperature ($^{\circ}\text{C}$) was calculated from ambient air temperature and relative humidity using the approximation proposed by Stull (Stull 2011). Because WBT incorporates both temperature and atmospheric moisture, it was selected as a composite environmental index that may better reflect the thermal conditions experienced during exercise and the evaporative cooling capacity of the environment.

Heart rate monitoring and processing

Heart-rate responses were recorded during all training sessions using chest-strap sensors (10 Hz; Polar Team Pro, Kempele, Finland). Data were subsequently extracted and processed in R (version 4.5.3) (R Core Team 2026). All HR traces were visually inspected to identify and remove obvious artefacts or physiologically implausible values ($n = 27$ of 1,022 observations removed; 2.6%). H_{Rex} was calculated as the mean heart rate during the final 30 s of the 4-min run (Shushan et al. 2024a). H_{Rex} values were expressed relative to each athlete's maximum HR (%HR_{max}), defined as the highest HR recorded across the competitive season, obtained from either maximal field-based assessments, high-intensity interval training exposures, matches, and routine training drills. The resulting player-specific HR_{max} value was then applied consistently across all observations.

Data analysis

Descriptive statistics are presented as the mean $\pm$ standard deviation (SD), and uncertainty around model estimates is reported using 90% confidence (compatibility) intervals (CIs). All data analyses and reporting were performed in R using the RStudio integrated development environment (version 2025.05.1) (Posit team 2025). A full and transparent description of the exploratory workflow and data analysis steps is accessible in the supplementary materials. The corresponding analysis code and data are openly available on the Open Science Framework (OSF repository; <https://osf.io/jczb6/overview>).

We followed recommendations for exploratory research in sport and exercise science to guide both our statistical analyses and data interpretation (Ditroilo et al. 2025). Given the observational and exploratory nature of the study, the analyses were intended to characterise associations rather than establish causal relationships (Stovitz et al. 2019; Haber et al. 2022). Consequently, the findings were interpreted as hypothesis-generating, and emphasis was placed on estimation and uncertainty rather than dichotomous significance testing.

The sample was first subset to include only players with at least three SMFT observations to avoid estimation instability at the individual level, and sessions flagged for exclusion were removed. These criteria excluded 24 observations, yielding the final 971 observations (supplementary materials, Step 1). Next, individual associations between H_{Rex} and WBT were visualised and quantified (Pearson's r) to assess the general shape and direction of the relationship across the observed

temperature range, and to determine whether it deviated meaningfully from linearity (supplementary materials, Steps 2–4). We then compared linear, quadratic and spline (knot at 16 °C) models of WBT. Both non-linear specifications increased Akaike Information Criterion (AIC) relative to the linear model (quadratic +1.8, spline +1.7 units). As these differences were small, all three specifications retain empirical support (Burnham et al. 2011). WBT was therefore modelled as a linear predictor in all subsequent analyses, being the more parsimonious and interpretable estimate (supplementary materials, Step 3).

Together, these exploratory analyses indicated a small but consistent directional relationship that appeared approximately linear. Given this pattern and the hierarchical, repeated-measures nature of the data, we proceeded with linear mixed-effects modelling using the *lme4* package to quantify the association between WBT and SMFT HRex.

To aid interpretation of fixed-effect estimates, WBT was mean-centred and scaled such that a one-unit change represented a 10 °C difference. Associations between WBT and HRex were subsequently examined using linear mixed-effects models to account for repeated observations within players and testing sessions. Three candidate models of increasing complexity were specified a priori (Table 1), which were all initially fit using Restricted Maximum Likelihood (REML) estimation (supplementary materials, Steps 5).

Model 1 was fit with player identification as a single random intercept to account for repeated observations within athletes. Model 2 expanded this structure by incorporating random intercepts for both players and unique testing sessions (i.e., dates nested within centres; Supplementary Figure S1). Because player identification codes were globally unique and players never crossed between locations, this architecture also implicitly respects the nesting of players within their respective centres. This structure avoids pseudoreplication by isolating and partitioning the shared day-to-day environmental variance experienced by players testing on the same day at the same location. We specifically chose to nest dates within centres, rather than including a standalone random intercept for centre, because the low number of participating centres ($n = 3$) violates the

mathematical assumption of sufficient levels required to reliably estimate cluster-level variance. Finally, Model 3 was fit using the same random intercept structure as Model 2, but with the addition of a correlated random slope for WBT to account for between-player variability in the association to HRex.

Model fit was evaluated using the AIC. An AIC reduction of >10 units was considered as strong empirical support for the competing model (Burnham et al. 2011). Where differences were smaller, we considered models to retain some support and preferred the simpler or more parsimonious specification (Burnham et al. 2011). We also compared models using nested likelihood ratio tests to determine if the addition of specific random parameters significantly improved model fit. The best-fitting model was retained as the final model; its fixed and variance components were extracted along with their 90% CIs, which were calculated using the profile likelihood method to account for asymmetry in random effect distributions.

Results

The final dataset included 971 SMFT observations from 84 female football players, recorded over 58 testing sessions. Players contributed 12 ± 6 observations each (median: 11; range: 3–25 observations). Across all repeated assessments, the grand mean ($\pm$ pooled SD) HRex was $88.4 \pm 4.5\%$ HRmax. Ambient temperature was 15 ± 4 °C (range: 7–23), relative humidity was $77 \pm 16\%$ (range: 34–100%), and WBT was 12 ± 4 °C (range: 5–22).

Raw data together with the fitted intercepts and slopes from each candidate model are presented in Figure 1. Model 3, which included random intercepts for players and testing sessions nested in centres plus a random slope for WBT, provided a significantly better model fit when compared with the other two candidate models (Table 2).

The estimated association between WBT and SMFT HRex is presented in Figure 2. A 10 °C within-player change in WBT was associated with a 1.0% point change in HRex (90% CIs: 0.1 to 1.9% points). Although the magnitude of this association remained consistent across all candidate models, progressively accounting for additional sources of variability increased the

Table 1. Candidate mixed-effects model structures used in the exploratory analyses.

Model	Effects		Syntax
	Fixed	Random	
1	WBT	Player intercepts	$hr \sim wbt + (1 id)$
2	WBT	Player & session intercepts	$hr \sim wbt + (1 id) + (1 centre:date)$
3	WBT	Random slopes + player & session intercepts	$hr \sim wbt + (1 + wbt id) + (1 centre:date)$

HR: heart rate, WBT: wet-bulb temperature.

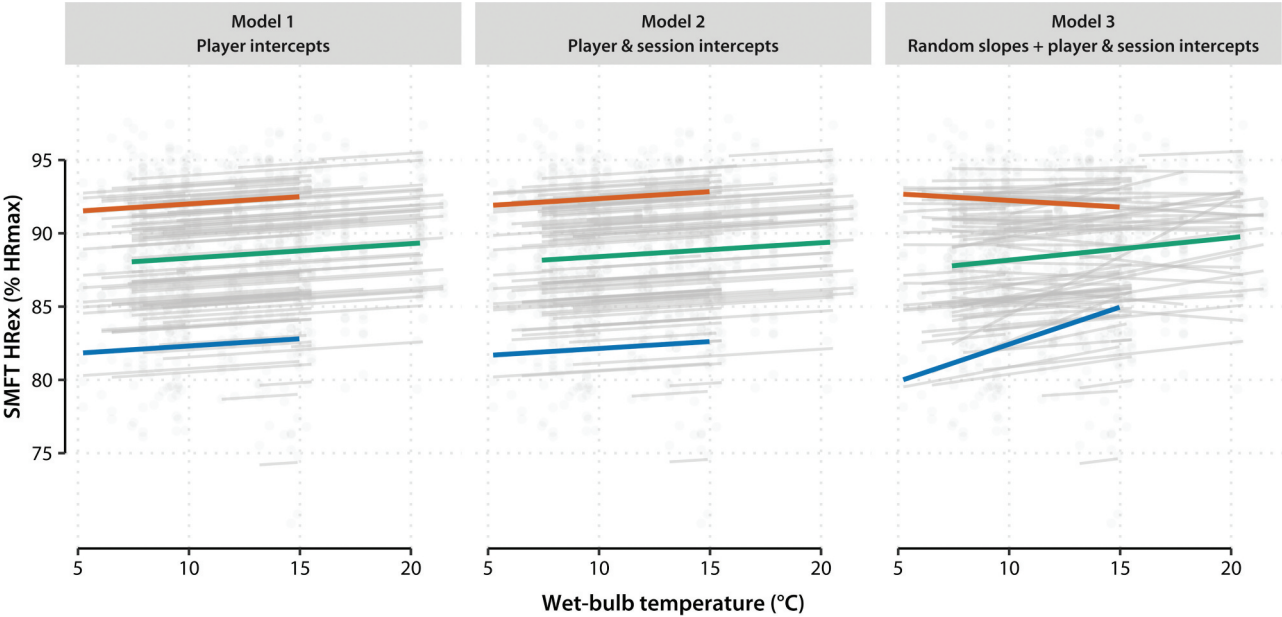


Figure 1. Within-player raw observations and fitted relationships from the three candidate mixed-effects models examining associations between WBT and HRex during SMFT. Grey points represent individual observations, and grey lines represent the fitted trajectory for each player. Coloured trajectories illustrate three representative players exhibiting positive, stable, and negative WBT–HRex associations, highlighting the increasing flexibility afforded by the more complex random-effects structures.

Table 2. Comparison of mixed-effects models performance quantifying the relationship between WBT and SMFT HRex.

Model	AIC	BIC	ICC	Residual SD (% points)	Δ AIC	χ^2	P value
1	4756.6	4776.1	0.76	2.4			
2	4624.3	4648.7	0.81	2.1	–132.3	134.3	<0.001
3	4593.3	4627.4	0.83	2.0	–31.0	35.0	<0.001

AIC: Akaike information criterion, BIC: Bayesian information criterion, ICC: Intraclass correlation coefficient, SD: standard deviation, WBT: wet-bulb temperature.

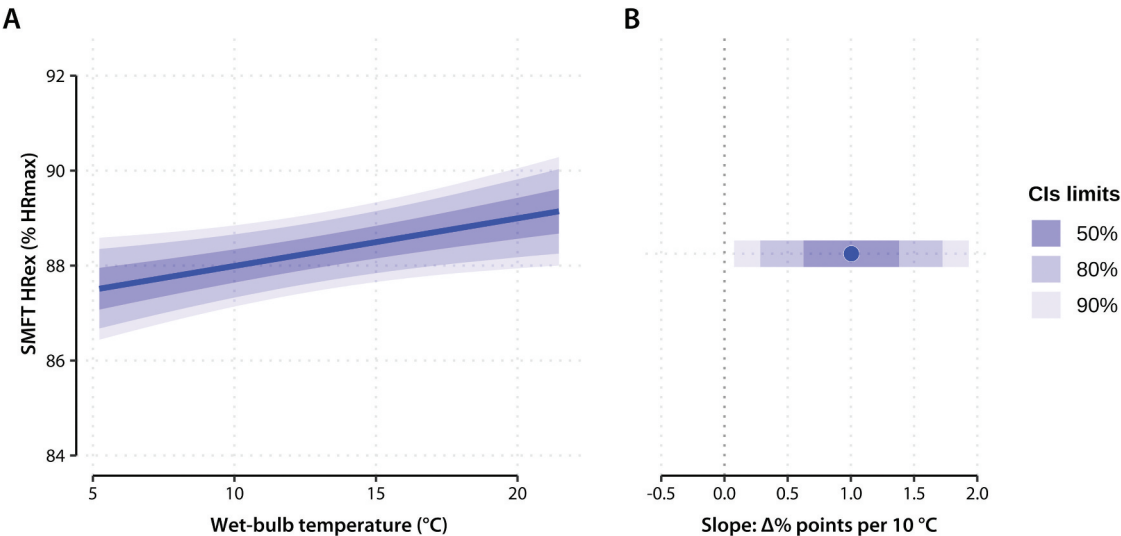


Figure 2. Population-average association between WBT and HRex during SMFT estimated from the final mixed-effects model (Model 3). Panel A presents the predicted HRex response across the observed WBT range, with shaded regions representing 50%, 80%, and 90% confidence (compatibility) intervals (CIs). Panel B presents the corresponding fixed-effect estimate expressed as the change in HRex associated with a 10 °C increase in WBT, together with the associated 50%, 80%, and 90% CIs.

uncertainty surrounding the average estimate (Supplementary Figure S2).

Between-player variability in this slope was $\pm 2.2\%$ points (1.7 to 2.8). Additional random effects representing the HRex variability between athletes, between sessions and residual noise are presented in Figure 3. The correlation (r) between individual intercept and slope values was -0.29 (-0.57 to 0.02).

Discussion

Our primary aim was to examine the association between wet-bulb temperature (WBT) and HRex during routine field-based SMFT in female football players. Across almost 1000 observations, we observed a positive association, with a 10°C increase in WBT corresponding to a $\sim 1\%$ point increase in HRex. This association was consistent across multiple model specifications and aligns closely with estimates previously reported in male team-sport cohorts (Lacome et al. 2018; Buchheit et al. 2025). Importantly, substantial within-athlete variability remained unexplained, indicating that environmental conditions represent only one of several factors contributing to longitudinal fluctuations in HRex.

The WBT range observed in the present study (5 to 22°C) reflects relatively moderate environmental conditions.

Within this range, the WBT–HRex relationship appeared approximately linear. It remains possible that more extreme environmental conditions, characterised by greater thermal strain and reduced evaporative cooling capacity, may induce non-linear cardiovascular responses or alter the magnitude of the WBT–HRex association. For example, under warmer and more humid conditions, evaporative heat loss may become insufficient to maintain thermal balance (Sawka et al. 2011) and the resulting accumulation of body heat could place progressively greater demand on the cardiovascular system (Wingo et al. 2012). Evaluating such departures from linearity, however, requires observations spanning a wide environmental range rather than simply a larger sample within a narrow range. Consequently, the present findings should be interpreted within the environmental range observed in this cohort. Nevertheless, the positive association observed across these moderate WBT values is consistent with previous investigations conducted across broader environmental ranges (e.g., <0 to $>30^\circ\text{C}$) that have reported similar directional relationships between environmental conditions and submaximal HR responses (Lacome et al. 2018).

To explore potential individual differences in environmental responses, we allowed the WBT slope to vary between players. Incorporating this additional random effect improved model fit ($\Delta\chi^2 = 35.0$, $p < 0.001$; Table 2), indicating some heterogeneity in the association

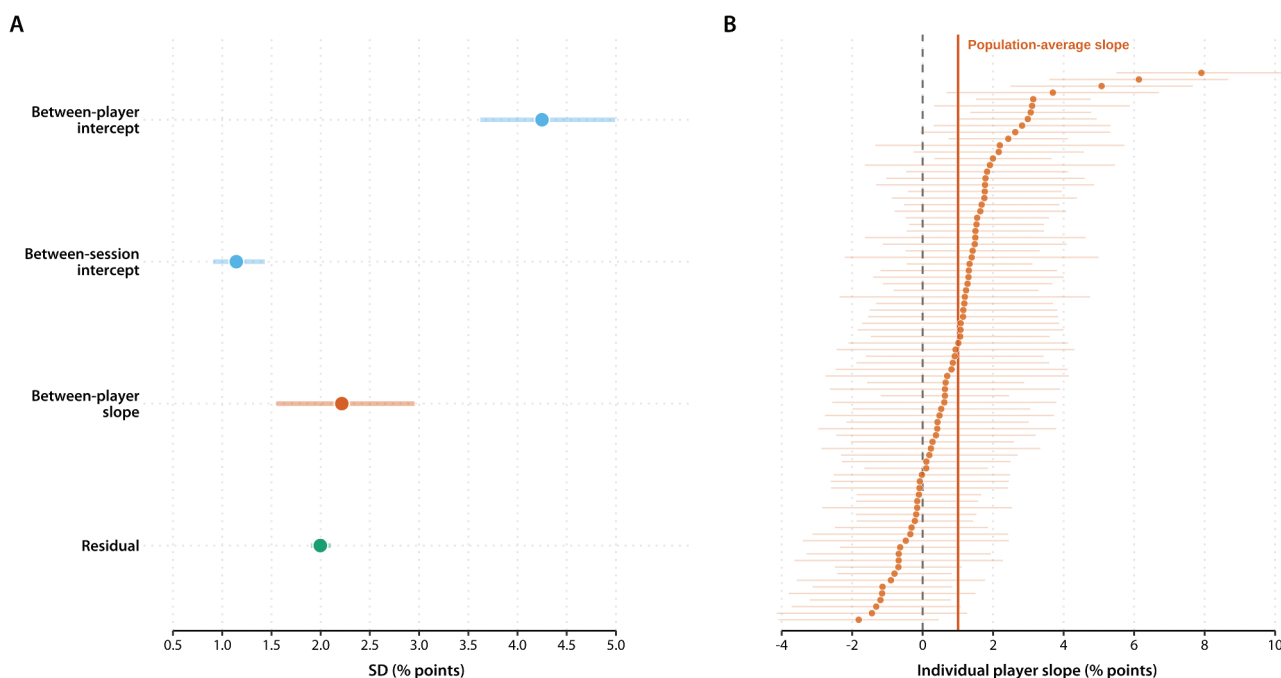


Figure 3. Variance components and individual WBT responses estimated from the final mixed-effects model. Panel A shows the estimated standard deviations (with 90% CIs) for between-player variability in baseline HRex (intercept), between-session variability, between-player variability in the WBT response (slope), and residual within-athlete variability. Panel B shows each player's WBT slope with 90% CIs, ranked from lowest to highest. The dashed vertical line marks no WBT association, and the solid orange line marks the population-average WBT association.

between WBT and HRex. Although the average WBT–HRex association remained remarkably consistent across all models (~1% point HRex per 10 °C), the final model had increased the uncertainty surrounding the population-average estimate. Both the estimated variation in WBT slopes between players ($SD = 2.2\%$ points) and the residual within-athlete variability ($SD = 2.0\%$ points) were approximately two-fold greater than the average WBT association itself. Collectively, these findings suggest that while a positive association between WBT and HRex was generally observed, substantial variability exists around this estimate. It may suggest that athletes differ in their responsiveness to environmental conditions, and this should be investigated in future experimental studies spanning a broader range of environmental exposures.

The correlation between estimated mean-centered intercepts (i.e., players' mean HRex) and slopes (i.e., players' individual WBT ~ HRex linear trends) was negative (-0.29), suggesting that players with typically higher HRex values tended to have weaker associations with WBT. However, uncertainty surrounding this estimate was substantial, and the confidence interval was compatible with both a moderate inverse association and no meaningful relationship (90% CIs: -0.57 to 0.02). One possible explanation is a physiological ceiling effect, whereby players operating closer to maximal cardiovascular strain have less capacity for further HR increases in response to environmental stress. Alternative explanations, including uncertainty in the estimation of individual slopes and unmeasured sources of variability, are also plausible. Therefore, given the exploratory and observational nature of this analysis, the relatively moderate range of environmental conditions observed, and the uncertainty surrounding the estimated individual responses, these findings should be interpreted cautiously and considered hypothesis-generating (Stovitz et al. 2019; Bailey et al. 2024).

Despite the consistent positive association between WBT and HRex, substantial variability remained unexplained. The average WBT association (~1% point HRex per 10 °C) was considerably smaller than both the between-athlete variability in HRex responses ($SD = 4.2\%$ points) and the residual within-athlete variability that remained after accounting for environmental conditions ($SD = 2.0\%$ points). These findings could support the generation of the hypothesis that SMFT HRex is a biologically contextual measure that is influenced by transient environmental factors, not simply physiological state alone (Schneider et al. 2018; Shushan et al. 2022). Although the present study was observational and not designed to establish causal relationships, several physiological mechanisms have been proposed that

may explain associations between environmental conditions and cardiovascular responses during exercise (Racinais et al. 2014; O'Connor et al. 2020). As environmental heat stress increases, thermoregulatory demands rise, leading to increased skin blood flow and peripheral vasodilation to facilitate heat dissipation. This redistribution of blood volume away from the central circulation can transiently reduce central venous return and stroke volume (Wingo et al. 2012). To preserve cardiac output at a fixed external workload, HR increases as a compensatory response. Accordingly, it is pragmatic to suggest that higher HRex values in warmer conditions should also be interpreted within the context of thermoregulatory cardiovascular adjustments rather than assumed changes in fitness status.

Several limitations require acknowledgment. Environment data were obtained from publicly available meteorological sources rather than direct on-field measurements and therefore may not fully reflect the environmental conditions experienced by players, including surface temperature, solar radiation, direct sun exposure, and evaporative cooling capacity of the environment. Additionally, the present analyses did not account for other factors that may contribute to variability in physiological responses, such as training load, recent match exposure, recovery status, hydration, or accumulated physical fatigue. Although hormonal status was reported for most players, cycle phase at the time of testing was not determined and this status was not verified. Given that ovarian hormones may influence thermoregulatory set points and resting body temperature, variation in hormonal status and cycle phase represents an unmeasured source of within-athlete variability that may contribute to the residual variability observed here. Any such influence may be attenuated in the present context, as the SMFT was for 4-min and performed early within the session warm-up, before substantial thermal load had accumulated, although this was not examined directly. The environmental conditions observed in this cohort represented a relatively moderate WBT range, limiting extrapolation to hotter environments or more extreme heat exposure. Lastly, as already reiterated and as with all observational studies, residual confounding cannot be excluded, and the observed associations should not be interpreted as evidence of causality.

Because this study was exploratory and based on observational monitoring data, the associations identified here should be interpreted as hypothesis-generating and require confirmation in future studies using appropriate confirmatory designs and error control strategies (Ditroilo et al. 2025). Future work should examine whether environmental indices that better

capture evaporative cooling capacity, such as WBT or wet-bulb globe temperature, provide stronger or more consistent explanations of longitudinal H_{REx} variation than ambient temperature alone. In addition, controlled, pre-registered experimental studies manipulating environmental conditions (e.g., climate chamber protocols) may help establish whether the observed associations reflect causal thermoregulatory responses during standardised submaximal exercise. Such information will likely be useful to practitioners interpreting changes in SMFT cardiovascular responses across a competitive season as proxies of physiological state.

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Author contributions

CRedit: **Tzili Shushan**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing; **Ric Lovell**: Conceptualization, Methodology, Writing – review & editing; **Shaun J. McLaren**: Conceptualization, Formal analysis, Methodology, Software, Visualization, Writing – review & editing; **Martin Buchheit**: Conceptualization, Methodology, Writing – review & editing.

Disclosure statement

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Data and code availability statement

The datasets and analysis code supporting the findings of this study are openly available via the Open Science Framework (OSF) at: <https://osf.io/jczb6/overview>.

Data deposition

Data and analysis code have been deposited in the Open Science Framework (OSF) repository.

Ethics approval and informed consent

This study was approved by the University of Wollongong Human Research Ethics Committee (Approval No. 2023/334). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to participation in the study.

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