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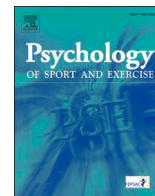
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Physical activity intensity and older adolescents' stress: The 'STress-Reactivity after Exercise in Senior Secondary Education' (STRESSED) 3-arm randomised controlled trial

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ABSTRACT

Objectives: Late adolescence (15–19 years) is a period of heightened susceptibility to stress, but regular physical activity may attenuate reactivity to stressors. We aimed to explore the effects of physical activity intensity on older adolescents' stress-reactivity and self-reported mental health.

Design and Methods: Three-arm randomised controlled trial in New South Wales, Australia (April–June 2021). Thirty-seven older adolescents (16.1 ± 0.2 years, 59.5 % female) were randomised to: i) non-active control (CON), ii) light-intensity physical activity (LPA), or iii) moderate-to-vigorous-intensity physical activity (MVPA). Physical activity groups participated in 2 x 20-min sessions/week for 6 weeks. Salivary cortisol (sCort) reactivity to induced stress was assessed using the Trier Social Stress Test for Groups and quantified as area under the curve (sCort_{AUC}; Primary outcome). Secondary outcomes included peak cortisol (sCort_{Peak}), subjective-reactivity, perceived stress, and non-specific psychological distress. Group differences were assessed using multiple linear regression and quantified using Cohen's *d*.

Results: No statistically significant effects were observed for sCort_{AUC} or sCort_{Peak} and the pattern of effects for subjective-reactivity was inconsistent. Effects for self-reported mental health were also non-significant ($p > .05$ for all) but of meaningful magnitude, favouring LPA and MVPA over CON (d 's = -0.38 to -0.54). Delivery fidelity was high, satisfaction was moderate-to-high, and there was no evidence of harm. However, recruitment, retention for sCort measures, and adherence were lower than expected.

Conclusion: Suboptimal recruitment, retention, and adherence limited our ability to conclude on the effect of physical activity intensity on older adolescents' sCort-reactivity to induced stress. We observed potentially meaningful effects on self-reported mental health for both physical activity conditions, which could be confirmed in a future powered trial.

1. Background

The final years of secondary school are highly stressful for many students, with the normal pressures of maturation compounded by high stakes examinations (Wuthrich et al., 2021). Evidence suggests there have been global increases in stress and anxiety among young people in recent decades and school-related stress appears to be a major contributor (Sweeting et al., 2010). Indeed, 'school challenges' and 'mental

health challenges' are among the most frequently cited personal challenges identified by older adolescents (15–19 years) (Leung et al., 2022). Chronic stress has substantial adverse impacts on students' well-being and predicts future mental illness (Brady & Sinha, 2005). Stress also affects executive functioning (Shields et al., 2016), undermining academic achievement during a critical stage of education. Given the importance of school performance for university entrance and post-school employment, there is a need to identify and understand

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modifiable factors that promote resilience to stress during the final years of secondary school.

A large body of evidence supports the importance of physical activity and physical fitness for young peoples' mental health (Biddle et al., 2019). However, physical activity declines precipitously during adolescence (Dumith et al., 2011) and fewer than 1 in 5 adolescents globally accrues the recommended 60 min or more of daily moderate-to-vigorous intensity physical activity (MVPA) (Guthold et al., 2020). There have also been global declines in adolescents' physical fitness (Tomkinson et al., 2021), which could have meaningful implications for population mental health. A recent meta-analysis of >1 million participants found incident mental disorders and suicidality were ~40 % lower among those with higher cardiorespiratory fitness (CRF) during the peak risk period for onset of adult-pattern mental disorders (Tacchi et al., 2019). Whether or not declining MVPA and fitness are a leading cause of the global rise in young peoples' psychological distress remains unclear. But the link is plausible and interventions to support older adolescents' physical activity participation are justified, particularly if the most effective prescription(s) can be identified and deployed at scale.

Despite widespread recognition of the psychological benefits of physical activity, there remains a lack of consensus on the underpinning mechanisms (Lubans et al., 2016). However, a growing literature is linking physical activity-induced changes in cognitive and mental health with various neurobiological factors (Heinze et al., 2021). Among these is cortisol, a primary end-product of the physiological stress response, which may be meaningfully implicated in the increased incidence of common mental disorders during adolescence. For example, there are age-related differences in cortisol-reactivity to an acute stressor, with evidence from one study ($n = 82$) indicating post-pubertal youth have heightened reactivity relative to pre-pubertal youth (Gunnar et al., 2009). This effect tracks closely with the timing of sex-specific increases in rates of mental ill-health between childhood and adolescence (Lawrence et al., 2016). In addition, cortisol level in childhood has been shown longitudinally to predict adolescent girls' susceptibility to depression following negative life events (LeMoult et al., 2015). LeMoult and colleagues found that the association between exposure to negative life events and depression onset at age 18 depended on girls' ($n = 62$) level of diurnal salivary cortisol at age 9–14 (i.e., negative life events were unrelated to depression in girls with lower baseline cortisol) (LeMoult et al., 2015).

While there is compelling support for the role of (particularly chronically) elevated cortisol production in the onset of common mental disorders, there are also nuances that make the association difficult to disentangle. For example, evidence suggests mental health status is meaningfully associated with cortisol-reactivity during adolescence, but in opposite directions for males and females. In a study of 111 participants, Mazurka and colleagues (Mazurka et al., 2018) found that 12–18 year old boys with depression demonstrated an elevated cortisol response to a lab-based stressor (relative to non-depressed boys and girls), whereas depressed girls had a *blunted* cortisol response. This study highlights the importance of considering mental health status and sex when exploring the effects of interventions on cortisol-reactivity. Lifestyle factors are also important to consider, with studies showing sleep ($n = 31$) (Capaldi et al., 2005) and dietary behaviours ($n = 60$) (Shearrer et al., 2016) (and by extension adiposity, $n = 63$ (Roemmich et al., 2007)) are also related to cortisol level in youth. However, for the purposes of the present investigation, it is the role physical activity in supporting healthy cortisol regulation that is the lifestyle factor of interest. There is consistent evidence supporting a link between youths' physical activity and self-reported 'perceived' stress (Moljord et al., 2011; Wright et al., 2023), which could be explained by the effects of physical activity on underlying stress physiology.

Almost three decades ago, Sothmann and colleagues (Sothmann et al., 1996) suggested the stress-buffering effects of physical activity could be the result of adaptations to the systems regulating the stress

response. Coined the 'Cross-Stressor Adaptation hypothesis' (hereafter CSA), regular exposure to the 'physical' stress of sufficiently intense exercise is posited to modify the stimulation of the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, leading to blunted cardiovascular- and adrenocortical- (i.e., cortisol) reactivity to 'psychosocial' stressors (Sothmann et al., 1996). Despite considerable research attention, empirical support for CSA remains somewhat inconsistent. For example, in a recent systematic review (Mücke et al., 2018) higher physical activity and CRF were associated with attenuated salivary cortisol (sCort) reactivity in ~60 % of studies (median $n = 84$) using the Trier Social Stress Test (TSST), a highly valid and widely-used psychosocial stress induction paradigm. However, very few included studies were conducted with school-aged youth (none with older adolescents) and almost all were observational.

To our knowledge, there has not been a single randomised controlled trial (RCT) assessing the effects of physical activity on adolescents' cortisol-reactivity to induced psychosocial stress, despite this being a highly salient and consequential issue for this group. The dearth of experimental research makes it difficult to judge the suitability of CSA as a guiding framework for designing physical activity-based stress-management interventions. Therefore, the aim of our exploratory study was to interrogate the assumptions of CSA by testing the moderating role of physical activity intensity on older adolescents' sCort-reactivity to a standardised stressor. Consistent with the predictions of CSA, we hypothesised that adolescents participating in MVPA would show a meaningful decline in sCort-reactivity to induced psychosocial stress, but that no effect would be found for light-intensity physical activity.

2. Materials and methods

2.1. Study design and participants

The conduct and reporting of our study complies with the consolidated standards for reporting trials (CONSORT) extension for multi-arm parallel-group RCTs (Juszczak et al., 2019) and the template for intervention description and replication (TIDieR) (Hoffmann et al., 2014). Ethical approval was obtained from the University of Newcastle (H-2019-0410) and the Catholic Schools Office of the Diocese of Maitland-Newcastle. All participants/parents provided informed assent/consent prior to enrolment. A 3-arm parallel-group RCT was conducted at a single non-government secondary school in New South Wales (NSW), Australia (ACTRN12621000672853). Eligible participants were students in Grade 11 (i.e., penultimate year of secondary school; ~16–17 years old) without an injury/illness that would preclude participation in MVPA. Given the lack of prior research on cortisol-reactivity to induced stress with this study population, we conducted an exploratory (rather than confirmatory) trial that is intended to inform a future powered trial. Thus, we aimed to recruit a total of 60 participants (20 per treatment arm), which represents the minimum recommended sample size for a preliminary trial designed to inform a subsequent main trial (with 90 % power, two-sided 5 % significance, assuming a standardised effect size of 0.5) inflated by 5 participants per arm to account for possible drop-out (Whitehead et al., 2016). Recruitment occurred during a 5-week period in the school term prior to the commencement of the trial (March–April 2021).

2.2. Randomisation and blinding

Given the importance of sex and mental health status on adolescents' cortisol responses to induced stress (Mazurka et al., 2018), participants were stratified prior to randomisation by sex (male/female) and baseline psychological distress (i.e., K-10 score <25 [likely to be well/likely mild mental disorder] or ≥25 [likely moderate/severe mental disorder]) (Andrews & Slade, 2001). After baseline assessments, the lead investigator used an online random number generator to allocate stratified participants in blocks of three (1:1:1 ratio) to either a non-active control

group (usual care; CON), a light-intensity physical activity group (LPA), or a moderate-to-vigorous intensity physical activity group (MVPA). Outcome assessors were blind to group allocation and analyses were conducted by a blinded statistician independent of the investigator team. Participants were partially blinded, in that those allocated to LPA and MVPA were not aware of the primary hypothesis but were aware of their allocation to an intervention group rather than control. Personnel delivering the exercise sessions were not blinded.

2.3. Physical activity interventions

Participants in the LPA and MVPA groups were encouraged to attend 2 x 20-min sessions/week (from four offered) for 6 weeks and given a small incentive each week if they met this target (i.e., free hot beverage). They also received a financial incentive (\$50AUD) for attending ≥ 10 sessions in total over the 6-week intervention period. Although this exercise dose is relatively low, there is evidence that short, twice-weekly exercise sessions delivered at a sufficiently high intensity can alter adrenocortical markers of stress in older adolescents (Lubans et al., 2021). Moreover, time is a key barrier to the implementation of exercise programs in schools, and so it is important to evaluate an intervention model that is realistic for a typical school (and which might reflect a minimally effective dose). Finally, a 6-week exercise intervention enabled baseline and post-test assessments to occur immediately prior to and following program delivery, without being impacted by the subsequent 2-week school holiday break, which might have resulted in a 'wash out' of immediate post-intervention effects if study measures were collected afterwards. Sessions were delivered on school premises at the same time of day for both LPA and MVPA by two Physical Education-qualified members of the research team, who regularly alternated between groups. Sessions were delivered in accordance with a published framework of pedagogical principles (Lubans et al., 2017) with which both facilitators had extensive prior experience.

The physical activity modalities for each group were selected to elicit the intended cardiovascular response, and because they were practically feasible for delivery in schools. LPA received two types of group-based sessions involving light-intensity physical activity (i.e., $< 64\%$ of age-predicted maximum heart rate [HR_{max}]): i) yoga-inspired stretching delivered indoors with relaxing slow-tempo music, and ii) leisurely walking conducted outdoors around the external perimeter of the school. Participants in the MVPA condition completed group-based interval training sessions designed to elicit a HR response in the moderate-to-vigorous intensity band (i.e., $\geq 64\%$ HR_{max}). Sessions were delivered indoors with high-tempo music and included a combination of aerobic (e.g., jumping jacks) and bodyweight resistance (e.g., push-ups) exercises.

2.4. Assessment procedure

Study assessments and intervention delivery occurred within a single 10-week school term between April–June 2021. Baseline and post-test assessments occurred in the two weeks either side of intervention delivery. Participants were provided with a moderate financial incentive (\$50AUD) for completing study assessments at each time point. Standard demographic information and self-report outcomes were evaluated using an online survey. sCort-reactivity measures were collected by trained research assistants on school premises.

2.5. Study measures

2.5.1. sCort-reactivity to acute psychosocial stress (primary outcome)

Stress-reactivity was assessed using the Trier Social Stress Test for Groups [TSST-G] (Von Dawans et al., 2011). Although the TSST has been used at baseline and post-test in an RCT (Gerber et al., 2020), research shows dampened sCort-reactivity with subsequent administrations implying habituation to the stressor (Kothgassner et al., 2021). To

preserve the efficacy of the stress induction at post-test as well as accounting for baseline differences, we employed an alternative protocol at baseline - the Socially Evaluated Cold Pressor Task (SECPT) (Schwabe et al., 2008). The SECPT and TSST-G are distinct protocols, but both incorporate motivated performance, uncontrollability, and social-evaluative threat to elicit HPA-axis activation (Schwabe et al., 2008; Von Dawans et al., 2011). Both protocols were conducted on school premises between 1300h and 1530h to control for diurnal cycle of cortisol, and participants were instructed not to eat or drink (water accepted) or engage in exercise for at least 2 h prior. Saliva samples were frozen and stored at $-20\text{ }^{\circ}\text{C}$ until shipped for analysis by a commercial laboratory (Dresden Lab Service GmbH, Germany). After thawing, Salivettes were centrifuged at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. sCort concentrations (nmol/L) were measured in duplicate using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). The intra- and inter-assay coefficients of variation for sCort were both below 9%. Further details of the SECPT and TSST-G protocol are provided below.

Socially Evaluated Cold Pressor Task. Upon arrival, participants entered a small room with a male experimenter wearing a white lab coat and provided a saliva sample (-5 min from stressor onset [T0]) using commercially available low density polyethylene swabs (Salivette®; Sarstedt, Romelsdorf, Germany). The participant was informed they would be video recorded so their facial expressions could be analysed later by experts and then asked to immerse their hand in cold water ($0-4\text{ }^{\circ}\text{C}$) for as long as they could tolerate (pre-specified end point [T0 +3 min] not disclosed). The experimenter observed from a position visible to the participant, periodically making notes to induce social-evaluative threat, while the participant looked continuously at the camera (Apple iPad 6). After 3 min, participants were told to remove their hand and immediately rated how 'unpleasant' and 'stressful' the experience was using an 11-point scale (0 = *not at all* to 100 = *very much*) before providing a second saliva sample (T0 +5 min). They then moved to a nearby waiting room for the recovery period where another four saliva samples were collected (T0 +15, +25, +35, and +45 min). Participants were given non-stimulating reading material to pass the time and were instructed not to interact with anyone. To prevent them from inferring the objectives of the stress-induction at post-test, they were not debriefed on the purpose of the SECPT.

Trier Social Stress Test for Groups. The TSST-G is a standardised group-based stress induction protocol that consists of three phases: i) introduction, preparation and anticipation, ii) public speaking (mock job interview), and iii) challenging mental arithmetic. Upon arrival, groups of 5 participants entered a room with a male experimenter where they were told they would have 10 min to prepare a 2 min speech for a panel of judges on why they would be a suitable candidate for a self-selected ideal job. They were also informed of an unspecified second task for which they could not prepare. Following this, participants moved to a nearby room to commence the public speaking task. They stood facing the panel (one male and one female wearing white lab coats), separated by mobile dividing walls that restricted eye contact and social interaction with other participants. The panel had been instructed not to provide any verbal or non-verbal feedback during the task. Participants were told they would be video recorded for later expert review, after which the panel selected participants in random order to complete their speech. For the arithmetic task, participants were given a unique 4 digit starting number and asked to serially subtract 13 as many times as possible for 80 s. If they made a mistake, the panel instructed them to start again. Once all participants had completed the arithmetic task, they reported how 'unpleasant' and 'stressful' the experience was using an 11-point scale (0 = *not at all* to 100 = *very much*) before returning to the preparation room for the recovery period. Saliva samples were collected upon arrival (T0 -12 mins), post-preparation (T0), post-speech (T0 +12 min), post-arithmetic (T0 +20 min), and at five points during recovery (T0 +30, +40, +50, +60 and +70 min). Once the final saliva sample was

collected, the experimenter debriefed participants on the purpose of the TSST-G (and SECPT at baseline), revealing the tasks were intended to elicit stress and emphasising that they had performed well.

2.5.2. Perceived stress

Perceived stress was assessed using the Perceived Stress Scale (PSS) (Cohen et al., 1983), which includes 10 items relating to the frequency of respondents' experience of stress over the past month (e.g., *How often have you felt that you were on top of things?*). Participants responded using a 5-point scale (0 = *Never* to 4 = *Very often*) and responses were summed to produce a total score (possible range = 0 to 40). The internal consistency among the present sample at baseline was good (Cronbach α = .87).

2.5.3. Psychological distress

Non-specific psychological distress was assessed using the Kessler-10 (K-10) (Kessler et al., 2003), which is a widely-used psychological screening instrument valid for use with adolescents. The K-10 includes 10 items relating to the frequency of internalising symptoms experienced over the past month (e.g., *In the past 4 weeks, about how often did you feel hopeless*). Participants responded using a 5-point scale (1 = *None of the time* to 5 = *All of the time*), and items were summed to produce a total psychological distress score (possible range = 10 to 50). Internal consistency among the present sample at baseline was good (Cronbach α = .93).

2.5.4. Process measures

The following process data were collected: i) *recruitment*, proportion of the target sample size enrolled; ii) *retention*, participant drop-out rate and proportion completing post-test assessments; iii) *adherence*, total sessions completed and proportion satisfying attendance target (instructor log); iv) *satisfaction*, satisfaction with exercise sessions ("*I enjoyed participating in the exercise sessions*") from 1 [*Strongly disagree*] to 5 [*Strongly agree*]; v) *fidelity*, mean/peak physical activity intensity using chest-worn Polar™ H9 Bluetooth HR monitors worn during all sessions; and vi) *potential harms*, injuries/adverse events from physical activity sessions or assessments (adverse event register).

2.6. Statistical analysis

All analyses were conducted using SAS software (SAS inc. Cary, NC, USA) by an independent statistical service operated by the Hunter Medical Research Institute. Prior to the main analysis, we first tested the efficacy of the stress induction using: i) an increase of $\geq 15.5\%$ in raw sCort values (nmol/L) from baseline to individual peak to distinguish responders from non-responders at each timepoint (Miller et al., 2013), and ii) one-way analyses of variance (ANOVAs) with repeated measures to determine the main effect of time on sCort ($p < .05$). We tested the efficacy of the TSST-G at post-test using participants in CON and LPA only, as our primary hypothesis assumed cortisol-reactivity would be attenuated for those allocated to the MVPA group.

To quantify stress-reactivity, sCort data were first log-transformed to account for skewness then converted to area under the curve with respect to ground (sCort_{AUC}; Primary outcome). Peak cortisol (sCort_{Peak}) was calculated as the difference between log-transformed sCort concentration for SECPT samples 1 and 4 at baseline and TSST-G samples 1 and 5 at post-test, which correspond with the expected timing of peak cortisol (Dickerson & Kemeny, 2004). Consistent with the intention-to-treat principle, missing sCort_{AUC} values were imputed so all enrolled participants were included in the analysis of the primary outcome. Missing values of sCort_{AUC} (baseline or follow-up) were multiply imputed and combined using Rubin's Rules. The fully conditional specification (with regression propensity mean matching) method was used, and data were imputed 25 times. Baseline sCort_{AUC} was calculated using sex and the non-missing values at baseline as predictors. Post-test sCort_{AUC} was calculated using the baseline values, sex,

and the non-missing sCort_{AUC} at follow-up as predictors. Complete case analyses were conducted for all other outcomes. Group differences were analysed using multiple linear regression, with baseline values of the dependent variable and treatment group as covariates. Cohen's *d* effect size was calculated with values of <0.2, 0.2, 0.5, and 0.8 representing negligible, small, medium, and large effects, respectively.

3. Results

The flow of participants appears in Figure 1 and baseline characteristics are provided in Table 1. A total of 37 participants (Mean \pm SD age = 16.1 \pm 0.2; 59 % female) were assessed at baseline and randomised to treatment groups. Three participants (all female) withdrew from the study after baseline assessments, resulting in 34 participants (92 %) retained at post-test. Based on K-10 scores, the full sample was generally mentally well at baseline. However, 4 (10.8 %) participants were considered likely to have a moderate mental disorder and 7 (18.9 %) participants were considered likely to have a severe mental disorder.

3.1. Effects on stress-reactivity

At baseline, 9/31 (29.0 %) participants were classified as non-responders to the SECPT. The main effect of time was not statistically significant $F(1,28) = 2.42$, $p = .131$, $\eta^2 = 0.08$, suggesting the stress induction was not as robust as intended (Figure 2). At post-test, 7/19 (36.8 %) participants were classified as non-responders to the TSST-G. The main effect of time was statistically significant $F(1,17) = 5.31$, $p = .034$, $\eta^2 = 0.24$, suggesting sCort increased as expected in response to the stressor (Figure 3). Baseline-adjusted post-test mean values by group and adjusted mean differences (with 95 % confidence intervals [CI]) between LPA, MVPA and CON are provided in Table 2. No statistically significant or practically meaningful group differences in sCort_{AUC} were observed. Compared with CON, sCort_{AUC} at post-test ($n = 37$) was 6 % lower (95%CI = -53 % to 89 %; $p = .863$; $d = -0.08$) for LPA and 1 % higher (95%CI = -55 % to 123 %; $p = .974$; $d = 0.02$) for MVPA. Group differences for sCort_{Peak} ($n = 25$) were also non-significant, negligible in size and showed no benefit of LPA or MVPA over CON. Differences in subjective-reactivity ($n = 29$) were non-significant, mostly negligible-to-small and inconsistent in direction. Although, there was a small-to-medium effect size ($d = -0.30$) for perceived stressful favouring MVPA over CON, and a large effect size ($d = 0.86$) for perceived unpleasant favouring CON over MVPA.

3.2. Effects on self-reported mental health

Intervention effects for the PSS and K-10 ($n = 34$ for both) were all not statistically significant. This said, the effect sizes favoured the physical activity groups and were of small-to-moderate magnitude. For example, perceived stress was lower among LPA (-2.0 units, 95%CI = -6.7 to 2.6; $p = .378$; $d = -0.38$) and MVPA (-2.8 units, 95%CI = -7.2 to 1.7; $p = .219$; $d = -0.52$) relative to CON. Similarly, differences in K-10 scores also favoured LPA (-2.7 units, 95%CI = -7.1 to 1.7; $p = .222$; $d = -0.54$) and MVPA (-1.9 units, 95%CI = -6.1 to 2.3; $p = .357$; $d = -0.38$) over CON, though again these effects were not statistically significant.

3.3. Process findings

We enrolled less than two thirds of the target sample size (37/60; 61.7 %) and while the drop-out rate was low (8.1 %), the retention rate for post-test measures was variable, ranging from 67.6 % of completers for sCort measures to 100 % for PSS and K-10. Participants in the LPA and MVPA groups completed a mean \pm SD of 9.1 \pm 3.7 and 6.6 \pm 4.3 physical activity sessions, respectively, with fewer than half in each group completing ≥ 10 sessions (i.e., $n = 4$ for LPA and $n = 5$ for MVPA). LPA and MVPA indicated high (4.5 \pm 0.5 out of 5) and moderate (3.7 \pm

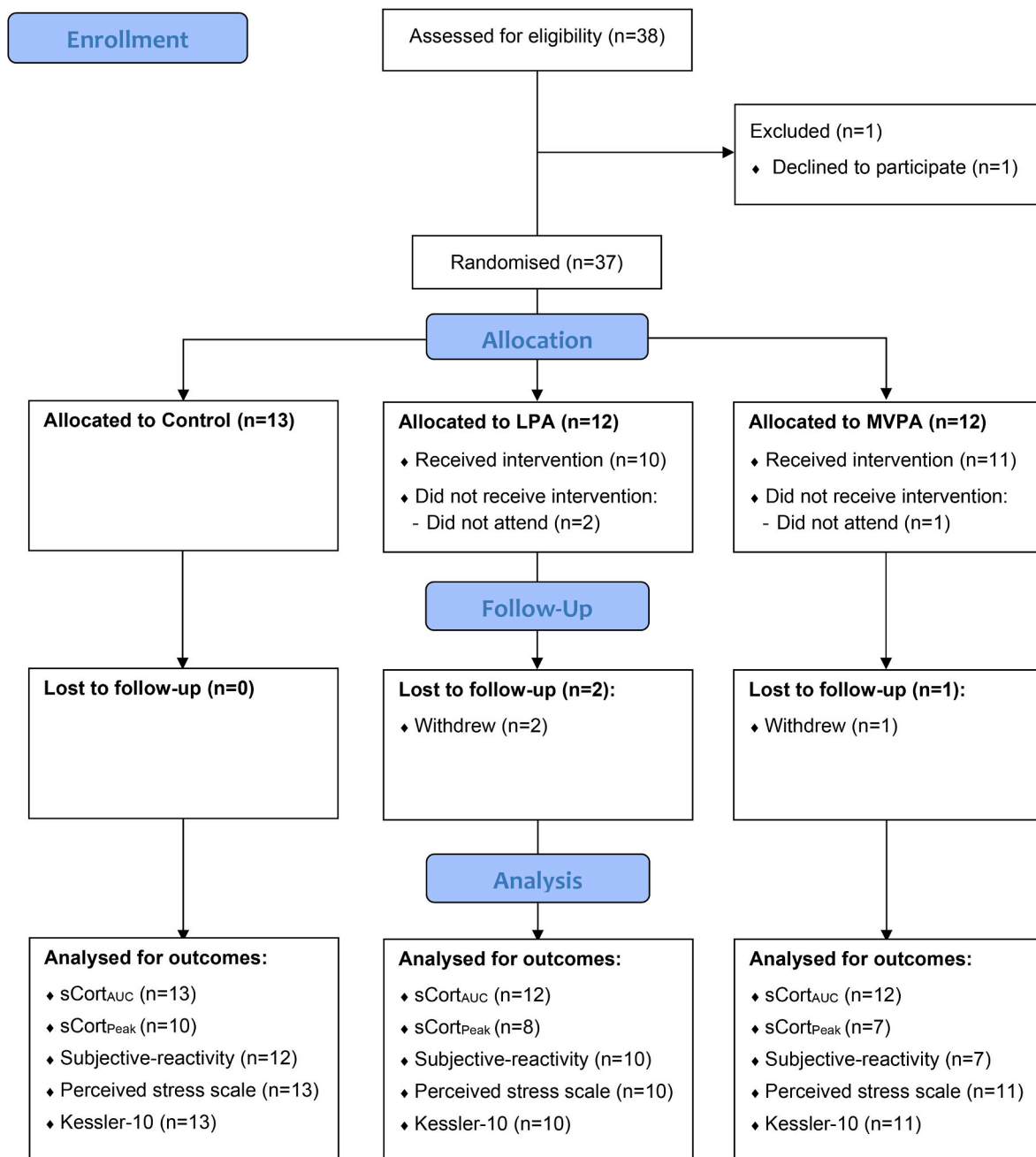


Figure 1. Flow of participants through the study.

1.1 out of 5) satisfaction with the physical activity sessions, respectively. HR data showed the intensity manipulation was effective, with mean \pm SD session intensity values of $54.6 \pm 0.6\%$ and $68.8 \pm 8.7\%$ HR_{Max} for LPA and MVPA, respectively. A similar pattern was found for peak session intensity, with mean \pm SD values of $68.8 \pm 8.7\%$ HR_{Max} for LPA and $83.4 \pm 7.7\%$ HR_{Max} for MVPA. No injuries or adverse events were recorded. Conversations with school staff identified that two of the three study dropouts had withdrawn from school due to mental health concerns, but staff confirmed this was unrelated to their participation in the trial.

4. Discussion

Stress-related mental ill-health is a pervasive and consequential issue for older adolescents (Leung et al., 2022), and the final years of secondary school are an opportune time to intervene. The aims of our

exploratory study were to investigate the influence of physical activity intensity on adolescents' sCort-reactivity to induced psychosocial stress (primary outcome) and self-reported mental health. Encouragingly, study drop-out was low, satisfaction was moderate-to-high, there were no adverse events, retention for self-report outcomes was high, and delivery fidelity was strong. However, there was also suboptimal participant recruitment, substantive missing data for the primary outcome, and poor participant adherence with the physical activity interventions (particularly for the MVPA condition). As a result, our ability to conclude on the effect of intensity on adolescents' stress-reactivity was limited. Of note, the trial was conducted in the context of the COVID-19 pandemic and concluded immediately prior to a second round of extended stay-at-home orders in the state of New South Wales, which likely influenced study recruitment and participation.

We did not observe any intervention effects for adolescents' adrenocortical-reactivity to acute stress, as measured by sCort_{AUC} ($n =$

Table 1
Characteristics of study sample at baseline.

Characteristics	All (N = 37)	CON (n = 13)	LPA (n = 12)	MVPA (n = 12)
Age, years, mean (SD)	16.1 (0.2)	16.1 (0.3)	16.0 (0.0)	16.1 (0.3)
Female sex, n (%)	22 (59.5)	7 (53.8)	7 (58.3)	8 (66.7)
Country of birth, n (%)				
Australia	33 (89.2)	9 (69.2)	12 (100)	12 (100)
Other	4 (10.8)	4 (30.8)	0 (0.0)	0 (0.0)
Primary language spoken at home, n (%)				
English	37 (100)	13 (100)	12 (100)	12 (100)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cultural background, n (%)				
Australian	28 (75.7)	8 (61.5)	10 (83.3)	10 (83.3)
European	4 (10.8)	2 (15.4)	1 (8.3)	1 (8.3)
Asian	2 (5.4)	0 (0.0)	1 (8.3)	1 (8.3)
African	2 (5.4)	2 (15.4)	0 (0.0)	0 (0.0)
Middle Eastern	1 (2.7)	1 (7.7)	0 (0.0)	0 (0.0)
Indigenous descent, n (%)	1 (2.7)	0 (0.0)	1 (8.3)	0 (0.0)
Maternal educational attainment, n (%)				
Unsure	4 (10.8)	1 (7.7)	2 (16.7)	1 (8.3)
Didn't complete high school	2 (5.4)	1 (7.7)	1 (8.3)	0 (0.0)
Grade 10 school certificate	9 (24.3)	3 (23.1)	3 (25.0)	3 (25.0)
Grade 12 higher school certificate	3 (8.1)	2 (15.4)	1 (8.3)	0 (0.0)
Vocational certificate	3 (8.1)	0 (0.0)	2 (16.7)	1 (8.3)
University undergraduate degree	7 (18.9)	3 (23.1)	2 (16.7)	2 (16.7)
University postgraduate degree	9 (24.3)	3 (23.1)	1 (8.3)	5 (41.7)
K-10 likelihood of mental disorder, n (%)				
Likely to be well (score = 10–19)	15 (40.5)	6 (46.2)	5 (41.7)	4 (33.3)
Likely mild disorder (score = 20–24)	11 (29.7)	3 (23.1)	3 (25.0)	5 (41.7)
Likely moderate disorder (score = 25–29)	4 (10.8)	1 (7.7)	1 (8.3)	2 (16.7)
Likely severe disorder (score = 30–50)	7 (18.9)	3 (23.1)	3 (25.0)	1 (8.3)

Note. CON, non active control group; K-10, Kessler-10; LIGHT, light-intensity physical activity group; MVPA, moderate-to-vigorous intensity physical activity group; SD, standard deviation.

37) and sCort_{Peak} (n = 25). The adjusted post-test mean differences between both physical activity groups and CON were negligible. There are several potential explanations for these results. First, it is not clear whether HPA-reactivity in typically developing adolescents is

‘dysfunctional’ enough that it can be altered. Klaperski et al. (Klaperski et al., 2014) found reduced cortisol- and HR-reactivity in response to the TSST among a group of 149 adult men participating in a 12-week cardiovascular training program (2 × 60 min/week). Conversely, Gerber et al. (Gerber et al., 2020) reported no effect of a 6-week (3 x 40–50 min/week) aerobic exercise program on cortisol-reactivity among a mixed-sex group of 25 adults with major depressive disorder. To our knowledge, these are the only two trials that have tested the effects of an exercise program on cortisol-reactivity to the TSST. Cortisol-reactivity to the TSST following an acute bout of physical activity has been shown to vary by intensity in a recent trial with 83 adult males (Caplin et al., 2021), but the generalisability of these findings to adolescents, females, or chronic physical activity is unclear. More research examining the effects of exercise on markers of HPA-axis function in school-aged youth is needed.

Due to the priority placed on curricular time, in the present trial school staff limited the physical activity sessions to the before school, lunch time, and after school periods. This restricted the duration to 20 min to allow sufficient travel time to/from school and to prevent students being late for classes. According to a recent meta-analysis (Rodriguez-Ayllon et al., 2019), the effects of physical activity interventions on youth mental health are moderated by duration, with programs facilitating ≥60 min/week of physical activity achieving an effect size approximately three times larger than those facilitating <60 min. Whether this finding extends to stress-reactivity is unclear, but as only 40 min/week was prescribed in our trial, it is plausible this was an insufficient dose to stimulate HPA-axis habituation. Beyond the weekly dose and frequency of exercise sessions, it is also plausible that 6-weeks was an insufficient overall duration to elicit improvements in cortisol-reactivity. In a meta-analysis of the effects of exercise interventions on anxiety in college students (Lin & Gao, 2023), the strongest effects were found for interventions delivered for 8–14 weeks, whereas the pooled effect for trials <8 weeks long was non-significant. Similar findings were recently reported by Singh and colleagues (Singh et al., 2023) in their umbrella review of physical activity interventions for depression, anxiety and distress. Though focused on healthy adults (not youth), they reported that the optimal intervention duration was ≤12 weeks. We therefore suggest a 6-week exercise period may be insufficient to meaningfully improve markers of stress in older adolescents (particularly given suboptimal participant adherence), and that a minimum of 8-weeks is prudent for future trials.

Reductions in older adolescents’ (n = 298) hair cortisol concentrations were found in the Burn 2 Learn (B2L) trial (Lubans et al., 2021)

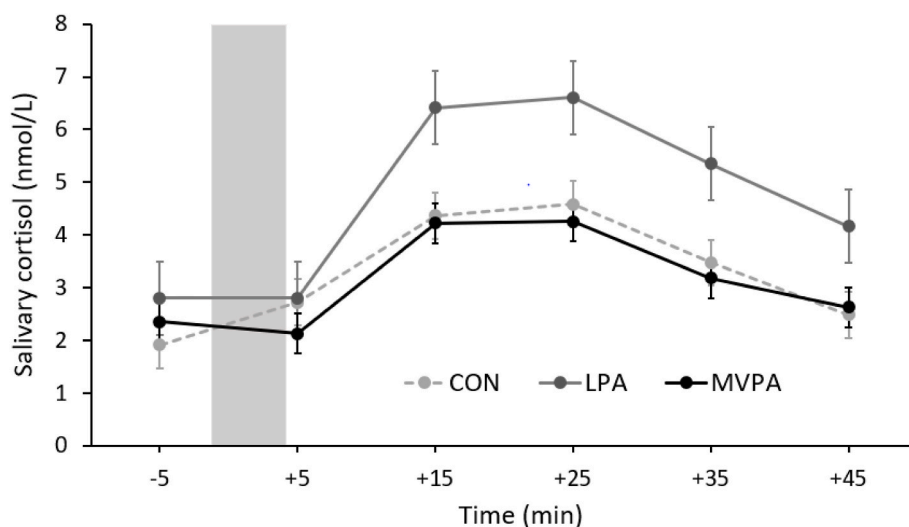


Figure 2. Salivary cortisol response curves for the Socially Evaluated Cold Pressor Task at baseline **Note.** Shaded area represents the stress induction period. Time is given relative to stressor onset (i.e., 0 min). Error bars represent standard error of the mean.

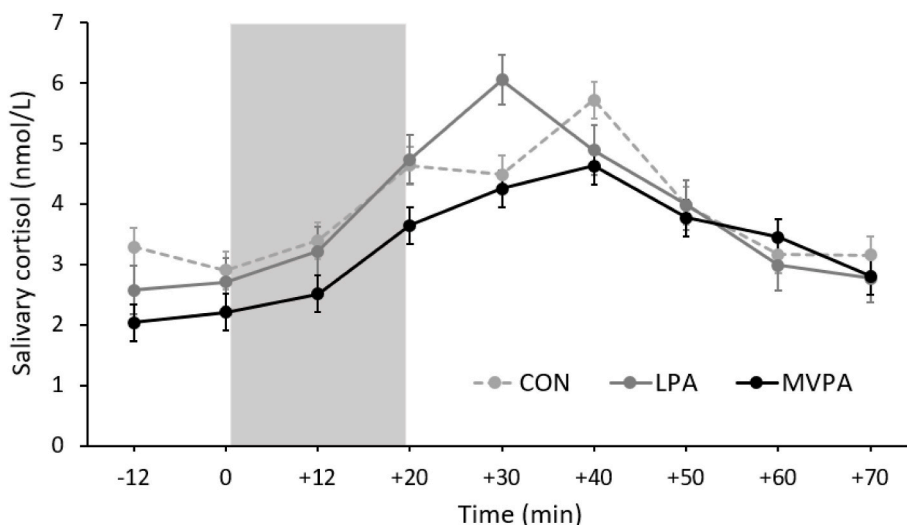


Figure 3. Salivary cortisol response curves for the Trier Social Stress Test for Groups at post-test **Note.** Shaded area represents the stress induction period. Time is given relative to stressor onset (i.e., 0 min). Error bars represent standard error of the mean.

Table 2
Effects of physical activity interventions on stress-reactivity and self-reported mental health.

Outcomes	Post-test mean (95%CI) values ^a			LPA v CON ^b			MVPA v CON ^b		
	CON	LPA	MVPA	Mean difference (95% CI)	<i>p</i>	<i>d</i>	Mean difference (95% CI)	<i>p</i>	<i>d</i>
sCort _{AUC} ^{c,d}	5.73 (5.20, 6.26)	5.67 (5.17, 6.16)	5.74 (5.17, 6.32)	-0.06 (-0.76, 0.64)	0.863	-0.08	0.01 (-0.80, 0.83)	0.974	0.02
sCort _{peak} ^c	0.41 (-0.23, 1.05)	0.48 (-0.28, 1.23)	0.57 (-0.16, 1.30)	0.07 (-0.94, 1.07)	0.890	0.08	0.16 (-0.81, 1.13)	0.728	0.19
Perceived <i>stressful</i>	60.8 (44.8, 76.8)	65.0 (47.5, 82.5)	52.9 (31.9, 73.8)	4.2 (-19.6, 27.9)	0.721	0.15	-8.0 (-34.3, 18.4)	0.539	-0.30
Perceived <i>unpleasant</i>	55.2 (38.8, 71.7)	59.6 (40.6, 78.5)	78.8 (57.5, 100)	4.4 (-21.0, 29.7)	0.725	0.16	23.6 (-3.4, 50.6)	0.084	0.86
Perceived stress scale	17.8 (14.8, 20.8)	15.8 (12.3, 19.3)	15.1 (11.8, 18.4)	-2.0 (-6.7, 2.6)	0.378	-0.38	-2.8 (-7.2, 1.7)	0.219	-0.52
Kessler-10	23.0 (20.2, 25.8)	20.3 (17.0, 23.6)	21.1 (18.0, 24.2)	-2.7 (-7.1, 1.7)	0.222	-0.54	-1.9 (-6.1, 2.3)	0.357	-0.38

Note. AUC, area under the curve; CON, control group; CI, confidence intervals; LPA, light-intensity physical activity group; MVPA, moderate-to-vigorous intensity physical activity group; sCort, salivary cortisol.

^a Least square mean (95%CI) values adjusted for baseline.

^b Mean difference between groups adjusted for baseline values. Negative values represent favourable differences relative to CON.

^c Log-transformed prior to analysis to account for skewness.

^d Adjusted mean difference calculated following multiple imputation to account for missing data. All other outcomes based on complete-case analysis.

that employed 2 × 10 min of high-intensity interval training per week, suggesting that short duration vigorous intensity activity can reduce adrenocortical markers of stress. However, B2L was delivered over 16 weeks and the difference in dose received might explain the discordant findings. In addition, B2L was delivered during curricular time and adherence was likely higher than in our trial, given sessions were delivered during mandatory classes. The MVPA group in the present study completed only 6.6 sessions (out of 12) on average, and fewer than half satisfied the 80 % attendance target. This is likely to have influenced the findings for sCort-reactivity and reinforces the value of delivering physical activity during mandated periods of the school day. While schools might more readily adopt programs that do not interfere with existing parts of the school day (i.e., before/after school, or break-time), such programs may be less effective given they rely on youth to opt-in to a non-mandatory opportunity and so might appeal mostly to those with an existing interest in sport and exercise. We suggest future trials explore ways to provide physical activity opportunities that reach most (or all) youth during mandated periods that do not rely heavily on student motivation.

Finally, the inclusion of both females and males in the study sample could have contributed to the null sCort effects. Often, studies exploring adrenocortical-reactivity to induced stress use male-only samples to

avoid the confounding effects of menstrual cycle and/or hormonal contraceptives (Caplin et al., 2021). Alternatively, researchers may include females but collect information on menstrual timing and contraceptive use so this can be accounted for in the inclusion criteria, randomisation or analyses. We felt limiting the study to males was problematic because: i) females report worse mental health than males during adolescence (Naninck et al., 2011), and so should be included in stress-prevention research; ii) focusing on males for practical scientific reasons has historically resulted in gender inequities in health and medical knowledge, and iii) limiting recruitment to a single sex group would have impacted our recruitment potential given we enrolled only a single school. We did not ask females to report their menstrual status or use of hormonal contraceptives, as they were minors and this would not have been permitted by our institutional review board.

Despite a near zero effect for sCort-reactivity, the effect sizes for perceived stress (PSS) and non-specific psychological distress (K-10) (*n* = 34 for both) were of meaningful magnitude (though importantly these were also not statistically significant). Prior research suggests higher-intensity physical activity may produce larger effects on adolescents' mental health compared with lower intensity physical activity (Ahn & Fedewa, 2011). However, the effect sizes for PSS and K-10 in the present trial were similar for both physical activity groups. Given the small

sample size and lack of statistical significance, inferences regarding these outcomes must be treated with caution. This said, our findings are consistent with a large body of literature demonstrating reliable benefits of physical activity on self-reported mental health among youth (Biddle et al., 2019).

Surprisingly, the largest effect size observed was for subjective-reactivity, with participant ratings of the TSST-G as ‘*unpleasant*’ favouring CON over MVPA ($d = 0.86, p = .084$). While not statistically significant, this finding is notable given the effect size is large, in the opposite direction to our hypothesis, and inconsistent with the between-group difference for ratings of the TSST-G as ‘*stressful*’ (which favoured MVPA over CON; $d = -0.30, p = .539$). Explaining this inconsistency is challenging, but one possibility is that these findings are actually in line with the CSA. For example, it is possible that participation in MVPA might ‘shift’ individuals from experiencing a more aversive affective state (i.e., ‘*stressful*’) in response to a stressor, to a less aversive (albeit still negative) affective state (i.e., ‘*unpleasant*’). In this sense, greater perceptions of the stressor as ‘*unpleasant*’ could actually be viewed as a positive finding, given they are coupled with lower perceptions of the experience as ‘*stressful*’ (though not the same degree). This explanation is speculative, and it may be that these differences are simply chance effects that would not reproduce in a subsequent trial. Still, this preliminary result raises an interesting question relevant to interpreting group differences in subjective-reactivity to the TSST-G, that would be good to explore further in a properly powered trial.

Strengths of the present study include the individually randomised RCT design, and use of a robust biomarker of stress collected in response to the most highly regarded psychosocial stress-induction paradigm, as well as strong delivery fidelity. The use of an alternative stressor task at baseline is also a notable innovation that to our knowledge has not been employed before. Additional strengths include controlling for potential bias in the intervention design, with physical activity sessions occurring on the same days and times, in the same setting, and with equivalent exposure to research staff. Finally, the experimental manipulation was successful, as demonstrated by clear differences between LPA and MVPA for mean/peak session HR responses collected for every participant across all sessions delivered. There are also several important limitations, many of which have been discussed. These include the small sample size, suboptimal participant retention for the primary outcome, and poor participant adherence with the physical activity programs. In addition, we did not collect data on sleep, general physical activity, dietary behaviours, or body composition, which have all been linked to cortisol levels in youth. While randomisation should provide rough balance between treatment groups on these factors, the small sample size means there is a risk of imbalance between conditions on potentially important confounders.

4.1. Implications for future research

Despite our null results, the novelty of the present trial alongside many robust features of our methodology provide a useful template for others working on this research question, which we feel is in urgent need of greater attention. To address the limitations of the present trial, future research might consider the following improvements: (i) increase sample size by enrolling students from a larger number of schools; (ii) improve participant adherence by providing exercise sessions during mandatory periods of the school day. This might require substantive preparatory work with school leaders to convince them of the advantages of this intervention when it might come at the cost of existing curricular time; (iii) increase the exercise dose by extending the intervention over a longer duration (and perhaps increasing session frequency/duration). While determining the minimally effective dose of exercise to support older adolescent stress remains important, an intervention of at least 8 weeks (with a weekly dose ≥ 60 min) may be needed to stimulate adaptations to HPA-axis functioning; (iv) consider additional confounders in the randomisation process or analysis, which

might include sleep, physical activity, body composition, and dietary behaviours; and (v) while a point of novelty/innovation in the present trial that we view as a strength, the SECPT used at baseline did not appear to produce as robust a change in participants’ cortisol as expected (and less so than the TSST-G). Whether this was limited to our study sample or is representative of how this population respond to the SECPT remains unclear. Further research examining the utility of the SECPT as a stress induction paradigm for older adolescents would therefore be informative. Having the ability to adjust for baseline differences in cortisol-reactivity without undermining the efficacy if the stress-induction at the primary end-point (due to habituation from repeated exposure) remains a tension and a challenge in this field.

5. Conclusion

Due to suboptimal participant recruitment, retention, and adherence, we were unable to draw firm conclusions on the influence of physical activity intensity on adolescents’ sCort-reactivity. Importantly, the lack of an effect for sCort markers does not indicate a lack of support for CSA, which remains a potential explanation for the often-reported association between physical activity and stress. Group differences for self-reported mental health were also non-significant but favoured physical activity conditions and were of meaningful magnitude, which is consistent with the extant literature. Thus, further examination of these outcomes in a properly powered trial is warranted. Understanding the mechanisms underpinning the effects of physical activity on youth mental health remains an important area for future research. Elucidating these mechanisms may aid the design of physical activity focused stress-management interventions for this priority group.

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CRedit authorship contribution statement

Jordan J. Smith: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mark R. Beauchamp:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Eli Puterman:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Angus A. Leahy:** Writing – review & editing, Resources, Project administration, Investigation, Data curation. **Sarah R. Valkenborghs:** Writing – review & editing, Methodology, Investigation. **Levi Wade:** Writing – review & editing, Investigation. **Frances Chen:** Writing – review & editing, Methodology. **David R. Lubans:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.psychsport.2024.102754>.

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