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Abstract

Physical activity participation is associated with effective stress coping, indicated by decreases in both physiological stress reactivity and perceived stress. Quantifying the effect of physical activity on the diurnal regulation of one key physiological stress indicator, the stress hormone, cortisol, across studies may demonstrate the extent to which physical activity participation is associated with diurnal HPA axis regulation. We meta-analyzed studies examining relations between physical activity participation and indices of HPA axis regulation: the diurnal cortisol slope and the cortisol awakening response. We also examined moderators of the relation. The analysis revealed a small, non-zero negative averaged correlation between physical activity and the diurnal cortisol slope ($r = -0.043$, 95% CI $[-0.080, -0.004]$).

Examination of sample sociodemographic differences, study design characteristics, cortisol measurement methods, and physical activity variables as moderators revealed few effects on the relation between physical activity and diurnal cortisol slope. We did not observe lower levels of variability in the mean cortisol awakening response at higher levels of physical activity participation, and moderator analyses showed little evidence of reductions in heterogeneity for this effect. We found some evidence of systematic publication bias. Findings suggest higher physical activity is associated with a steeper diurnal cortisol slope. However, the cortisol awakening response did not differ by physical activity level. Future studies testing the physical activity and cortisol regulation association should use standardized physical activity measures, follow guidelines for better quality cortisol sampling collection and analysis, and test relations in large-scale empirical studies to confirm the direction and causality of the effect.

Keywords: Diurnal cortisol slope; Cortisol awakening response; Physical activity; Exercise; HPA axis regulation; Health behavior

Physical Activity and Cortisol Regulation: A Meta-Analysis

Introduction

Chronic psychological stress¹ is associated with the development or exacerbation of multiple physical and psychological health conditions including cardiovascular disease, type II diabetes, stroke (Rosmond & Bjorntorp, 2000), cancer (Abercrombie et al., 2004), premature cellular aging (Parks et al., 2009), autoimmune disorders and inflammation (Silverman & Sternberg, 2012), systemic hypertension (Wirtz et al., 2007), depression (Stetler & Miller, 2005), and anxiety (Vedhara et al., 2003). Exposure to psychological stress is ubiquitous, impacting individuals worldwide. For example, in 2018 about one third of the worldwide population, and more than half of Americans, reported experiencing elevated stress during the day (Gallup, 2019). Therefore, identifying the correlates of psychological stress and its development, as well as the mechanisms involved, is considered a priority area of research that may inform the development of interventions to minimize the deleterious effects of stress on health (O'Connor et al., 2020).

One mechanism that has been implicated in the association between stress and stress-related health outcomes is cortisol, a steroid hormone that is reactive to stressful stimuli. Physical (e.g., physical activity) and psychological (e.g., work stress) forms of stress can activate stress-related responses, which, in turn, activate cortisol secretion to provide the energy and biological substrate necessary to cope with stress-provoking stimuli or escape threats (Jankord & Herman, 2008). Although cortisol secretion is an adaptive short-term response to stress, chronic stress-related cortisol secretion can cause the system that produces cortisol, the hypothalamic-pituitary-

¹ Psychological stress is defined as the perception that the demands of a task or situation exceed available personal and social resources (Lazarus & Folkman, 1984) and is associated with characteristic cognitive and somatic responses (Ulrich-Lai & Herman, 2009).

adrenal axis (HPA axis), to become *dysregulated*. This means that cortisol secretion does not follow the expected secretion pattern.

There are two main patterns of dysregulated cortisol secretion: tonic and phasic. Tonic cortisol actions generally refer to effects from the long-term presence, ranging from a few hours to days, of basal circulating cortisol, and produce a somewhat constant modulation of cortisol secretion (Sapolsky et al., 2000). In contrast, phasic cortisol actions can be attributed to the effects that stem from a rapid and transient increase in cortisol elicited by exposure to stress in-the-moment (Sapolsky et al., 2000). Both phasic and tonic cortisol secretion patterns contribute to the regulation of basal and stimulated HPA axis activity (Dallman et al., 1987; Sapolsky et al., 2000). However, for the purpose of the current review, where we refer to HPA axis regulation we mean tonic HPA axis regulation patterns. Tonic HPA axis dysregulation is typically measured through parameters that capture the circadian diurnal rhythm of cortisol secretion, such as the diurnal cortisol slope and the cortisol awakening response (Chida and Steptoe, 2009; Saxbe, 2008).

The diurnal cortisol slope refers to the naturally occurring decline in cortisol throughout the waking day from awakening until bedtime, and the cortisol awakening response is a measure of the increase in cortisol levels during the first 30 minutes to 1 hour after awakening. Deviations from the expected rhythm of the diurnal cortisol slope and the cortisol awakening response are indicative of HPA axis dysregulation. Research has demonstrated associations between HPA axis dysregulation and increased risk of many chronic physical and mental health conditions such as cardiovascular disease (Whitworth et al., 2005), obesity-related metabolic disorders (Baudrand & Vaidya, 2015), insomnia (Rodenbeck & Hajak, 2001), hypertension (Whitworth et al., 1995), and uni- and bi-polar depression (McIsaac & Young, 2009).

In addition, meta-analytic evidence has shown that deviations from the expected steep daily decline in the diurnal cortisol slope (i.e., a ‘flatter’ slope) are associated with many stress-related mental and physical health outcomes such as depression, fatigue, immune/inflammatory outcomes, obesity and adiposity, cancer, and all-cause mortality (Adam et al., 2017). Stress-related deviations in HPA axis regulation may, therefore, play a role in mediating associations between stress exposure and health outcomes (Chrousos & Gold, 1992; Davis & Sandman, 2010; Lupien et al., 2009), including both the onset and progression of mental and physical health disorders (Heim et al., 2008). Based on these data, it is imperative to identify modifiable targets for interventions that promote HPA axis regulation, and one target may be physical activity behavior. Physical activity participation is associated with improvements in, and prevention of, many health conditions that are also associated with a flatter diurnal cortisol slope such as depression (Dunn et al., 2005), fatigue (Puetz, 2006), elevated inflammation levels (Abramson & Vaccarino, 2002), cancer (McTiernan et al., 2019), and all-cause mortality (Lear et al., 2017). Therefore, examining the overall relations between physical activity and diurnal cortisol indices are an important first step in exploring the potential of promotion of physical activity as an efficacious means to promote HPA axis regulation.

In the current study, we aimed to examine the association between physical activity participation and HPA axis regulation, as well as factors that moderate this association across the extant literature. Physical activity participation, defined as participation in exercise, sport, or physical activity as part of daily living, occupation, leisure, and active transportation (Garber et al., 2011), encompasses activities at light, moderate, and vigorous intensities and of any duration. We expected our study to make an important contribution to knowledge by examining the strength of the evidence across studies of physical activity participation as a potential correlate of

cortisol regulation, a variable that provides a good indicator of effective stress coping. We also aimed to synthesize evidence on the conditions that affect the association between physical activity participation and HPA axis regulation.

Physical Activity and HPA axis Regulation

HPA axis regulation is one potential mechanism by which physical activity participation may impact stress-related outcomes (Tsatsoulis & Fountoulakis, 2006). Previous research supports the psychological stress-reducing effects of regular physical activity participation (Nguyen-Michel et al., 2006). Physical activity participation² is associated with lower levels of physiological stress reactivity after exposure to psychosocial stress (heart rate, blood pressure; Forcier et al., 2006; Hamer et al., 2006; Wipfli & Ramirez, 2013). These findings brought about the development of the cross-stressor adaptation hypothesis, which suggests that regular physical activity leads to adaptations in the stress response systems that induce decreased physiological responses to psychological stressors (Sothmann, 2006). While these data provide robust evidence that physical activity participation is related to effective in-the-moment physiological stress coping to stressful psychosocial stimuli, the underlying mechanisms that mediate this effect have yet to be fully elucidated. Developing an understanding of *how* physical activity interventions produce these changes and the mechanisms involved may provide targets for future interventions that will reliably produce adaptive changes in stress-related health parameters.

HPA axis regulation has been identified as a proposed mechanism by which physical activity relates to better stress coping (Chen et al., 2017). Chronic physical activity is proposed to affect the medial prefrontal cortex and the hippocampus, two brain regions that are key to

² For the purposes of this review, physical activity is defined as participation in exercise, sport, or physical activity as part of daily living, occupation, leisure, and active transportation (Garber et al., 2011)

regulating the HPA axis negative feedback loop (Diorio et al., 1993; Herman et al., 2003; Mizoguchi et al., 2003), which can be thought of as the ‘off-switch’ that stops cortisol output after a stressor subsides (Zschucke et al., 2015; for a detailed explanation, see the review Chen et al., 2017). The diurnal cortisol slope has been proposed to represent an intact HPA axis negative feedback loop as a steep diurnal cortisol slope models an individual’s physiological ability to recover and disengage from stressful events over the course of a day (Heim et al., 2000; Miller et al., 2007). Physical activity participation may facilitate optimal HPA axis recovery to disengage from daily stress (Kim et al., 2008; van Hooff et al., 2019; Zschucke et al., 2015). Confirming that physical activity participation is associated with indices of diurnal HPA axis regulation may provide important evidence of this proposed mechanism.

While many studies have found an association between physical activity and parameters related to HPA axis regulation such as the diurnal cortisol slope (Gubelmann et al., 2018; Ho et al., 2020; Vreeburg et al., 2009) and the cortisol awakening response (Calogiuri et al., 2016; Gubelmann et al., 2018; Tortosa-Martínez et al., 2015), findings across studies have been inconclusive. For example, some studies have reported statistically significant or non-zero effects (Tortosa-Martínez et al., 2015; Vreeburg et al., 2009), while others have reported effects that do not differ from the null (Foss et al., 2014; Lederbogen et al., 2010; McHale et al., 2014; Menke et al., 2014). Reviews of research examining relations between physical activity on parameters and HPA axis regulation have suggested an association (Anderson & Wideman, 2017; Pascoe et al., 2017), but, to date, no research synthesis has provided an estimate of the size of the relation between physical activity participation, broadly defined, and indices of HPA axis regulation. Quantifying the relation between physical activity participation and these indices across studies may provide important evidence to support the size of the effect and its degree of

heterogeneity across studies, and, in so doing, provide a reliable estimate of the true variability in the effect. It may also assist in identifying possible conditions or factors that may affect it through an analysis of moderators. The research is expected to provide valuable information for those developing stress coping interventions to reduce stress and improve health outcomes.

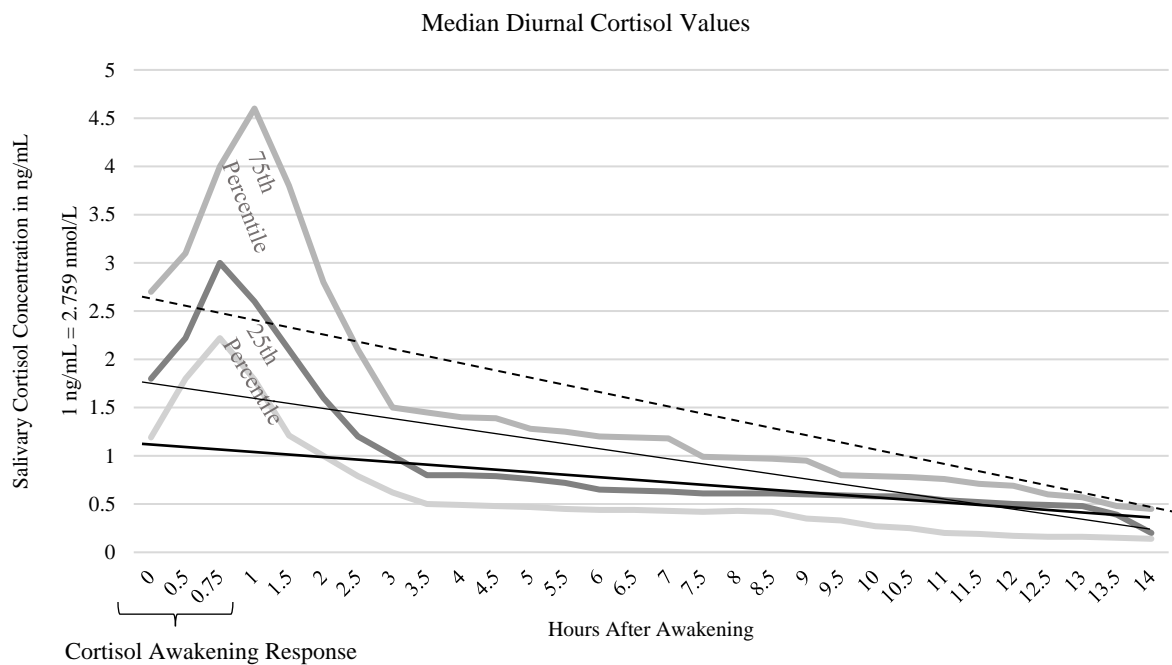
The Present Study

Study Overview

In the present study, we aimed to synthesize research testing relations between physical activity participation and indices of effective HPA axis regulation and estimate the variability and heterogeneity in the effect. Specifically, we aimed to estimate the average size and heterogeneity of the relation between physical activity participation and two independent indices of HPA axis regulation, the diurnal cortisol slope and the cortisol awakening response, across studies in existing literature using multi-level meta-analysis. These indices were selected because they are among the most commonly used means to indicate HPA axis dysregulation in the extant literature (Adam & Kumari, 2009), enable accurate estimation of the variability in cortisol across key diurnal timepoints of interest, and represent independent aspects of HPA axis dysregulation (Wilhelm et al., 2007). We aimed to evaluate the extent to which the observed variability in the correlation between physical activity and the diurnal cortisol slope, and the mean and standard deviation for salivary cortisol awakening responses at different levels of physical activity participation, across studies was attributable to methodological artifacts corrected for in meta-analysis (i.e., sampling error), or to true variability in the effect across studies and the extent of that variability.

Figure 1

Median diurnal cortisol patterns based on population norms from the CIRCORT database, adapted from Miller et al., 2016. The linear lines from awakening (0 hours after awakening) to bedtime (14 hours after awakening) represent the degree of decline in the diurnal cortisol slope. Flatter diurnal cortisol slopes are considered dysregulated, whereas steeper diurnal cortisol slopes are considered regulated.



The Association Between Physical Activity and Indices of HPA axis Regulation

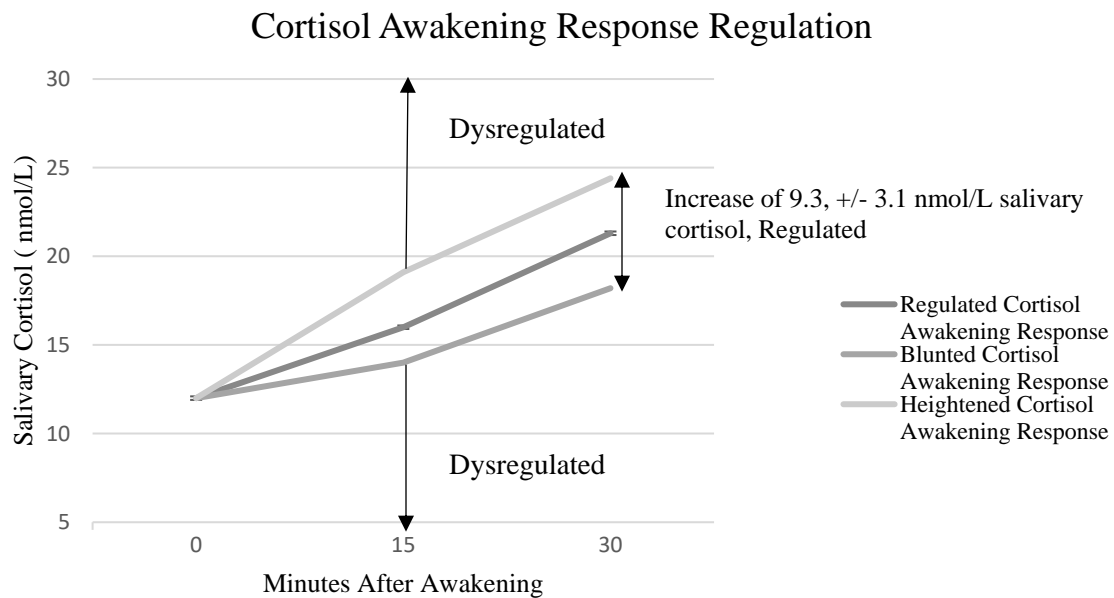
Consistent with prior theory and research (Gubelmann et al., 2018; Ho et al., 2020; Vreeburg et al., 2009), we hypothesized that physical activity participation would be associated with steeper diurnal cortisol slopes across studies (Figure 1). We also hypothesized that physical activity would be related to the cortisol awakening response. Both heightened and blunted cortisol awakening responses are indicative of HPA axis dysregulation, while a mid-range cortisol awakening response, indicated by rise in cortisol from awakening to peak secretion in the range of 9.3 ± 3.1 nmol/l, reflects adequate regulation (Clow et al., 2004; Wüst et al., 2000). We therefore hypothesized that there would be less variability about the mean of the cortisol awakening response in those who are more physically active compared to those who are less active. This is because those who are less physically active may be more likely to exhibit a dysregulated cortisol awakening response, either heightened or blunted, which is expected to be manifested in higher variability about the mean cortisol awakening response across studies. By comparison, those who are active are more likely to exhibit HPA axis regulation, which is likely to be manifested in less variability in the mean response across studies (Figure 2).

Moderators of the Physical Activity-HPA axis Regulation Effect

In addition to quantifying the relation between the physical activity participation and HPA axis regulation, we aimed to test effects of several key moderators on the relations for each index: sociodemographic variables (age, sex, BMI, clinical sample), cortisol measurement methods (type of diurnal cortisol slope, number of cortisol samples taken over the relative time period, number of days cortisol was measured, whether samples were taken on a resting day or not reported, cortisol sampling quality), physical activity assessment methods (physical activity intensity, intensity assessment type, physical activity duration, level of physical fitness, fitness

Figure 2

Cortisol awakening response norm values, adapted from Clow et al., 2004. Any value above the “Heightened Cortisol Awakening Response” and any value below the “Blunted Cortisol Awakening Response” values signals cortisol awakening response dysregulation, whereas a rise of $9.3, \pm 3.1$ nmol/L signals cortisol awakening response regulation.



assessment type, time of day physical activity was performed, physical activity type, physical activity measurement, and physical activity frequency), and general study design (time lag and study design)³. These moderators were assessed using categorical moderator analyses and meta-regression. Specific predictions relating to the effects of each moderator on the relations between physical activity and indices of cortisol regulation are briefly summarized in Table 1 with a more detailed treatment provided in Appendix A (supplemental materials).⁴ In addition to pre-registered moderator analyses, we also explored the possibility of examining the effects of race/ethnicity and season in which the data were collected as moderators of the relationship. This is based on studies that have observed racial/ethnic (Adam et al., 2015; DeSantis et al., 2007) and seasonal (Miller et al., 2016) differences in diurnal cortisol patterns.

Method

Literature Search

We located studies for inclusion in the current analysis via a search of five electronic databases: Web of Science, PsycINFO, EBSCO, PubMed, and ProQuest Dissertations and Theses. The search was not limited by language or publication year, and included research published on, or prior to, October 31, 2020. We also conducted manual searches of the reference lists of pertinent review and overview studies. In addition, we contacted prominent authors in the field to locate unpublished data. In addition, requests for unpublished data were circulated on the listservs of two relevant organizations: the Society for Behavioral Medicine and the International Society of Psychoneuroendocrinology. The search strings for the data base searches are provided

³Detailed coding for moderator categories is available in Appendix H. Absolute intensity is the measurement of physical activity intensity on a standardized scale, usually as a metabolic equivalent, or MET. By contrast, relative exercise intensity is a measure of intensity relative to an individual's own physiological maximum capacity for physical activity.

⁴Both a brief (Table 1) and detailed (Appendix A) explanation for moderator inclusion is available along with supporting references..

in Appendix B (supplemental materials) and the study protocol was pre-registered on the Prospero registry of systematic reviews:

https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=247847.

Inclusion and Exclusion Criteria

Published research, abstracts, dissertations, and unpublished theses and manuscripts were eligible for inclusion. Studies were included if they fulfilled two main criteria: (1) Inclusion of at least two measures of time-specific cortisol secretion in one day sufficient to assess either the diurnal cortisol slope (i.e., at least one cortisol measurement in the morning between waking and noon and one at night between 4pm-bedtime) or the cortisol awakening response (i.e., at least one cortisol measurement upon awakening and one measurement 30 minutes to 1 hour after awakening); and (2) Inclusion of at least one measure of physical activity participation, defined as participation in exercise, sport, or physical activity as part of daily living, occupation, leisure, and active transportation (Garber et al., 2011). This definition encompasses activities at light, moderate, and vigorous intensities and of any duration. Studies adopting both self-report and non-self-report (e.g., physical activity measured using accelerometers, pedometers, or other devices) measures of physical activity were included. Studies were included if physical activity participation and diurnal cortisol slope and/or cortisol awakening response were measured irrespective of design (e.g., cross-sectional, longitudinal).

Case studies, n-of-1 studies, qualitative studies, reviews, and methods studies were excluded. Studies that examined the cortisol awakening response and/or the diurnal cortisol slope as a predictor of subsequent physical activity participation were excluded. Studies that only measured the cortisol awakening response within two days before or after competition were excluded, but if studies included measurements of the cortisol awakening response three days

Table 1.*Moderator Hypotheses with Supporting Evidence for Inclusion*

Moderator Category	Moderator	Hypothesis	Supporting Evidence
Study design and sample characteristics	Age	Larger PA-DCS in younger vs. older participants.	Therrien et al., 2007; Vreeburg et al., 2009
		Mean variability of CAR in high and moderate PA levels vs. low PA levels larger in older vs. younger participants.	
	Sex	Larger PA-DCS in male vs. female participants.	Therrien et al., 2007; Vreeburg et al., 2009
		Mean variability of CAR in high and moderate PA levels vs. low PA levels larger in female participants vs. male participants.	
	Body Mass Index (BMI)	Larger PA-DCS in participants with an under or normal weight BMI ($BMI \leq 25$) vs. participants with an overweight or obese BMI ($BMI > 25$).	Rodriguez et al., 2015
		Mean variability of CAR in high and moderate PA levels vs low PA levels larger in high mean BMI samples vs. low mean BMI samples.	
	Clinical sample	Smaller PA-DCS in clinical samples vs. non-clinical samples.	Adam et al., 2017
		Mean variability of CAR in high and moderate PA levels vs. low PA levels larger in studies with clinical samples relative to studies with non-clinical samples.	
Study design	Study design	Larger PA-DCS in studies adopting cross-sectional designs than experimental or intervention designs.	Cross-sectional studies use similar methods and measure constructs in close proximity which may inflate relations due to common method variance and measurement correspondence
		Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in correlation designs vs. experimental or intervention designs.	
		Larger PA-DCS in experimental and intervention studies in longer vs. shorter periods of physical activity	
			Anderson & Wideman, 2017

Physical activity measurement	Time lag	Mean variability of CAR in high and moderate PA levels vs. low PA levels in experimental and intervention studies smaller in longer vs. shorter periods of physical activity. Greater heterogeneity in PA-DCS in larger time lag in longitudinal studies vs. shorter time gaps.	Longitudinal studies that measure constructs in close proximity may inflate relations
	Intervention components	Greater heterogeneity in mean cortisol awakening response in the physical activity-CAR in studies with longer time lag vs. studies with shorter time lag. Single (physical activity only) vs. multiple intervention components.	Exploratory
	Season	Greater heterogeneity in PA-DCS in fall/winter months vs. spring/summer	Miller et al., 2016
	Race/Ethnicity	Greater heterogeneity in mean cortisol awakening response in the physical activity-CAR in studies that collected data in fall/winter months vs. spring/summer months. Larger PA-DCS in white vs. non-white participants.	Adam et al., 2015; DeSantis et al., 2007
	Measure type	Mean variability of CAR in high and moderate PA levels vs. low PA levels larger in non-white participants vs. white participants. Greater precision and less variability in the PA-DCS effect in non-self-report measures vs. self-report measures.	Adams et al., 2005; Sallis & Saelens, 2005
		Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in non-self-reported physical activity measures vs. self-reported physical activity measures	Milton et al., 2011
		Greater precision and less variability in the PA-DCS effect in validated scales vs. bespoke scales.	
	Physical activity	Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller validated scales vs. bespoke physical activity scales. PA-DCS larger when bouts of physical activity exceeded an individuals' 50% VO ₂ max threshold.	Duclos et al., 2003; Hackney & Viru, 1999; Hill et al., 2008;

intensity		Viru, 1992
Physical activity intensity derivation	PA intensity was not assessed in the PA-CAR effect because intensity is inherent in the classification of study effects into PA subgroups. Greater averaged PA-DCS in studies that measured intensity as relative PA intensity vs. those based on absolute values	Freedson et al., 1998 Ozemek et al., 2013
Physical activity duration per bout	Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in in relative exercise intensity measures vs. absolute values. Larger PA-DCS when bouts of PA were 30 minutes or greater in duration vs. less time per bout.	Duclos et al., 2003; Hackney & Viru, 1999; Hill et al., 2008; Viru, 1992
Level of physical fitness	PA duration was not assessed in the PA-CAR effect because duration is inherent in the classification of study effects into physical activity subgroups. Larger PA-DCS in athletes and individuals with high levels of physical fitness vs. lower levels of fitness.	Duclos et al., 2003
Fitness assessment type	Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in high fitness vs. lower fitness. Studies utilizing non-self-reported measures of physical fitness are less heterogeneous than studies that utilized self-reported fitness assessment.	Tortosa-Martínez et al., 2015 Non-self-reported measures may be less prone to error self-report.
Physical activity time of day	Larger PA-DCS in morning PA vs. afternoon or evening PA. Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in morning PA compared to afternoon or evening PA.	Kanaley et al., 2001
Physical activity type	Larger PA-DCS in aerobic PA vs. anaerobic PA.	Beserra et al., 2018; Vrinceanu et al., 2019
Physical activity frequency	Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in aerobic PA vs. anaerobic PA. Larger PA-DCS in three or more PA sessions a week compared to fewer than three sessions per week. PA frequency was not assessed in the PA-CAR effect because is inherent in the	Beserra et al., 2018

Cortisol Measurement	Diurnal cortisol slope measurement	classification of study effects into PA subgroups.	
		Larger PA-DCS in studies using amplitude measures and wake-bed slopes vs. other methods.	Adam et al., 2017
	Cortisol awakening response measurement	Larger PA-DCS in waking vs. fixed time point cortisol measurements.	Wilhelm et al., 2007
		Mean variability of CAR in high and moderate PA levels vs. low PA levels smaller in waking vs. fixed time point cortisol measurement.	Wilhelm et al., 2007
		Larger PA-DCS in studies with more than two samples in one day vs. studies that took only two samples.	Adam et al., 2017 ; Chida & Steptoe, 2009
		Mean variability of CAR in high and moderate PA levels vs. low PA levels greater in studies with two samples of cortisol vs. more than two.	
	Number of days cortisol was sampled	Greater variability in the PA-DCS in one day vs. more than one day of cortisol samples.	Adam & Kumari, 2009
		Mean variability of CAR in high and moderate PA levels vs. low PA levels larger in one day vs more than one day of cortisol samples.	
	Cortisol sampled on resting day	Less variability in the PA-DCS in studies with a rest period prior to diurnal cortisol measurement vs. those that do not instruct participants to rest prior to cortisol measurement.	Viru, 1992
		We assumed that participants would not be likely to engage in PA between awakening and 30-45 minutes after awakening samples.	
	Methodological quality of cortisol sampling	Lower quality studies were likely to exhibit greater error variance in the PA-DCS vs. studies of acceptable quality.	Johnson et al., 2014
		Greater error variance in the variability of the mean CAR between high and moderate versus low levels of PA.	

Note. PA = physical activity participation; DCS-PA = the physical activity-diurnal cortisol slope association; CAR= the cortisol awakening response.

before or after competition, these measurements were included. Studies that measured the diurnal cortisol slope on a day when physical activity participation was performed were excluded as such measures are not likely to effectively capture HPA axis regulation but, instead, may reflect diurnal cortisol reactivity to physical activity participation. Studies focusing on daily average cortisol, area under the curve measures of salivary cortisol, or other integrated cortisol measures such as overnight urinary cortisol or hair cortisol were excluded, as these measures do not provide information on daily cortisol variability (Badrick et al., 2017; Fekedulegn et al., 2007). Studies on participants with endocrine disorders, genetic disorders or polymorphisms, or impaired capacity to provide responses on self-report questionnaires were excluded. Studies with proxy measures (e.g., parent, caregivers) of study variables were also excluded.

We located ‘fugitive’ data (Rosenthal, 1994) by sending email requests to authors of eligible studies that did not report sufficient data to compute an effect size, and gave them a four-week time frame to respond. This included studies that reported taking measures of physical activity participation and two time-dependent cortisol secretion measurements in the same day, but did not calculate the cortisol awakening response and/or the diurnal cortisol slope (disaggregate values reported only); did not calculate the cortisol awakening response as the raw mean difference (e.g., the peak cortisol value minus the awakening cortisol value); did not report the physical activity participation-HPA axis regulation association; or reported the association within a multivariate analysis from which a unique physical activity participation-HPA axis regulation effect size could not be isolated. Furthermore, authors were contacted if the study only included a statistic reporting the association between physical activity participation and either the diurnal cortisol slope or the cortisol awakening response, and sufficient data was gathered to assess the other outcome, but it was not reported. Authors were also contacted if physical activity

data were collected as part of a global ‘health behavior’ measure or as a covariate, but isolated effects of physical activity participation were not reported. In cases where interventions or experimental studies employed a manipulation of physical activity participation and measured changes in HPA axis regulation parameters, then the effect of the manipulation on the outcome was taken as the estimate of the effect. If the experimental manipulation did not consist of physical activity participation (e.g., pharmacological administration, psychological stress exposure), targeted an outcome other than HPA axis regulation, or did not specify the target construct, then data at baseline or in the control group were used to estimate the effect where possible, otherwise the authors were contacted to provide the relevant data.

Screening

After removal of duplicate studies, 16,558 studies were identified for inclusion. Titles and abstracts of the studies retrieved in search were screened against inclusion/exclusion criteria. This comprised an initial title screen, followed by abstract and full text screening, conducted by the lead researcher and two trained research assistants. During title and abstract screening, studies were divided into retained, excluded, or potentially eligible categories. Queries raised during screening were discussed between the research team and resolved through mutual agreement, with 250 of the studies screened by all researchers to validate the screening procedure. Of these, 10,270 were excluded after title screening and a further 6,269 studies excluded after abstract screening. Agreement between the three reviewers was calculated for each screening phase using the AC₁ coefficient (Gwet, 2008). While Cohen’s kappa statistic is used more widely in meta-analyses to determine inter-rater reliability, Gwet’s AC₁ coefficient has been shown to provide a more stable inter-rater reliability estimate than Cohen’s kappa, and it is also less affected by high agreement prevalence and marginal probability than Cohen’s

kappa (Wongpakaran et al., 2013). Further, Gwet's AC1 is the statistic of choice for the case of two raters because it does not depend upon the assumption of independence between raters (Gwet, 2008). Therefore, we opted to utilize the AC1 coefficient over Cohen's kappa in this meta-analysis. During title screening there was 90.03% average agreement ($AC_1 = .844$, [0.819, 0.909], $p < .001$), and with 92.71% average agreement ($AC_1 = .897$, [0.874, 0.941], $p < .001$) during abstract screening. Main reasons for exclusion included: studies were theoretical or conceptual reviews, systematic reviews, and off-topic (not pertaining to any content related to physical activity participation or HPA axis regulation). The remaining studies ($k = 1,559$) were subjected to full-text analysis for inclusion in the final sample, of these, 1,462 did not meet criteria for inclusion. Full text screening was conducted by the lead and senior researchers, with 25% of the studies screened by both researchers to validate the screening procedure.

Disagreements were also resolved through discussion and inclusion/exclusion criteria were modified and reapplied if necessary. Average inter-rater reliability across raters for inclusion-exclusion decisions during full-text screening was acceptable ($AC_1 = .750$, [0.471, 1.00], $p < .001$). Studies were excluded at this stage for the following reasons: cortisol was only measured one time in a day, cortisol was measured twice, but data were insufficient to calculate either the diurnal cortisol slope or the cortisol awakening response; cortisol was measured twice, but physical activity was performed in between sampling times; no measure of physical activity participation was included; study design included physical activity with other stressful components (e.g., hiking in hypoxic conditions, combat military training, overtrained athletic competitors) that may influence the cortisol awakening response and/or the diurnal cortisol slope; or insufficient data were available in the article to compute effect sizes and authors could not be contacted or were unable to supply the required data. Study selection procedures are

summarized in the PRISMA (Moher et al., 2009) flow diagram presented in Appendix C (supplemental materials).

Data Extraction

Study characteristics, effect size data, and data for moderator variable coding were extracted from all eligible studies by the lead and senior researchers. The following study characteristics were extracted from each study: author names, publication year, sample size, HPA axis regulation measure to be included (diurnal cortisol slope/cortisol awakening response). Extracted data for moderator coding were: sociodemographic variables (sex, age, race/ethnicity, BMI, clinical sample); type of calculation for the diurnal cortisol slope (levels: amplitude, wake-bed slope, fixed timepoint slope, and peak and late decline slopes); number of cortisol samples taken to assess the diurnal cortisol slope and/or the cortisol awakening response (levels: 2 samples and >2 samples); number of days cortisol was sampled (levels: 1 day and >1 day); cortisol sampling quality rating (score range: 0-9); report of physical activity performed on cortisol sampling day (levels: cortisol sampled on resting day and not reported); physical activity measure (levels: previously-validated physical activity scales, bespoke physical activity related questions, non-self-report physical activity measure, longitudinal grouped physical activity, and experimentally assigned physical activity); physical activity intensity (levels: low intensity and moderate-to-vigorous intensity); physical activity intensity assessment type (levels: absolute measure of intensity based on metabolic equivalents and relative measure of intensity based on maximal oxygen consumption); duration (levels: more than 30 min per activity bout and less than 30 min per activity bout); frequency of activity (levels: <3 times a week and 3 or more times a week); time of day physical activity was performed (levels: morning and afternoon or evening); physical activity type (levels: aerobic and anaerobic) and level of fitness (levels: athlete, high fit,

and low fit); fitness assessment type (levels: self-report and non-self-report); study design (levels: correlational, longitudinal, and experimental); intervention components (level: physical activity intervention only and physical activity intervention plus one or more additional intervention component/s); season of data collection; and time lag.

The zero-order correlation coefficient (r) was selected as the effect size metric for the diurnal cortisol slope outcome as the majority of the studies were correlational in design and the correlation coefficient was expected to be the most frequently adopted effect size. Where effect sizes were not expressed as a correlation, available effect size data including computed effect sizes (Cohen's d or f , eta-squared) and tests of difference (e.g., t and F -ratios, chi-square values) were converted to r using published formulae. All formulae utilized are reported in Appendix D (supplemental materials; Borenstein et al., 2009; Digby, 1983). To assess the relation between physical activity participation and the cortisol awakening response, the variability in the mean cortisol awakening response was compared in groups of studies reporting low, moderate, or high levels of physical activity participation. Physical activity level classification was based on the International Physical Activity Questionnaire (IPAQ) categories, representing low, moderate, and high levels of physical activity participation (Sjöström et al., 2002). Activity level was considered moderate if the level of physical activity participation met at least one of the following criteria: (a) 3 or more days of vigorous-intensity physical activity of at least 20 minutes per day; (b) 5 or more days of moderate-intensity and/or walking of at least 30 minutes per day; or (c) 5 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities achieving a minimum of at least 600 MET-min/week of physical activity. Activity level was considered high if the level of physical activity participation met at least one of the following criteria: (a) vigorous-intensity physical activity participation on at least 3 days

and accumulating at least 1500 MET-min/week; or (b) 7 or more days of any combination of walking, moderate- or vigorous-intensity physical activity accumulating at least 3000 MET-min/week. Activity was considered low if neither of these criteria were met.

Data for relations between physical activity and HPA axis regulation were extracted from included studies by two researchers. In cases where data for one of the target variables was expressed as a categorical variable (e.g., regular vs. irregular slope), with means and standard deviations for a measure of physical activity participation for each category, we computed a standardized mean difference using the available data and converted it to r . For experimental or intervention studies with a manipulation of physical activity participation, we computed an effect size using baseline and follow-up data for the dependent variable for either the experimental or control group (controlled designs), or baseline to follow-up (pre-post designs) manipulations of the independent variable (Borenstein et al., 2009). Multiple measures and comparisons between multiple levels of physical activity participation within the same study were utilized, where available, and effects were treated as dependent effects.

Where studies reported multiple effect sizes for the physical activity participation-HPA axis regulation relation within each study, we treated each effect size according to a pre-defined protocol. Studies reporting separate effect sizes estimated in two or more independent samples were treated as separate studies (e.g., male/female, low/moderate/high physical activity subgroups). Where multiple effect sizes were reported in the same study, such as when studies reported correlations between a measure of HPA axis regulation and two or more levels of physical activity intensity, these data were treated as multiple effects from the same study. However, when studies reported statistics between a measure of physical activity participation and both outcomes of interest, both were included in the separate diurnal cortisol slope and

cortisol awakening response analyses. We coded the data according to whether data were from independent samples or multiple effect sizes within a single study, and this coding was used as input for subsequent data analysis. Some studies that met inclusion criteria utilized the same data set (e.g., multiple published studies from the Midlife in the United States data set (MIDUS)), and the published studies had varying degrees of sample overlap. Therefore, all articles which met inclusion criteria from the same data set were added, but we treated them as a single data point, to avoid inflating relations. Also, in many of these instances, we directly accessed the publicly available data to perform the analyses to include the data point for these studies, and then coded moderators and study quality from the available data across the published articles. Our protocol outlining how data were treated for each outcome is summarized in Appendix E (supplemental materials), with coding of effect size for each study for the diurnal cortisol slope and the cortisol awakening response provided in Appendixes F and G, respectively (supplemental materials). The moderator coding protocol is reported in Appendix H (supplemental materials).

Assessment of Quality of Cortisol Sampling Methods

We assessed the quality of the cortisol sampling methods in each study based on criteria from previous meta-analyses and systematic reviews of HPA axis regulation (Adam et al., 2017; Chida & Steptoe, 2009). The assessment was based on whether studies reported accounting for the following participant characteristics and conditions during cortisol sampling: age; gender; smoking status; use of steroid-based medications; wake time; sampling day (weekday or weekend); self-reported adherence with sampling times; objective adherence to sampling times based on electronic monitoring; and clear sampling instructions provided to participants (e.g., to refrain from brushing their teeth, drinking, or eating 15 minutes prior to sampling). Scores were summed and both dichotomous and continuous quality of cortisol sampling methods rating

scores were included in moderator analyses.

Meta-Analytic Methods

The effect sizes of interest were the average sample-weighted correlation (r) between the diurnal cortisol slope and physical activity participation, and the average sample-weighted raw mean for the cortisol awakening response in groups of studies at different levels of physical activity participation. Effect sizes from each study were synthesized using random-effects meta-analysis implemented using the metafor package (Viechtbauer, 2010) in R. Some studies reported data from multiple measures or levels of physical activity participation within the same sample rather than independent samples, so including multiple effects from the same sample in the averaged effect size violates the assumption of independence. To address the dependency issue, we applied a multi-level meta-analytic model. The multi-level meta-analysis provides averaged sample-weighted effect size estimates and compartmentalizes variance into between-participants (level 1), between-effect sizes (level 2) and between-study (level 3) components separately. It enables estimation of the degree to which each variance component contributes to overall variability across the studies (Assink & Wibbelink, 2016). We coded studies as independent effects or as effects within a single study (see Appendix E, supplemental materials) to designate studies according to the different variance components in the multi-level meta-analytic model. Contribution of the between-effect size and between-study variance to the total variance in the physical activity participation-HPA axis regulation relation across studies, as well the proportion of total variance attributable to sampling error, is provided by Cheung's (2014) formula.

The analysis yielded averaged sample-weighted correlation corrected for variance components with 95% confidence intervals, and the alpha level was set at .05. In addition, we

computed Cochran's Q and I^2 coefficients as estimates of effect size variability (Higgins & Thompson, 2002). Values for Q and I^2 that were non-zero and exceeded 25%, respectively, were used as indicators of the possible presence of moderators of the effect size (Higgins & Thompson, 2002).

We assessed small-study bias in the effect size using analyses based on 'funnel' plots of the physical activity-HPA axis effect size from each study against a measure of study precision (e.g., the reciprocal of the study sample size). If the studies deviate from the expected 'funnel' shape, particularly on one or the other side of the averaged effect, it may indicate bias. This can be verified by a regression test in which the effect size of each study is regressed on the precision estimate (Egger & Sterne, 2005). Two versions of the test were computed for each outcome: the precision effect test (PET), in which uses the standard error as the precision estimate, and the precision effect estimate with standard error (PEESE), which uses the square of the standard error as the precision estimate (Stanley & Doucouliagos, 2014). The intercept provides an estimate of the effect size under conditions of no bias. PET and PEESE estimates were computed using the PETPEESE function in R (Carter et al., 2019). Following Stanley and Doucouliagos' (2014) rule, if the PET estimate for the effect size is no different from zero, the PET estimate is used as the corrected value of the effect size, however when the PET estimate is significantly different from zero, the PEESE estimate is used.

We assessed moderator variable effects on the averaged correlation between physical activity participation and the diurnal cortisol slope by conducting separate meta-analyses at each level of the moderator. Effects of moderators on the variability of the cortisol awakening response at each physical activity subgroup was assessed by computing averaged means of the cortisol awakening response in each physical activity subgroup and at each level of the

moderator. Moderator group comparisons were made using 95% confidence intervals about the averaged sample-weighted correlations for the physical activity-diurnal cortisol slope analysis, and about the difference in the variability of the averaged sample-weighted mean of the cortisol awakening response between high and moderate physical activity subgroups compared to the low physical activity subgroup between the levels of the moderator (Schenker & Gentleman, 2001). We also conducted multivariate meta-regression analyses to examine the effects of moderators that were continuous in format (e.g., time lag), and also allowed for the examination of unique effects of categorical and continuous moderator variables for the physical activity-diurnal cortisol slope analysis.

We assessed the presence of outliers by conducting a leave-one-out analysis for each outcome, in which the meta-analysis of the effect of interest is estimated iteratively leaving out exactly one study on each iteration. This provides an estimate of the extent to which each individual study affects the averaged effect size (Iyengar & Greenhouse, 2009).

Results

Study Characteristics

Forty-one studies were included in the analysis reporting a total of 51 independent samples testing the association between physical activity participation and the diurnal cortisol slope. Some studies also included multiple effect size estimates within studies (e.g., studies reporting correlations between the diurnal cortisol slope and multiple measures of physical activity; see Appendix E, Table E1, supplemental materials), resulting in a final sample of 98 effect sizes available for analysis ($N = 19,744$). For the analysis of the relations between physical activity and the cortisol awakening response we segregated samples into three physical activity subgroups; low, moderate, and high and extracted the mean cortisol awakening response for each

study in each subgroup. In the low physical activity subgroup, 30 studies were included reporting a total of 42 independent samples. After including sample dependencies resulting from multiple measures of physical activity and/or multiple timepoints in the same sample (see Appendix E, Table E2, supplemental materials), a final sample of 57 means was available for analysis ($N = 5313$). In the moderate physical activity subgroup, 26 studies were included reporting a total of 30 samples, and after including dependencies, a final sample of 44 means was available for analysis ($N = 2794$). In the high physical activity subgroup, 30 studies were included reporting a total of 33 independent samples, and, after adding dependencies, resulted in a final sample of 52 means available for analysis ($N = 3416$).

In some cases, the samples in each physical activity subgroup (low/moderate/high physical activity) differed within studies across multiple physical activity measures or timepoints, or both. For example, participants in a longitudinal study may have increased their physical activity between baseline and follow up measurement occasions and were therefore classified in different physical activity subgroups within the same study. As a consequence, modeling all sampling dependencies in each study for each physical activity subgroup was not possible, as it was unclear which participants changed physical activity level, and therefore subgroup, between measures or timepoints. For this reason, model comparisons of different sampling dependencies were analyzed for the cortisol awakening response outcome (see Appendix I for sampling dependency coding in each tested model and Appendix J for model comparison results, supplemental materials). There were no differences between models with different sampling dependencies, so the most conservative sampling dependency modeling scheme was selected, wherein dependencies in samples under the same physical activity subgroup were accounted for, whenever possible.

Study characteristics, details of the outcome measures, moderator coding, and raw effect sizes in each study are provided in Appendices F and G (supplemental materials). A full list of studies included in each meta-analysis is presented in Appendix K and L (supplemental materials), and a PRISMA checklist (Moher et al., 2009) is provided in Appendix M (supplemental materials).⁵

Overall Meta-Analytic Effects

Results of the multi-level meta-analysis of the association between physical activity participation and the diurnal cortisol slope are reported in Table 2, and a forest plot is displayed in Figure 3. Results indicated that the model correcting for within-study sampling variance between participants (level 1) accounted for a significant proportion of the variance in the effect across studies, while the variance accounted for by differences between effect sizes within studies (level 2) did not. This was confirmed by performing separate meta-analyses and comparing the variance accounted for in each restricted model with the overall model that included both variance components (Table 2). We therefore took the analysis correcting only for within-study sampling variance as the most precise estimate and variability of the effect size based on the current set of studies. The analysis revealed a small, negative, non-zero overall sample-weighted average correlation between physical activity participation and the diurnal cortisol slope ($r = -0.043$, 95% CI $[-0.080, -0.004]$). The proportion of the total variance in the correlation across studies attributable to between-study (49.46%) variance components was larger than the proportion attributable to between-participant variance (27.48%) and within-study variance (23.06%). Heterogeneity statistics indicated medium-to-high heterogeneity in the effect size and suggested the presence of moderators of the effect size.

⁵The data file used in the meta-analysis including effect sizes and moderator coding is available online: <https://osf.io/ebpy2/>

Results of the multi-level meta-analysis of the mean cortisol awakening response at each level of physical activity participation are reported in Table 3 and corresponding forest plots are displayed in Figures 4-6 . In the analyses for all physical activity subgroups (low, moderate, and high), we selected the relevant model based on whether the within- or between-study sampling variance components, or both, accounted for a significant proportion of the total variance in the mean across studies. Although the variance estimate associated with the mean cortisol awakening response was smaller in the moderate physical activity subgroup, compared to the low and high physical activity subgroups, there was substantive overlap in the confidence intervals about the variance estimates across subgroups. These data therefore do not provide definitive evidence to support lower levels of variability in the mean cortisol awakening response at higher levels of physical activity. Substantive heterogeneity was also observed in the averaged cortisol awakening response mean across studies in each activity subgroup.

Moderator Analyses

Results of the categorical moderator variable analysis for the association between physical activity and the diurnal cortisol slope are presented in Table 4 (see Appendix N, supplemental materials, for a complete summary of methods used to compute effect sizes in moderator analyses for the physical activity-diurnal cortisol slope that correspond with table subscripts). Despite observed differences in the averaged effect sizes across moderator groups, there was substantive overlap in the confidence intervals about the correlation at each level of the moderator in most of the analyses. There was also substantive heterogeneity associated with the effect sizes in each moderator group, which indicated that considerable variability remained in the effect sizes estimated in most of the moderator groups and suggested that the moderator

Figure 3

Forest plot of the physical activity and diurnal cortisol slope meta- analysis

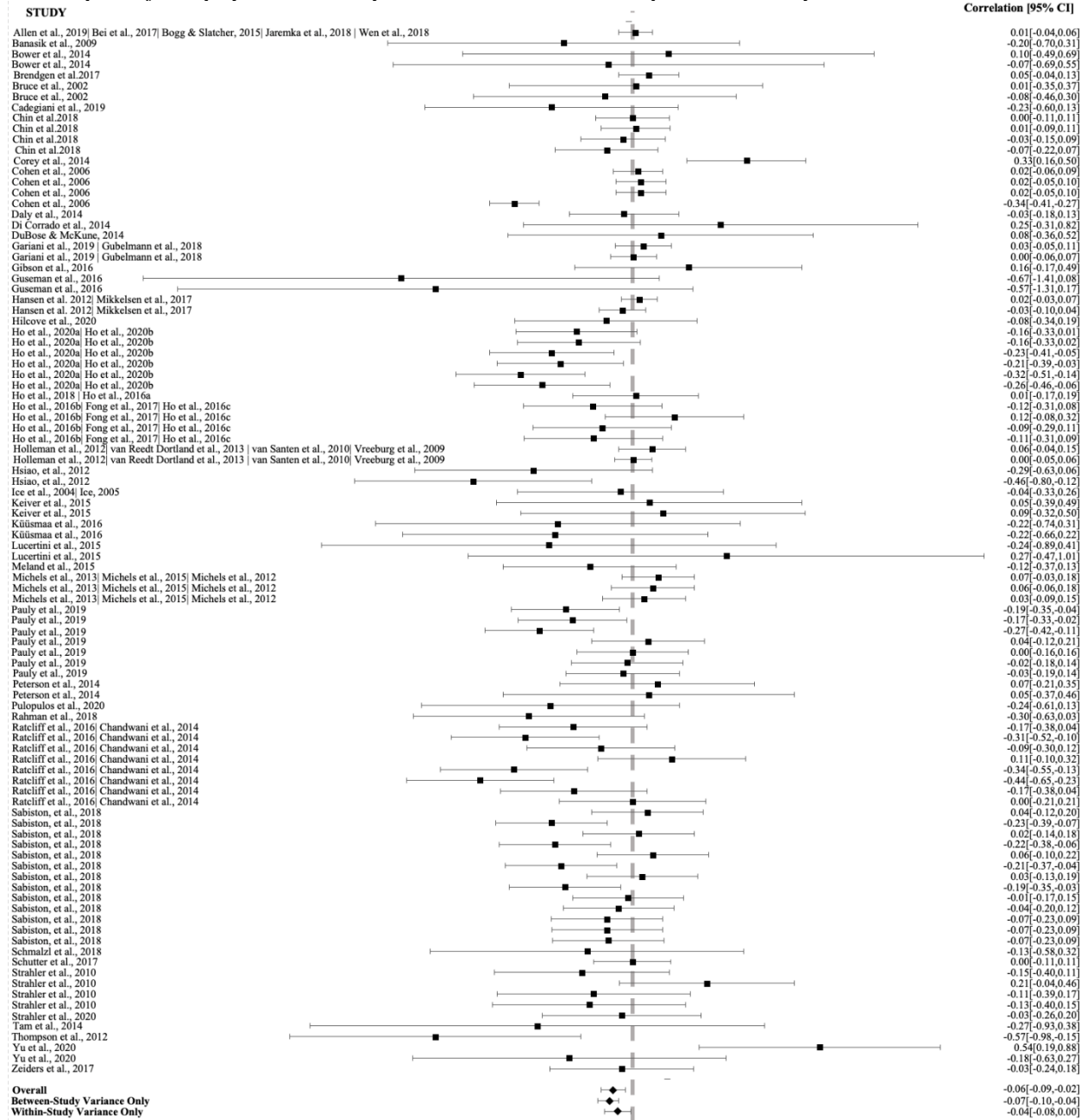


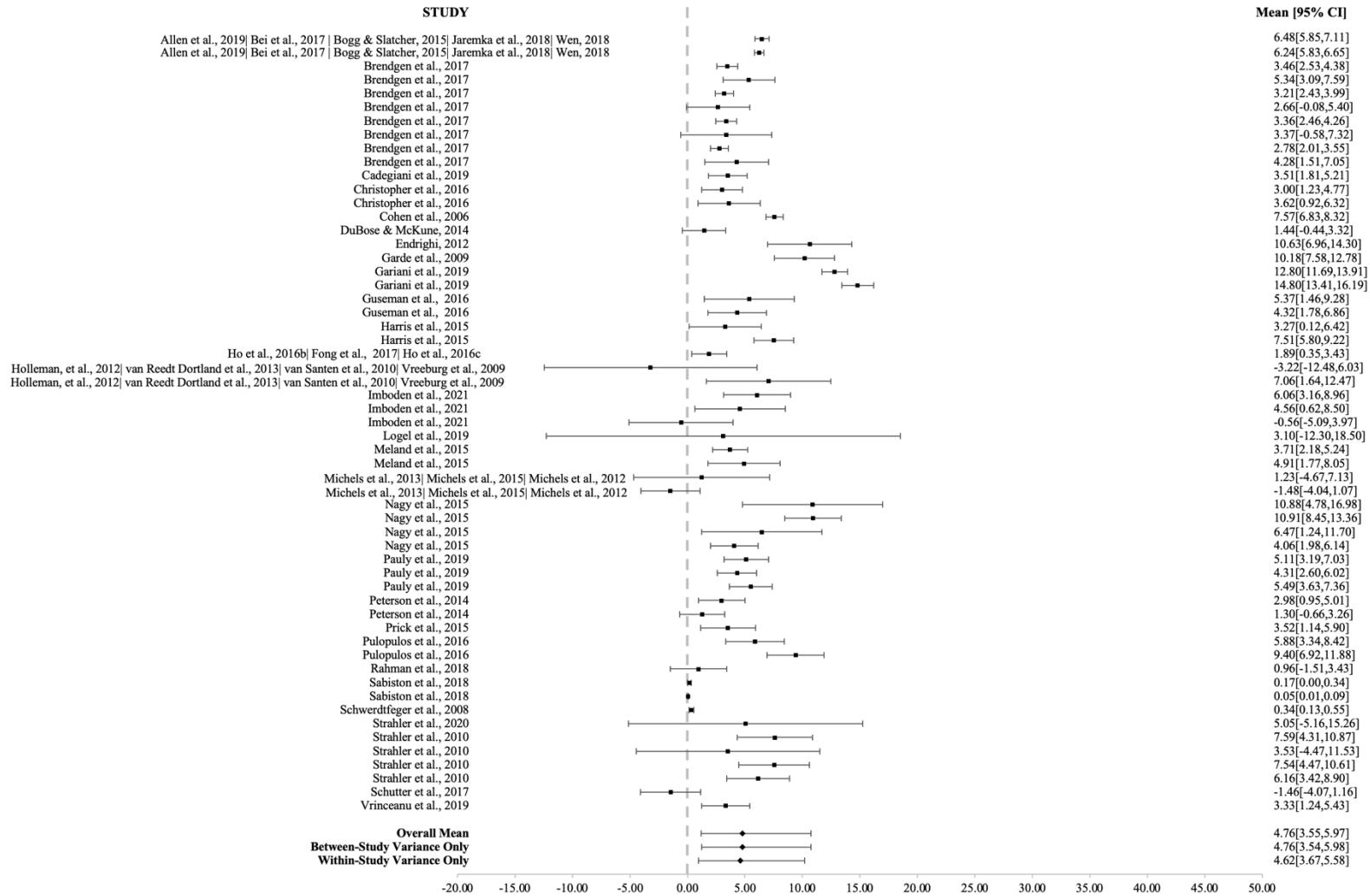
Figure 4*Forest plot of the mean cortisol awakening response in the low physical activity subgroup*

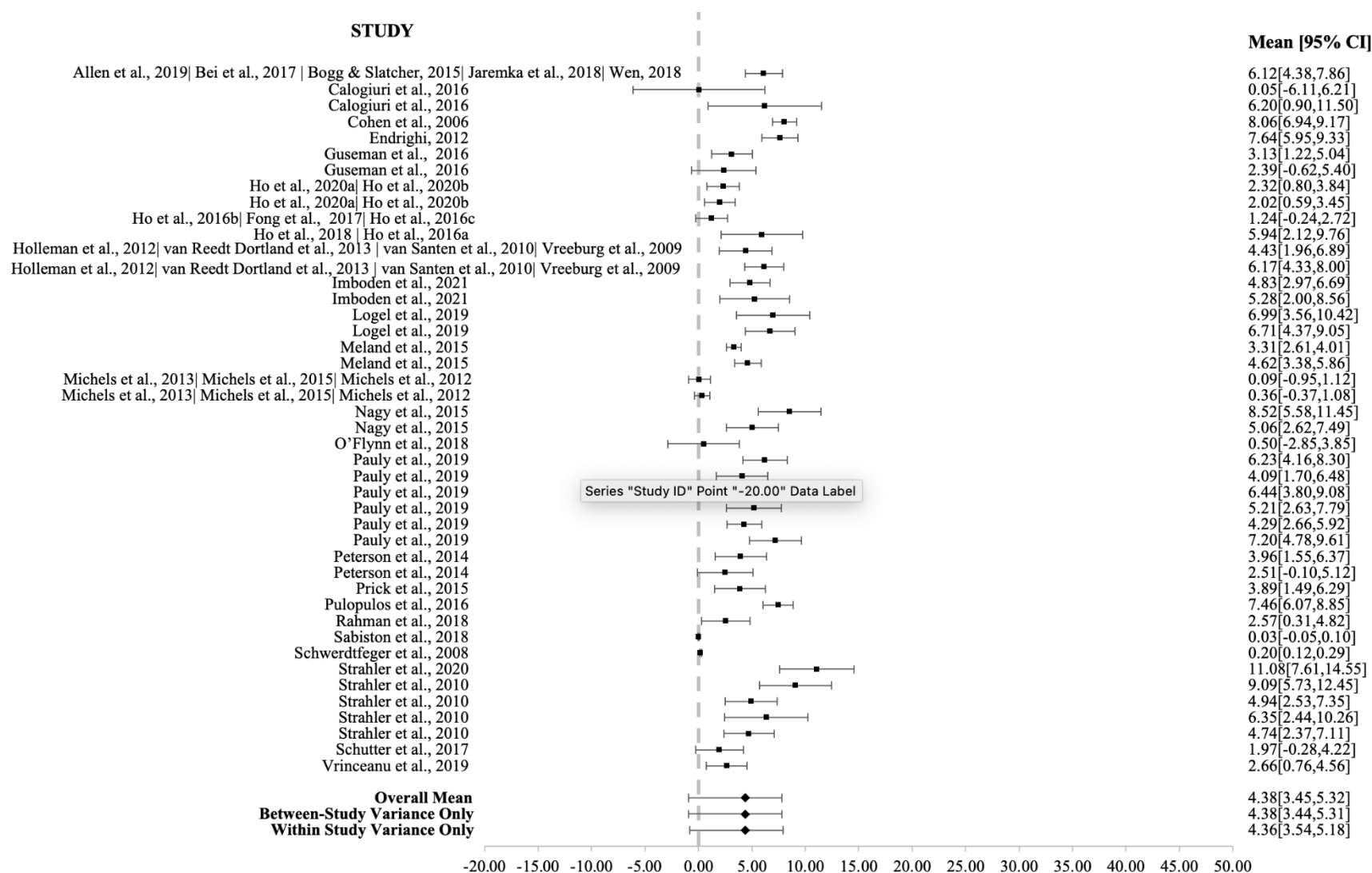
Figure 5*Forest plot of the mean cortisol awakening response in the moderate physical activity subgroup*

Figure 6

Forest plot of the mean cortisol awakening response in the high physical activity subgroup

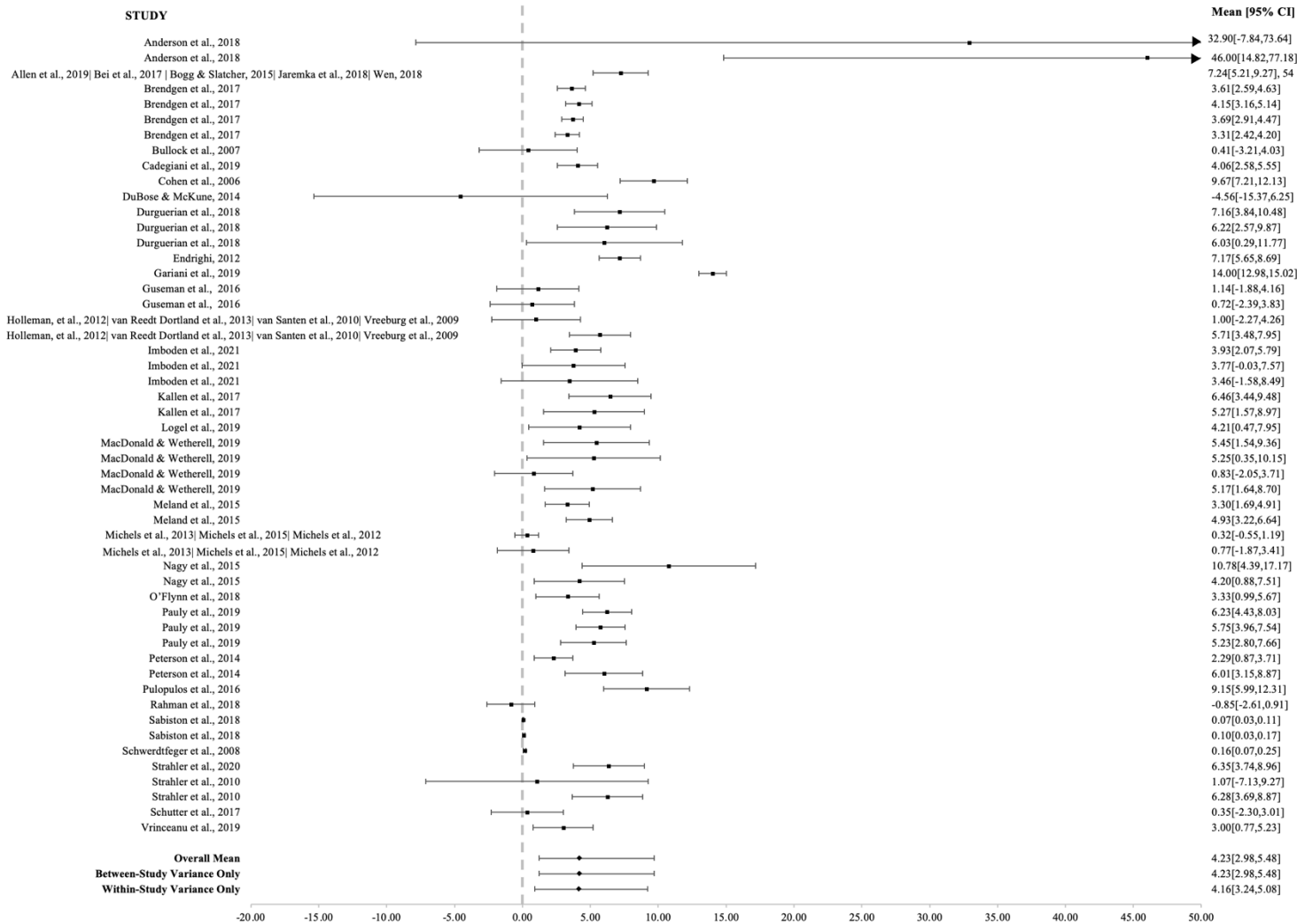


Table 2*Results of Multi-Level Meta-Analysis of the Association Between Physical Activity Participation and the Diurnal Cortisol Slope*

Model	r	95% CI		σ^2 within	σ^2 between	Q	df	AIC	Model comparisons			I^2	95% CI	
		LL	UL						χ^2	LRT	p		LL	UL
Overall	-.06**	-0.09	-0.02	0.01	0.01	298.84***	97	-62.34	34.17	—	—	70.75	60.72	82.35
Between-study variance (σ^2) only	-.07***	-0.10	-0.04	—	0.01	298.84***	97	-62.20	33.10	2.14	0.14	—	—	—
Within-study variance (σ^2) only	-.04*	-0.08	-0.004	0.01	—	298.84***	97	0.96	1.52	65.29	<.001	—	—	—

Note. Number of studies is 98, and total sample size 19,744 in all analyses. N = Number of participants; r = Average sample-weighted correlation; σ^2 between = Between-study variance; σ^2 within = Within-study variance; Q = Cochran's Q statistic; df = Degrees of freedom; AIC = Akaike's Information Criterion; χ^2 = Chi-square (log likelihood); LRT = Likelihood ratio test; p = Significance level of the LRT; I^2 = Index of the dispersion of effect sizes.

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 3

Results of Multi-Level Meta-Analysis of the Association Between Physical Activity Participation and the Cortisol Awakening Response

Moderator	k	N	M	95% CI		σ^2 within	σ^2 within 95% CI		σ^2 between	σ^2 between 95% CI		σ^2 absolute difference	Q	df	AIC	Model comparisons			I ²	95% CI	
				LL	UL		LL	UL		LL	UL					χ^2	LRT	p		LL	UL
Low Physical Activity Subgroup																					
Overall Between-study variance (σ^2) only	57	5313	4.76***	3.55	5.97	1.17	0.00	5.89	11.63	5.13	20.65	4.89 ^a	3382.48***	56	300.88	-147.44	–	–	99.11	98.61	99.41
Within-study variance (σ^2) only	57	5313	4.76***	3.54	5.98	–	–	–	12.87	8.08	21.72	2.34 ^b	3382.48***	56	300.45	-148.22	1.57	.21	–	–	–
	57	5313	4.62***	3.67	5.58	10.72	7.12	16.75	–	–	–	2.55 ^c	3382.48***	56	306.44	-151.22	7.56	.01	–	–	–
Moderate Physical Activity Subgroup																					
Overall Between-study variance (σ^2) only	44	2794	4.38***	3.45	5.32	0.32	0.00	3.40	0.56	0.00	1.84		1045.99***	43	205.95	-99.98	–	–	98.90	98.17	99.35
Within-study variance (σ^2) only	44	2794	4.38***	3.44	5.31	–	–	–	6.11	3.57	11.06		1045.99***	43	204.66	-100.33	0.71	.40	–	–	–
	44	2794	4.36***	3.54	5.18	5.83	3.59	9.89	–	–	–		1045.99***	43	211.03	-103.52	7.08	.01	–	–	–
High Physical Activity Subgroup																					
Overall Between-study variance	52	3416	4.23***	2.98	5.48	.000	0.00	0.05	10.61	0.00	19.38		1587.66***	51	257.90	-125.95	–	–	99.70	99.56	99.86
	52	3416	4.23***	2.98	5.48	–	–	–	10.61	6.27	19.38		1587.66***	51	255.90	-125.95	0.000	1.00	–	–	–

(σ^2) only Within- study variance (σ^2) only	52	3416	4.16***	3.24	5.08	8.38	5.38	13.63	–	–	–	1587.66***	51	281.27	-138.64	25.37	<.01	–	–	–
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Note. ^aAbsolute difference of the variance of the mean cortisol awakening response between low and moderate physical activity subgroups;

^bAbsolute difference of the variance of the mean cortisol awakening response between low and high physical activity subgroups; ^cAbsolute difference of the variance of the mean cortisol awakening response between moderate and high physical activity subgroups; k = Number of studies; N = Number of participants; M = Average sample-weighted mean cortisol awakening response; σ^2 between = Between-study variance; σ^2 within = Within-study variance; Q = Cochrane's Q statistic; df = Degrees of freedom; AIC = Akaike's Information Criterion; χ^2 = Chi-square (log likelihood); LRT = Likelihood ratio test; p = Significance level of the LRT; I^2 = Index of the dispersion of effect sizes.

* $p < .05$ ** $p < .01$ *** $p < .001$

analyses did not resolve the observed variability or lead to a narrowing of the confidence intervals about the effect size.

However, there were a few noteworthy exceptions. Difference tests in the effect sizes across moderator categories revealed that the physical activity-diurnal cortisol slope size was larger in mixed age groups relative to older age groups ($t(68) = -2.04, p < .05$); larger in studies that grouped participants based on physical activity level when compared to a validated physical activity measure ($t(32) = 3.57, p < .001$); larger in studies that experimentally assigned physical activity when compared to a validated physical activity measure ($t(53) = 2.38, p < .05$); and larger in studies that utilized a non-self-report physical activity measure when compared to a bespoke physical activity measure ($t(34) = 2.80, p < .01$). Importantly, the effect was non-zero in experimental studies, providing evidence that changing physical activity participation behavior produced changes in the diurnal cortisol slope. However, in each of the cases above, heterogeneity statistics remained substantive and confidence intervals about the mean differences were wide. Taken together, these findings suggest that although there was some evidence of moderation, the differences did not lead to homogenous cases or a narrowing of confidence intervals about the correlation in each moderator group. We found homogenous cases for the effect sizes in studies with mixed age groups, studies utilizing bespoke physical activity measures, and studies measuring the diurnal cortisol slope as a peak-to-bed or late decline slope, although effect sizes and comparisons with other moderator groups were no different from zero. We also found homogenous effect sizes for studies with mostly male participants, studies utilizing physical activity only as the intervention design, studies on vigorous-intensity physical activity only, and studies that took two samples of cortisol to determine the diurnal cortisol slope, with the correlation in studies using physical activity only as the intervention the only non-

zero effect size. It must be stressed, however, that the number of studies in each of these cases was small and are unlikely to have provided a precise estimate of effect size heterogeneity in each group.⁶

Results of the moderator analyses for the cortisol awakening response for each physical activity subgroup are reported in Table 5. We found large absolute differences in the variability about the mean cortisol awakening response between physical activity subgroups in some analyses. For example, the comparison between low and moderate, and between low and high, physical activity subgroups in studies with samples with low and high BMI. However, in all instances, the confidence intervals about the variance estimates of the effect size at each level of the moderator were wide and overlapped substantially, so differences across moderator groups were not supported. Numbers of effect sizes at each level of the moderator groups comparing averaged cortisol awakening response values across the physical activity subgroups numbered few (typically $k < 10$) in most cases precluding a meaningful comparison of the effect at levels of each moderator for many of the planned moderator variables: clinical status, intervention components, study design, physical activity type, cortisol awakening response measurement type, and our exploratory moderator variables: race/ethnicity and season of data collection. It should also be noted that moderator analyses for the mean cortisol awakening response significantly reduced the heterogeneity of the effect in some instances. For example, the effect was homogenous in the analysis of male participants in the low and moderate physical activity subgroups. This was also the case in some other moderator analyses, but the number of studies in the level of each of these moderators were small, and the confidence intervals largely overlapped across subgroups (see Appendix P, supplemental materials, for a complete summary of methods

⁶Meta-regressions were conducted to examine the effects of categorical and continuous moderators simultaneously and mirrored the above results (see Appendix O, supplemental materials).

used to compute effect sizes in moderator analyses for the physical activity-cortisol awakening response relation that correspond with table subscripts).

Analysis of Bias

Regression tests for the diurnal cortisol slope analysis revealed non-zero effects of the precision estimate on the effect size for the PET ($B_0 = 0.01$, 95% CI $[-.01, .04]$, $p = 0.27$) and PEESE ($B_1 = -.02$, 95% CI $[-.04, -.01]$, $p = 0.007$) tests. As the PET estimate for the

Table 4*Moderator Analyses of the Association Between Physical Activity Participation and the Diurnal Cortisol Slope*

Variable	<i>k</i>	<i>r</i>	95% CI		<i>Q</i>	σ^2 between	σ^2 within	^a Difference tests				
			LL	UL				MD	95% CI		<i>t</i>	<i>p</i>
									LL	UL		
Sample Age (years)												
Older (<i>M</i> >= 40, <i>SD</i> < 10)	41	-.10*	-0.18	-0.02	112.83***	0.01	0.01	-.09 ^b	-0.17	-0.01	-2.04	.04
Mixed (younger to older)	29	-.01	-0.04	0.02	40.06	0.00	0.00	-.07 ^c	-0.17	0.04	-1.27	.20
Younger (<i>M</i> < 40, <i>SD</i> < 10)	28	-.03	-0.10	0.04	121.28***	0.00	0.02	-.02 ^d	-0.05	0.09	0.54	.59
Sex												
Female (>= 75% female)	42	-.09*	-0.15	-0.02	107.69***	0.01	0.01	-.06 ^e	-0.13	0.02	-1.34	.18
Mixed (26%-74% female)	49	-.03	-0.08	0.01	180.02***	0.00	0.01	-.02 ^f	-0.17	0.13	-0.23	.82
Male (>= 75% male)	7	-.07	-0.23	0.10	5.91	0.00	0.00	.03 ^g	-0.10	0.17	0.39	.70
Sample BMI												
Underweight/Normal Weight (<i>M</i> BMI >= 25)	21	-.03	-0.10	0.04	101.09***	0.00	0.01	-.04 ^h	-0.14	0.06	-0.75	.45
Overweight/Obese (<i>M</i> BMI < 25)	37	.01	-0.07	0.09	92.19***	0.01	0.01					
Clinical Status												
General population	46	-.04*	-0.08	0.00	144.53***	0.00	0.01	-.02 ⁱ	-0.18	0.14	-0.24	.81
Physical Health Diagnosis	36	-.02	-0.18	0.14	107.05***	0.05	0.01	.08 ^j	-0.02	0.18	1.47	.14
Mental Health Diagnosis	13	-.12*	-0.22	-0.02	32.18**	0.01	0.00	.10 ^k	-0.08	0.28	1.56	.12
Study Design												
Cross-Sectional	38	-.02	-0.07	0.02	141.48***	0.00	0.01	.04 ^l	-0.02	0.10	1.32	.19
Longitudinal	29	-.06**	-0.10	-0.02	54.69**	0.00	0.01	.10 ^m	0.01	0.20	1.96	.05
Experimental	31	-.12*	-0.22	-0.03	80.16***	0.01	0.02	.06 ⁿ	-0.04	0.16	1.20	.23
Intervention Components												
Physical Activity Only	13	-.19**	-0.29	-0.09	15.58	0.00	0.01	-.13 ^o	-0.29	0.02	-1.55	.12
Physical Activity Plus Other Components	16	-.06	-0.19	0.07	44.90***	0.02	0.02					
Physical Activity Measure												
Validated Scale	26	-.02	-0.07	0.03	109.65***	0.00	0.09	-.02 ^p	-0.08	0.03	-0.76	.45
Bespoke Measure	19	.01	-0.02	0.03	22.86	0.00	0.00	.09 ^q	-0.04	0.21	1.29	.20
Non-Self-Report Measure	17	-.10	-0.22	0.02	44.43**	0.01	0.01	.21 ^r	0.11	0.30	3.57	.00

Longitudinal Grouped PA	7	-.23**	-0.33	-0.12	5.35	0.00	0.00	.11 ^s	0.02	0.20	2.38	.02
Experiment Assigned PA	29	-.11*	-0.20	-0.02	70.33	0.04	0.02	.12 ^t	0.04	0.20	2.80	.01
								.12 ^u	-0.02	0.26	1.51	.13
								.00 ^v	-0.14	0.15	0.05	.96
								.11 ^w	-0.01	0.23	1.64	.10
								-.01 ^x	-0.13	0.11	-0.15	.88
Physical Activity Intensity								-.12 ^y	-0.24	0.00	-1.70	.09
Low Intensity	14	-.04	-0.19	0.12	41.20***	0.02	0.01	.05 ^z	-0.11	0.21	0.61	.55
Moderate Intensity	16	-.09*	-0.18	-0.01	37.56*	0.01	0.00	-.04 ^{aa}	-0.19	0.11	-0.51	.61
Vigorous Intensity	6	-.01	-0.06	0.08	1.96	0.00	0.00	-.03 ^{ab}	-0.12	0.18	0.40	.69
Mixed Intensity	40	-.07*	-0.13	-0.02	151.87***	0.00	0.01	.06 ^{ac}	-0.23	0.35	0.36	.72
Moderate-Vigorous Intensity	11	-.10	-0.39	0.20	38.54***	0.11	0.00	-.10 ^{ad}	-0.19	0.00	-1.75	.08
								-.02 ^{ae}	-0.12	0.07	-0.41	.69
								.01 ^{af}	-0.27	0.28	0.04	.97
								.08 ^{ag}	0.00	0.15	1.67	.09
								.10 ^{ah}	-0.16	0.37	0.68	.50
Physical Activity Type								.03 ^{ai}	-0.24	0.29	0.18	.86
Aerobic PA	24	-.13	-0.31	0.05	52.44***	0.05	0.00	-.05 ^{aj}	-0.28	0.19	-0.36	.72
Anaerobic PA	18	-.08	-0.26	0.09	64.45***	0.02	0.02					
Physical Activity Frequency												
Less Than 3 Days Per Week	14	-.05	-0.22	0.11	30.27**	0.03	0.00	.11 ^{ak}	-0.05	0.28	1.25	.21
3 Or More Days Per Week	20	-.17***	-0.24	-0.10	39.33**	0.00	0.01					
Diurnal Cortisol Slope Type												
Wake-to-Bed Slope	60	-.07*	-0.12	-0.01	238.40***	0.01	0.01	-.05 ^{al}	-0.11	0.01	-1.66	.10
Peak-to-Bed or Late Decline Slope	18	-.01	-0.04	0.02	18.95	0.00	0.00	-.05 ^{am}	-0.28	0.18	-0.32	.75
Fixed Timepoint Slope	7	-.02	-0.30	0.26	14.62*	0.03	0.03	.01 ^{an}	-0.22	0.23	0.04	.97
Number of Cortisol Samples Per Day												
Two Samples Only	5	.00	-0.04	0.05	3.42	0.00	0.00	.07 ^{ao}	0.01	0.12	2.04	.04
More than Two Samples Per Day	93	-.06**	-0.10	-0.02	287.90***	0.01	0.01					
Number of Days Cortisol Sampled												
One Day Only	41	-.07*	-0.13	-0.01	168.47***	0.00	0.01	-.03 ^{ap}	-0.11	0.05	-0.76	.45
More than One Day	57	-.04	-0.09	0.02	130.30***	0.01	0.01					

Instructed Rest Before Cortisol Sampling												
No Instructions to Rest Given	61	-.042	-0.10	0.01	215.100***	0.01	0.01	.04 ^{aq}	-0.04	0.11	0.92	.36
Instructions To Rest Given	37	-.079**	-0.13	-0.02	80.471***	0.00	0.00					
Methodological Quality of Cortisol Sampling												
Questionable Quality (0-6)	71	-.057*	-0.11	-0.01	238.809***	0.00	0.01	-.40 ^{ar}	-0.09	0.06	-0.40	.69
Acceptable Quality (7-9)	27	-.042	-0.10	0.01	53.305**	0.01	0.00					

Note. ^aDifference tests comparing effect sizes across levels of moderator using Schenker and Gentlemen's (date) standard method; k =

Number of studies; r = Average sample-weighted correlation for the effect; 95% CI = 95% confidence intervals of the correlation; LL =

Lower limit of the 95% confidence interval; UL = Upper limit of the 95% confidence interval; Q = Cochrane's Q statistic; σ^2 between =

Between-study variance; σ^2 within = Within-study variance.

* $p < .05$ ** $p < .01$ *** $p < .001$

Table 5*Moderator Analyses of the Sample-Weighted Raw Mean Cortisol Awakening Response in Low, Moderate, and High Physical Activity**Subgroups*

Variable ^a	<i>k</i>	<i>M</i>	95% CI		<i>Q</i>	σ^2 between	σ^2 between 95% CI		σ^2 within	σ^2 within 95% CI		σ^2 absolute difference
			LL	UL			LL	UL		LL	UL	
Sample Age (years)												
Older (<i>M</i> ≥ 40, <i>SD</i> < 10)	16	4.83***	2.37	7.29	269.50***	0.00	0.00	5.02	15.97	7.41	41.64	6.29 ^b
	18	3.93***	2.36	5.51	307.57***	0.84	0.00	10.58	5.37	0.00	16.56	3.95 ^c
	10	5.20*	1.41	9.00	278.51***	0.00	0.00	8.96	20.20	6.94	75.99	—
Mixed (younger to older)	22	5.16***	3.30	7.02	2071.48***	15.16	2.42	31.97	0.00	0.00	>10.00	—
	14	4.17***	2.80	5.54	344.43***	0.94	0.00	8.55	2.70	0.00	10.16	—
	12	2.93*	0.83	5.04	144.56***	0.00	0.00	0.06	7.62	0.00	0.25	—
Younger (<i>M</i> < 40, <i>SD</i> < 10)	19	3.75***	1.90	5.61	137.06***	0.00	0.00	0.78	5.66	1.92	20.60	—
	12	5.31***	2.95	7.66	232.70***	0.00	0.00	3.08	9.97	3.79	32.70	—
	30	4.51***	3.10	5.92	156.31***	0.00	0.00	0.89	5.93	2.48	15.96	—
Sex												
Female (≥ 75% female)	16	4.93***	2.64	7.22	314.56***	14.65	1.90	37.53	0.64	0.00	27.72	9.54 ^d
	9	4.69**	2.22	7.15	198.10***	4.51	0.00	27.96	3.79	0.00	27.86	4.09 ^e
	9	3.14*	0.30	5.98	113.47***	0.00	0.00	0.06	7.78	2.78	40.79	—
Mixed (26%-74% female)	34	5.13***	3.36	6.90	1790.39***	0.00	0.00	0.56	15.16	8.28	30.88	—
	33	4.37***	3.27	5.47	688.74***	0.00	0.00	2.58	6.10	2.81	12.44	—
	28	4.39***	2.51	6.27	1214.50***	0.00	0.00	0.57	14.29	7.46	31.51	—
Male (≥ 75% male)	7	3.44***	2.43	4.44	4.22	0.00	0.00	1.96	0.00	0.00	2.48	—
	2	3.86	-4.36	12.07	3.26	0.59	0.00	>59.44	0.00	—	—	—
	15	4.47***	2.82	6.11	37.65**	1.28	0.00	10.12	2.78	0.00	18.22	—
Sample BMI												
Underweight/Normal Weight (<i>M</i> BMI ≤ 25)	14	6.06***	2.49	9.62	209.31***	0.00	0.00	>10.00	23.72	0.00	75.22	8.40 ^f
	15	5.09***	3.05	7.14	429.61***	0.00	0.00	>10.00	10.31	1.05	28.00	11.16 ^g

	20	4.78***	2.90	6.65	359.65***	0.00	0.00	4.75	8.34	2.74	25.02	—
Overweight/Obese (<i>M</i> BMI > 25)	20	4.44***	2.34	6.54	842.60***	4.59	0.58	25.35	10.12	0.00	31.60	—
	18	4.35***	2.82	5.87	368.70***	0.24	0.00	7.11	5.12	0.00	15.08	—
	14	3.94**	1.27	6.61	831.53***	2.97	0.00	35.10	14.34	0.00	44.55	—
Clinical Status												
General population	40	5.63***	4.12	7.13	1891.37***	1.04	0.00	6.02	12.00	4.89	24.25	—
	26	5.14***	3.75	6.52	671.29***	0.00	0.00	5.86	7.37	1.59	16.63	—
	37	4.94***	3.34	6.54	1301.88***	0.00	0.00	0.70	11.64	6.15	24.88	—
Physical Health Diagnosis	7	3.24	-1.21	7.69	60.96***	0.07	0.00	>10.00	15.54	0.00	105.37	—
	6	2.61***	-0.34	5.57	35.30***	0.00	0.00	>10.00	4.27	0.00	46.94	—
	6	1.30	-1.14	3.74	26.97***	0.00	0.00	0.06	2.24	NA	44.04	—
Mental Health Diagnosis	9	3.34***	1.85	4.82	16.34*	1.69	0.00	11.68	0.00	0.00	7.09	—
	11	3.92***	2.47	5.36	53.48***	0.73	0.00	9.69	2.65	0.00	11.18	—
	7	3.22*	0.32	6.12	29.87***	0.31	0.00	15.65	5.32	0.00	39.07	—
Study Design												
Cross-Sectional	38	5.13***	3.33	6.93	1395.12***	1.17	0.00	6.00	16.06	7.31	33.33	—
	24	4.57***	3.28	5.86	688.24***	0.17	0.00	3.81	6.12	2.21	13.84	—
	32	4.22***	2.60	5.83	1246.74***	0.00	0.00	1.64	12.18	6.56	25.10	—
Longitudinal	9	3.62*	0.57	6.67	1321.48***	0.00	0.00	>10.00	9.34	0.00	43.91	—
	11	5.72***	3.02	8.43	274.28***	0.92	0.00	15.30	8.40	0.00	40.14	—
	17	4.98***	2.74	7.23	238.19***	0.00	0.00	0.05	6.73	2.29	NA	—
Experimental	10	4.31***	2.32	6.30	54.61***	3.15	0.00	21.60	3.15	0.00	4.65	—
	9	2.37***	1.57	3.17	10.02	0.00	0.00	3.64	0.00	0.00	3.64	—
	3	1.99	-4.42	8.41	14.90**	2.84	0.00	113.90	2.84	0.00	113.90	—
Intervention Components												
Physical Activity Only	0	—	—	—	—	—	—	—	—	—	—	—
	5	1.90*	0.60	3.19	4.00	0.00	0.00	7.82	0.00	0.00	7.82	—
	3	1.99	-4.42	8.41	14.90**	2.84	0.00	113.90	2.84	0.00	113.90	—
Physical Activity Plus Other Components	7	3.74**	1.53	5.95	32.23***	2.19	0.00	20.96	2.19	0.00	20.96	—
	3	2.76*	0.27	5.25	2.99	0.00	0.00	>10.00	0.00	0.00	>10.00	—
	0	—	—	—	—	—	—	—	—	—	—	—
Physical Activity Scale												
Validated Scale	11	2.79*	0.11	5.46	408.55***	0.47	0.00	27.80	9.80	0.00	37.86	0.92 ^h
	14	4.56***	2.97	6.15	407.98***	0.00	0.00	>10.00	5.78	0.00	15.93	0.06 ⁱ
	12	3.70**	1.83	5.57	143.95***	6.67	0.00	19.90	0.00	0.00	>10.00	23.35 ^j
Bespoke Measure	24	5.52***	3.96	7.07	1015.97***	1.85	0.00	9.14	4.96	0.00	16.45	1.67 ^k
	12	5.50***	3.07	7.94	462.03***	1.49	0.00	25.44	9.91	0.00	34.83	1.50 ^l
	16	4.66***	2.68	6.65	473.76***	0.00	0.00	1.30	8.03	3.28	24.56	3.04 ^m

Non-Self-Report Measure	10	5.10*	0.41	9.80	1018.93***	0.00	0.00	>10.00	33.59	11.59	120.93	22.43 ⁿ
	7	3.47*	0.29	6.66	56.94***	0.00	0.00	5.61	6.22	0.07	57.32	1.61 ^o
	8	3.52	-2.49	9.52	796.82***	0.00	0.00	>10.00	35.05	11.29	182.55	2.42 ^p
Longitudinal Grouped PA or Experiment Assigned PA	12	4.67***	2.95	6.39	61.49***	2.93	0.00	17.92	2.93	0.00	17.92	2.97 ^q
	11	2.94***	1.92	3.97	17.11	0.41	0.00	6.02	0.41	0.00	6.02	24.85 ^r
	3	1.99	-4.42	8.41	14.90**	2.84	0.00	113.90	2.84	0.00	113.90	1.36 ^s
Physical Activity Type												
Aerobic PA	10	4.22*	0.01	8.42	505.37***	0.00	0.00	>10.00	25.37	0.00	90.77	—
	19	4.12***	2.90	5.33	324.18***	0.50	0.00	8.34	3.69	0.00	10.29	—
	25	3.81**	1.70	5.92	986.02***	0.00	0.00	0.07	14.81	12.00	36.59	—
Anaerobic PA	9	4.62**	2.29	6.94	50.29***	0.00	0.00	>10.00	6.08	0.00	27.03	—
	9	4.62**	2.29	6.94	50.29***	0.00	0.00	>10.00	6.08	0.00	27.03	—
	9	4.62**	2.29	6.94	50.29***	0.00	0.00	>10.00	6.08	0.00	27.03	—
Cortisol Awakening Response Measurement Type												
Fixed Awakening Time	0	—	—	—	—	—	—	—	—	—	—	—
	0	—	—	—	—	—	—	—	—	—	—	—
	6	4.58	-0.16	9.32	9.10	0.00	0.00	>10.00	8.23	0.00	27.03	—
Natural Awakening Time	57	4.76***	3.55	5.97	3382.48***	1.17	0.00	5.89	11.63	5.13	20.65	—
	44	4.38***	3.45	5.32	1045.99***	0.32	0.00	3.40	5.82	2.29	10.83	—
	46	4.20***	2.86	5.54	1531.70***	0.00	0.00	0.05	11.13	6.44	20.99	—
Number of Cortisol Samples Per Day												
Two Samples Only	39	5.43***	3.83	7.03	3184.20***	0.68	0.00	4.32	13.61	6.67	27.19	8.79 ^t
	28	4.48***	3.38	5.59	762.66***	0.77	0.00	6.66	4.17	0.00	10.26	3.04 ^u
	35	4.92***	3.05	6.79	1446.90***	0.00	0.00	0.05	13.49	6.79	31.50	—
More than Two Samples Per Day	18	3.34**	1.53	5.14	183.90***	9.22	0.00	23.28	0.00	0.00	>10.00	—
	16	4.27***	2.48	6.06	282.41***	0.00	0.00	6.47	8.58	2.47	22.48	—
	17	3.29***	1.70	4.88	138.04***	0.00	0.00	5.88	6.06	0.29	16.94	—
Number of Days Cortisol Sampled												
One Day Only	17	5.98***	3.08	8.90	1219.66***	0.00	0.00	3.31	20.42	9.08	57.83	15.35 ^v
	18	4.72***	3.23	6.21	452.18***	0.59	0.00	8.51	4.75	0.00	14.60	0.11 ^w
	14	4.60**	1.43	7.76	905.17***	0.00	0.00	4.46	20.26	8.43	62.48	—
More than One Day	40	4.16***	2.95	5.37	1918.51***	2.49	0.02	11.40	6.27	0.00	15.06	—
	26	4.19***	2.93	5.45	586.72***	0.23	0.00	5.72	6.58	1.29	14.46	—
	38	3.89***	2.68	5.10	663.15***	0.00	0.00	0.05	6.21	3.06	NA	—

Methodological Quality of Cortisol Sampling												
Questionable Quality (0-6)	25	3.11***	1.84	4.38	663.19***	0.05	0.00	7.93	5.52	0.00	12.83	6.84 ^x
	27	3.81***	2.60	5.03	601.98***	0.21	0.00	2.77	5.06	1.86	11.92	0.43 ^y
	29	3.10***	1.72	4.49	339.23***	0.00	0.00	0.07	6.20	2.81	15.44	—
Acceptable Quality (7-9)	32	6.13***	4.31	7.95	1708.09***	1.79	0.00	10.13	13.67	3.35	30.61	—
	17	5.13***	3.63	6.63	437.68**	6.37	0.00	17.05	0.73	0.00	15.42	—
	23	5.54***	3.46	7.63	1186.21***	0.00	0.00	0.98	13.41	6.38	33.90	—

Note. ^aValues reported on the upper, middle and lower lines for each moderator level are for the low, moderate, and high physical activity

subgroups, respectively; k = Number of studies; M = Average sample-weighted mean; 95% CI = 95% confidence intervals of the average sample-weighted mean; LL = Lower limit of the 95% confidence interval; UL = Upper limit of the 95% confidence interval; Q = Cochrane's Q statistic; σ^2 between = Between-study variance; σ^2 between 95% CI = 95% confidence intervals of the between-study variance; LL = Lower limit of the 95% confidence interval of the between-study variance; UL = Upper limit of the 95% confidence interval of the between-study variance; σ^2 within = Within-study variance; σ^2 within 95% CI = 95% confidence intervals of the within-study variance; LL = Lower limit of the 95% confidence interval of the within-study variance; UL = Upper limit of the 95% confidence interval of the within-study variance.

* $p < .05$ ** $p < .01$ *** $p < .001$

effect size was no different from zero, the PET estimate was taken as the corrected estimate for the physical activity participation-diurnal cortisol slope effect size. These findings provided some evidence of small study bias for this effect in the sample of studies. Although there was an observed difference in the corrected estimate from the PET analysis from the uncorrected estimate, the difference was modest, although the corrected estimate did not differ from zero. The tests revealed non-zero effects for the precision estimate on the cortisol awakening response mean for the PET and PEESE versions in all physical activity subgroups. These analyses suggested the presence of small study bias in each subgroup, however, observed differences in the corrected effect size estimates from the tests did not differ substantially from the original estimates in each subgroup and did not lead us to alter our overall conclusions on the size and variability of mean cortisol awakening response in each physical activity subgroup. ‘Funnel’ plots and full details of the bias analyses are presented in Appendix Q (supplemental materials).

Sensitivity Analyses

The leave-one-out analysis for the physical activity-diurnal cortisol slope analysis revealed two effect sizes that may have affected the overall effect size. Omitting each study from the analysis did not substantively change the overall estimate of the effect and its variability (see Appendix R, supplemental materials). The leave-one-out analysis for the mean cortisol awakening response in each physical activity subgroup identified a few influential studies in each physical activity subgroup, and in all cases, omission of those studies did not change the overall estimate of the mean effect. Full details of the analysis are presented in Appendix R.

Discussion

The aim of the present study was to estimate the average size and variability of the relation between physical activity participation and two independent indices of HPA axis regulation, the diurnal cortisol slope and the cortisol awakening response, across studies using multi-level meta-analysis. In line with our hypotheses, findings revealed a small, negative, non-zero averaged correlation between physical activity and the diurnal cortisol slope across studies, suggesting that the diurnal cortisol slope is steeper at higher levels of physical activity participation. However, contrary to our hypotheses, confidence intervals about the average mean variance estimates for the cortisol awakening response overlapped considerably across physical activity subgroups and did not provide definitive evidence supporting lower levels of variability in the mean cortisol awakening response at higher levels of physical activity participation.

Significant heterogeneity in the averaged correlation between physical activity and diurnal cortisol slope suggested the presence of moderators. Tests of moderators revealed some differences in the effect in some age, clinical status, and physical activity measure moderator subgroups, but no clear pattern emerged and significant heterogeneity in the effect sizes in each moderator group remained. Significant heterogeneity in the averaged mean estimate of the cortisol awakening response in each physical activity subgroup also suggested the presence of moderators. However, the small number of studies in each subgroup precluded a meaningful comparison for many planned moderators. Where there were sufficient studies at each level of the moderator across subgroups, we found large absolute differences in the variability about the mean cortisol awakening response between physical activity subgroups in the comparison between low and moderate, and between low and high, physical activity subgroups in studies with samples with low and high BMI; the comparison between low and moderate physical

activity subgroups, comparing validated and non-self-report physical activity measures; the comparison between low and moderate physical activity subgroups, comparing bespoke and non-self-report physical activity measures; the comparison between low and moderate physical activity subgroups comparing non-self-report and experimental assignment or longitudinal grouped physical activity measures; and the comparison between low and moderate physical activity subgroups comparing studies that sampled cortisol on one day only and studies that sampled cortisol on more than one day. Further, the effect was homogenous in the analysis of male participants in the low and moderate physical activity subgroups, experimental studies in the moderate physical activity subgroup, experimental studies that assigned physical activity as the only intervention component and experimental studies that assigned physical activity plus other intervention components in the moderate intensity subgroup, studies that measured physical activity as longitudinal grouped or experimentally assigned in the moderate intensity subgroup, and studies that based the cortisol awakening response measurement on a fixed awakening time in the high physical activity subgroup.

Physical Activity and the Diurnal Cortisol Slope

Current findings provide evidence of a small overall association between physical activity participation and the diurnal cortisol slope, indicative of better overall HPA axis regulation and cortisol secretion throughout the day among those participating in physical activity corroborating findings elsewhere (e.g., Gubelmann et al., 2018; Ho et al., 2020; Vreeburg et al., 2009). However, it must be stressed that applying bias-correction analyses yielded a corrected estimate that did not differ from zero, which indicates that the effect may be inflated due to selective reporting in studies. However, this finding should not be considered definitive given that the bias correction analyses are less precise in small samples and under

conditions of heterogeneity (Stanley, 2017). So, results from the bias analyses should be interpreted with this caveat in mind.

Analysis of Moderators

Although the candidate moderators of the physical activity-diurnal cortisol slope association tested in the current analysis did not confirm the presence of moderators of the effect, some of the correlations for specific moderator groups were non-zero and worth noting. Of particular note is the non-zero the averaged correlation observed in studies adopting experimental or intervention designs. This suggests studies using these designs are associated with changes in the diurnal cortisol slope such that it becomes steeper, an indication of HPA axis regulation. Furthermore, we also observed a non-zero correlation when comparing intervention studies that assigned physical activity as the sole intervention component. Taken together, these findings provide evidence that systematically and intentionally changing physical activity behavior leads to changes in a key index of effective stress coping. This finding is important because, unlike interventions, other physical activity measurement methods such as self-report questionnaires inflate levels of physical activity due to the phrasing of the measurement items, compromising their accuracy. If intentionally and systematically changing one's physical activity behavior produces a steeper diurnal cortisol slope, this may lead to an inverse of the expected effect, that is a flatter slope, if individuals who engage in regular physical activity were to intentionally limit their physical activity levels. Further, since follow-up periods of the included intervention studies were short, the greatest change in cortisol indices may be observed during the initial stages of physical activity intervention prescriptions but may later return to baseline levels. We therefore encourage researchers to adopt longitudinal designs in future studies, including multiple sampling periods over longer timeframes to monitor diurnal cortisol indices.

However, it should be noted that these findings are associated with considerable heterogeneity, indicating a highly variable effect, and we could not identify moderators that accounted for this variance. This points to a need for large-sample experimental or intervention studies that not only provide further robust evidence for the causal effect of physical activity change on the diurnal cortisol slope, but also systematically tests the effects of salient moderators. For example, researchers should consider testing whether effects of intervention or experimental manipulation of physical activity levels on cortisol regulation changes according to types of measurement methods (e.g., type of diurnal cortisol slope used, number of cortisol samples taken over the relative time period, number of days cortisol was measured, cortisol sampling quality, biochemical analysis procedure), type of physical activity assessment method (physical activity intensity, intensity assessment type, physical activity duration, level of physical fitness, fitness assessment type, time of day physical activity was performed, physical activity type, physical activity measurement, and physical activity frequency), and general study design (time lag and study design). Assessment of these moderators may assist in resolution of the heterogeneity observed in the effect size observed in studies adopting these designs.

Physical Activity and the Cortisol Awakening Response

Findings from the current analysis did not support the hypothesized relations between physical activity participation and adequate regulation of the cortisol awakening response. Although there were some observed differences in the variability estimates for the mean cortisol awakening response in the different physical activity subgroups, they were associated with substantial variability and our analysis did not enable us to verify any significant differences. For example, the moderate physical activity subgroup had the lowest absolute degree of variability in the mean cortisol awakening response relative to the low physical activity subgroup, which was

expected. However, the high physical activity subgroup exhibited higher absolute variability than the moderate physical activity subgroup. The findings from this review corroborate the proposed ‘inverted-U’ response as described by Anderson and Wideman (2017) – individuals participating in moderate physical activity have less variability about the mean cortisol awakening response while those participating in low or high levels of physical activity participation have higher variability about the mean (Anderson & Wideman, 2017). Although the high variability in the observed mean in each physical activity subgroup suggested the presence of moderators, none of the moderator tests yielded differences in the effects, some non-significant observed differences notwithstanding.

One explanation for the higher degree of variability about the mean cortisol awakening response in high versus moderate physical activity subgroups may be that the high physical activity subgroup contains highly trained athletes and individuals participating in particularly high levels of physical activity. Previous research has shown that athletes exhibit blunted cortisol awakening responses after periods of intense physical training and competition (Filaire et al., 2013). Therefore, those engaging in very high levels of physical activity participation likely have a higher percentage of intense training days, whether they are an athlete or not, and this may be why much higher variability was observed in the mean cortisol awakening response in this subgroup. Although we excluded studies that measured the cortisol awakening response within two days before or after competition, highly trained individuals may exhibit more variability on intense training days, possibly contributing to the high degree of heterogeneity and more variability about the mean cortisol awakening response in the high physical activity subgroup.

Importantly, the mean estimate for the cortisol awakening response at each level of physical activity participation was outside of the range of cortisol awakening response values

that are considered adequately regulated based on population norms (i.e., a rise in cortisol from awakening to peak secretion of 9.3 nmol/l \pm 3.1). Findings suggest that, on average, each subgroup displayed a somewhat blunted cortisol awakening response (Clow et al., 2004; Wüst et al., 2000), which has been associated with fatigue, burnout, and exhaustion (Chida & Steptoe, 2009). However, our analysis adopted normative values for healthy adults presented in previous research – but many studies have reported cortisol awakening response values that lie well outside of the ranges suggested in this literature (e.g., Calogiuri et al, 2016; Gubelmann et al., 2018). Therefore, as moderators of the cortisol awakening response are revealed, accounting for extraneous variables may explain more of the variance in the cortisol awakening response and as the literature proliferates, updating population norms for an adequately regulated cortisol awakening response for use in future research will be necessary.

Due to the variability in reported cortisol awakening response population norms, many researchers choose to examine the overall association between physical activity participation and the cortisol awakening response, however this negates the conceptual understanding that a mid-level cortisol awakening response is considered regulated whereas both a heightened and blunted cortisol awakening response have been associated with maladaptive psychological states (Chida & Steptoe, 2009). For example, previous findings have shown that higher levels of physical activity participation are associated with a higher cortisol awakening response values (Gubelmann et al., 2018), but without comparing values to a population norm, it adds little in the overall understanding of the meaning of this association for clinically relevant health purposes. It is, therefore, imperative to account for known moderating factors and re-establish population norms. Further, establishing population norms for samples that may not be classified as healthy

adults will also contribute to an understanding of the cortisol awakening response and associated health outcomes.

A number of factors may have contributed to the high degree of heterogeneity in both the physical activity-diurnal cortisol slope effect and the physical activity-cortisol awakening response effect in the present analysis that were not accounted for in moderator analyses. For example, various environmental and contextual factors have been shown to influence cortisol awakening response, such as the time of awakening, ambient light exposure, prior day experiences, anticipation of the day ahead, ovulation, jet lag, and alcohol consumption (Adam et al., 2006; Doane et al., 2010; Edwards et al., 2001; Stalder et al., 2009, 2010; Wolfram et al., 2011). We were unable to account for these factors in the current analysis because these data were not reported or unavailable in the pool of studies. These factors may have contributed to high heterogeneity in the mean estimates of the cortisol awakening response in each subgroup.

Furthermore, recent evidence has shown that sleep moderates the association between prior-day physical activity and the cortisol awakening response the next morning (Anderson et al., 2021). Sleep may, therefore, have been a further factor contributing to the heterogeneity in the effect. In addition, a wide range of immunoassay methods were utilized to determine cortisol values for the cortisol awakening response, and each has different sensitivities and cortisol estimates from different methods do not compare favorably, which is likely to have contributed to the observed variability in effects (Kirschbaum & Hellhammer, 1989; Miller et al., 2013). Taken together, environmental extraneous variables and the methods used to measure cortisol may have introduced additional error variance to the mean cortisol awakening response in each physical activity subgroup. Results should be interpreted with these potential confounding variables in mind. Future studies need to broaden knowledge of conditions likely to affect the

physical activity-cortisol regulation association by systematically examining the effect of these factors in adequately powered studies of the effects using strong experimental design and measures.

Key Contributions and Considerations for Future Research

This is the first meta-analysis to examine the association between physical activity participation and indices of diurnal cortisol regulation. A key contribution of the current research is that it provides initial empirical support of the link between physical activity participation and the diurnal cortisol slope across multiple studies, an association which is implicated in the mechanism by which physical activity assists in stress coping (Adam et al., 2017; Heim et al., 2008). Since physical activity is associated with a reduction of stress indices and regulation of the diurnal cortisol slope, it may mitigate risk of long-term chronic illness (Pedersen & Saltin, 2015; Warburton & Bredin, 2017; Warburton et al., 2006) and mental health problems (Biddle et al., 2019; Dunn, Trivedi, & O'Neal, 2001) that have been associated with chronic stress.

Clinical Implications

Although we found a non-zero effect size between physical activity participation and the diurnal cortisol slope, particularly for intervention and experimental studies, the effects were small in size. This has implications for whether changes in the diurnal cortisol slope are sufficient to produce clinically meaningful changes in health outcomes that have been associated with 'flatter' diurnal cortisol slopes. Future studies should test whether the effect of physical activity participation on the diurnal cortisol slope also translates to concomitant, clinically meaningful change in health outcomes that are associated with the diurnal cortisol slope. While meta-analytic evidence supports an association between the diurnal cortisol slope and health outcomes (Adam et al., 2017), evidence is needed to evaluate the mediating role of the diurnal

cortisol slope in the physical activity-health outcome association. Based on current evidence, we cannot unequivocally conclude that the small association between physical activity and the diurnal cortisol slope is of practical or clinical significance, but the initial evidence provided in this analysis justifies future investigations that aim to quantify what constitutes a clinically significant effect in this context and employ appropriate research designs with optimal sampling to evaluate whether effect sizes of this magnitude will lead to clinically significant effects in health.

By comparison, we found no clear evidence for an association between physical activity and cortisol awakening response in the current analysis. The high heterogeneity in the samples and the methods used in the included studies (e.g., participants with physical and mental health conditions, utilization of various immunoassay types with varying sensitivities in detecting cortisol concentrations) is likely to have impacted the cortisol awakening response data, as discussed elsewhere (Miller et al., 2013). These factors may have masked any differences as they are likely to have contributed substantially to the observed variability in the cortisol awakening response. Therefore, future studies would benefit from utilizing homogenous samples and standardizing cortisol awakening response measurement methods so that like-for-like comparisons may be made across studies.

Cortisol Measurement Considerations

Overall, the substantive heterogeneity observed in the relations between physical activity and indices of cortisol regulation highlights some imperatives for future research. Specifically, the analysis highlighted the need for more precise measures of the indices of cortisol regulation – only about a third of the studies in the current sample were classified as having high methodological quality in cortisol sampling. There is also a need for uniformity in the collection,

calculation, immunoassay type, analysis, and reporting of cortisol regulation indices to allow uniform comparisons in findings across studies. Following consensus guidelines for cortisol regulation measurement and reporting, and adherence to these standards in peer review should be strictly enforced to ensure greater precision in effects of physical activity on indices of the cortisol awakening response (Stadler et al., 2016).

Most important, given the high variability in the methods used to measure cortisol regulation across studies, there is also a need for systematic, large-sample tests of how key measurement components might affect estimates of the physical activity-cortisol regulation relations. For example, utilizing various immunoassay methods across studies limits effect size comparison across studies in a meta-analysis. Standardization of immunoassay methods across studies would allow for more precise comparisons, and there is a need to determine what may be the most precise immunoassay to use while also accounting for the assay cost and necessary skill level of the assayist across studies. The current study also highlighted the need to standardize the way in which HPA axis indices are calculated and operationalized. In the current sample of studies, researchers calculated the diurnal cortisol slope and reported data for the slope in different ways. Future research should systematically evaluate the effect of standardizing calculations and data reporting on the association between physical activity and the diurnal cortisol slope.

Physical Activity Measurement Considerations

Current findings also suggest there may be value in adopting better measurement and reporting of physical activity participation in studies testing the relations between physical activity and cortisol regulation. While we were able to code for a large number of candidate measurement-related moderators of the effect, in many cases the moderators lacked precision

due to insufficient information or data available to develop precise, fine-grained moderator groups, limiting the ability to detect moderation effects. For example, physical activity intensity was predicted to be a key moderator of the physical activity-diurnal cortisol slope correlation, but a large number of studies ($k = 40$) did not specify or measure physical activity intensity and precluded a meaningful moderator analysis of the effect of intensity on the correlation in these studies. Researchers should consider reporting physical activity participation using a standardized metric that includes an overall estimate of the intensity, frequency, and duration of physical activity participation over a specific amount of time, such as METs-min/week. While some studies did report physical activity in this metric in the current study, there was considerable variability, requiring estimates to be made based on close-as-possible conversions (e.g., using the Adult Compendium of Physical Activities to estimate the intensity of a certain type of physical activity that was mentioned in a study, such as cycling), or by requesting the data from authors. Standardized reporting would allow for more precision in comparing effects across studies.

Further, while this review defined physical activity to be as inclusive as possible and, therefore, utilized a broad definition of physical activity participation; as participation in exercise, sport, or physical activity as part of daily living, occupation, leisure, and active transportation (Garber et al., 2011), inevitably, it included studies in which individuals may have performed physical activity during leisure time, occupational work, and/or household chores. Previous literature demonstrates that leisure-time physical activity and occupational physical activity function differently to impact stress and health (Wolff et al., 2021; Holtermann et al. 2010; Holtermann et al., 2012; Holtermann et al., 2018); whereas leisure-time physical activity reduces the risk of many health conditions, but occupational physical activity exacerbates risk.

Therefore, we considered including physical activity context as a moderator or excluding occupational forms of physical activity, but many of the physical activity measures that were utilized in the included studies did not separate leisure-time from other forms of physical activity. For example, the International Physical Activity Questionnaire (IPAQ; Booth, 2000) prompts individuals to self-report their vigorous, moderate, and low intensity physical activity over the previous seven days, regardless of the context that it was performed in (e.g., leisure time, occupational, household and daily living, transport). In our meta-analysis of the diurnal cortisol slope, only three of the included studies utilized scales that specifically focus on leisure-time physical activity (e.g., the Godin-Shephard Leisure Time Physical Activity Questionnaire, GSLTPAQ; Godin, 2011). Also, we included non-self-report measures of physical activity participation (e.g., pedometers). Such non-self-report devices do not distinguish between activity done during leisure-time or as part of daily living, active transportation, or occupation. Limiting our inclusion criteria to only assess the effect of leisure-time physical activity would have substantially narrowed the pool of available studies for the meta-analysis. However, the inclusion of physical activity performed as a part of occupation, household chores, or active transportation may contribute to the substantial heterogeneity observed in the current study. Therefore, results should be interpreted with this in mind, and future research should aim to differentiate and report physical activity context and explore whether context moderates the relation.

Strengths and Limitations

The current study had numerous strengths: (a) Use of data from multiple studies and populations to provide the first cumulative synthesis estimates of the size and variability of the relations between physical activity participation and two key indices of cortisol regulation, the diurnal cortisol slope and the cortisol awakening response, using meta-analysis; (b) Use of multi-

level random-effects meta-analytic methods to correct effect sizes for variability attributable to within- and between-effect size variance components; and (c) Testing the effects of key moderator variables of the association. Overall, current findings provide estimates of association between physical activity participation and cortisol regulation indices based on the currently available evidence. This review is expected to provide researchers with an overview of the currently available evidence for physical activity as a key correlate of cortisol regulation and the extent to which extraneous variables may affect it, and, most importantly, identify the gaps in current evidence and signal where future research efforts should be directed.

However, a number of limitations should be highlighted. For the analysis of the correlation between physical activity and the diurnal cortisol slope, while we found no moderator effects in the current set of studies, this should not be taken as definitive evidence for the null effects of these moderators. A number of caveats to the current analysis and the available data should be considered when interpreting these findings. First, imprecision in moderator coding may have impacted the results. Many of the moderators were based on self-report (e.g., physical activity scales, BMI), which may have introduced error in classification of studies due to imprecision (e.g., affirmation bias and socially desirable responding). Bias due to self-report may have introduced error variance in the physical activity-diurnal cortisol slope association itself given that many studies used self-report measures of physical activity, which may have had the effect of inflating or attenuating effect. Second, moderator categories were produced to ensure that moderator analyses were feasible (e.g., sufficient numbers of studies within moderator groups). This may have resulted in some loss of fidelity in the moderator variables. For example, the BMI moderator variable was coded as high ($\geq 25 \text{ kg/m}^2$) or low ($<25 \text{ kg/m}^2$). As these classifications were made at the study level, some participants in the samples of these studies

may have been on the border of the cutoff values. These classifications may have resulted in a reduced ability to detect moderator effects. As the research in this domain expands, future analyses in which moderator groups with greater precision may be enabled and may provide more rigorous tests of moderator effects in future meta-analyses.

Another caveat is that while we coded to assess moderation of race/ethnicity, fitness level and fitness assessment type, intensity assessment type, physical activity time of day, and season of data collection, we were unable to analyze these variables with moderator analyses. Very few studies reported sufficient race data to conduct a moderator or sensitivity analysis. For example, most of the samples reported either did not report race at all, or samples were comprised of mostly white participants. Many studies have found differences in diurnal cortisol patterns between racial/ethnic groups (Adam et al., 2015; DeSantis et al., 2007). Future research should always account for the race/ethnicity of samples and report the demographics clearly. While research suggests that physical fitness is related to the diurnal cortisol slope (Lucertini et al., 2015), future studies should consider assessment of fitness, as well as current levels of physical activity participation in relation to the diurnal cortisol slope to enable future moderator analyses to be conducted. Future research in this domain should determine how the use of self-reports and non-self-reported fitness levels impacts this association. Further, given that absolute intensity assessments were extensively used in the current studies, but have been shown to be less precise than relative intensity assessments, this should be another moderator that is accounted for in future research. The time of day that physical activity was performed was only reported in one study (Küüsmaa et al., 2016). Future studies should include a measure of the time of day that participants engage in physical activity and consider examining whether the time of day that physical activity is performed moderates the physical activity-diurnal cortisol slope association.

Similarly, most studies did not report the time of year that their data were collected. In those that did, a large majority collected data over a span of a year or several years, so all seasons were included. While there is evidence that season of data collection impacts diurnal cortisol patterns (Miller et al., 2016), future studies should report which season(s) data were collected in to control for possible influences.

With respect to the cortisol awakening response, we divided effect sizes from the included studies into physical activity subgroups based on physical activity level in order to provide a fit-for-purpose test of the physical activity-cortisol awakening response relations. However, this classification limited the numbers of studies in each activity subgroup, reducing the scope of the moderator analyses due to small numbers of studies in moderator groups. Like the moderator analyses for the physical activity-diurnal cortisol slope association, there were also studies with participants on the boundaries of the cut-off for certain moderators (e.g., age) and participants who changed physical activity subgroup classification over the course of the study. Second, while physical activity subgroup classification was based on the IPAQ classification scheme, physical activity measurements were highly variable, and some studies did not provide sufficient information to classify the sample into the defined subgroups with high precision. Considering the above limitations, caution should be exercised in interpreting current results. Future studies examining the physical activity-cortisol awakening response relations should evaluate the cortisol awakening response using standardized measures that adequately capture the response (Stalder et al., 2016) and measure physical activity using measures with appropriate precision, and adequately report the contextual factors that likely moderate the effect.

Conclusion

We set out to determine the average size and degree of heterogeneity of the relations between physical activity participation and indices of cortisol regulation, the diurnal cortisol slope and the cortisol awakening response, across available studies using meta-analysis. Consistent with theory and research, findings revealed a non-zero negative correlation between physical activity and the diurnal cortisol slope across studies, supporting the hypothesis that the diurnal cortisol slope is correlated with physical activity participation. For the cortisol awakening response, we did not find strong support that lower levels of variability in the mean cortisol awakening response would be observed at higher levels of physical activity participation. Moderator analyses did not provide clear evidence of moderation and did not resolve the substantive observed heterogeneity in the effect for both indices. There were some noteworthy findings, foremost among them was the non-zero, negative effect of experimental or intervention manipulations of physical activity on the diurnal cortisol slope, providing initial evidence that physical activity interventions may have efficacy in promoting diurnal cortisol regulation.

Results also serve to highlight some important gaps in the current literature, such as the need for more precision in physical activity assessment (e.g., including the time of day, current fitness level of participants, intensity, duration, and frequency of participation); more precise cortisol data collection and analysis (e.g., based on consensus guidelines, Stalder et al., 2016); and reporting of sufficient data among all included variables including covariates and cortisol regulation parameters to compute effect sizes for synthesis. Findings also suggest the importance of more research examining the mediating effect of the diurnal cortisol slope between physical activity participation and the subsequent impacts on health outcomes. There is also a need for review studies to examine how other health behaviors relate to cortisol regulation. Studies with factorial designs are also advocated to determine how multiple health behaviors may relate to the

diurnal cortisol slope. Further, it is important to note that research in this field is receiving much attention and is evolving (e.g., Anderson, 2021; Ogasawara et al., 2022), and as research in this field expands, future syntheses may directly address some of the knowledge gaps highlighted here, particularly the analysis of moderators of the effects. This research is expected to provide valuable data on the relative contribution of health behaviors in cortisol regulation, which may be utilized to intervene to improve cortisol regulation, and, indirectly, health conditions associated with cortisol dysregulation.

Declaration of Interest

The authors have no conflicts of interest to disclose.

Data Availability

Study materials, data files, data analysis scripts, and analysis output presented in this manuscript are available online: <https://osf.io/ebpy2/>.

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