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A Longitudinal Study Assessing the Influence of Salivary Cortisol, Alpha Amylase, and Interleukin-1β Levels on Sleep and Physical Activity Among Emerging Adults

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A Longitudinal Study Assessing Bidirectional Relationships between Salivary Cortisol and Salivary Alpha Amylase and Physical Activity Among Emerging Adults

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Biography

The author was born in Illinois in May 1994. Bernardo graduated from James B. Conant High School, Hoffman Estates, Illinois in 2012. Bernardo received his Bachelor of Arts, summa cum laude, from DePaul University in Chicago, Illinois in 2016. He received his Master of Arts with distinction in Clinical-Child Psychology in 2021 from DePaul University and is currently pursuing a Ph.D. in Clinical-Child Psychology at DePaul University. He is completing his American Psychological Association accredited pre-doctoral clinical internship at the Childhood Trauma Treatment Program through Advocate Health Care.

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Abstract

Emerging adulthood can be marked by psychological disorders and distress. Stress exposure activates several bodily responses involving the hypothalamic pituitary axis (HPA) and autonomic nervous system (ANS). Research into these systems involves the examination of several biomarkers including cortisol and alpha amylase. Basal values of these biomarkers have been linked to well-being and health outcomes. Also, stress biomarkers have been shown to influence physical activity (PA) which is salient because it is linked to chronic illness and disease (e.g., obesity, cancer, depression, diabetes). The present study aimed to explore the bidirectional relationships between basal stress biomarkers and daily PA in a diverse sample of emerging adults (45.6% non-White). Multilevel models were estimated with biomarkers and PA counts (level 1) nested within individuals (level 2). All models were estimated using the Restricted Maximum Likelihood method with a random intercept only. There were no bidirectional relationships between same- or previous-day PA and alpha amylase or cortisol production. Daily hassles predicted average cortisol production and cortisol awakening response (CAR). Future research should focus on increasing observations and/or duration of data collection to better assess the relationship between daily PA and basal biomarkers. Also, researchers should assess intensity of physical activity on biomarkers over longer durations. Overall, emerging adulthood continues to be marked by high levels of uncertainty and stress; therefore, identifying and providing tools to manage stress within this earlier stage of life is still warranted for its potential to produce adaptable, and healthier, individuals and communities as these individuals age into adulthood and beyond.

Abbreviations

AA-AR – amylase awakening response
AIC – Akaike's Information Criterion
AICC – Hurvich and Tsai's Criterion
ANS – autonomic nervous system
APA – American Psychological Association
BIC – Schwarz's Bayesian Criterion
CAIC – Bozdogan's Criterion
CAR – cortisol awakening response
CHOICE – Challenging Health Outcome Inequities through Community Engagement
Contra – contraceptive use
HPA – hypothalamic-pituitary-adrenal axis
IBM – International Business Machines Corporation
M – mean
ML – Maximum Likelihood
PA – physical activity
PD – previous day
PNS – parasympathetic nervous system
REML – Restricted Maximum Likelihood
sAA – salivary alpha amylase
SAM – sympathetic-adrenal-medullary
SD – standard deviation
SE – standard error
SPSS – statistical package for the social sciences
VIF – variance inflation factor
-2LL – -2 Restricted Log Likelihood

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Introduction

Project Summary

Emerging adulthood, marked by ages 18 to 25, has been identified as one of the most unstable developmental periods due to the strain and stress from feeling "in-between" an adolescent and adult (Arnett, 1998; Arnett, 2000; Arnett, 2004; Arnett, 2007; Arnett, 2014; Arnett et al., 2014). This sentiment is supported by the American Psychological Association's yearly nationwide survey which has been measuring "stress in America" and its impact since 2007. Specifically, Gen Z adults (ages 18-23) reported significantly higher levels of stress compared to all other generations (e.g., millennials (ages 24-41), Gen X (ages 42-55), boomers (56-74), and older adults (75+); American Psychological Association (APA), 2020). More recently, this group of adults (ages 18 to 34) reported the highest rate of mental illnesses at 50% in 2023 (APA, 2023). The main sources of stress among 18- to 34-year-olds in the United States in 2023 were money and health related (both 82%; APA, 2023). Of the categories comprising the health-related concerns (family related, physical health, and mental health), mental health stressors were the highest for this age range at 72% (APA, 2023). Additionally, the largest increase in stress for these ages were due to the economy (52% in 2019 to 72% in 2023) and housing prices (52% in 2019 to 70% in 2023; APA, 2023). The stress experienced by emerging adults is significant because stress, when chronic, has been linked to short- and long-term adverse health outcomes and disease (e.g., obesity, cancer, depression, diabetes; Garfin et al., 2018; Liu et al., 2017; Matud et al., 2020; O'Connor et al., 2021; Yu et al., 2007). Stress

biomarkers have been shown to respond to physical activity and, when physical activity is engaged in consistently, it reduces biomarker reactivity to stress, which is salient for confronting stress-related chronic illness and disease (Fleshner, 2005). Thus, emerging adulthood may be an ideal population to study the relationships between stress biomarkers and physical activity. Much of the existing literature in this area focuses on older adults, children, or involves samples that include a broad age range. Additionally, previous research tended to focus on predominantly white samples, rely heavily on self-report measures for health behaviors, or use a cross-sectional design despite biomarkers being known to fluctuate temporally. Finally, few studies incorporate more than one stress biomarkers even though they are known to assess different physiological processes within the stress response and relate differently to physical activity. There currently exists a substantial body of literature investigating psychosocial stress and health behaviors in emerging adults (Arnett, 2014; Auerbach et al., 2018; Bonnie et al., 2015; Lane et al., 2017; Schiller et al., 2016; Stroud et al., 2015; Twenge et al., 2019) but far fewer studies using biological, objective measures. Thus, the purpose of the current study was to address gaps in the literature by exploring the longitudinal relationships between objective biomarkers of stress and objective physical activity in a diverse sample of emerging adults.

Stress and Stress Responses

Due the prevalence of stress in daily life, the term ‘stress’ has developed multiple connotations over time. Ursin and Eriksen (2004) developed the cognitive activation theory of stress (CATS) which provided a working definition of stress that identified four distinct facets: stress exposure (stressor, stimulus), experience and feelings of a situation (based on self-reports), psychoneuroendocrinological activation, and experience and feelings of the somatic response. Moreover, these facets can and should be measured separately to clearly define what is meant by

stress (Brown et al., 1991; Ursin & Eriksen, 2004). Additionally, it is important to note that stress is a nuanced construct that can be defined differently across disciplines depending on its conceptualization and measurement (Cohen et al., 2016). Cohen and colleagues (2016) identify three facets when studying stress: the epidemiological, psychological, and biological. The epidemiological lens focuses on which events should be defined as stressful to well-being, the psychological involves investigation into someone's perception of a stressor and their ability to cope, and the biological concerns itself with physiological processes that occur during stress as it relates to homeostasis and metabolic control. There can be overlap between how these separate stress definitions impact someone in daily life. For example, physical activity is a physical stressor that can biologically activate the body's stress response, but the individual engaging in exercise may not feel psychologically stressed during this time. Furthermore, not all situations perceived as stressful result in a biological, cortisol response (Dickerson & Kemeny, 2004). In this regard, stress is not inherently "bad" nor "good" and an immediate response to stress is shown to be a dynamic process that changes over time (Russell & Lightman, 2019). The body's stress response is an evolutionarily advantageous set of systems that prepare the body to conquer a stressful task: whether that be running a marathon or delivering a speech (Russell & Lightman, 2019). It is the activation of these systems repeatedly over long periods of time that result in negative health effects (Russell & Lightman, 2019; van der Kooij, 2020). Research methods have included self-report stress measures, behavioral observation, and physiological response, but few have used objective measures such as cortisol and alpha amylase (Crosswell & Lockwood, 2020). Regardless of how stress is conceptualized, the activation of the stress response, as well as resting levels of biomarkers of stress, have been linked to health outcomes (Ali & Nater, 2020; Hoyt et al., 2021; Russell & Lightman, 2019) and are therefore both salient when considering

ways to support emerging adults during this phase of life. Of note, understanding basal biomarker levels is important because past research has suggested they assess an individual's stress sensitivity in a different way from their stress-induced levels (Henckens et al., 2016). This finding is due to basal biomarker levels mostly being involved in the activation of receptors to reduce disturbances to stress (nuclear mineralocorticoid receptors) compared to stress-induced biomarkers which activate receptors that increase arousal (low-affinity mineralocorticoid receptors and glucocorticoid receptors).

Basal Stress Biomarkers and Mental Health Outcomes in Adults and Emerging Adults

Common biomarkers used to assess biological stress are cortisol and alpha amylase and are important for objectively measuring the body's stress response (Dhama et al., 2019). Resting or basal cortisol profiles via saliva sampling have been shown to relate to mental health risk and well-being over time among young adults ages 18 to 25 years (Hoyt et al., 2021). A systematic review and metaanalysis have also suggested that naturally steeper diurnal cortisol decline (diurnal slope) is associated with improved physiological functioning, while a flat diurnal slope is related to poorer health outcomes (Adam et al., 2017). Other parameters of cortisol, such as the cortisol awakening response (CAR), have been associated with chronic stress and the development of depression and anxiety symptoms, although the directionality of these relationships is mixed within the literature (Adam et al., 2014; Kudielka & Wüst, 2010; Stetler & Miller, 2005; Vrshek-Schallhorn, 2013). Finally, elevated cortisol secretion has been associated with those experiencing depression (Wai & Bond, 2004). Similarly, a review of basal and reactive alpha amylase levels indicate that this biomarker is related to health outcomes across samples of various ages (Ali & Nater, 2020), and that salivary alpha amylase secretion patterns (e.g., blunted levels at awakening, hyper- or hypo-secretions over a day, average daily alpha

amylase) have been associated with nervous system dysregulation and health outcomes (e.g., cardiovascular disease, tinnitus, cancer, depression, and anxiety) in emerging adult and adult samples (Alsalman et al., 2016; Ikeda et al., 2021; Limm et al., 2011; Lipschitz et al., 2013). Understanding basal levels of stress biomarkers are equally important compared to observing stress-induced levels of cortisol and alpha amylase. The current study provided more understanding into basal cortisol and alpha amylase within emerging adults.

Stress Biomarkers and Physical Activity in Emerging Adulthood: A rationale

It is important to understand behaviors that may support regulation of these biomarkers within emerging adults due to stress' prevalence during this period of life. Presently, research has shown that certain health behaviors such as physical activity can mitigate stress' impact on the body (O'Connor et al., 2021). Conversely, increased levels of stress can lead to less engagement in physical activity (Brockmann & Ross, 2020). Understanding the interplay between biological stress measures and physical activity during emerging adulthood is worthwhile given the notable stress and health statistics for this age group (APA, 2023; APA, 2020; Twenge et al., 2019). Currently, our understanding of these biological markers of stress and their relationship to physical activity within emerging adults is not fully understood.

Health behaviors, such as physical activity, are a reasonable area of focus due to the theory of emerging adulthood and research demonstrating that it is a critical time for individuals to adopt or abandon many important health behaviors (Daw et al., 2017; Kwan et al., 2012; Nelson et al., 2008). Specifically, a longitudinal study involving over 600 Canadian adolescents (12 to 15 years old) found that involvement in physical activity decreased significantly throughout emerging adulthood (until 24 to 27 years old) (Kwan et al., 2012). A more recent longitudinal study found that involvement in common health behaviors and health outcomes

(e.g., cigarette smoking, binge drinking, obesity, sedentary behavior) were malleable as adolescents transitioned into emerging adulthood (Daw et al., 2017). These variable findings, as well as the heterogeneity within the emerging adult experience (Arnett, 2004), highlight the need to continue researching health behaviors over time among emerging adults. Further research among this population is especially warranted considering health habits acquired during this period of life have been known to shape future health behaviors and influence rates of disease and mortality later in life (Daw et al., 2017; Hawkins et al., 1992; Irwin, 2010).

Researching physical activity of emerging adults is important because emerging adulthood may be among the most self-efficacious, and realistic, times for a person to engage in behavior change (Nelson et al., 2008). Compared to other life stages, emerging adults are often still experiencing increasing levels of autonomy, undergoing identity development, and not fully burdened by the responsibilities of adulthood (Arnett, 1998; Arnett, 2000; Arnett, 2004; Arnett, 2007; Nelson et al., 2007). These characteristics are advantageous when attempting long-term behavior change, especially when considering the role of self-identity (Nelson et al., 2008). Specifically, identity and behavior reciprocally reinforce each other (e.g., someone's identity of being an 'active' person would be influenced by their outward behaviors (i.e., running, biking) and ultimately feed back into their self-image). Therefore, the identity/behavior relationship is important for creating deep-rooted health behavior change and patterns (Miller et al., 2002). The great potential for behavior changes during emerging adulthood requires knowledge of internal and external factors that influence behavior. The following sections will focus on the biology of the stress biomarkers and their relationship to the health behavior of interest: physical activity.

Cortisol and Physical Activity

Cortisol is a steroid hormone (glucocorticoid) that is involved throughout several bodily systems from supporting synthesization of cells into new compounds to reducing inflammation (Levine et al., 2007). Specifically, cortisol is a byproduct of the hypothalamic-pituitary-adrenal (HPA) axis. When the HPA axis is stimulated, the hypothalamus produces corticotrophin-releasing hormone which then prompts the pituitary gland to secret adrenocorticotrophic hormone. This adrenocorticotrophic hormone triggers the cortex of the adrenal gland to secrete cortisol. Among humans, the secretion of cortisol occurs diurnally with levels peaking in the morning around waking, known as the cortisol awakening response (CAR), and lowering throughout the day. Cortisol is widely known for its role in the body's response to both physical and psychological stress. It should be noted that cortisol is just one mediator in a network of several that respond during allostasis (McEwen, 2019). During the stress response, cortisol's presence in the body will increase to support the body in regaining homeostasis. In this regard, cortisol is vital for a body to maintain a healthy immune system and adapt to stressors. Problems arise when the stress response is engaged too often or becomes unstable resulting in too much or too little cortisol production. When this disruption occurs, it is referred to as allostatic overload. This phenomenon causes other systems in the body (e.g., metabolism, sleep patterns, mood) to become affected detrimentally and can lead to disease, such as obesity, diabetes, and psychopathology. Cortisol has often been used to assess HPA axis activity in research and is a preferred biomarker due to it being reliably measured via saliva sampling with little burden on participants and researchers (Hellhammer et al., 2009).

Cortisol response to physical activity seems to have an intensity threshold in which vigorous and high intensity exercise, but not lower intensity activity, results in increased cortisol levels (Anderson & Wildeman, 2017; Duclos & Tabarin, 2016). This pattern is expected given

that the body's stress response is meant to support regaining homeostasis and is therefore mainly activated during a notable stressor such as vigorous activity compared to lower levels of activity. In fact, the positive effect of regular exercise is supported by Duclos and Tabarin (2016) who note the body adapts to repeated and prolonged cortisol secretion from exercise such that it becomes less sensitive to the negative effects of glucocorticoids (i.e., cortisol). These "acute elevations" of cortisol support the fight-or-flight response and are also beneficial to surviving and coping with day-to-day challenges (Russell & Lightman, 2019). Cross-sectionally, research among young adults (M_{age} =21.2 years) found vigorous physical activity to be significantly related to increased cortisol levels via hair sample after controlling for age, sex, and perceived stress (Gerber et al., 2013). Similarly, a study including emerging adults (ages 18 to 35 years) demonstrated that vigorous, but not moderate, physical activity resulted in increased salivary cortisol concentrations to the same extent as acute social stress (Ponce et al., 2019). The physical activity occurred within a laboratory-controlled setting and cortisol was measured at 0, 15, and 35 minutes after the end of the experimental condition. This pattern of increased cortisol levels immediately following high intensity activity was also observed in an earlier study involving healthy males ages 18 to 30 years (Hill et al., 2008). Hill and colleagues (2008) also noted that low intensity exercise reduced circulating cortisol levels. Physical activity also influences future cortisol activity. Previous day physical activity has been shown to partially predict next day CAR among young adults (M_{age} = 19.1 years, SD = 1.89; Anderson et al., 2021). Specifically, high activity levels and short sleep duration produced an interaction effect and resulted in a larger CAR the following morning. Overall, activity's relationship to cortisol appears intensity dependent, with high intensity physical activity increasing cortisol levels within emerging and young adult samples. Moreover, this relationship tends to be studied across short amounts of

time in which cortisol is measured closely following activity completion (i.e., 30 minutes post exercise, 1 hour post exercise). Given that the stress response is dynamic and changes over time (Russell & Lightman, 2019), more research is needed to understand activity throughout the day and its relation to cortisol production within the same day and in subsequent days.

Alpha Amylase and Physical Activity

As explained above, the HPA axis is a prominent system within the body's stress response. The HPA axis operates in tandem with the autonomic nervous system (ANS) to make up the sympathetic-adrenal-medullary (SAM) and parasympathetic nervous system (PNS). These networks work together to provide an immune response to stress. However, different enzymes and hormones have emerged as trusted biomarkers for these various systems (Ali & Nater, 2020). Specifically, salivary alpha amylase (sAA) has been shown to be a reliable and valid indicator of ANS functioning within stress research (Ali & Nater, 2020; Nater & Rohleder, 2009). Alpha Amylase is a salivary enzyme active in carbohydrate and starch digestion (Sun et al., 2019). Additionally, sAA is a useful biomarker in research due to being easier to measure compared to other ANS markers such as norepinephrine which require blood sampling.

Similar to cortisol, sAA operates diurnally with levels sharply dropping within the initial 30 minutes of waking and subsequently rising throughout the day. This pattern has been researched as the amylase awakening response (AAR). Within a dysregulated ANS response, the AAR produces a smaller, "blunted" decline following the first 30 minutes after waking with higher sAA production over the course of the day. Ali and Nater (2020) highlight that sAA is a valuable biomarker of the stress response because it has been shown to successfully identify individuals with anxiety disorders from other groups including healthy controls. Notably, cortisol showed no statistical difference within these samples while sAA did, suggesting sAA provides a

more comprehensive view of the body's stress system compared to cortisol alone. The need for both biomarkers has been shown in stress research in which alpha amylase patterns differed from cortisol among children (*M*age = 11.29 years, *SDage* = 0.67) after experiencing acute stress (Wunsch et al., 2019). For cortisol, there was an increase, peak, and gradual decrease following the stressful experience; however, alpha amylase maintained levels over time after stress was induced in the sample. Despite this difference, both biomarkers displayed significant interindividual variance with some children experiencing high levels of biomarker activity and some experiencing little, non-significant, changes over time. Another study observed that interindividual changes along with mean cortisol did not predict levels of alpha amylase (Nater et al., 2007). Of note, a review of the literature indicates that the ANS (often assessed by sAA) and HPA axis (often assessed by cortisol) are differentially affected depending on the clinical context of stress within many samples; however, few studies within behavioral medicine measured sAA (Ali & Nater, 2020).

Physical activity has been shown to generally increase alpha amylase production in the short term (i.e., over a few hours) although findings are mixed. Previous research has shown that intermittent bouts of exercise can increase alpha amylase levels by five times and require two hours and thirty minutes to return to pre-activity levels (Walsh, 1999). A systematic review of salivary alpha amylase and exercise also showed that many of the studies reported increases in alpha amylase following exercise (Koibuchi & Suzuki, 2014). Specifically, increases were seen across various male samples (i.e., healthy, endurance-trained, elite wheelchair athletes, cyclists, taekwondo athletes, swimmers) and various activities (i.e., walking, biking, running, tournament competitions). However, a handful of studies from the review reported no change in alpha amylase production. One study of college students observed no change following twenty-minute

walks (Yamaguchi et al., 2006). An older literature review also reported rises in alpha amylase following physical (e.g., exercise, heat/cold stress) and psychological stress across ages and sex (Granger et al., 2007). Among a sample ages 18 to 58 years (*M*age = 26.7 years, *SDage* = 8.8), there was no effect of physical activity on alpha amylase production (Nater et al., 2007); however, physical activity was measured via self-report. This discrepancy compared to most other research may be due to the need for objective measures of activity. Specifically, objective measures allow for more precise evaluation of various intensities of activity and their effect on alpha amylase. Overall, alpha amylase production tends to increase shortly after physical activity with no studies observing activity and alpha amylase throughout the day over longer periods of time (i.e., over several days). Given that alpha amylase levels change diurnally, tracking levels over time is needed to understand its relationship to typical daily levels of activity.

Considerations when Measuring Stress Biomarkers and Physical Activity

Accounting for the amount of daily stress participants experience is important when measuring biological stress levels in order to understand the relationship, and unique variance accounted for, between physical activity and stress biomarkers unrelated to (perceived) stress itself. Daily hassles, as defined as daily living conditions that an individual has identified as harmful to their well-being (Lazarus, 1986), is a common metric used to approximate how much stress an individual is experiencing in their life (Wright et al., 2019). These conditions or events are often irritating, cause distress, or frustrate the individual as they navigate tasks and interpersonal relationships of daily living (Wright et al., 2019). Past research has also evaluated daily hassles among emerging adult populations to understand health outcomes (Bottos & Dewey, 2004) and health complaints (Tran et al., 2021).

Beyond accounting for perceived stress, certain grouping variables have also shown to be important when evaluating the relationship between stress biomarkers and physical activity. Specifically, relationships between race, sex, chronic illness status, and contraceptive use have been observed regarding levels of salivary biomarkers and engagement in physical activity. Regarding physical activity, previous literature noted differences in physical activity by race (Bantham et al., 2021; Elgaddal et al., 2022; Powell et al., 2004) with individuals of color engaging in less activity than their White counterparts, by sex with males engaging in more physical activity compared to females (Elgaddal et al., 2022), and by chronic illness status with chronic illness status negatively impacting physical functioning (Oris et al., 2018). Regarding salivary biomarker values, previous literature noted oral contraceptive use influencing the intensity of the values of salivary cortisol and alpha amylase (Høgsted et al., 2021; Lucas et al., 2019; Nielsen et al., 2013).

Purpose

Currently, studies that measure biomarkers and health behaviors typically do so over a short amount of time, such as a 15 to 30 minutes, an hour, or next day; however, the findings from the current study provide insight into how cortisol and alpha amylase production throughout the day relates to daily, typical physical activity as they occur among emerging adults. Specifically, the current study identified if these relationships remain consistent or are malleable over multiple days. In this regard, the data contributed to the literature by identifying possible trends between biomarkers and physical activity as they occur throughout an entire day for three consecutive days and has the ability to predict next day biomarker level based on previous day physical activity. Due to the dearth of research involving these variables among

emerging adults, the current study looked at these relationships bidirectionally to understand if basal biomarker levels influence the amount of physical activity someone engages in.

Presently, little research exists regarding the relationship between more than one biomarkers of stress (cortisol, alpha amylase) and physical activity as they naturally occur among emerging adults. The current literature on the biomarkers of stress focuses on cortisol above alpha amylase; however, cortisol provides a limited view into the physiological stress response. Specifically, alpha amylase can respond differently to stressors than cortisol and, therefore, can augment our understanding of the body's immune and stress response by including them in research. Involving both biomarkers has been recommended to assess HPA axis, ANS, and immune response (Nater et al., 2013a). For some of the previous research focusing on one biomarker and health behavior, the study designs were cross-sectional and lacked the possibility to assess predictive relationships between variables. Additionally, most of the available research on these topics involve predominantly white samples. This is an important distinction given that health behaviors (Hughes et al., 2019; St-Pierre et al., 2019) and exposure to stressors and stress impact differs by race and ethnicity (Brown et al., 2020; Williams, 2018). Specifically, people of color often experience more stressors compared to their white counterparts and engage in less physical activity due to lack of access to resources (i.e., parks and open spaces) because of systemic barriers (Braveman et al., 2011; Braveman & Gottlieb, 2014). Also, little research exists specifically looking into the well-defined age range that comprises emerging adults. Beyond demographic differences, several reviewed studies relied heavily on self-report measures for physical activity and highlighted the need for more objective measurement of this health behavior.

The current study addresses these gaps by 1) adding to the existing body of literature the relationship between cortisol, alpha amylase, and physical activity among emerging adults, 2) understanding these variables within a diverse sample of emerging adults, 3) solely utilizing objective measures for all variables of interest, and 4) utilizing a longitudinal design with the ability to assess predictive relationships between biomarkers and health behaviors.

The current study investigated the relationships between salivary cortisol and salivary alpha amylase concentration over three consecutive days with objective measures of physical activity and aimed to:

1: Examine the longitudinal relationship between physical activity on cortisol and alpha amylase indices over a three-day period (full model details in analyses section).

Hypothesis 1a – Same- and previous-day physical activity would be predictive of same- and next-day cortisol levels.

Hypothesis 1a1 - There would be a significant positive association between total physical activity and average total cortisol.

Hypothesis 1a2 - There would be a significant inverse association between total physical activity and cortisol diurnal slope (i.e., more activity resulting in steeper slope).

Hypothesis 1a3 - There would be a significant inverse association between total physical activity and cortisol awakening response (i.e., more total physical activity resulting in smaller awakening response).

Hypothesis 1b – Same- and previous-day physical activity would be predictive of same- and next-day alpha amylase levels.

Hypothesis 1b1 - There would be a significant positive association between total physical activity and total alpha amylase.

Hypothesis 1b2 - There would be a significant inverse association between total physical activity and alpha amylase awakening response (i.e., more total physical activity resulting in smaller awakening response).

2: Examine the longitudinal relationship between cortisol and alpha amylase indices on physical activity over a three-day period (full model details in analyses section).

Hypothesis 2a – Same- and previous-day cortisol levels would be predictive of same- and next-day total physical activity.

Hypothesis 2a1 - There would be a significant positive association between average total cortisol and total physical activity.

Hypothesis 2a2 - There would be a significant inverse association between cortisol diurnal slope and total physical activity (i.e., steeper slope resulting in more next day total activity).

Hypothesis 2a3 - There would be a significant inverse association between cortisol awakening response and total physical activity (i.e., smaller awakening response resulting in more total physical activity).

Hypothesis 2b – Same- and previous-day alpha amylase levels would be predictive of same- and next-day total physical activity.

Hypothesis 2b1 - There would be a significant positive association between average total alpha amylase and total physical activity.

Hypothesis 2b2 - There would be a significant inverse association between alpha amylase awakening response and total physical activity (i.e., smaller awakening response resulting in more total physical activity).

3: Determine the strongest biomarker predictor of physical activity outcomes.

Hypothesis 3a – Same-day average total cortisol would be the strongest predictor of same-day physical activity outcomes compared to same-day alpha amylase.

These findings helped inform our understanding of the relationship between stress biomarkers and common health behaviors among an underrepresented sample of emerging adults. Further, this study addressed an important gap in the literature given that the cumulative influence of these biomarkers on physical activity has yet to be fully examined over multiple days. Finally, significant results have potential for highlighting the value of engaging in regular activity to support stress biomarker functioning and offset the impact of stress during emerging adulthood.

Method

Participants

The current sample is derived from a larger study involving 265 emerging adults from a large Midwestern university. Participants were ages of 18-24 years at the time of consent, currently enrolled at the university, proficient in English, and having a cell phone that could receive text-messages to complete daily assessments. Outside of not meeting inclusion criteria, participants were excluded if they participated in a varsity sport.

Eighty-eight participants completed saliva data collection; however, six were removed from analyses due to submitting inadequate data or no data. Another 14 participants were removed due to not having submitted activity data that corresponded to days in which saliva sampling occurred. Therefore, the valid number of participants included in analyses was 68. This sample is notably smaller than the original 265 participants due to salivary data collection being added later, after the study had begun. Therefore, not every participant from the original 265 person sample had the opportunity to provide salivary data. Participants were aged 18 to 24 with a sample average age of M=19.7, SD=1.6. Based on self-reported sex, 58.8% of participants were female (41.2% male). The sample is more diverse than the university's population as a whole, with 4.4% identifying as African-American, 20.6% as Asian/Pacific Islander, 29.4% as Hispanic, 54.4% as White, and 17.6% as Other.

Procedure

Two strategies were utilized for recruitment: flyers were posted around campus and research assistants attended classes and student organizations to disseminate study information. The study design was longitudinal and involved an initial in-person baseline assessment, two weeks of daily assessment tracking, and a final in-person follow-up assessment at the end of the two-week period. Saliva samples were collected four times daily across three days of the two-week period. During baseline, participants were consented, provided their mobile number to receive text reminders, completed self-report surveys, and measured to obtain their anthropometric data. Text reminders were sent daily as a way to prompt participants to complete their questionnaires across the two-week study period. Participants were compensated $15 for baseline participation. During daily assessment, participants completed questionnaires regarding various health behaviors and compensated $5 each day surveys were completed. They earned an

additional $70 if they had completed surveys for all 14 days of the two-week period. Additionally, participants were asked to wear ActiGraph devices around their non-dominant wrist during the two-week daily assessment period. ActiGraphy was used to objectively monitor participant daily activity and sleep patterns. During follow-up assessment, participants completed the same batteries from baseline and were compensated $20.

Saliva Samples

Participants were asked to provide saliva samples via spittle into a tube. To facilitate correct saliva sampling, participants were given written and verbal instructions on how to use their saliva collection kit. Saliva collection kits contained the following: insulated lunch bag, ice pack, straws, and 12 2mL salivettes. Written directions were also accompanied by photos so participants could view each step prior to reading. Prior to providing a saliva sample, participants were instructed to refrain from brushing their teeth, eating a large meal, and smoking and drinking for thirty minutes to an hour. A study-developed schedule was created and distributed to participants, so they knew which days to collect saliva samples.

Participants were asked to provide a total of 12 saliva samples: four daily samples for three consecutive days. The three days were weekdays that occurred within the two weeks of data collection. The three days were chosen by participants which provided them flexibility and prevented attrition. Four saliva samples across three days are in accordance with recommendations for repeated measurements of biomarkers to support accurate findings (Nater et al., 2013b; Stalder et al., 2016). The four daily samples occurred upon waking, +30 minutes after waking, mid-day, and evening for participants. In addition, participants tracked the time of day they provided their four daily samples using a study-developed log. On the log, participants

also answered questions such as whether they ate, drank, or brushed their teeth closely in time to when they provided their sample.

Following saliva collection, participants were asked to bring their samples to their follow-up assessment session. Research assistants kept collected saliva samples frozen by storing them in a freezer set to -20°C. Saliva samples were sent to the Salimetrics SalivaLab (Carlsbad, CA) and analyzed using the Salmetrics Salivary Alpha-Amylase Assay Kit (Cat. No. 1-1902). To evaluate the participant's adherence to the saliva protocol, they self-reported how closely they followed the procedures on a scale from 0-10 (10 indicating they followed the saliva protocol 100% of the time). This adherence score was recorded by participants outside of the presence of research staff. After which, the participant placed the form containing their score in a locked box. Compensation was given after participants self-reported their adherence score. For each day participants completed their four saliva samples, they were compensated $15.

ActiGraphy

ActiGraph devices provide "activity count" data in one-minute epochs via vertical acceleration movements to measure the user's intensity and quantity of activity as well as sleep patterns (Loiacono et al., 2020). For the current study, participants were asked to wear the ActiGraph as a watch on their non-dominant wrist for the entire two-week daily assessment period. Participants were only asked to remove the ActiGraph during activities that would get the device wet (e.g., showering or swimming). Non-wear time is calculated and accounted for separately by the ActiGraph and participants missing more than 10 hours of data for any of the three saliva sampling days were excluded from analyses. Although no firm cut-offs exist for wear time of accelerometry, research has suggested that at least 12 hours of wear data for around 3 to 4 days is sufficient for assessing physical activity and sedentary behavior (Di et al., 2022).

Participants returned devices at their follow-up session appointment for analysis. Research assistants downloaded ActiGraph data from the watch into ActiLife. ActiLife is ActiGraph's data analysis platform used to process and score collected data based on “independently developed and validated algorithms.” The present study utilized features to assay wear time, activity and sedentary bouts, and sleep. Accelerometer protocol details for ActiGraph are available (*National Health and Nutrition Examination Survey LABORATORY PROCEDURES MANUAL*, 2004).

Measures

Physical Activity

Actigraphy was used to measure physical activity and provided summaries of daily time engaged in sedentary behavior, quantity of activity, and intensity of activity. Specifically, values for time spent engaged in light, moderate, and vigorous activity were available. Of note, a vast majority of participant activity counts fell into the light activity category, followed by moderate, and very few if any activity counts in the vigorous category. This is likely due to 1) the nature of the study in which the participants were asked to wear the ActiGraphs during typical daily activities and not during a specific exercise and 2) the standard factory cut-offs for these intensity qualifiers being strict in determining what constitutes vigorous activity (e.g., high performance training). Therefore, light, moderate, and vigorous activity counts were combined to produce a total physical activity score per day. As stated previously, 14 days of activity data was recorded; however, only the three days corresponding to the participant’s saliva sample days were used in the current study to assess previous- and next-day relationships.

Biomarkers

All biomarkers were measured via salivary data which has been shown to be efficacious in diagnosing and monitoring disease (Melguizo-Rodríguez et al., 2020), advantageous for

measuring physiological processes that change throughout the day, and can be collected non-invasively to provide reliable and valid data of internal bodily functioning (Granger et al., 2012). As stated previously, saliva samples were collected upon waking, +30 minutes after waking, mid-day, and evening across three days.

Cortisol. Each participant who has submitted complete data has provided 12 cortisol data points across the three days of sampling. Therefore, CAR, total cortisol, and diurnal slope was computed for each day of saliva sampling.

CAR is defined as the increase of cortisol within the first hour after waking. CAR was computed using the area under the curve (AUC) formulas derived from the trapezoid formula outlined in Pruessner et al. (2003). Specifically, the AUC1 formula was used because it places a stronger emphasis on a variable's change over time by focusing on how each measurement differs from each other and ignoring the specific measurement's distance from zero (Pruessner et al., 2003). Because we are interested in the change in cortisol from wake to +1 hour, understanding the distance from zero or "the level at which changes occur over time" at each time point is not necessary. Pruessner et al. (2003) provides the following equation:

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2} \right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i \right)$$

m_i - single time point measurement of cortisol; t_i - the time distance between the measurements; and n - denoting the total amount of saliva measurements being considered, which was two because calculating the area under the curve requires measuring the distance incrementally from time point one to two, two to three,

and three to four across the four sample times: upon waking, +30 minutes after waking, mid-day, and evening.

Conversely, understanding the saliva sample's distance from zero is beneficial for measuring total cortisol in that it provides a summation of the trapezoids. For this index, calculation of all four saliva sample time points occurred. Pruessner et al. (2003) provides the following equation:

$$AUC_G = \sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2}$$

m_i - single time point measurement of cortisol; t_i - the time distance between the measurements; and n - denoting the total amount of saliva measurements being considered, which was two because calculating the area under the curve requires measuring the distance incrementally from time point one to two, two to three, and three to four across the four sample times: upon waking, +30 minutes after waking, mid-day, and evening.

Diurnal slope is understood as the linear degree of change in the biomarker throughout the day after excluding the awakening response (Ross et al., 2014). A linear regression line is subsequently calculated for each participant based on their saliva samples from waking and during the evening, effectively excluding the +30 minutes after waking sample. The formula for CAR was utilized again in which m_i represents a participant's cortisol sample from their time point 3 (mid-day) and $m_{(i+1)}$ represents their sample for time point 4 (evening).

Alpha Amylase. Enzymatic activity via spectrophotometric assays and reported as international units of sAA activity per milliliter (IU/mL) were derived from saliva samples

(Rohleder & Nater, 2009; Rohleder et al., 2006). Total sAA involved averaging the enzymatic values across all sample timepoints for each day. sAA awakening response was calculated as the delta change between enzymatic value of sample at timepoint 2 from the value of sample at timepoint 1, per previous research (Eddy et al., 2018; Filaire et al., 2013; Katz et al., 2016; Skoluda et al., 2016).

Control Variables

Daily Hassles

Daily hassles were measured using the 20-item Brief College Student Hassles Scale (BCSHS) which assesses stressors across several domains (academic, financial, social stress). The BCSHS has been shown to possess good internal consistency based on total scores (Cronbach's α = 0.81) (Blankstein & Flett, 1992; Blankenstein et al., 1991). This self-report measure uses a seven-point Likert scale ranging from 1 (No hassle, not persistent at all) to 7 (High occurrence; extremely persistent, high frequency or duration). Higher scores on the measure indicate more hassles.

Race

Race was recorded at baseline via self-report by participants. The survey item stated, 'race – select choice' and asked participants to select all that applied. The original race variable consisted of White, Black or African American, American Indian or Alaska Native, Asian or Asian American, Native Hawaiian or Pacific Islander, and Other; however, due to insufficient sample sizes across races, this variable was dummy coded with the reference group being those that identified as White.

Sex

Sex was recorded at baseline via self-report by participants. The survey item stated, 'biological sex – select choice: male; female.' This variable was dummy coded with the reference group being males.

Chronic Illness Status

Chronic illness status was recorded at baseline via self-report by participants and may refer to physical (e.g., diabetes, chronic pain) and/or psychiatric (e.g., depression, attention-deficit/hyperactivity disorder) illnesses. The survey item stated, 'are you currently diagnosed with any of the following health conditions?' This variable was dummy coded with the reference group being no chronic illness status.

Contraceptive Use

Contraceptive use was recorded via self-report by participants. The survey item stated, 'are you currently using contraceptives (i.e., birth control or intrauterine device)?' This variable was dummy coded with the reference group being no contraceptive use.

Preliminary Analyses

All analyses were conducted using SPSS (IBM Corp, 2023). Linear regression was conducted with total activity as the outcome variable and daily hassles and all biomarker variables (e.g., Average cortisol, CAR, Cortisol slope, Average AA, AA awakening response) as predictors to assess multicollinearity and prevent high overlap in shared variance (Reichwein Zientek & Thompson, 2006). Specifically, variance inflation factor (VIF) was calculated. No predictor combination produced a VIF value over 5, indicating an absence of multicollinearity (O'Brien, 2007). Additionally, Pearson correlations were conducted to verify that predictor variables were not highly correlated (i.e., two-tailed significance of $<.05$). Regarding missing data, participants without complete activity data, activity data that did not overlap with their

biomarker samples, or less than two complete consecutive days of bio samples were excluded (20 participants). Tolerance statistics were also conducted to measure the proportion of variance in a predictor that is not explained by other predictors in the model. This statistic ranges from 0 to 1, with values closer to 1 indicating low multicollinearity (Tolerance = $1-R^2$ for each predictor from a regression of that predictor on all other predictors) (Field, 2024; Tabachnick & Fidell, 2001).

Lag Variables: Due to the present study's longitudinal design, and the hypotheses aiming to predict future outcomes from past values of physical activity/biomarker, 'single lag' (X_{t-1}) lag variables were created to model temporal dependencies and understanding the dynamic relationships between physical activity, salivary cortisol, and salivary alpha amylase. Specifically, lagging variables allow analyses to incorporate values of a variable at a previous time point (previous day) relative to a current value. To this end, the study can understand the effect of past values of a predictor (e.g., physical activity) on a next day outcome (e.g., biomarker).

Transformations: Models that included participants' diurnal slopes of cortisol produced convergence errors. This error was likely due to the small values of the original slope data compared to the values of other variables within the model. To address this issue, the diurnal slopes were multiplied by 10,000 to support convergence and stabilize parameter estimates.

Analyses

Assumptions of Multilevel Modeling

Linearity: Scatterplots were created for each predictor variable (biomarker variables) against the outcome variable (physical activity) to visually assess if the pattern is roughly linear.

Homoscedasticity: A scatterplot was created with the residuals placed on the y-axis and a relevant predictor on the x-axis to see if the variance of residuals is constant across all levels of predictors (e.g., ideally, residuals are evenly spread around the horizontal line at zero).

Normality: the saved level 1 residuals from running the multilevel model were used to create a histogram and assess if they display a normal distribution.

Regarding the hierarchical structure of the analyses, Level 1 consisted of the biomarkers which were nested within individuals at Level 2. Physical activity was also considered at Level 1. Variables at level 1 vary within individuals across time, influencing biomarkers at each time point. Biomarkers also vary within individuals across time and level 1 captured these changes in individual characteristics. Level 2 (random intercept) accounted for baseline individual-specific differences in biomarker levels that were not explained by fixed effects. A conceptual formula is located below that also accounts for race, sex, chronic illness status, and contraceptive use.

Of note, types of multilevel modeling and multilevel model estimates are generally robust to violations of assumptions (Schielzeth et al., 2020) and skewness (Snijders & Bosker, 2012).

Level 1 (Within Model):

Individual-specific measurements Y_{ij} (biomarker) and X_{ij} (previous day physical activity) for each individual *i* and each time point *j*.

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 \text{Race}_i + \beta_3 \text{Sex}_i + \beta_4 \text{Illness Status}_i + \beta_5 \text{Contraceptive Use}_i + \beta_6 \text{Stress/Hassles}_i + \epsilon_{ij}$$

Yij: Biomarker measurement for individual *i* at time *j*.

X_{ij}: Previous day physical activity level for individual *i* at time *j*.

Race_i: Indicator variable (0 White or 1 Non-White) for race of individual *i*.

Sex_i: Indicator variable (0 male or 1 female) for sex of individual *i*.

Illness Status$_i$: Indicator variable (0 no chronic illness or 1 chronic illness) for illness status of individual *i*.

Contraceptive Use$_i$: Indicator variable (0 no contraceptive or 1 contraceptive) for contraceptive use of individual *i*.

Stress/Hassles$_i$: Daily hassles rating (stress metric) of individual *i*.

β_0: Population mean intercept (average biomarker measurement when physical activity and all other predictors are zero).

β_1: Coefficient for the effect of previous day physical activity X_{ij} on biomarker Yij.

β_2, β_3, β_4, β_5, β_6: Coefficients for the effects of race, sex, illness status, contraceptive use, and stress/hassles on biomarker Yij.

ϵ_{ij}: Residual error term for measurement error and unexplained variability.

Level 2 (Between Model):

Random effects assessed to account for individual-specific variability in biomarker measurements.

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 \text{Race}_i + \beta_3 \text{Sex}_i + \beta_4 \text{Illness Status}_i + \beta_5 \text{Contraceptive Use}_i + \beta_6 \text{Stress/Hassles}_i + u_{0i} + \epsilon_{ij}$$

$u_{0i} \sim N(0,\sigma^2_{u0})$: Random intercept to assess individual-specific deviations in biomarker measurements.

Analytic Plan

Aims 1-2: Examine the bi-directional relationship between salivary cortisol and salivary alpha amylase indices and physical activity over a three-day period.

Multilevel models were computed with same- and previous- day physical activity as the predictor variables and biomarker as the outcome variable. The current study sample size of 68 participants observed over three days is acceptable based on past research that has suggested that

small sample sizes (50 or less) at level two leads to biased estimates (Maas & Hox, 2005). The lag variable for physical activity was used to assess the relationship between previous day physical activity and next day biomarker. Race, sex, chronic illness status, and contraceptive use were added to the model as grouping variables due to previous research demonstrating that these person-level variables are often associated with these biomarkers and health behavior, as described in the measures section. Daily hassles were also added to the models as a predictor variable and proxy for stress. This inclusion allowed the model to account for the unique variance that physical activity has related to biomarker outcomes regardless of stress level.

Similar multilevel models were estimated to assess same- and previous-day biomarker's predictability for physical activity. This involved using the biomarker variable (e.g., diurnal slope, awakening response, total) and its corresponding lag variable as predictors. Physical activity became the outcome variable. The same individual-specific variables and daily hassles were also included for the reasons previously stated. Finally, for models in which biomarker are the predictors, separate models were created for each biomarker index (awakening response, diurnal slope, total) to avoid issues of multicollinearity and prevent high overlap in shared variance between biomarker data.

The Restricted Maximum Likelihood (REML) method was used for all multilevel models. REML is advantageous for running final models compared to the maximum likelihood (ML) method because REML provides unbiased estimates of the variance parameters and includes the fixed effects in the likelihood function, producing a more accurate model output (Pal & Chakravarty, 2020).

Aim 3: Determine the strongest biomarker predictor of physical activity.

Linear regression was used with total physical activity as the criterion variable and average total alpha amylase and average total cortisol as predictor variables. Standardized beta coefficients of the predictors were used to assess the strength (magnitude of the effect) and direction of the relationship between biomarker and physical activity.

Results

Descriptive Statistics

See Table 1 for descriptive statistics. For the overall sample (n = 68), mean and standard deviation across variables of interest were: Total Activity (M = 668.61, SD = 159.87); Daily Hassles (M = 34.20, SD = 11.25); CAR (M = 12.43, SD = 6.21); Cortisol Diurnal Slope (M = -3.05, SD = 2.27); Average Cortisol (M = 0.25, SD = 0.09); Alpha Amylase Awakening Response (M = -23.98, SD = 61.45); Average Alpha Amylase (M = 98.20, SD = 76.45). Frequencies for the grouping variables used within models were: Race (44.1% Non-White); Sex (58.8% female); Chronic Illness Status (51.5% experiencing chronic illness); Contraceptive Use (25% use, 30.9% do not use, 44.1% did not respond).

Relevant significant two-tailed Pearson correlations were observed between chronic illness status and total physical activity (r = 0.27, p <0.001); chronic illness status and average cortisol (r = -0.16, p = 0.033); chronic illness status and CAR (r = -0.31, p <0.001), chronic illness status and diurnal slope of cortisol (r = 0.18, p = 0.021); race and daily hassles (r = -0.17, p = 0.028); race and diurnal slope of cortisol (r = -0.22, p = 0.003); race and average alpha amylase (r = -0.19, p = 0.008); and daily hassles and average alpha amylase (r = 0.21, p = 0.006). As expected, significant correlations were observed between the cortisol indices. The alpha amylase indices also correlated significantly with one another. None of the cortisol and alpha amylase variables were significantly correlated. See table 2 for correlation matrix.

Multilevel Models (Aim 1)

Model 1: Total Activity on Average Cortisol

Regarding the fixed effects of total activity on average cortisol production, neither same-day physical activity (β = -2.21E-5, *SE* = 6.09E-5, p = 0.72) nor previous-day physical activity (β = 1.63E-5, *SE* = 6.08E-5, p = 0.79) significantly predicted average cortisol production. Conversely, average daily hassles did significantly predict average cortisol production (β = 2.83E-3, *SE* = 0.009, p = 0.002). Specifically, participants with more daily hassles displayed higher levels of cortisol production. No significant results were observed between grouping variables: race (β = 0.03, *SE* = 0.04, p = 0.40); sex (β = -0.05, *SE* = 0.05, p = 0.36); contraceptive use (β = 1.37E-3, *SE* = 0.03, p = 0.97); illness status (β = 0.05, *SE* = 0.04, p = 0.26). Model statistics include: -2 Restricted Log Likelihood (-2LL) = -118.37; Akaike's Information Criterion (AIC) = -114.37; Hurvich and Tsai's Criterion (AICC) = -114.21; Bozdogan's Criterion (CAIC) = -107.68; Schwarz's Bayesian Criterion (BIC) = -109.68 [see table 3].

Model 2: Total Activity on CAR

Regarding the fixed effects of total activity on cortisol awakening response (CAR), neither same-day physical activity (β = -4.53E-3, *SE* = 0.004, p = 0.28) nor previous-day physical activity (β = -5.66E-4, *SE* = 0.004, p = 0.90) significantly predicted CAR. Conversely, average daily hassles did significantly predict CAR (β = 0.17, *SE* = 0.06, p = 0.01). Specifically, participants with more daily hassles displayed a larger CAR. No significant results were observed between grouping variables: race (β = 3.72, *SE* = 2.28, p = 0.12); sex (β = 1.32, *SE* = 3.47, p = 0.71); contraceptive use (β = 0.27, *SE* = 2.23, p = 0.91); illness status (β = 4.23, *SE* = 2.54, p = 0.12). Model statistics include: -2LL = 517.85; AIC = 521.85; AICC = 522.01; CAIC = 528.48; BIC = 526.48 [see table 4].

Model 3: Total Activity on Diurnal Slope of Cortisol

Regarding the fixed effects of total activity on diurnal slope of cortisol, neither same-day physical activity (β = -9.19E-5, *SE* = 0.002, p = 0.96) nor previous-day physical activity (β = 2.33E-4, *SE* = 0.002, p = 0.89) significantly predicted diurnal slope of cortisol. Similarly, average daily hassles did not significantly predict diurnal slope of cortisol (β = -0.01, *SE* = 0.02, p = 0.54). No significant results were observed between grouping variables: race (β = -0.19, *SE* = 0.62, p = 0.76); sex (β = 0.58, *SE* = 0.97, p = 0.56); contraceptive use (β = 0.53, *SE* = 0.60, p = 0.38); illness status (β = -0.97, *SE* = 0.74, p = 0.20). Model statistics include: -2LL = 388.39; AIC = 392.39; AICC = 392.56; CAIC = 399.08; BIC = 397.08 [see table 5].

Model 4: Total Activity on Average Alpha Amylase

Regarding the fixed effects of total activity on alpha amylase, neither same-day physical activity (β = -0.11, *SE* = 0.02, p = 0.62) nor previous-day physical activity (β = 0.04, *SE* = 0.02, p = 0.10) significantly predicted average alpha amylase production. Similarly, average daily hassles did not significantly predict alpha amylase production (β = 0.33, *SE* = 0.32, p = 0.31). No significant results were observed between grouping variables: race (β = -2.58, *SE* = 13.05, p = 0.85); sex (β = -1.33, *SE* = 19.89, p = 0.95); contraceptive use (β = 5.63, *SE* = 12.82, p = 0.66); illness status (β = -16.39, *SE* = 14.41, p = 0.26). Model statistics include: -2LL = 843.99; AIC = 847.98; AICC = 848.13; CAIC = 854.79; BIC = 852.79 [see table 6].

Model 5: Total Activity on Average Alpha Amylase Awakening Response

Regarding the fixed effects of total activity on alpha amylase awakening response (AA-AR), neither same-day physical activity (β = -0.03, *SE* = 0.05, p = 0.56) nor previous-day physical activity (β = -0.02, *SE* = 0.05, p = 0.73) significantly predicted AA-AR. Similarly, average daily hassles did not significantly predict AA-AR (β = -0.25, *SE* = 0.69, p = 0.72). No

significant results were observed between grouping variables: race (β = 30.76, *SE* = 19.49, *p* = 0.13); sex (β = -29.13, *SE* = 31.18, *p* = 0.36); contraceptive use (β = 20.84, *SE* = 19.20, *p* = 0.29); illness status (β = -19.87, *SE* = 23.23, *p* = 0.40). Model statistics include: -2LL = 891.04; AIC = 895.04; AICC = 895.20; CAIC = 901.70; BIC = 899.70 [see table 7].

Multilevel Models (Aim 2)

Model 6: Average Cortisol on Total Activity

Regarding the fixed effects of average cortisol production on total activity, neither same-day cortisol production (β = -2.71, *SE* = 203.91, *p* = 0.99) nor previous-day cortisol production (β = -198.69, *SE* = 194.95, *p* = 0.31) significantly predicted total activity. Similarly, average daily hassles did not significantly predict total activity (β = -2.07, *SE* = 1.61, *p* = 0.21). Regarding grouping variables, race did not significantly relate to total physical activity (β = -1.46, *SE* = 44.69, *p* = 0.97). However, sex did significantly predict total physical activity (β = 201.38, *SE* = 65.76, *p* = 0.005) with females engaging in significantly more physical activity than males. Contraceptive use was not associated with physical activity (β = 32.02, *SE* = 42.67, *p* = 0.46); however, illness status did significantly relate to total physical activity (β = -177.50, *SE* = 45.54, *p* <0.001) with those with chronic illness engaging in significantly less activity compared to those with no chronic illness status. Model statistics include: -2LL = 942.10; AIC = 946.10; AICC = 947.17; CAIC = 953.55; BIC = 951.55 [see table 8].

Model 7: CAR on Total Activity

Regarding the fixed effects of CAR on total activity, neither same-day CAR (β = -1.75, *SE* = 3.28, *p* = 0.60) nor previous-day CAR (β = -1.38, *SE* = 3.14, *p* = 0.66) significantly predicted total activity. Similarly, average daily hassles did not significantly predict total activity (β = -2.38, *SE* = 1.76, *p* = 0.18). Regarding grouping variables, race did not produce significant

results (β = 2.88, *SE* = 50.56, p = 0.96); however, sex did significantly predict total physical activity (β = 203.39, *SE* = 85.29, p = 0.03) with females engaging in significantly more physical activity than males. Contraceptive use did not produce significant results (β = 24.35, *SE* = 48.96, p = 0.62); however, illness status did significantly relate to total activity (β = -155.24, *SE* = 53.07, p = 0.007) with those with chronic illness engaging in significantly less activity compared to those with no chronic illness status. Model statistics include: -2LL = 924.01; AIC = 928.01; AICC = 928.19; CAIC = 934.48; BIC = 932.48 [see table 9].

Model 8: Diurnal Slope of Cortisol on Total Activity

Regarding the fixed effects of diurnal slope of cortisol on total activity, neither same-day diurnal slope of cortisol (β = 3.30, *SE* = 6.85, p = 0.63) nor previous-day diurnal slope of cortisol (β = 7.02, *SE* = 6.28, p = 0.27) significantly predicted total activity. Similarly, average daily hassles did not significantly predict total activity (β = -1.92, *SE* = 1.40, p = 0.18). Regarding grouping variables, no significant differences were observed by race (β = -5.95, *SE* = 42.54, p = 0.89). Sex, however, was significantly related to total physical activity (β = 180.69, *SE* = 61.01, p = 0.006) with females engaging in significantly more physical activity than males. Contraceptive use did not produce significant results (β = 5.50, *SE* = 41.48, p = 0.90); however, illness status did significantly relate to total activity (β = -163.37, *SE* = 45.04, p = 0.001) with those with chronic illness engaging in significantly less activity compared to those with no chronic illness status. Model statistics include: -2LL = 923.52; AIC = 927.52; AICC = 927.70; CAIC = 934.05; BIC = 932.05 [see table 10].

Model 9: Average Alpha Amylase on Total Activity

Regarding the fixed effects of average alpha amylase production on total activity, neither same-day alpha amylase production (β = -0.14, *SE* = 0.47, p = 0.76) nor previous-day alpha

amylase production (β = 0.51, *SE* = 0.35, *p* = 0.15) significantly predicted total activity. Similarly, average daily hassles did not significantly predict total activity (β = -2.67, *SE* = 1.49, *p* = 0.08). Regarding grouping variables, no significant differences were observed by race (β = -4.11, *SE* = 41.49, *p* = 0.92); however, sex did significantly predict total physical activity (β = 204.85, *SE* = 61.80, *p* = 0.002) with females engaging in significantly more physical activity than males. Contraceptive use did not produce significant results (β = 19.63, *SE* = 40.63, *p* = 0.63); however, illness status did significantly relate to total activity (β = -180.24, *SE* = 43.10, *p* <0.001) with those with chronic illness engaging in significantly less activity compared to those with no chronic illness status. Model statistics include: -2LL = 1092.12; AIC = 1096.12; AICC = 1096.27; CAIC = 1102.93; BIC = 1100.93 [see table 11].

Model 10: Alpha Amylase Awakening Response on Total Activity

Regarding the fixed effects of AA-AR on total activity, neither same-day AA-AR (β = -0.26, *SE* = 0.30, *p* = 0.40) nor previous-day AA-AR production (β = -0.28, *SE* = 0.25, *p* = 0.91) significantly predicted total activity. Similarly, average daily hassles did not significantly predict total activity (β = -2.91, *SE* = 1.67, *p* = 0.09). Regarding grouping variables, race did not produce significant results (β = -1.86, *SE* = 48.26, *p* = 0.97); however, sex did significantly predict total physical activity (β = 204.95, *SE* = 70.50, *p* = 0.006) with females engaging in significantly more physical activity than males. Contraceptive use did not produce significant results (β = 37.67, *SE* = 46.35, *p* = 0.42); however, illness status did significantly relate to total activity (β = -202.24, *SE* = 47.70, *p* <0.001) with those with chronic illness engaging in significantly less activity compared to those with no chronic illness status. Model statistics include: -2LL = 985.76; AIC = 989.76; AICC = 989.93; CAIC = 996.34; BIC = 994.34 [see table 12].

Linear Regression (Aim 3)

Neither average total alpha amylase (β = 0.089, t = 1.17, p = 0.24) nor average total cortisol (β = -0.012, t = -0.15, p = 0.88) significantly predicted total physical activity. The model did not predict a significant portion of variance in total activity counts (R^2 = 0.008, F(2, 172) = 0.72, p = 0.49. Model statistics: Durbin-Watson = 1.34; Tolerance of predictors = 0.991; VIF of predictors = 1.01.

Discussion

The primary aims of the current study were to analyze the relationships between previous- and same-day physical activity on same- and next-day cortisol and alpha amylase indices, and vice versa. For aims one through three, the results did not support the hypotheses. Specifically, for all models in which same- and previous- day physical activity were predictors and biomarker indices (total, awakening response, diurnal slope) were outcomes, neither same- nor previous-day physical activity significantly predicted same- or next-day biomarker. This finding is inconsistent with previous literature that demonstrated physical activity significantly predicting cortisol (Anderson & Wildeman, 2017; Duclos & Tabarin, 2016; Gerber et al., 2013; Hill et al., 2008; Ponce et al., 2019) and alpha amylase (Granger et al., 2007; Koibuchi & Suzuki, 2014; Walsh, 1999) levels. Of note, the significant findings between physical activity and stress biomarkers from previous research often involved inducing a specific physical activity intensity and/or measuring biomarker levels closely after physical activity ceased (Hill et al., 2008). Specifically, vigorous, but not lower intensities such as light or moderate, have been shown to increase cortisol levels (Anderson & Wildeman, 2017; Duclos & Tabarin, 2016) and CAR (Ponce et al., 2019). Despite the lack of findings between physical activity and alpha amylase indices, these results are not completely inconsistent with previous research due to some studies also reporting no change in alpha amylase production due to physical activity (Nater et al., 2007;

Yamaguchi et al., 2006). The lack of significant results still provide value to this area of research due to the novelty of the current study's design. The current study worked to better understand how total activity (light, moderate, and vigorous intensities combined) measured throughout the day influenced cortisol and alpha amylase, and vice versa, over longer periods of time (i.e., the next day) compared to previous research designs. Within these models where physical activity and daily hassles were predictors and biomarker index was an outcome, no significant differences were observed for any of the grouping variables (race, sex, contraceptive use, and chronic illness status). Despite these lack of findings for physical activity, daily hassles did significantly predict same day average cortisol and CAR. Specifically, higher reported daily hassles resulted in individuals having higher levels of cortisol on average and a larger cortisol awakening response. This finding aligns with previous literature in which daily hassles and related stressors produce higher levels of stress hormones (cortisol) (Figueroa et al., 2021; Newman et al., 2007; Sher, 2004; Weber et al., 2022). This finding is expected due to the body's use of cortisol to achieve homeostasis during perceived and physical stress (McEwen, 2019): if an individual is reporting more hassles (i.e., more stressors across multiple areas of life), then their biomarker response should also be elevated to support them in meeting the challenges of overcoming the stressors.

When assessing whether same- or previous-day biomarker index predicts same- or next-day physical activity, no biomarker index significantly predicted physical activity for any model. This lack of significance was unexpected due to past systematic review of the literature suggesting higher levels of stress and stress biomarkers significantly lowers the amount of engagement in physical activity (Stults-Kolehmainen & Sinha, 2014). These studies highlight the connection between those with higher levels of stress hormones likely deprioritizing engagement

in health behaviors, such as physical activity, because of the time needed to address current stressors. Similar to biomarker indices, daily hassles did not significantly predict amount of physical activity in any of the models. There were significant differences between grouping variables and physical activity, however. Specifically, females engaged in significantly more physical activity compared to males. This relationship between sex and physical activity is not aligned with most previous research that shows males being more physically active compared to females (Feraco et al., 2024; Portela-Pino et al., 2020; Santos et al., 2003) and specifically when at university (Cahuas et al., 2020; Dikmen et al., 2020; Fagaras et al., 2015). It is possible this overall trend is flipped for the narrow section of emerging adults within the present study. Specifically, participants were from a university located in a major city which may have provided the built environment and walkability (e.g., access to public spaces, parks, gyms, recreational centers, and intramural clubs) to reduce sex-related barriers (e.g., stereotypes, cultural acceptability, male dominated activities) to exercise and physical activity (Cla, 2018; Sallis et al., 2016). Chronic illness status also differed significantly regarding involvement in physical activity. Specifically, those with an identified chronic illness engaged in significantly less physical activity compared to those with no chronic illness status. This finding is consistent with previous research in which those with a chronic illness experiencing a reduction in physical functioning overall compared to pre-illness functioning (Mackenbach et al, 2001; Oris et al., 2018). Unlike sex and chronic illness status, physical activity engagement did not differ significantly by race nor contraceptive use. When considering both alpha amylase and cortisol in the same model with total activity count as the outcome, neither biomarker significantly predicated activity counts. This was unexpected given the previously sited research above linking physical activity to biomarker response, especially for cortisol. The lack of findings

within this regression model may also highlight the need for analyzing the intensity of physical activity when increasing the duration of physical activity and biomarker measurement (i.e., throughout the day).

Limitations

The current study had several limitations. Firstly, although the 68 participants were observed over three days for a total of 204 data points, the smaller sample size negatively impacts statistical power and reduces the precision of parameter estimates (Akobeng, 2016; Baguley, 2004; Prajapati et al., 2010). Although past research used similar durations of data collection to demonstrate significant relationships between these variables in the short-term (e.g., 5 minutes up to an hour), three days of biomarker and physical activity data may be too few to fully model the trend of previous day onto next day for the study's sample size. Increasing the number of participants, the number of days of data collection, or both would benefit future studies. A statistical power analysis is warranted for future research to assess appropriate sample sizes for study replication and/or computing more complex models.

In addition to sample size and length of data collection, lack of activity counts and variability within the physical activity intensities (light, moderate, vigorous) resulted in collapsing activity intensities into one variable - total activity. Having the ability to assess how various intensities of physical activity predict biomarker would produce a more thorough understanding of how activity relates to biomarker production. This is especially important given previous research has demonstrated that intensity of physical activity influences levels of stress biomarkers (Anderson & Wildeman, 2017; Duclos & Tabarin, 2016; Ponce et al., 2019).

Finally, generalizability of results is limited. Although a notable percentage (39%) of individuals ages 18 to 24 years within the United States of America are enrolled in university

(National Center for Education Statistics, 2024), the current study does not capture a large majority of emerging adults (i.e., those individuals who do not attend university). This is important due to this stage in life being known for heterogeneity of experiences as these individuals work toward an adult identity (Arnett et al., 2014; Arnett, 2004). The current sample was also derived from a university located in a major Midwestern metropolitan area and therefore only partially captures the 39% of emerging adults at university. Finally, attending university has been shown to be a protective factor against mental health concerns (Han et al., 2016; Kovess-Masfety et al., 2016; Yorgason et al., 2008) and would warrant incorporating emerging adults that are not attending university to fully capture the various levels of stress this group experiences.

Future Directions

The current set of data used for the study was not ideal for analyzing more complex models nor comparing intensities of physical activity due to a smaller sample size and lack of variability across physical activity intensities. The lack of findings may suggest that the intensity of physical activity (e.g., vigorous) is most important and influential over biomarker values over a longer a period of time; however, this is something for future research to address. Specifically, researchers should evaluate whether individuals who engage in more vigorous physical activity throughout the day, compared to other intensities, are able to influence their biomarker levels over longer periods of time (e.g., the next day).

Statistically, the current study focused on the direct relationship between physical activity and cortisol/alpha amylase production, and vice versa (i.e., only fixed effects with random intercept). With more data, more complex models could be estimated to accurately assess the relationships and interactions between these variables. Therefore, a more comprehensive

understanding is possible by incorporating random effects and understanding between-group dynamics by comparing nested models. Future research should start with multilevel models that are estimated using the maximum likelihood (ML) method in order to assess the inclusion of, and examine, random effects of predictors. Maximum likelihood is desirable when comparing multilevel models because of its ability to evaluate the statistical significance of adding additional parameters (likelihood ratio) to identify if more model complexity results in a better fit for the data; and, ML includes insights into fixed effects and variance components compared to REML which removes fixed effects from estimation (McNeish, 2017).

Specifically, models should be created with all predictors and grouping variables as fixed effects (with random intercept). Then, a second model should be created with all predictor variables as fixed *and* random effects. Within models, the inclusion of random effects of the continuous predictors supported the understanding of their variability at different levels of the model.

To assess if the inclusion of random effects produce a better fitting model to the data, negative two log likelihood (-2LL) values from the original, all fixed effects, model and the expanded random effects model should be compared. The negative two log likelihood statistic should be used to compare models to assesses a model's level of misfit to the data. When comparing models, lower -2LL values indicate better fit (i.e., less misfit). To compare -2LL values between models statistically, the likelihood ratio test should be performed (Peugh, 2010).

This likelihood ratio value can be assessed for statistical significance by comparing its value to the critical values of a Chi-squared distribution (Peugh, 2010). For example, if the difference score of the -2LL values between model 1 (fixed effects) and, the full, model 2 (fixed and random effects) is greater than the critical value of 3.841, then the model with the lower -

2LL value is a statistically better fitting model to the data at the <.05 level. After the best model is identified using ML, it should be re-run using the REML method for reasons stated above in the analytic plan section.

Beyond model fit, the current study was unable to address physical activity intensity and next day biomarker due to lack of variability across intensities, with an overwhelming majority of participant activity counts falling into the light intensity category. It is possible the effects of various intensities of physical activity on stress biomarker last longer (i.e., into the next day) than what current literature has focused on. Currently, research on the intensities of physical activity have demonstrated that higher intensities have the most influence over stress biomarkers in the short term (Anderson & Wildeman, 2017; & Duclos & Tabarin, 2016; Ponce et al., 2019). Of note, physical activity that is engaged in at a vigorous intensity on a consistent schedule has been shown to benefit mental health and lower the perceived stress within emerging adults (VanKim & Nelson, 2013). Therefore, physical activity remains a salient health behavior that supports stress management.

Clinical implications. The heterogenous experience of emerging adulthood produces a homogenous experience of high levels of stress (Arnett, 1998; Arnett, 2000; Arnett, 2004; Arnett, 2007; Arnett, 2014; Arnett et al., 2014) and therefore requires stress management techniques that a variety of lifestyles can engage in. Similar to coping strategies such as mindfulness, meditation, and deep breathing that support stress management (Chi et al., 2018; Kumar et al., 2022; Reangsing et al., 2022), physical activity is a common and accessible intervention that has potential for supporting the majority of individuals through this stage of life. Beyond stress, physical activity has the potential to modulate other physical health outcomes more than the above coping skills, and more research is needed to identify ways to encourage

emerging adults to engage in physical activity to support overall health and functioning. Investment in supports for emerging adults is advantageous at the societal level because of these individuals soon becoming the leaders, caregivers (e.g., directly or indirectly contributing to the care of children and/or of older individuals), and main workforce for their local communities. Due to much of the societal responsibilities transferring to emerging adults as they enter adulthood, identifying and providing tools to manage stress within this earlier stage of life has a greater potential to produce adaptable, and healthier, individuals and communities.

Overall, the present study aimed to explore the bidirectional relationships between basal stress biomarkers and daily physical activity in a diverse sample of emerging adults. Results indicated that neither same- nor previous-day physical activity significantly related to alpha amylase or cortisol production, and vice-versa. Significant results were observed between daily hassles and cortisol indices in which experiencing more daily hassles resulted in higher daily average cortisol and a larger CAR. Future research should focus on assessing the intensity of physical activity on these biomarkers over longer durations due to physical activity intensity significantly influencing cortisol and alpha amylase production in the short term. Findings may support interventions for emerging adults as they progress through this highly stressful developmental period and into adulthood.

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Table 1: Sample Demographics

Sample Demographics (n = 68)		
	M	***(SD)***
Age (years)	19.71	*(1.58)*
Total Activity	668.61	*(159.87)*
Daily Hassles	34.2	*(11.25)*
Average Cortisol	0.25	*(0.09)*
CAR	12.43	*(6.21)*
Slope of Cortisol*	-3.05	*(2.27)*
Average AA	98.20	*(76.01)*
AA-AR	-23.98	*(61.45)*
	%	***(n)***
Sex		
Male	41.2	*(28)*
Female	58.8	*(40)*
Chronic Illness		
Yes	51.5	*(35)*
No	47.1	*(32)*
Race		
Non-White	44.1	*(30)*
White	54.4	*(37)*
Contraceptive Use		
Yes	25.0	*(17)*
No	30.9	*(21)*

CAR – Cortisol Awakening Response
Slope of Cortisol* – Diurnal Slope of Cortisol linearly transformed
AA – Alpha Amylase
AA-AR – Alpha Amylase Awakening Response

Table 2: Correlations

Correlations

	Race	Sex	Contra Use	Chronic Illness Status	Total Activity	Avg. Cortisol	CAR	Slope Cort	Avg. AA	AA-AR	DH
1	-										
2	0.128	-									
3	-0.418**	-0.036	-								
4	-0.149*	0.220**	0.257**	-							
5	-0.083	-0.102	0.084	0.266**	-						
6	0.042	-0.049	0.036	-0.163*	-0.020	-					
7	0.069	-0.042	-0.045	-0.312**	0.013	0.773**	-				
8	-0.223**	0.069	-0.056	0.175*	0.049	-0.556**	-0.621**	-			
9	-0.191**	-0.025	-0.016	0.077	0.072	-0.095	-0.093	0.132	-		
10	-0.006	0.007	-0.074	0.039	-0.084	0.008	-0.005	-0.088	-0.347**	-	
11	-0.166*	-0.079	0.167	0.143	-0.001	0.021	-0.013	0.058	0.211*	-0.027	-

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Contra Use - Contraceptive Use
CAR - Cortisol Awakening Response
Slope Cort – Diurnal Slope of Cortisol
AA - Alpha Amylase
AA-AR - Alpha Amylase Awakening Response
DH – Daily Hassles
Race (0 – White; 1 – Non-White); Sex (0 – Male; 1 – Female)

Table 3: Model 1 – Physical Activity on Average Cortisol

MLM Estimates of Same- and Previous-Day Physical Activity Predicting Average Cortisol with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	0.13	0.09	1.50(74.05)	0.14
	Race	0.03	0.04	0.86(26.54)	0.40
	Contra	1.37E-03	0.03	0.04(25.88)	0.97
	Sex	-0.05	0.05	-0.93(31.37)	0.36
	Illness Status	0.05	0.04	1.14(33.32)	0.26
	Total Activity	-2.21E-05	6.09E-05	-0.36(72.32)	0.72
	Total Activity_PD	1.63E-05	6.08E-05	0.27(70.49)	0.79
	Hassles	2.83E-03	8.89E-04	3.19(73.44)	0.002
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	6.86E-03	2.31E-03	2.98	0.003
	Residual	3.32E-03	6.83E-04	4.86	<.001

PD – Previous Day
Contra – Oral Contraceptive Use

Table 4: Model 2 – Physical Activity on Cortisol Awakening Response

MLM Estimates of Same- and Previous-Day Physical Activity Predicting CAR with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	6.45	6.10	1.06(70.87)	0.29
	Race	3.72	2.28	1.64(23.66)	0.12
	Contra	0.27	2.23	0.12(23.48)	0.91
	Sex	1.32	3.47	0.38(28.57)	0.71
	Illness Status	4.23	2.54	1.66(30.45)	0.12
	Total Activity	-4.53E-03	0.004	-1.09(69.07)	0.28
	Total Activity_PD	-5.66E-04	0.004	-0.13(71.23)	0.90
	Hassles	0.17	0.06	2.65(72.58)	0.01
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	28.28	10.44	2.71	0.007
	Residual	15.88	3.39	4.69	<0.001

CAR – Cortisol Awakening Response
PD – Previous Day
Contra – Oral Contraceptive Use

Table 5: Model 3 – Physical Activity on Diurnal Slope of Cortisol

MLM Estimates of Same- and Previous-Day Physical Activity Predicting Slope of Cortisol with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	-2.32	2.22	-1.04(45.41)	0.30
	Race	-0.19	0.62	-0.31(20.55)	0.76
	Contra	0.53	0.60	0.90(19.11)	0.38
	Sex	0.58	0.97	0.60(24.28)	0.56
	Illness Status	-0.97	0.74	-1.32(26.35)	0.20
	Total Activity	-9.19E-05	0.002	-0.05(72.48)	0.96
	Total Activity_PD	2.33E-04	0.002	0.14(76.60)	0.89
	Hassles	-0.014	0.02	-0.61(60.98)	0.54
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	0.99	0.89	1.12	0.264
	Residual	3.83	0.81	4.74	<0.001

Slope of Cortisol – Diurnal Slope
PD – Previous Day
Contra – Oral Contraceptive Use

Table 6: Model 4 – Physical Activity on Average Alpha Amylase

MLM Estimates of Same- and Previous-Day Physical Activity Predicting Average AA with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	61.69	32.32	1.91(78.45)	0.06
	Race	-2.58	13.05	-0.19(28.68)	0.85
	Contra	5.63	12.82	0.45(28.15)	0.66
	Sex	-1.33	19.89	-0.07(32.49)	0.95
	Illness Status	-16.39	14.41	-1.14(37.06)	0.26
	Total Activity	-0.11	0.02	-0.51(76.35)	0.62
	Total Activity_PD	0.04	0.02	1.69(77.38)	0.10
	Hassles	0.33	0.32	1.01(76.77)	0.31
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	1002.55	319.63	3.14	0.002
	Residual	469.64	92.10	5.10	<0.001

AA – Alpha Amylase
PD – Previous Day
Contra – Oral Contraceptive Use

Table 7: Model 5 – Physical Activity on Alpha Amylase Awakening Response

MLM Estimates of Same- and Previous-Day Physical Activity Predicting AA-AR with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	-0.61	60.93	-0.01(56.48)	0.99
	Race	30.76	19.49	1.58(25.41)	0.13
	Contra	20.84	19.20	1.09(25.25)	0.29
	Sex	-29.13	31.18	-0.93(31.58)	0.36
	Illness Status	-19.87	23.23	-0.86(30.77)	0.40
	Total Activity	-0.03	0.05	-0.59(75.95)	0.56
	Total Activity_PD	-0.016	0.05	-0.35(75.97)	0.73
	Hassles	-0.25	0.69	-0.36(70.09)	0.72
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	1438.69	745.21	1.93	0.054
	Residual	2662.76	545.14	4.89	<0.001

AA-AR – Alpha Amylase Awakening Response
PD – Previous Day
Contra – Oral Contraceptive Use

Table 8: Model 6 – Average Cortisol on Physical Activity

MLM Estimates of Same- and Previous-Day Average Cortisol Predicting Physical Activity with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	823.37	88.44	9.31(53.43)	<0.001
	Race	-1.46	44.69	-0.03(21.10)	0.97
	Contra	32.02	42.67	0.75(19.51)	0.46
	Sex	201.38	65.76	3.06(23.23)	0.005
	Illness Status	-177.50	45.54	-3.90(21.33)	<0.001
	Average Cortisol	-2.71	203.91	0.01(65.45)	0.99
	Average Cortisol_PD	-198.69	194.95	-1.02(66.52)	0.31
	Hassles	-2.07	1.61	-1.29(51.37)	0.21
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	5280.39	4380.09	1.21	0.228
	Residual	16944.76	3784.13	4.48	<0.001

PD – Previous Day
Contra – Oral Contraceptive Use

Table 9: Model 7 – Cortisol Awakening Response on Physical Activity

MLM Estimates of Same- and Previous-Day CAR Predicting Physical Activity with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	806.47	90.60	8.90(51.31)	<0.001
	Race	2.88	50.56	0.06(23.01)	0.96
	Contra	24.35	48.96	0.50(22.59)	0.62
	Sex	203.39	85.29	2.39(25.41)	0.03
	Illness Status	-155.24	53.07	-2.93(23.70)	0.007
	CAR	-1.75	3.28	-0.53(64.25)	0.60
	CAR_PD	-1.38	3.14	-0.44(67.50)	0.66
	Hassles	-2.38	1.76	-1.35(61.00)	0.18
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	9403.04	5081.67	1.85	0.064
	Residual	15738.54	3451.99	4.56	<0.001

CAR – Cortisol Awakening Response
PD – Previous Day
Contra – Oral Contraceptive Use

Table 10: Model 8 – Diurnal Slope of Cortisol on Physical Activity

MLM Estimates of Same- and Previous-Day Slope of Cortisol Predicting Physical Activity with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	815.22	72.44	11.25(56.33)	<0.001
	Race	-5.95	42.54	-0.14(27.53)	0.89
	Contra	5.50	41.48	0.13(26.39)	0.90
	Sex	180.69	61.01	2.96(27.22)	0.006
	Illness Status	-163.37	45.04	-3.63(28.91)	0.001
	Slope of Cortisol	3.30	6.85	0.48(70.82)	0.63
	Slope of Cortisol_PD	7.02	6.28	1.12(70.51)	0.27
	Hassles	-1.92	1.40	-1.37(69.47)	0.18
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	7172.22	3331.86	2.15	0.031
	Residual	11231.92	2320.37	4.84	<0.001

Slope of Cortisol – Diurnal Slope
PD – Previous Day
Contra – Oral Contraceptive Use

Table 11: Model 9 – Average Alpha Amylase on Physical Activity

MLM Estimates of Same- and Previous-Day Average AA Predicting Physical Activity with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	767.03	80.05	9.58(47.67)	<0.001
	Race	-4.11	41.49	-0.10(29.39)	0.92
	Contra	19.63	40.63	0.48(28.78)	0.63
	Sex	204.85	61.80	3.31(32.10)	0.002
	Illness Status	-180.24	43.10	-4.10(33.68)	<0.001
	Average AA	-0.14	0.47	-0.31(74.50)	0.76
	Average AA_PD	0.51	0.35	1.47(78.67)	0.15
	Hassles	-2.67	1.49	-1.79(74.09)	0.08
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	5799.05	3284.87	1.77	0.077
	Residual	15675.05	2991.51	5.24	<0.001

AA – Alpha Amylase
PD – Previous Day
Contra – Oral Contraceptive Use

Table 12: Model 10 – Alpha Amylase Awakening Response on Physical Activity

MLM Estimates of Same- and Previous-Day AA-AR Predicting Physical Activity with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)	*p*
Fixed Effects	Intercept	796.32	76.88	10.36(47.90)	<0.001
	Race	-1.86	48.26	-0.04(29.06)	0.97
	Contra	37.67	46.35	0.81(28.23)	0.42
	Sex	204.95	70.50	2.91(32.86)	0.006
	Illness Status	-202.24	47.70	-4.24(29.24)	<0.001
	AA-AR	-0.26	0.30	-0.85(68.15)	0.40
	AA-AR_PD	-0.28	0.25	-0.11(72.25)	0.91
	Hassles	-2.911	1.67	-1.74(66.37)	0.09
		Estimate	S.E.	Z	*p*
Random Effects	Intercept	6929.26	3895.68	1.78	0.075
	Residual	17000.95	3446.07	4.93	<0.001

AA-AR – Alpha Amylase Awakening Response
PD – Previous Day
Contra – Oral Contraceptive Use