# An evaluation of metabolic energy mechanisms and their role in Kaqchikel Mayan adolescent girls' reproductive maturation

**by**

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## Declaration of Committee

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# Ethics Statement

The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

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or

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# Abstract

Adolescence is a critical life history transition during a woman's reproductive lifespan in which important developmental changes occur, including somatic growth, cognitive development, social and emotional maturation, and reproductive maturation. According to life history theory, socio-ecological challenges requiring more immediate attention are prioritized over less urgent tasks like reproduction. Shifts in reproductive investments during adolescence can cause delayed or accelerated maturation, including variation in the timing of menarche, a girl's first menstrual bleed. In humans, energy allocation is mediated by the hypothalamic-pituitary-adrenal axis (HPAA). Energy is also recuperated through sleep. To understand how socio-ecological factors impact adolescent development, it is important to understand how energy allocation mechanisms function across the transition. Yet, how these mechanisms mediate energy allocation between growth and reproduction remains unclear. To address this, I present three papers that test hypotheses regarding how metabolic energy is regulated across the adolescent transition using data from Indigenous Mayan adolescents from Guatemala.

In Chapter 2 I examine how the HPAA mediates energetic trade-offs between growth and reproduction in pre-menarche girls. In Chapter 3 I use longitudinal, within-participant data to evaluate how energy allocation strategies change from pre- to post-menarche. In Chapter 4 I examine sleep as an energy regulation mechanism during adolescence. The first study indicates that energy storage, dependent on HPAA activation, is associated with variation in menarche timing. These results coincide with the association between percentage of somatic growth attained and variation in menarche timing. The second study shows that, while HPAA activation continues to promote energy uptake and mobilize energy from storage as girls transition from pre- to post-menarche, pre-menarche girls have higher energy uptake, while post-menarche girls have higher levels of energy storage, likely reflecting a decrease in energy demands of growth with maturation. The third study suggests that low levels of energy storage prompt increases in sleep quality, to recuperate energy used during the pre- to post-menarche transition. Taken together, these findings suggest that biological mechanisms, including HPAA activation and sleep, modulate metabolic energy to support reproductive maturation across the pre- post-menarche transition. These studies advance our understanding of potential pathways shaping complex developmental processes during adolescence.

**Keywords**: Adolescence; reproductive maturation; menarche; hypothalamic-pituitary-adrenal axis; metabolic energy; sleep

# Dedication

“That is so adolescent. – But we are adolescent’ – Grease (1978)

This dissertation is dedicated to all the Kaqchikel adolescent girls who were involved in this project.

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Although my name appears as the sole author is this dissertation, it could not have been done without the enormous amount of support I received on all aspects of this dissertation.

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# List of Acronyms

| | |
|---|---|
| **ACTH** | Adrenocorticotropic Hormone |
| **ADHD** | Attention Deficit Hyperactivity Disorder |
| **ATP** | Adenosine Triphosphate |
| **BMI** | Body Mass Index |
| **BMR** | Basal Metabolic Rate |
| **COPE** | Consequences of Peri-conceptional Events (study) |
| **CRH** | Corticotropin-Releasing Hormone |
| **CRP** | C-reactive protein |
| **DAG** | Directed Acyclic Graph |
| **DHEA** | Dehydroepiandrosterone |
| **E1G** | Estrone-3-glucuronide |
| **EEG** | Electroencephalogram |
| **ELISA** | Enzyme-Linked Immunosorbent Assay |
| **FMU** | First Morning Urine |
| **FSH** | Follicle-Stimulating Hormone |
| **GnRH** | Gonadotropin-Releasing Hormone |
| **GR** | Glucocorticoid receptor |
| **hCG** | Human Chorionic Gonadotropin |
| **HPAA** | Hypothalamic-Pituitary-Adrenal Axis |
| **HPGA** | Hypothalamic-Pituitary-Gonadal Axis |
| **IL-6** | Interleukin-6 |
| **LH** | Luteinizing Hormone |
| **LHT** | Life History Theory |
| **LMICs** | Low- and middle-income countries |
| **NREM** | Non-Rapid Eye Movement |
| **PdG** | Pregnanediol Glucuronide |
| **REM** | Rapid Eye Movement |
| **RMR** | Resting Metabolic Rate |
| **SER** | Society, Environment and Reproduction (study) |
| **SES** | Socio-economic status |

# Glossary

| | |
|---|---|
| **Adiponectin** | A protein hormone produced by white adipose tissue, inversely related to visceral adipose fat mass; higher levels indicate lower fat storage. |
| **Adolescent transition** | The transitional period from childhood to adulthood, characterized by changes in somatic growth patterns, secondary sexual characteristics, sleep patterns, reproductive maturation and hormonal profiles. |
| **Adrenarche** | The maturation of the adrenal glands, which begins between 6-8 years of age. |
| **Anovulation** | The absence of an ovulation. |
| **Basal metabolic rate (BMR)** | The amount of energy being used to maintain metabolic tasks when an organism is at rest. |
| **C-peptide** | A segment of proinsulin released in 1:1 equimolar amounts with insulin, used as a proxy for insulin levels. |
| **Corpus luteum** | The remainder of the ruptured follicle that develops after ovulation and releases progesterone. |
| **Cortisol** | A glucocorticoid hormone released by the adrenal glands as part of the hypothalamic-pituitary-adrenal axis response to physiologic stress |
| **Enzyme-linked immunosorbent assays (ELISAs)** | A laboratory method used to measure biomarkers. |
| **Fat oxidation** | The release of free fatty acids from adipose fat stores. |
| **Follicular phase** | The phase of the menstrual/ovulatory cycle during which estrogen levels increase and build up the endometrium, and FSH stimulates the growth and recruitment of ovarian follicles. |
| **Glucocorticoids** | Hormones, including cortisol, that are released as part of HPAA activation. |
| **Gonadarche** | The development and maturation of the hypothalamic-pituitary-gonadal axis. |
| **Gynecological age** | The time since age at menarche. |
| **Hypothalamic-pituitary-adrenal axis (HPAA)** | A neuroendocrine system that is activated in response to physiologic stress, and regulates many physiological processes. |

| | |
|---|---|
| **Hypothalamic-Pituitary-Gonadal Axis (HPGA)** | A system involved in regulating reproductive function and development, including the production of gonadal steroids such as estrogen and progesterone. |
| **Insulin Resistance** | A condition of decreased insulin sensitivity, in which cells become less responsive to insulin. |
| **Life History Theory (LHT)** | A theoretical framework that explains how organisms allocate energy between growth, maintenance, and reproduction. |
| **Lipolysis** | A metabolic process in which triacylglycerol breaks down into either glycerol or free fatty acids. |
| **Luteal phase** | The phase of the menstrual/ovulatory cycle that begins after ovulation, during which the corpus luteum releases progesterone. |
| **Menarche** | The first menstrual bleed in girls. |
| **Metabolic energy** | The energy available for an organism's physiological processes. |
| **Non-REM (NREM) sleep** | A phase of sleep characterized by lower brain activity, further divided into light and slow-wave stages. |
| **Ovulation** | The release of an egg from an ovary, triggered by an increase in luteinizing hormone, |
| **Ovulatory cycle** | A menstrual cycle in which ovulation occurs. |
| **Peak Height Velocity** | The fastest rate of growth in height stature during adolescence. |
| **Pubarche** | The development of pubic hair and other secondary sex traits. |
| **Rapid Eye Movement (REM) sleep** | A phase of sleep characterized by faster brain waves and higher neural activity. |
| **Resting metabolic rate (RMR)** | Similar to BMR, the amount of energy used by the body at rest. |
| **Sleep architecture** | The organization of NREM and REM sleep phases during a sleep cycle. |
| **Somatic Growth** | The physical growth and development of the body during adolescence. |
| **Thelarche** | The onset of breast development during puberty. |
| **Visceral Adipose Fat Mass** | Fat stored within the abdominal cavity. |

# Chapter 1. Introduction

# Overview of adolescence

The adolescent transition is defined as the transitional period from childhood to adulthood, in which a shift from somatic growth and development towards reproductive function occurs. This transition is characterized by changes in somatic growth patterns, secondary sexual characteristics, sleep patterns, and hormonal profiles, including changes in cortisol, a stress hormone associated with metabolic energy allocation, and gonadotrophins and gonadal steroids associated with reproductive maturation (Belsky, 2012; Ellison, 2017; Ellison et al., 2012; Stearns & Koella, 1986).

## Adolescent transition stages

The adolescent transition (Figure 1-1) for human females (hereafter referred to as 'girls') is preceded by adrenarche, the maturation of the adrenal glands, which begins between 6-8 years of age (Ellison, 2017). The adrenal glands secrete androgen dehydroepiandrosterone (DHEA), which triggers the development of pubic hair, changes in body odour, and other secondary sexual traits that occur during later stages of maturation, such as during pubarche (Ellis & Essex, 2007). Thelarche, defined as breast budding, follows adrenarche and marks the true beginning of adolescent development between the ages of 8 and 13 years old (Moodie et al., 2020; Parent et al., 2003). The average age of thelarche is between 8.9-10.9 years among girls from Western, post-industrial countries (Parent et al., 2003), and 10.4 years among girls from low- and middle-income countries (LMICs) (Moodie et al., 2020). Gonadarche, defined as the development and maturation of the hypothalamic-pituitary-gonadal axis (HPGA), occurs simultaneously with thelarche, on average between the ages of 9-10 years (Ellis, 2004). The onset of HPGA maturation is characterized by increases in the production of reproductive hormones. An increase in pulsatile gonadotrophin-releasing hormone (GnRH) prompts the production and secretion of gonadotrophins (follicular-stimulating hormone (FSH) and luteal hormone (LH)), which leads to increases in gonadal steroid production (estrogen, progesterone) (Huhmann, 2020; Martin & Valeggia, 2018; Sharma et al., 2016).

Somatic growth rates, such as peak height velocity (the peak in linear height growth rate) and skeletal maturation, including an increase in bone mass, bone mineral density, and pelvic widening, increase towards the latter end of thelarche (Gomula et al., 2024;

Sharma et al., 2016). As somatic growth progresses, the development of pubic hair and other secondary sex traits as part of pubarche occurs. The onset of menarche occurs once the growth and maturation stages described above have either slowed (i.e., peak height velocity) or have progressed (i.e., pubarche). Menarche is an important marker of maturation, as it reflects ovarian function and thus further progression of HPGA development.

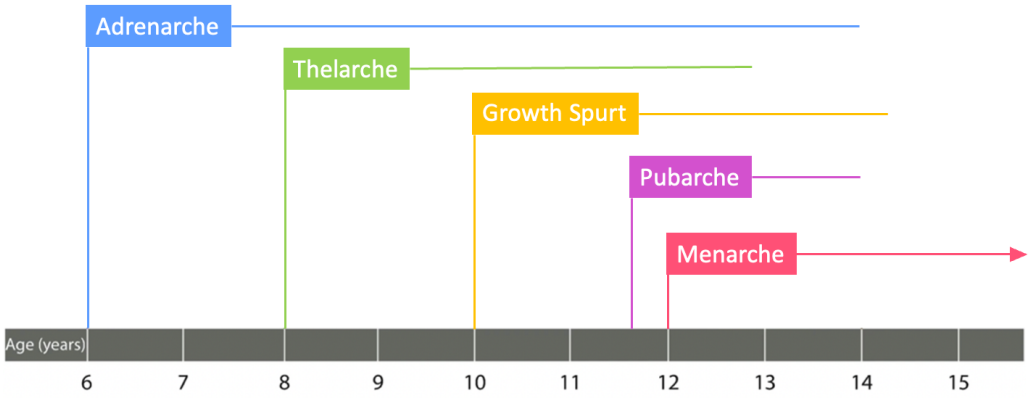


**Figure 1-1:** Maturation stages of the adolescent transition among girls (Mathur, 2022)

## Menarche and the menstrual-ovulatory cycle

Menarche is defined as the first menstrual bleed in girls, and occurs towards the latter end of the adolescent transition. Evidence suggests that menarche occurs 2-2.5 years after the onset of thelarche (Cabrera et al., 2014) and once other developmental milestones have commenced or have been established, such as breast development, skeletal maturation, and other secondary sexual characteristics (Ellis, 2004). In high-income, Western countries, where most of the data on age at menarche is generated, the average age at menarche is between 12-13.5 years old (Cabrera et al., 2014; Hvidt et al., 2019; Papadimitriou, 2016; Parent et al., 2003). Pooled age estimates from several studies in LMICs report the average age of menarche is 12.3 years old (Moodie et al., 2020), with significant variation occurring between populations. For example, in certain populations in Sub-Saharan Africa, age at menarche was estimated at 13.8 years, whereas age at menarche in the Western Pacific Region was 11.8 years (Moodie et al., 2020).

*Characteristics of the ovulatory cycle*

When sufficient levels of gonadotrophins and gonadal steroids are produced and secreted, an ovulatory cycle occurs. Menstrual/ovulatory cycles are characterized by two phases: the follicular phase and luteal phase. Throughout the follicular phase, estrogen levels gradually increase, building up the endometrium (uterine lining), and an increase in follicle-stimulating hormone (FSH) stimulates the recruitment and growth of a few ovarian follicles. A surge in luteinizing hormone (LH) levels triggers the dominant follicle to rupture, resulting in ovulation, the release of an oocyte (Bull et al., 2019). After ovulation occurs, the luteal phase begins, during which the remainder of the ruptured follicle develops as the corpus luteum. The corpus luteum releases progesterone, which maintains the endometrium in preparation for the potential implantation of a fertilized oocyte (Bull et al., 2019). If implantation occurs, the embryo produces human chorionic gonadotropin (hCG), which maintains the endometrium and promotes placentation. If implantation does not occur, the corpus luteum disintegrates, causing a drop in progesterone levels and subsequent breakdown of the uterine lining – i.e., menstruation – marking the start of the next follicular phase (Bull et al., 2019).

Menstrual cycle length is highly variable among adolescents. Most cycles range from 20-45 days, and menstrual bleeding lasts on average 2-7 days among the majority (80-90%) of adolescent girls (Adams Hillard, 2008). There is a broader range of cycle length observed within and among girls who are in the earlier stages of their transition (Adams Hillard, 2008), and this is often attributed to anovulation – the absence of an ovulation (Adams Hillard, 2008). Cycles generally become more regular with gynecological age, the time since age at menarche, as girls advance through their transition and reach reproductive maturity (DiVall & Radovick, 2008). The end of the transition is generally marked by stable ovulatory cycles within an individual (Adams Hillard, 2008). Most individuals' normal cycle lengths are established by their 5th to 6th gynecological year (Adams Hillard, 2008). However, cycle length, frequency, and regularity are highly variable, even among women who are reproductively mature (Jasienska & Ellison, 2004).

### Secular trends in menarche timing

In general, the average age at menarche has gradually declined over time in both low-middle- and high-income countries. Menarche was estimated to occur between 7-13 years of age during the paleolithic period, based on paleoanthropological data (Papadimitriou, 2016). It then increased to an average age of 15-16 years around the time of the industrial revolution. Since then, the average age at menarche has decreased to around 12-13.5 years of age (Papadimitriou, 2016).

The gradual global decline in age at menarche is proposed to be the result of improved living conditions and access to nutrition and healthcare, thus having a positive effect on girls being able to achieve optimal growth and development earlier. Studies evaluating age at menarche among populations of different socio-ecological contexts, reflecting differences in factors such as access to nutrition and improved hygiene, find significant differences in age at menarche. In a study conducted by Marván and colleagues (2020), findings suggest that in the last 80 years in Mexico, overall age at menarche has declined, but with significant discrepancies in Indigenous versus non-Indigenous, urban versus rural, and low versus high socio-economic status (SES) girls (Marván et al., 2020). Other studies have also reported declines in age at menarche from the 1800s to 1950s, with a greater trend in earlier age at menarche observed in developed, compared to developing, countries (Adams Hillard, 2008). Accelerated or delayed timing of developmental outcomes, such as menarche, can reflect adaptations in response to different environmental contexts, which can be understood through the application of life history theory.

## Theoretical Frameworks

*Life History Theory and the adolescent transition*

According to life history theory (LHT), when energy is limited, organisms face continuous trade-offs in energy allocation between growth, maintenance, and reproduction (Chisholm et al., 2005; Stearns & Koella, 1986). These trade-offs can result in energy being withdrawn from lower priority tasks, such as reproduction, and invested in response to urgent socio-ecological challenges such as poor nutrition, high mortality, or infectious diseases (Ellison et al., 2012; Stearns, 1976).

During the adolescent transition, a critical life history transition, girls are still investing in growth and development, but they are also beginning to invest in reproductive function. It is possible that energetic trade-offs imposed by socio-ecological challenges may affect physical and psychosocial development over the adolescent transition, including the onset, timing, pace, and quality of reproductive maturation (Belsky, 2012; Ellison, 2017; Mishra et al., 2009; Stearns et al., 2000; Stearns & Koella, 1986). In this dissertation I evaluate the role of energy regulation mechanisms across the adolescent transition, to better understand the biological pathways through which environmental contexts can affect development, which I ground in life history theory (LHT).

Accelerated or delayed life history strategies reflect adaptations in response to different environmental contexts. Risky environments are associated with accelerated life history strategies and appear to affect the timing of growth and development (Griskevicius et al., 2011). In contexts of high mortality, accelerated reproductive development has been proposed to be an adaptive strategy, where individuals who are at risk of dying at an earlier age maximize their chances of reproducing before dying by developing faster, maturing earlier, and engaging in sexual activity earlier (Belsky, 2012; Chisholm et al., 2005). This pattern has been observed in both human and non-human populations. For example, Szekely and colleagues (2017) studying the effects of different stressors on tadpole development, reported that tadpoles exposed to different water level treatments had different rates of growth and metamorphosis. Tadpoles exposed to low or fast drying water levels went through metamorphosis faster and had resulting smaller body sizes, as the risk of losing their aquatic environment was greater than tadpoles in stable high-water environments who demonstrated slower growth rates, later age at metamorphosis, and larger adult body sizes (Crespi & Denver, 2005; Székely et al., 2017). In this example, tadpoles were able to adopt a shorter developmental period when there was a perceived risk to the environment in which they were developing. Similarly, experimental treatments of higher extrinsic mortality led to a phenotypic change in fruit flies and a shift towards earlier development and faster life history strategies (Stearns et al., 2000).

In humans, exposure to high risk or high mortality environments can also lead to earlier and faster reproductive development (Chisholm et al., 2005). Exposure to high rates of violent crime, which are directly associated with high mortality rates and, thus, infer a risky environment, are associated with earlier life history strategies, including earlier age at first birth (Griskevicius et al., 2011). Contexts that lead to earlier and faster

development are often associated with exposure to early childhood adversity, unstable or disrupted early family environments, and lack of parental support during childhood (Ellis, 2004; Ellis & Essex, 2007; Henrichs et al., 2014). Magnus and colleagues (2018) reported that specific types of childhood adversity, such as sexual abuse, were associated with earlier age at menarche (Magnus et al., 2018). Other study findings are consistent with these results, and further report that compounding effects of adversities, such as neglect, family violence, parental substance use, and physical and sexual abuse, significantly increase the odds of earlier age at menarche (Henrichs et al., 2014). Early childhood exposure to adversities may signal risk in the environment. From an LHT perspective, adversity experienced during childhood accelerates maturation as a developmental strategy, which is often associated with earlier age at first birth. Biological mechanisms that accelerate reproductive maturation may be an adaptive trait that increases the chance of reproducing in high mortality-risk environments (Chisholm et al., 2005; Lawn et al., 2020).

Conversely, under conditions of poor resource availability but lower risk of mortality, it may be more advantageous to delay reproductive maturation and slowly invest in growth compared to faster growth and earlier reproductive maturation in higher resource availability contexts. Significant evidence suggests that low socioeconomic status (SES), which is often associated with contexts of low resource availability but can be lower mortality risk, is associated with a later age at menarche. Overall, girls with high SES reach menarche at an earlier age, compared to girls with lower SES (Khah et al., 1995; Marván et al., 2020; Wronka & Pawlińska-Chmara, 2005). Many factors associated with SES may impact the onset, timing, and pace of development. SES is strongly associated with lifestyle factors such as nutrition availability, dietary habits, access to healthcare, parental education and income, parental support, and other variables that may impact an individual's context in which they live, and, thus, play a role in mediating their developmental trajectory (Wronka & Pawlińska-Chmara, 2005). For example, girls who experience poor nutrition or food scarcity, within lower mortality risk environments, exhibit significantly delayed reproductive maturation of up to 3-4 years and have overall smaller body sizes compared to girls with adequate and stable resources (Stearns & Koella, 1986). This delayed development and smaller body size may be adaptive to maximize survival in conditions of low food access, as it takes longer to accrue enough resources to invest in reproductive function (Stearns & Koella, 1986; Tahirovicâ, 1998).

Disparities associated with lower SES may be driving variation in age at menarche among populations. Indigenous women in Mexico experience lower SES than non-Indigenous women, and their mean age at menarche is not only later than non-Indigenous women, but has actually been increasing over time, which is in contrast to global trends (Marván et al., 2020). Insufficient access to nutrition, adequate health care, and sanitation are all more prevalent among Indigenous women in Mexico, which may be affecting their transition from prioritizing growth to reproduction (Marván et al., 2020).

While LHT predicts that contexts of high mortality would lead to earlier development, and low mortality would lead to later development, with energetic stress contributing complexity to these predicted relationships, variation in the timing of these exposures can result in significant variation in the timing and pace of maturation. As LHT would predict, Amir and colleagues (2016) report that perceptions of danger in the environment were associated with an earlier age at menarche, yet this association was examined in addition to poor access to economic resources (Amir et al., 2016). While they did not examine access to nutrition specifically, economic resource access may shed light on how, in this example, risk overrides the consequences of access to energetic availability on the pace of development. However, several studies evaluating young girls' exposure to high mortality, such as contexts of war, have reported a later mean age at menarche and a slower pace of development (Ivka Prebeg et al., 2000; Tahirovicâ, 1998). These conflicting results may be related to maturation thresholds - a minimum size, stage, or state (such as metabolic energy state) that an individual much reach before they can commence a life history transition (Nilsson-Örtman & Rowe, 2021; Stearns & Koella, 1986). The timing of a risky exposure in relation to these maturation thresholds, or the predictability of the high-risk period, influences the variation in delayed or accelerated transitions (Nilsson-Örtman & Rowe, 2021). Exposures, such as limited nutrition availability, that occur before an individual has met a minimum size threshold may delay the transition, as it will now take longer to achieve that critical size for entering the transition. In contrast, an exposure that occurs after a threshold has been met may accelerate maturation. The temporal relationship between risk and energetic challenges is thus an important scenario to consider. Given the discrepancy of age at menarche in different risk contexts, more research is needed on maturation thresholds in terms of size, developmental stage, or metabolic energy state among humans, in combination with risk perception.

The accelerated or delayed onset of reproductive development as a response to risk may reflect a trade-off with other components of growth. For example, pelvic growth may slow down if energy is re-allocated towards reproductive function and, as a result, a girl's pelvis may be smaller than her potential final pelvic size as she reaches reproductive maturation and achieves regular ovulatory cycles (Malabarey et al., 2012; Sharma et al., 2016). Reaching reproductive maturation early but at the cost of an underdeveloped pelvis can lead to significant health risks, including a higher risk of complications during pregnancy (Malabarey et al., 2012). Yet this trade-off between reproductive maturation and pelvic development may be considered adaptive if it means that an individual increases their chance of reproducing before dying. Another example of a trade-off between growth and reproduction was found when girls who reached menarche at a later age were taller than those who reached menarche at an average age, suggesting that the former continued to invest in growth for a longer period of time while slowly allocating energy towards reproductive function (Sheppard et al., 2016). Trade-offs between growth and reproduction are mediated by the amount of energy available to an individual, and the contexts of risk as described above may influence the proportion of that energy that is allocated to reproduction.

Yet, how these socio-ecological contexts drive variation in age at menarche is still not fully understood. Much of the research testing factors that would theoretically influence age at menarche have done so by evaluating associations between environmental stressors and menarche age, without necessarily examining the biological pathways through which these socio-ecological contexts lead to developmental outcomes. Thus, I contribute important information on how these associations may be biologically linked to age at menarche. I examine changes in metabolic energy across the transition, and examine two different biological mechanisms, the hypothalamic-pituitary-adrenal axis and sleep, that have been proposed to play a role in mediating energy allocation and recuperation.

# Metabolic energy and energy regulation mechanisms

## Metabolic energy during adolescence

The adolescent transition represents a shift in energy allocation from growth to reproductive function. During this transition, patterns in energy storage and expenditure

shift to meet the concurrent energetic requirements of growth and reproductive maturity, including concurrent skeletal growth and other somatic development processes. In a review conducted by Cheng and colleagues (2016), there was consistent evidence across several studies showing higher absolute basal metabolic rate (BMR) and resting metabolic rate (RMR) in adolescents who were in later stages of their transition (i.e., post thelarche, and are both growing and maturing reproductively) compared to adolescents who had not yet reached thelarche (who are only growing) (Cheng et al., 2016). An increase in BMR and RMR reflects these increased energy needs, as they reflect the amount of energy being used to maintain metabolic tasks when an organism is at rest.

As girls progress towards reproductive maturity, they tend to show increases in energy uptake and storage levels. Insulin secretion patterns, facilitating glucose uptake into tissue cells either for immediate use or long-term storage in adipose fat, change during the adolescent transition (Böttner et al., 2004; Emery Thompson & Knott, 2008). Adipose fat storage also appears to increase in girls as they advance towards reproductive maturity, with higher levels of overall fat mass observed among girls who have reached the end of their transition compared to pre-menarche girls (Ellison, 2017; O'Keeffe et al., 2020; Reiches et al., 2013). These changes in metabolic energy uptake and storage patterns are proposed as strategies that support increased energy investment in reproductive maturation.

Variation in energy availability has been proposed as a key factor impacting variation in the onset, timing, and pace of the transition. Variation in energy availability is often a reflection of environmental and socio-ecological conditions. In contexts of high energy availability, individuals have, on average, an earlier onset of reproductive development. In girls specifically, adiposity gained during childhood and higher levels of fat mass pre-transition are associated with earlier onset and timing of reproductive development, including earlier age at thelarche and menarche (Calcaterra et al., 2021; O'Keeffe et al., 2020). Findings suggest that overweight girls reach thelarche at a significantly earlier age compared to girls within normal BMI ranges, with an average of 8.4 years compared to 10.4 years, respectively (Moodie et al., 2020). Higher BMI during childhood is also associated with an earlier age at menarche (Le-Ha et al., 2018; Moodie et al., 2020).

In contexts of low energy availability, girls typically reach reproductive development at a later age and go through their transition at a slower rate. Low energy availability may

delay maturation, as there are insufficient energy levels available to invest in reproductive development and function while simultaneously supporting somatic growth, including growth in height stature, bone mass and bone mineralization accrual (Huhmann, 2020; Reiches et al., 2013). Adolescents diagnosed with anorexia nervosa, exhibiting extremely low adipose fat stores, are at risk of poor bone mass accumulation, which coincides with a later age at menarche (15-16 years old) (Huhmann, 2020). During the transition, inadequate energy availability leads to hypothalamic suppression, leading to lower levels of gonadotrophins and gonadal steroid production and secretion, exacerbating the delayed onset of reproduction and slower development of secondary sexual characteristics (Huhmann, 2020).

High energy expenditure also leads to variation in the timing and onset of maturation. Adolescents who experience high levels of energy expenditure, such as high-level athletes that engage in excessive exercise, may be in chronic negative energy balance and are at risk of delayed maturation. Under conditions of high energy expenditure, adolescent girls cannot meet adequate energy levels needed to support the development of the HPGA concurrently with somatic growth (Huhmann, 2020). As a result, this leads to a delay in HPGA maturation and, subsequently, lower levels of gonadal steroid production (Kapczuk, 2017). This longer and slower period of reproductive development may reflect trade-offs in energy allocation between growth and reproductive function. Maintenance and growth are prioritized in this context of negative energy balance, as reproductive function can be delayed until improved conditions and sufficient energy is met.

## The hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal axis (HPAA) is involved in regulating metabolic energy allocation, and as such mediates variation in the timing of reproductive developmental outcomes, as described above. The HPAA regulates individuals' physiological responses to socio-ecological challenges by mediating energy allocation between physiological processes that support responses to those challenges in addition to maintenance, growth, and reproductive function (Chisholm et al., 2005). The HPAA regulates metabolic energy strategies through the interaction of glucocorticoids, cortisol in humans, with glucocorticoid receptors located in the hypothalamus, pituitary, and

ovaries, and other tissues such as muscle, skeletal, and adipose tissue (Comuzzie et al., 2001; Worthman, 2003).

With HPAA activation, corticotrophin-releasing hormone (CRH) is released by the hypothalamus. CRH secretion triggers the release of adrenocorticotrophin hormone (ACTH) from the anterior pituitary, which, in turn, triggers the release of glucocorticoids, such as cortisol in humans, by the adrenal glands (Joseph & Whirledge, 2017). Cortisol mediates insulin secretion to aid in promoting uptake of glucose from the bloodstream into tissue cells, and induces the release of free fatty acids from adipose fat stores, which provides energy to tissues involved in responding to said physiological needs (Di Dalmazi et al., 2012; Nepomnaschy et al., 2011). However, how the HPAA mediates metabolic energy allocation across the adolescent transition to meet the energetic requirements at different stages of maturation remains unclear.

## Sleep

Sleep is another critical energy regulation mechanism that plays a role in energy availability and allocation (Schmidt, 2014; Schmidt et al., 2017). In humans, a regular sleep cycle is comprised of two phases: the REM phase (Rapid Eye Movement) and the Non-REM phase (NREM). The NREM phase is further broken into two stages: the light sleep stage, which has a lower arousal threshold and is easier to wake up from (higher electroencephalogram (EEG) activity), and the slow-wave sleep stage, which has a deeper arousal threshold (i.e., is more difficult to wake up from) (lower EEG activity) (Carskadon & Dement, 2017; Samson, 2021). The REM phase typically has faster waves, a higher active neural pattern and faster electrocortical activity. Sleep patterns typically cycle between the NREM and REM phases, with increasing time in REM happening later in the night or further into the sleep period (Carskadon & Dement, 2017).

Sleep is involved in metabolic energy recuperation and conservation by prompting changes in metabolic rate (energy utilization) during different sleep stages (Kayaba et al., 2017; Nicolaides et al., 2000; Schmidt et al., 2017). Specifically, during slow-wave sleep phases, or deep sleep, metabolic rate is reduced which reduces energy expenditure, thus supporting greater energy conservation (Schmidt et al., 2017). Sleep supports traits that are imperative for survival, maintenance, reproduction, and development, such as tissue growth and repair, cognitive function, behavioural and

emotional regulation, immune function, and efficient metabolism (Samson, 2021). To support said processes, sleep induces the secretion of growth hormone, which is especially important during periods of adolescent growth and maturation (Olarescu, 2019; Redwine et al., 2000). Growth hormone release is highest during slow-wave sleep, or deep sleep, periods and promotes tissue growth, and, specifically during adolescence, bone and cartilage growth (Kim et al., 2015). Humans exhibit shorter sleep cycles compared to other mammals and primates, yet they also present more intense and deeper sleep patterns compared to other mammals and primates. It has been proposed that the trade-off of shorter and more flexible but deeper sleep patterns enables more opportunities for developing skills, gaining and enhancing knowledge, increasing reproductive mating, and building stronger social and community networks (Samson, 2021).

Sleep deprivation and low-quality sleep are linked to increased risk of disease and poor health outcomes. Sleep disturbances, low-quality sleep, and chronic poor sleep among adolescents are associated with increased risk of disruption in cognitive processing and function, memory consolidation, increased risk of conditions such as ADHD, and other poor mental health outcomes including anxiety and depression (Brand & Kirov, 2011; Shochat et al., 2014). Low quality and low quantity sleep during adolescence are also associated with increased risk of cardiometabolic disease, obesity, poor cognitive development, psychosocial dysregulation, and hormone dysregulation (Shochat et al., 2014). However, in their systematic review, Shochat and colleagues (2014) point to the need for a better understanding of the underlying mechanisms, mediating factors, and causal pathways between sleep and adolescent health and development. Further, they highlight that to explore these pathways, it is important to better understand what constitutes, or what drives the sleep needs of adolescence (Shochat et al., 2014).

*Sleep and the HPAA*

The HPAA is intricately linked with sleep architecture (i.e., organization of NREM and REM sleep phases). Cortisol's normal circadian rhythm is linked to the sleep and wake patterns of the sleep cycle (Buckley & Schatzberg, 2005). Cortisol levels increase two to three hours after initial sleep onset, and continue to rise across the sleep period. A peak in cortisol (the cortisol awakening response) occurs 30 minutes to one hour after awakening. Evidence suggests that the relationship between HPAA activation and sleep

is bidirectional. An increase in cortisol secretion is linked with an increase in EEG frequency, which is associated with wakefulness and leads to less sleep or increased periods of awakening throughout the sleep period (Buckley & Schatzberg, 2005; Nicolaides et al., 2000). Sleep deprivation is proposed to increase HPAA reactivity, while increases in sleep are reported to have an inhibitory effect on cortisol secretion (Buckley & Schatzberg, 2005). This bidirectional pathway should prompt important considerations for how the HPAA and sleep may be functioning synergistically as energy regulation mechanisms to support adolescent development, which, to our knowledge, has not yet been examined.

## Rationale

Variation in maturation patterns during the adolescent transition can have significant implications. Earlier or later age at menarche, reflecting very early or very late maturation of ovarian function, is associated with reproductive risks such as earlier age of first sexual intercourse and first birth, and infertility, as well as an increase in cardiometabolic and mental health risks (Elks et al., 2013; Herva et al., 2004; Nettle et al., 2011; Warp et al., 2024), which can persist across the lifespan. It is, thus, important to better understand the biological mechanisms through which socio-ecological contexts, such as stress and inequities as described above, alter developmental outcomes that may contribute to inequitable reproductive and health outcomes throughout adulthood.

Improving our understanding of the biological mechanisms that mediate how environmental exposures can lead to health inequities, especially during critical windows of susceptibility across development, can be used to inform population and systems-level change to support optimal health and development during adolescence. In this dissertation I aim to contribute knowledge on how biological mechanisms function to potentially mediate variation in reproductive outcomes during the adolescent transition.

## Aims and Objectives

The central aim of this dissertation is to contribute knowledge on how HPAA activation and sleep act as biological mechanisms that regulate metabolic energy across the adolescent transition among girls (Figures 1-2 and 1-3). I evaluate several hypotheses and test predictions contributing to these hypotheses that are derived from the literature

on energetic trade-offs between growth and reproduction. **Objective 1:** Evaluate pre-menarche factors associated with variation in the timing of menarche; **Objective 2:** Evaluate metabolic energy changes within girls across their pre- to post-menarche transition; and **Objective 3:** Consider sleep's role in energy restoration and conservation across the transition. The analyses conducted as part of each of these objectives, and the selection of predictor and response variables for each set of analyses, were informed by a Directed Acyclic Graph (DAG) (Figure 1-3). The relationships between variables outlined in the DAG were guided by a conceptual model of how these variables are related (Figure 1-2).

## Conceptual model and Directed Acyclic Graph (DAG)

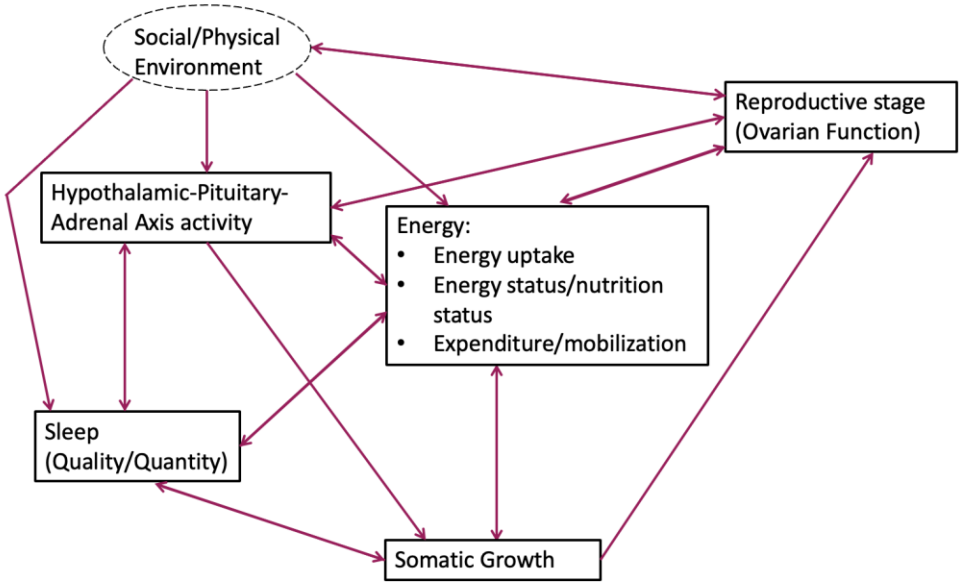


**Figure 1-2:** Conceptual model describing the relationships between variable constructs

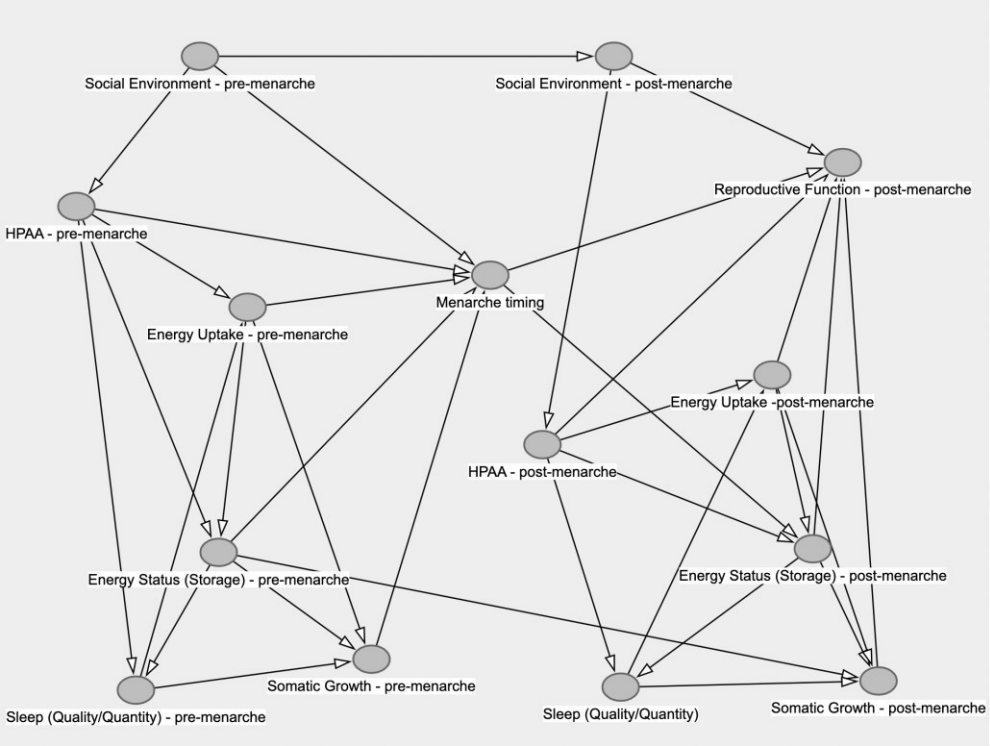


**Figure 1-3:** Directed Acyclic Graph (DAG) of relationships among variables

# Research methods overview

## The Consequences of Peri-conceptional Events (COPE) study

The three studies presented in this dissertation are part of a larger, longitudinal, naturalistic cohort study – the Consequences Of Peri-conceptional Events (COPE). COPE began in 2000 and has followed the life histories of Indigenous Kaqchikel Mayan girls since their conception. Participants live in two rural communities in the highlands of Guatemala, located on the north shore of Lake Atitlán, Guatemala, and are under the jurisdiction of the VII Health Region of the Sololá Department (Nepomnaschy et al., 2004).

The Kaqchikel communities in which these studies are based vary less with respect to genetics, social and cultural practices, socio-economic status, and lifestyle factors such as diet, physical activity, and tobacco and alcohol use, compared to people living in more urban or high-income settings (Barha et al., 2019). The majority of families in the

community live traditional, semi-subsistence lifestyles. The local economy is based primarily on small-scale agriculture, construction, and tourism and hospitality. Many families own or lease land, and produce corn, beans, coffee, fruit, and vegetables (personal communications). People's diets also consist of some chicken, meat, and freshwater fish. However, within the last 10 years, access to more economically accessible, packaged foods such as candy, chips, soda, and fried foods high in salt and fat has increased substantially, and consumption of these processed foods is now very high due to this 'market economy shift'. Social structures consist primarily of large family networks. Women and adolescent girls are primarily responsible for domestic activities, including food preparation (which are time consuming traditional practices, such as preparing corn tortillas), cooking, cleaning, caring for younger children, and textile work. Adolescent girls may also attend school or hold jobs in the hospitality and tourism sector, working in local hotels or restaurants. Daily stressors or environmental exposures that may contribute to socio-ecological challenges, and subsequently activate HPAA responses, include chronic, persistent exposure to infectious diseases or infections, poor- or low-quality energy intake leading to nutritional stress, strenuous or demanding physical activities, and exposure to psychosocial stressors such as financial stress or interpersonal conflicts with family and community members. These are all important socio-ecological conditions to consider when interpreting findings of the studies presented in this dissertation.

COPE data were collected during three field seasons: in 2013 from 21 Indigenous Kaqchikel girls (ages 10-11 years old) who were conceived as part of the original COPE study in 2000-2001; in 2017 for follow-up data from the original COPE participants (ages 14-15) and initial data for 25 additional girls (ages 12-15 years old), some of whom are sisters of the original COPE participants; and in 2023 for follow-up data from all girls (ages 18-21 years old) who participated in 2013 and/or 2017. During each field season, a team of researchers and local female field assistants collected biospecimen samples and anthropometry measures, and conducted demographic questionnaires with participants. First morning urine (FMU) samples, which provide information on hormone biomarkers of HPAA activity and metabolic energy, were collected every day except Sunday for 3 weeks, starting in January in 2013, and every other day for 4 months, starting in January in 2017 and in 2023. Anthropometry measures were collected two to four times during each field season (monthly or bi-monthly). Observational ethnographic

data was also recorded, but only considered in this dissertation for the purpose of contextualizing the findings of each paper within the lived experiences of COPE participants.

## Data sources for each study objective

For the first study, only data from participants who provided FMU samples when they were in their pre-menarche stage, and who provided anthropometry measures to evaluate progression of somatic growth, were included in this analysis. A total of 29 participants provided hormone data, anthropometry measures (including height) pre-menarche (from either 2013 or 2017), and their age at menarche. Age at menarche data collected in follow-up demographic questionnaires in subsequent field seasons (2017 and/or 2023) was used to calculate time remaining to menarche from the pre-menarche data collection period. Height data was also collected in the subsequent 2023 field season when girls were assumed to be at the end of their growth period, and was used to calculate the pace of somatic height growth by comparing girls' pre-menarche height as a proportion of their 'final' height.

For the second study, a total of 46 participants contributed pre-menarche and/or post-menarche hormone data. Twenty-nine participants provided both pre- and post-menarche hormone data to evaluate within-individual changes across the transition. The 17 participants who only participated during their pre-menarche stage (did not participate in follow-up field seasons) or post-menarche stage (i.e. began participating in COPE only after having gone through menarche) were also included in this study, to contribute additional information for inter-individual comparisons across the pre-post-menarche transition.

The third study uses a subset (n=20) of the COPE participants who participated in a collaborative and complimentary sleep project with Dr. David Samson from the University of Toronto during the 2017 field season. Participants' hormone data were matched to a 19-day sleep data collection period that began in January 2017. Participants were approximately equally distributed across three distinct maturation stages at the time of data collection. Among-participant analyses were conducted with this subset of participants, lending to a cross-sectional study design by nature.

## Structure of the Dissertation

This dissertation aims to fill gaps in knowledge on the factors that may contribute to variation in the onset, timing, and pace of the adolescent transition among girls. It does so through three independent, original studies that constitute chapters 2, 3, and 4 of this dissertation. Although the studies were designed to function as complementary chapters to make up the entirety of this project in answering questions about biological changes that occur during girls' adolescent transitions, they are also written as standalone scientific research papers. The papers appear in this dissertation as they are intended to appear as independent manuscripts published in an academic journal, with several exceptions. My supervisors and collaborators are not listed here as co-authors, but will be listed as co-authors in the manuscripts submitted for publication. I use language such as "we" throughout chapters 2 through 4 to reflect this co-authorship. As well, tables defining the same variables may appear in multiple chapters (such as definitions of metabolic energy hormones), as this information will be important for these papers as standalone manuscripts submitted for publication.

### Breakdown of Chapters

The first study, presented in Chapter 2, is titled "The hypothalamic-pituitary-adrenal axis, metabolic energy, and growth are associated with the timing of reproductive maturation in a group of Mayan adolescent girls". This study investigates factors associated with variation in the timing of menarche. It focuses on interactions between the HPAA and metabolic energy that are potentially mediating trade-offs between growth and reproductive maturation.

The second study, presented in Chapter 3, is titled "The hypothalamic-pituitary-adrenal axis and metabolic energy strategies pre- to post-menarche: A study based on a cohort of Mayan Indigenous adolescent girls". This study investigates how the HPAA mediates metabolic energy differently depending on whether girls are in their pre- or post-menarche stage, and in doing so aims to identify variation in energetic needs depending on maturation stage.

The third study, presented in Chapter 4, is titled "Sleep, hypothalamic-pituitary-adrenal axis activity, and metabolic energy patterns across the adolescent transition in a group of

Mayan girls". This study uses a subset of the cohort from study #2, who participated in the complimentary sleep project, and considers sleep as another mechanism mediating energy allocation across the pre- to post-menarche transition.

# Chapter 2. The hypothalamic-pituitary-adrenal axis, metabolic energy, and growth are associated with the timing of reproductive maturation in a group of Mayan adolescent girls

## Abstract

**Background:** Adolescence is a critical transition in which girls face energetic trade-offs between growth and reproductive development. These trade-offs can affect the onset and pace of this transition. The hypothalamic-pituitary-adrenal axis (HPAA) is a proposed to be an energy regulation mechanism, involved in mediating energy allocation between growth, maintenance, and reproduction. Yet, few studies have examined this role of the HPAA during girls' reproductive maturation.

**Hypothesis:** The HPAA modulates metabolic energy allocation between growth and reproductive maturation. We predict that energy storage levels and progress of somatic growth, reflecting how much energy is available for maturation, should be linked with the timing of menarche.

**Methods**: This study included 29 pre-menarche Mayan adolescent girls from Guatemala, who also provided data on their age at menarche. We evaluated biomarkers of HPAA activity (cortisol), energy uptake (c-peptide), energy storage (adiponectin), and measures of height growth (proportion of final height) as predictors of the time remaining to menarche.

**Results**: After controlling for age at pre-menarche and holding all other variables constant, we observed that, for each 1% increase in proportion of their final height, girls were 1.34 months closer to reaching menarche. We also observed the relationship between menarche timing and adiponectin depended on variation in cortisol. At high levels of cortisol pre-menarche, high levels of adiponectin (smaller fat stores) were associated with more time remaining to menarche relative to low levels of adiponectin (larger fat stores). At low levels of cortisol pre-menarche, high levels of adiponectin (smaller fat stores) were associated with significantly shorter time remaining to menarche.

**Discussion:** Consistent with our hypothesis, the association between time remaining to menarche and energy storage levels was a function of HPAA activity, which was also linked to the pace of somatic growth. Future studies should include the frequent collection of anthropometric data and biomarkers throughout the transition from childhood to adolescence to explore within-individual changes in HPAA activity, energy levels, somatic development, and the pace of reproduction, and draw among-individual

comparisons to further clarify the HPAA's role in modulating energy allocation between growth and reproduction.

# Introduction

Life history theory posits that organisms face continuous trade-offs in energy allocation among growth, maintenance, and reproduction (Chisholm et al., 2005; Stearns & Koella, 1986). These trade-offs can result in the withdrawal of energy from lower priority tasks, such as reproduction, to be invested in responding to more urgent socio-ecological challenges such as insufficient nutrition or infectious diseases (Ellison et al., 2012; Stearns & Koella, 1986). Adolescence, in particular, presents key energetic trade-offs between somatic growth and reproduction. These trade-offs can accelerate or delay the timing (including onset and pace) of reproductive maturation (Belsky, 2012; Ellison, 2017; Mishra et al., 2009; Stearns et al., 2000; Stearns & Koella, 1986).

In human females (hereafter referred to as 'girls'), reproductive maturation typically begins between the ages of 8 and 13 years old (Moodie et al., 2020). This transition is characterized by a relatively gradual shift from investment in somatic growth towards an investment in reproductive maturation. This process involves the maturation of the hypothalamic-pituitary gonadal axis (HPGA), including increases in gonadotropin and gonadal steroid hormone production and secretion (Huhmann, 2020; Martin & Valeggia, 2018; Sharma et al., 2016). This increase in HPGA activity induces thelarche, defined as breast budding, marking the beginning of adolescent development (Mathur, 2022; Parent et al., 2003). Thelarche is followed by pubarche, the development of pubic hair and other secondary sex traits, and the adolescent growth spurt. This growth spurt is characterized by peak height velocity, defined as the peak in linear growth rate, which generally occurs in girls between the ages of 11 and 12 (Chapter 1, Figure 1-1) (Mathur, 2022; Sharma et al., 2016).

Thelarche and pubarche are followed by menarche, the first occurrence of menstrual bleeding. Menarche typically occurs once other growth milestones have commenced or have been established. Thus, menarche is often used as key marker of reproductive maturation (Chapter 1, Figure 1-1) (Ellis, 2004). In post-industrial Western countries, where most of the research on developmental milestones has been conducted, the average age at menarche is reported between 12-13.5 years old (Cabrera et al., 2014; Hvidt et al., 2019; Papadimitriou, 2016; Parent et al., 2003). In low- and middle-income countries (LMICs), pooled age estimates of age at menarche report an average age of 12.3 years old (Moodie et al., 2020), with significant variation occurring among

populations. For example, average age at menarche was estimated at 11.8 years in the WHO Western Pacific Region compared to 13.8 years in the WHO Africa Region (Moodie et al., 2020).

Globally, the average age at menarche has declined over time, in both high- and low-income countries. Improved nutrition availability, living and hygiene conditions, and better access to healthcare are proposed as key factors in the global decline in age at menarche (Marván et al., 2020). These improved socio-structural and environmental conditions are associated with lower infectious disease risk, freeing up energetic resources to support growth and maturation (Marván et al., 2020). In a study conducted by Marván and colleagues (2020), findings suggest that over the last 80 years, age at menarche in Mexico has declined overall, but with significant differences between Indigenous versus non-Indigenous, urban versus rural, and low versus high socio-economic status (SES) populations, reflecting potential differences in socio-ecological conditions (Marván et al., 2020). Other studies also report a decline in age at menarche beginning as early as the 1800s, with this trend being more distinct in developed compared to developing countries (Adams Hillard, 2008).

Given that growth and reproductive maturation during adolescence are energetically demanding (Cheng et al., 2016), energy availability may influence the timing of reproductive maturation, including menarche. The trade-offs in energy allocation among physiological processes that support maintenance, growth, or reproductive function are hypothesized to be mediated by the hypothalamic-pituitary-adrenal axis (HPAA) (Chisholm et al., 2005; Joseph & Whirledge, 2017). HPAA secretion of glucocorticoids, including cortisol, promotes both uptake of glucose, through interactions with insulin secretion, and fat oxidation, which is the release of free fatty acids from adipose fat stores to be used as an energy source (Di Dalmazi et al., 2012; Huybrechts et al., 2014; Pou et al., 2007). During adolescence, fat oxidation is prioritized so that fat stores are used as the main metabolic energy source (Hannon et al., 2006). Evidence suggests that fat mobilized from storage changes approximately linearly with changes in cortisol levels (Djurhuus et al., 2002). Thus, the interaction between HPAA activity and energy storage is important to consider when exploring how energy storage is related to time remaining to menarche as a function of changes in HPAA activity. In this study we aim to contribute knowledge on mechanisms promoting energy availability during adolescence

and how variation in energy availability may impact the timing (pace and onset) of the transition.

We hypothesize that the HPAA modulates metabolic energy allocation between growth and reproductive maturation by shifting energy uptake and energy storage patterns, changing the amount of energy available for maturation and, thus, affecting the timing of menarche. We expect HPAA activity to increase during the adolescent transition, increasing access to metabolic energy by promoting more glucose uptake and converting more stored energy into glucose, making it available to target tissues that are critical to the transition. Thus, HPAA activation (indicated by higher cortisol levels), should interact with increased energy availability (indicated by higher c-peptide levels, a marker of insulin, and lower adiponectin levels, a marker inversely related to visceral adipose fat mass) to predict the time remaining to menarche. In conjunction with these expected HPAA-energy relationships, pre-menarche girls who have achieved greater proportions of their final somatic growth, as indicated by the proportion of final height achieved, should have a shorter time remaining to menarche. See variable definitions in Table 2-1.

# Methods

**Table 2-1:** Description of variables

| Variable name | Variable definition |
|---|---|
| Time remaining to menarche | Number of months until a participant reached menarche, calculated as the difference between age at menarche and age at which participants provided their pre-menarche samples/data |
| Pre-menarche age | Age at time of pre-menarche data collection |
| Cortisol (ng/mL) pre-menarche | Log 10 transformed measure of cortisol, which is a measure of HPAA activity; data collected every other day for 3 weeks during pre-menarche |
| C-Peptide (ng/mL) pre-menarche | Log 10 transformed measure of c-peptide, which is a measure of energy uptake activity; data collected every other day for 3 weeks during pre-menarche |
| Adiponectin (ng/mL) pre-menarche | Log 10 transformed measure of adiponectin, which is a measure of energy storage; data collected every other day for 3 weeks during pre-menarche |
| Proportion of final height | The amount of height growth a participant attained at the time of pre-menarche data collection as a percentage of their height measured at, or near, the end of their growth period |

## Study population

Our analyses are based on data from the Consequences of Peri-conceptional Events (COPE) study, a longitudinal, naturalistic cohort study derived from the Society, Environment and Reproduction (SER) Study, which began in 2000 and follows the life histories of Indigenous Kaqchikel Mayan women (Barha et al., 2019; Nepomnaschy et al., 2004). COPE follows the girls conceived by SER participants. COPE participants live in two rural communities in the highlands of Guatemala (Nepomnaschy et al., 2004). Most families from these communities live traditional, semi-subsistence lifestyles. Many families own or lease land and produce corn, beans, coffee, fruit, and vegetables, which comprise the majority of their diet (personal communications). People's diets also consist of small amounts of chicken, meat, and freshwater fish. Although families are able to produce some of their own food, food insecurity, which presents a nutritional and energetic challenge, is still present among most families. Due to a market economy shift

that has taken place over the last 15 years, access to processed, packaged foods such as candy, chips, and fried foods high in sugar, salt, and unhealthy saturated and trans fats has increased substantially due to their low cost and increased availability. Given that COPE participants are young and have lived through this nutritional transition, their diet has been a combination of traditional and processed foods which may have exposed them to nutritional challenges, including potential insufficient micronutrients and caloric surpluses.

## Data and biospecimen collection

Data used in this study were collected during three field seasons. The first part of the study was conducted in 2013 and included 21 COPE girls (aged 10-11 years old) conceived between 2000-2001, and the second, conducted in 2017, which included data from the original 21 participants (then aged 14-15) as well as data for 25 additional girls (ages 12-15), some of whom are sisters of the original COPE participants. The third part of the study was conducted in 2023, when participants were in more advanced stages or at the end of their adolescent transition (aged 18-21 years old).

Participants' reproductive maturity status was evaluated in each field season using reproductive hormone data (described below). Only participants who provided data and biological specimens in their pre-menarche stage (n=29 girls) were included in this analysis.

In the first two field seasons, 2013 and 2017, local field assistants administered questionnaires to, and collected biospecimen samples from, participants. In 2013, first morning urine (FMU) samples were collected every day for 3 weeks starting in January. In 2017, FMU samples were collected every other day over the course of four months starting in January. Participants self-collected their FMU samples in plastic, chemically inert urine collection containers. The 29 adolescent girls included in this analysis provided an average of 14.7 samples per person in 2013 and 37 samples in 2017. Aliquots (2 mL) of the FMU samples were transferred to cryo-vials and stored at -10°C in the local laboratory while in the field. At the end of each field season, all samples were shipped on dry ice to the Maternal and Child Health Laboratory at Simon Fraser University (SFU), where they are stored at -80°C.

We administered demographic questionnaires to each participant once during each of the 2013 and 2017 field seasons as well as during a third, follow-up field season in 2023; demographic questionnaires included information regarding participants' reproductive status and age at menarche. Time remaining to menarche, the difference between time at data collection in 2013 or 2017 when girls were pre-menarche and when they reached menarche, was calculated based on self-reported age at time of first bleed.

We collected anthropometry measures from participants two to three times during each field season, usually monthly or bi-monthly. Height was used as a representation of somatic linear growth. To minimize error in data collection variability, we calculated mean height from duplicate height measures collected when each participant was in her pre-menarche stage (in either 2013 or 2017). In 2023, all participants were aged 18-21 years old and presumed to be at or nearing the end of their adolescent transition, and thus we were able to measure their height during this advanced stage of maturation. Hereafter, "final height" will refer to this height measure collected in 2023, representing final or nearly final somatic height growth. We calculated the proportion of total somatic growth attained at the time of pre-menarche data collection as a percentage of this height measured in 2023.

## Hormone Assays

We quantified biomarkers (hormone concentrations) of HPAA activity and metabolic energy in FMU samples. We took the sample mean of each hormone measured over the course of each study period (2013 or 2017) for each participant. FMU cortisol levels were used to evaluate participants' basal HPAA activity (measured as urinary-free cortisol levels) throughout the previous night (Table 2-1). FMU c-peptide levels provide information on metabolic energy uptake. C-peptide is a segment of proinsulin that is released in 1:1 equimolar amounts with insulin during the conversion of proinsulin to insulin (Emery Thompson & Knott, 2008) and is, thus, a proxy for insulin levels. Higher c-peptide levels, thus higher levels of insulin, reflect more energy uptake as insulin facilitates glucose uptake from the bloodstream into cells. FMU adiponectin is used as a marker of an individual's stored energy. Adiponectin, produced by white adipose tissue, is inversely related to the amount of accumulated visceral adipose fat mass (Brochu-Gaudreau et al., 2010). Higher adiponectin levels reflect lower levels of adipose fat storage and suggest energy mobilization (Brochu-Gaudreau et al., 2010). Patterns of

urinary alpha sub-unit of follicle-stimulating hormone (FSH) and urinary conjugates of estradiol and progesterone (estrone-3-glucuronide (E1G) and pregnanediol glucuronide (PdG), respectively) were used to assess and confirm self-reported reproductive status (i.e., whether girls were pre- or post-menarche during each data collection period) (Baird et al., 1991; Kassam et al., 1996; O'Connor et al., 2006). We determined the presence or absence of ovulatory cycles by observing the dynamic profiles of all three reproductive hormones in relation to each other, following the methods developed by Baird and Kassam (Baird et al., 1991; Kassam et al., 1996).

Hormone analyses were conducted at the Maternal and Child Health Laboratory at SFU. Urinary biomarkers of HPAA activity (cortisol), energetic status (c-peptide and adiponectin), and reproductive hormones (FSH, E1G, and PdG) were measured using enzyme-linked immunosorbent assays (ELISAs) (Quansys Biosciences, Utah, USA), which our lab has previously validated (lower limits of detection for cortisol= 0.343 ng/mL; c-peptide= 0.090 ng/mL; and adiponectin = 0.023 ng/mL) (Salvante et al., 2012). To adjust for variation in dilution levels for each urine sample due to daily variation in the hydration state of participants, all hormone concentrations were corrected for specific gravity using refractometry (Miller et al., 2004). To control for variation among assays, each of the samples from the same participant was run on the same plate. All intra-and inter-assay coefficients of variation were below 7% and 11%, respectively.

## Statistical Analysis

We first inspected the data for errors. Cortisol, adiponectin, and c-peptide, which were all right-skewed, were log10-transformed to be approximately normally distributed on the logarithmic scale (Osborne, 2019). Datapoints well outside established average biological ranges, and outside the interquartile range for each variable, within and between subjects, were examined. We checked if extreme values were correlated with similarly extreme values of other hormones (suggesting an error in the sample) and if so, they were identified as outliers. All possible outliers were evaluated and, if considered suspect, were removed from further statistical analysis. A total of 9 (out of 2016 datapoints) associated with the same urine sample were identified as outliers and removed from the dataset.

To evaluate the relationships between time remaining to menarche with HPAA activity, energy uptake and storage, the interaction between HPAA and energy storage, and growth measures, we fit the following linear regression model:

*Time remaining to menarche = mean Cortisol + mean C-Peptide, + mean Adiponectin + mean Cortisol*mean Adiponectin + Proportion of final height + age.*

We also included age at pre-menarche data collection in our model to adjust for the time at which participants provided pre-menarche data in relation to when they reached menarche.

# Results

*Data description*

Descriptive statistics summarizing the sample means (standard deviation) and medians (minimum, maximum) of log10-transformed cortisol, c-peptide, and adiponectin, proportion of final height achieved, and ages at pre-menarche data collection and at menarche are described in Table 2-2.

**Table 2-2:** Data summary of hormone biomarkers[1], somatic growth (height), and age at pre-menarche data collection and age at menarche (n=29)

| Variable | Mean | (SD) | Median | [Min, Max] |
|---|---|---|---|---|
| **Cortisol (ng/mL)** | 3.44 | (0.741) | 3.63 | [1.50, 4.37] |
| **C-Peptide (ng/mL)** | 2.87 | (1.16) | 3.23 | [0.443, 4.57] |
| **Adiponectin (ng/mL)** | 0.811 | (1.47) | 1.47 | [-3.16, 2.67] |
| **Proportion of final height (%)** | 89.8 | (4.89) | 88.0 | [82.6, 100] |
| **Age at menarche** | 14 | (1.4) | 14 | [11, 17] |
| **Pre-Menarche age** | 11.6 | (1.55) | 11 | [8, 16] |

[1] *Log10-transformed cortisol, c-peptide, adiponectin*

*Model results*

Proportion of final height, the cortisol-adiponectin interaction, and pre-menarche age were all statistically associated with time remaining to menarche, after holding all other predictor variables constant (Table 2-3). Proportion of final height was negatively associated with time remaining to menarche (p=0.053); a 1% higher proportion of final

height attained was associated with a 1.37-month shorter estimated mean time remaining to menarche, holding all other variables constant (Table 2-3). The association between adiponectin and time remaining to menarche depends on cortisol levels, holding all other variables constant ($p<0.01$) (Table 2-3, Figure 2-1). At high levels of cortisol pre-menarche, the association between adiponectin and time remaining to menarche was more positive. In other words, with higher cortisol, high adiponectin levels (low fat stores) were associated with more time remaining to menarche (Figure 2-1, top right teal area) relative to low levels of adiponectin (greater fat storage) and a shorter time remaining to menarche. At low levels of cortisol pre-menarche, the association between adiponectin and time remaining to menarche was more negative. In other words, with lower cortisol levels, high levels of adiponectin (low fat stores) were associated with a shorter time remaining to menarche (Figure 2-1, bottom right orange area). Time remaining to menarche was negatively associated with age at pre-menarche data collection ($p<0.05$); a 1-year increase in age was associated with an estimated 3.7-month shorter mean time remaining to menarche holding all other variables constant. After adjusting for all other variables, there was no evidence that c-peptide was associated with time remaining to menarche.

**Table 2-3:** Estimated effects of cortisol, energy markers[1], and height on time remaining to menarche

| Model 1 | Effect estimate | 95% CI |
|---|---|---|
| **(Intercept)** | 212.352** | 69.01, 355.69 |
| **Mean Cortisol (ng/mL)** | -6.235 | -17.88, 5.41 |
| **Mean Adiponectin (ng/mL)** | -32.054** | -52.83, -11.28 |
| **Mean C-Peptide (ng/mL)** | 0.616 | -6.04, 7.27 |
| **Proportion final height** | -1.374* | -2.77, 0.02 |
| **Age pre-menarche** | -3.719* | -7.16, -0.28 |
| **Mean Cortisol*Mean Adiponectin** | 8.565** | 2.55, 14.58 |

*** p < 0.001; ** p < 0.01; * p < 0.05

[1] *Log10-transformed cortisol, c-peptide, adiponectin*

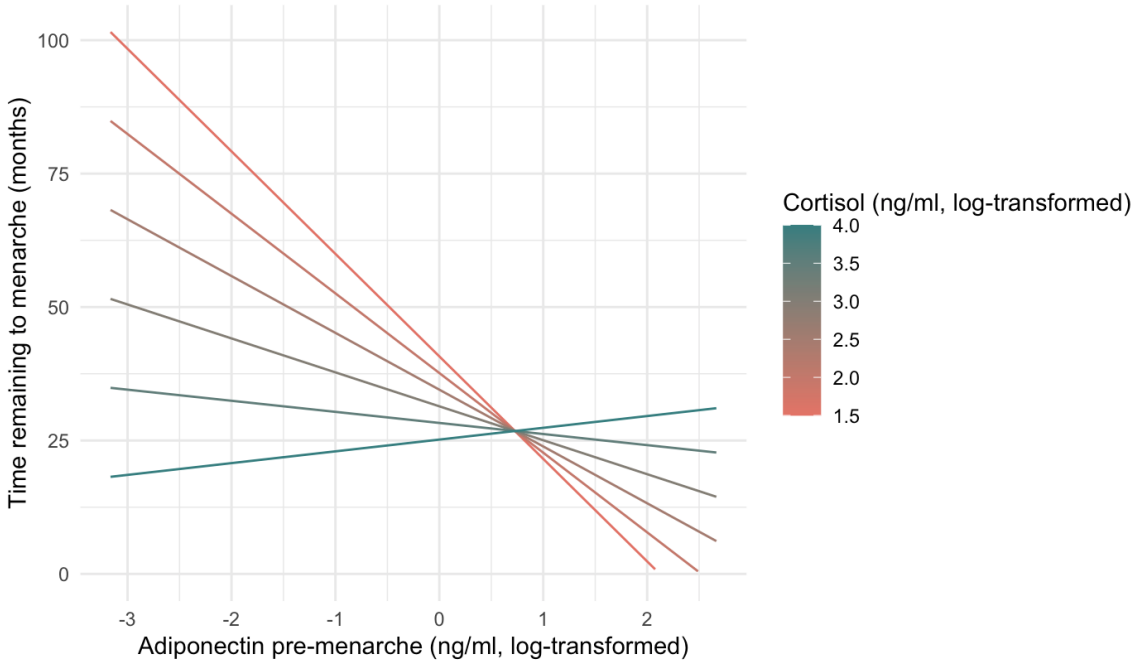


**Figure 2-1:** Select fitted regression lines showing the predicted relationship between the estimated mean time remaining to menarche and adiponectin depending on different values of cortisol, holding the mean of all other variables constant (see means in Table 2-2)

## Discussion

We examined the hypothesis that metabolic energy allocation between growth and reproductive maturation depends on HPAA activation, which will lead to shifts in energy available for maturation and, thus, variation in time remaining to menarche. To test predictions derived from this hypothesis, we examined possible associations between somatic growth, HPAA activity, metabolic energy, and time remaining to menarche among a group of Indigenous Mayan adolescent girls. We found that a higher proportion of final height attained was associated with a shorter time remaining to menarche. The association between adiponectin and time remaining to menarche depended on variation in participants' cortisol levels.

*Somatic growth and time to menarche*

The association we observed between greater proportion of final height achieved and being closer to menarche is consistent with our predictions. For every 1% increase in percentage of final height achieved, girls reached menarche on average an estimated 1.37 months sooner, holding all other variables, including pre-menarche age, constant. Our findings are consistent with reported timing of trade-offs between somatic growth and reproduction, and how they co-vary (Reiches & Ellison, 2022). Peak height velocity in adolescent girls in Western populations occurs between ages 11 and 12 when, on average, 88% of height growth has been achieved (Parent et al., 2003; Sharma et al., 2016). Pre-menarche girls in our sample had achieved a minimum of 84.8% of their final height. After controlling for age at the time of sampling, those girls who were closer to their final height had a shorter time remaining to menarche. In both Indigenous and non-Indigenous populations, menarche has been reported to occur approximately 10-18 months after peak height velocity occurs (Martin & Valeggia, 2018; Sharma et al., 2016). We interpret our findings to suggest that most somatic growth occurs prior to reproductive maturity, and these findings are consistent with the hypothesis that there is an energetic trade-off between investing in somatic development and reproductive maturation.

Bone growth and bone mineralization patterns, both of which are coordinated with height growth (McCormack et al., 2017), are promoted by increases in circulating gonadal steroids, and occur simultaneously with pelvic maturation in early and mid-transition. Estrogen plays a role in stimulating bone growth and bone mineral density acquisition; peak bone density accrual corresponds with increasing estrogen levels early in the transition (Hoyt & Falconi, 2015; Loomba-Albrecht & Styne, 2009). As skeletal maturation is achieved, estrogen used for bone growth decreases (Loomba-Albrecht & Styne, 2009). Indeed, estrogen receptor expression declines in bone cells and growth plates across the transition, suggesting estrogen plays a key role in the trade-off between growth and reproduction (Chagin & Savendahl, 2007, 2009). Our observation that girls closer to menarche had achieved a higher percentage of their final height is consistent with the proposition that during earlier stages of this transition, energy allocation is prioritized towards somatic growth over the maturation of ovarian function. These findings are consistent with the proposed interactions between skeletal

maturation and HPGA maturation mediating trade-offs between somatic growth and reproduction.

*HPAA activity, metabolic energy, and time remaining to menarche*

Our findings contribute novel insights into the HPAA's role in regulating the trade-offs described above through energy allocation during reproductive maturation. The cortisol and adiponectin interaction was a significant predictor of the time remaining for girls to reach menarche. Specifically, independent of their age, girls with low pre-menarche cortisol and higher fat stores (low adiponectin levels) were the furthest away from reaching menarche (Figure 2-1, top left orange area). This pattern may reflect girls who are in very early stages of their transition with large energy stores that have yet to be mobilized to prioritize fuelling growth before reproductive maturation. Girls with high pre-menarche cortisol and lower fat stores (high adiponectin levels) were also further away from menarche (Figure 2-1, top right teal area). This pattern may reflect that, among participants that are still in earlier stages of maturation, the HPAA may now be mobilizing more energy out of fat storage and using it towards supporting somatic growth or responding to challenges. In contrast, girls with low pre-menarche cortisol and lower fat stores (high adiponectin levels) were chronologically closest to menarche (Figure 2-1, lower right orange area). This pattern may reflect girls who have exhausted their energy stores to fuel both growth and reproductive maturation. In other words, as girls progress towards menarche and more somatic growth is achieved (i.e., they have reached a larger percentage of their final height), more of this mobilized energy was diverted towards investment in reproductive maturation, reflected by the imminent onset of menarche. These girls may no longer require high levels of cortisol to mobilize energy stores as they have finished their growth spurt and require less energy for growth, compared to earlier in their transition.

Our results suggest that changes in adiponectin, as a function of HPAA activation, reflect energy being mobilized out of storage and invested in growth pre-menarche. While somatic growth is prioritized, little energy is available for reproductive maturation, and thus individuals are further from reaching menarche. In sum, our observations are consistent with our hypothesis that the HPAA plays a regulatory role in reproductive maturation. Specifically, the HPAA may mediate the energetic trade-offs between growth

and reproduction by mobilizing energy from storage to meet the varying energetic needs of adolescent development (Joseph & Whirledge, 2017; Worthman, 2003).

Our results are comparable to those of previous studies that evaluate the relationship between adiposity and age at menarche. In a systematic review evaluating non-genetic determinants of age at menarche, lower body fat correlated with later age at menarche (Yermachenko & Dvornyk, 2014). These observations, while not testing HPAA modulation of energy specifically, are consistent with our observation that menarche is preceded by periods in which metabolic energy reserves are low. In the context of our findings, in the absence of sufficient energy to advance somatic growth and reproductive maturation, HPAA activation may mobilize these lower levels of energy for a longer period of time, resulting in delayed onset of menarche.

Our observation that the effects of energy storage is a function of HPAA activation is consistent with how energy mobilization occurs. Glucocorticoids, including cortisol, induce lipolysis, a metabolic process in which triacylglycerol breaks down into either glycerol or free fatty acids (Di Dalmazi et al., 2012; Peckett et al., 2011). Visceral adipose tissue has high concentrations of glucocorticoid receptors (GRs), allowing cortisol to bind to visceral adipose cells to mobilize energy from these adipose fat stores (Pou et al., 2007). Evidence from a control trial evaluating the effects of elevated cortisol among healthy men found an increase in serum free fatty acids, indicating an increase in fat mobilization (Djurhuus et al., 2002). Other studies examining the effects of cortisol-induced treatments, or chronically high cortisol levels (e.g. hypercortisolemia), report similar correlations with elevated circulating free fatty acids; however, these studies consist primarily of males, with only some including one female in their study sample (n=1 female), and do not evaluate whether these relationships differ between sex (Gravholt et al., 2002; Peckett et al., 2011; Samra et al., 1998). In another study using human males, experimentally elevated cortisol proportionately stimulated fat oxidation from femoral and abdominal adipose tissue in a linear fashion, which provides evidence that cortisol linearly leads to energy mobilization from fat stores (Djurhuus et al., 2002). In rat models looking at females specifically, chronic stress is reported to promote lipolysis and free fatty acid mobilization and uptake into mitochondria (Kovacevic et al., 2017). The release of free fatty acids from white adipose tissue (which includes visceral fat tissue) induces a metabolic switch of energy consumption from glucose to fatty acid use – i.e. fat oxidation (Grabner et al., 2021). Tissues with high energy requirements,

such as those supporting growth, utilize energy from fat stores through HPAA activation and subsequent fat mobilization. Together, this evidence supports our interpretation that the association we observed between cortisol and adiponectin and time remaining to menarche reflects the modulating effect of the HPAA in energy trade-offs between maintenance, somatic growth, and reproductive maturation.

*Trade-offs between growth and reproduction*

Our findings that a longer time remaining to menarche was associated with lower energy storage depending on greater HPAA activation, in conjunction with less final growth attained, is also consistent with the hypothesis that earlier in the transition, energy mobilized from storage is mostly allocated towards growth processes, and, or, towards responding to external challenges (Nilsson-Örtman & Rowe, 2021; Stearns & Koella, 1986).

The trade-offs imposed by external challenges on development have been previously documented. Jansen and colleagues (2015) report that from a national nutrition survey in Columbia, adolescent girls who reported experiencing food insecurity during childhood had a later age at menarche (Jansen et al., 2015). Energetic challenges in addition to other external socio-ecological challenges present added complexity in energetic trade-offs between growth and maturation. For example, chronic immune activation in response to infectious disease, which can increase morbidity and mortality, present more urgent and greater energy needs, which are prioritized over development. Indeed, children with inadequate nutrition (low body fat) and high CRP levels, a marker of immune activation, present stunted or delayed growth trajectories, compared to children with no immune activation present (Caldwell, 2016). Similarly, Grob and Zacharin (2020) report that children with chronic health conditions characterized by chronic inflammation experience not only slower or truncated height growth and less bone mass accrual but also decreased sex steroid production (Grob & Zacharin, 2020). In contrast, children with higher body fat percentage (reflecting better energetic conditions) do not experience stunted or delayed growth even with high levels of CRP (Caldwell, 2016). These findings suggest that when sufficient energy is available for allocation towards growth processes in addition to fueling immune system activation, children do not experience delayed development and reach more of their full growth and maturation potential. The direct pathways linking inflammation and timing of maturation are being investigated. Michels

and colleagues (2020), for example, reported that among a cohort of 397 adolescent girls in Chile, elevated levels of interleukin-6 (IL-6) during thelarche were associated with an earlier age at menarche, while higher CRP levels during later maturation stages (after thelarche) were associated with a later age at menarche (Michels et al., 2020).

Among high-performing adolescent athletes who experience high levels of energy expenditure, menarche also tends to occur at a later age (i.e., on average 16 years old, and significantly later compared to their moms and non-training sisters) (Georgopoulos et al., 2010; Huhmann, 2020; Kapczuk, 2017). In sum, under conditions of high levels of physical activity, girls are unable to meet sufficient energy levels needed to support HPGA maturation and experience lower levels of gonadal steroid production, which is associated with a longer time to reach menarche (Kapczuk, 2017). Our findings that a longer time remaining to menarche was associated with greater HPAA mobilization of energy from fat stores may reflect potential energetic trade-offs between socio-ecological and physical challenges, growth, and reproduction.

## Limitations

Our measure of time remaining to menarche should be interpreted with caution. We calculated time remaining to menarche as the difference between the age at which girls reached menarche and age at data collection when girls were pre-menarche. The participants' age at menarche was based on self-reports. Although studies validating self-reports of age at menarche report consistent, high levels of accuracy in recall of age at menarche (Lundblad & Jacobsen, 2017), these validation studies are conducted primarily among Western, industrialized populations. The reliability of self-reported age at menarche in non-Western populations needs to be established. In this study we asked about age at menarche on more than one occasion, and there were discrepancies in reported age at menarche among several participants between the two post-menarche data collection periods (2017 and/or 2023). To reduce potential recall errors in said data, we used the reported age at menarche that was closest to when they reached menarche. For example, if a participant went through menarche in 2016, then we used her reported age at menarche that was collected in 2017, rather than the reported age at menarche collected in 2023. Additionally, menarche time was calculated in months, yet some participants were only able to report when they reached menarche in years. This limited precision may further limit our ability to identify how variation in menarche time is

associated with HPAA activity, and metabolic energy strategies. While the interaction term between cortisol and adiponectin aims to assess how the HPAA may be mediating metabolic energy as a predictor of menarche timing, we should be cautious in our interpretations of this finding. Including an interaction term between two continuous variables introduces a strong assumption in the model that the change of one predictor variable occurs as a linear function of a change in the other predictor variable. As such, while the interaction term was significant in predicting time remaining to menarche, additional data would be required to check the validity of this assumption. Finally, our sample size is relatively small, which may limit our ability to identify relevant associations beyond our findings, yet the population in which this study was based has lower levels of heterogeneity which increases our statistical power.

## Significance and Implication of Findings

Most studies focus on the independent effects of either metabolic energy or HPAA activity on menarche timing (Glass et al., 2022). As well, studies evaluating the associations between HPAA activity and age at menarche often only consider variation in HPAA as a marker of stress, rather than an energy regulation mechanism. Our findings contribute novel insights about the role of the HPAA in mediating energetic trade-offs between growth and reproduction among girls as they approach menarche. It also improves our understanding of among-individual variability in the timing of this critical reproductive transition. Interpreted within a life history theory framework, these results suggest that energy availability plays an important role in reproductive maturation. If girls face energetic challenges that result in greater HPAA activation and subsequent mobilization of metabolic energy to respond to those challenges during growth, then less energy will be available for reproductive maturation, which may result in delays in reaching menarche.

Energy that is re-allocated towards reproductive function before growth processes are complete can lead to negative reproductive outcomes later in adulthood. Accelerated reproductive development, in some contexts, has been proposed as an adaptive strategy, where individuals who are at risk of dying at an earlier age maximize their chances of reproducing before dying by developing faster, and maturing earlier (Belsky, 2012; Chisholm et al., 2005). Achieving regular ovulatory cycles at a faster pace, however, can be associated with incomplete somatic development. This phenomenon

could include, for example, an underdeveloped pelvis, which is linked to significantly higher health risks including birth complications (Malabarey et al., 2012). Under conditions of energetic challenges (e.g., poor nutrition or resources), but lower risk of mortality, it may be more strategic to have a slower transition to ensure that growth and development can be fully achieved, to avoid the costs of 'stunted' or immature growth on reproduction. Our findings that a longer time remaining to menarche is associated with greater HPAA mobilization of energy from fat stores and a lower percentage of final height achieved may reflect this life history strategy. In contrast, greater food availability is proposed to have a positive impact on growth, leading to earlier reproductive maturation and menarche (Glass et al., 2022). Higher BMI during childhood is associated with both greater increases in height and earlier maturation (He & Karlberg, 2001). This results from a surplus of metabolic energy, which exceeds the energetic requirements for supporting growth. This excess energy is thus available to allocate towards maturation processes simultaneously with growth, resulting in faster growth and earlier age at menarche (Glass et al., 2022).

Impacts on growth and development during this critical transition, including very early or very onset of menarche, can have long term effects on reproductive and health outcomes across the lifespan. Early age at menarche is correlated with earlier age at coitarche and first birth (Chisholm et al., 2005; Coall & Sheppard, 2016; Nettle et al., 2011). Early age at menarche is also associated with increased risk of diabetes (Elks et al., 2013; Lakshman et al., 2008), cardiovascular disease and obesity (Remsberg et al., 2005), subfecundity (Warp et al., 2024), and mental health conditions such as depression and bipolar disorder (Rosso et al., 2020; Stice et al., 2001). On the other hand, extreme delays in menarche (often reported as 15 years and older) are associated with increased depression (Herva et al., 2004), subfecundity (Guldbrandsen et al., 2014; Warp et al., 2024), and cardiovascular disease risk (Luijken et al., 2017).

Our analysis focused on metabolic energy strategies that may regulate trade-offs between growth and the onset of menarche in pre-menarche girls. To further understand how the HPAA acts as an energy regulation mechanism during adolescence, future studies should evaluate associations between HPAA and metabolic energy availability across different stages of maturation, including post-menarche stages, to further elucidate the biological pathways that respond to the changing energetic demands as girls become more reproductively mature.

While we did not include specific measures of poor access to nutrition (food insecurity) or other energetic resources, our results suggesting that energy allocation is associated with the timing of menarche may have important implications in how socio-economic disparities or inequities may impact development. If adolescent girls experience disproportionate, inequitable access to resources, this could trigger greater HPAA activity to mobilize energy to meet the demands of these challenges, thus impacting growth and reproductive maturation. Our findings can thus be used to understand, mechanistically, how inequitable social environments may lead to inequitable health and development outcomes. This further supports the need to address socio-ecological inequities, to support optimal growth and reproductive development during adolescence and throughout adulthood.

# Chapter 3. The hypothalamic-pituitary-adrenal axis and metabolic energy strategies pre- and post-menarche: A study based on a cohort of Mayan Indigenous adolescent girls

## Abstract

**Background:** Adolescence is a critical transition in which girls face energetic trade-offs between growth and reproductive development. The hypothalamic-pituitary-adrenal axis (HPAA) is a key modulator of metabolic energy trade-offs across the lifespan, yet few studies have examined its role during girls' reproductive maturation.

**Hypothesis:** The HPAA is a major regulator of metabolic energy strategies at different stages of maturation, to meet the changing energy demands imposed by somatic growth and reproductive maturation, including the development of secondary sexual traits and the onset of ovarian function, within girls across their adolescent transition.

**Methods:** To test the predictions that contributed to our hypothesis, we evaluated biomarkers of HPAA activity (cortisol), energy storage (adiponectin) and energy uptake (c-peptide) quantified in urinary specimens from 46 Mayan adolescent girls from Guatemala collected longitudinally during their pre-menarche and post-menarche maturation stages. Longitudinal, mixed effects models were conducted to evaluate longitudinal differences in the associations between HPAA activity and metabolic energy pre- to post-menarche.

**Results:** Cortisol was positively associated with both c-peptide (0.21 ng/mL, $p<0.001$) and adiponectin (0.29 ng/mL, $p<0.001$). Adiponectin and c-peptide were significantly higher in pre-menarche compared to post-menarche stages ($p<0.001$). The magnitude of the relationships between cortisol and both c-peptide and adiponectin were not significantly different depending on menarche stage ($p>0.05$).

**Discussion**: The positive relationship between cortisol and c-peptide suggests that the HPAA may be involved in energy uptake, by prompting an increase in insulin secretion patterns, to meet the high energy needs of growth and reproduction. This is further reflected in higher energy uptake among pre- compared to post-menarche girls. The positive association we observe between cortisol and adiponectin may reflect the HPAA acting as a mechanism for triggering fat oxidation, the release of free fatty acids from adipose fat tissue, to mobilize energy to support the concurrent energy needs of both growth and reproduction. We interpret the decrease in adiponectin from pre- to post-menarche to reflect an increase in visceral fat (storage), which may suggest that more energy is being stored for investment in anticipation of future reproductive efforts.

# Introduction

Adolescence is a critical developmental transition in which human females (hereafter referred to as 'girls') face key energetic trade-offs between somatic growth and reproduction. Girls typically begin reproductive maturation between the ages of 8 and 13 years old (Moodie et al., 2020). Menarche, defined as the first occurrence of menses, reflects the onset of ovarian function and typically occurs towards the latter end of the adolescent transition, once other growth or maturation processes (e.g., pelvic widening, development of secondary sexual traits) have commenced and milestones (e.g., peak height velocity) have been achieved (Ellis, 2004; Sharma et al., 2016). Initially, ovulatory frequencies tend to be low, as adolescent girls' hypothalamic-pituitary-gonadal axes (HPGAs) are not yet fully mature; however, the presence of menstrual bleeding reflects the onset of ovarian production and secretion of gonadal steroids (estrogen and progesterone, among others) (Huhmann, 2020; Sharma et al., 2016).

Changes in metabolic energy requirements are proposed to support reproductive maturation processes, such as the onset of ovarian function. Consistent with this proposition, several studies report that adolescents in later stages of their transition have higher absolute basal metabolic rates (BMR) and higher resting metabolic rates (RMR) compared to pre-transition adolescents (Cheng et al., 2016). BMR and RMR reflect the energy used to maintain metabolic tasks when an organism is at rest and are measures of energetic needs. The observed increases in BMR and RMR among adolescents at later stages of maturation, compared to pre-transition adolescents, may thus reflect changes in energy needs that occur with reproductive maturation (Cheng et al., 2016). Consistent with these observations, emerging evidence suggests that as girls advance towards reproductive maturity, they tend to show increases in energy uptake and storage. An insulin resistant period characteristic of adolescence triggers a compensatory increase in insulin secretion levels (Kelsey & Zeitler, 2016). Insulin facilitates glucose uptake into cells, either for immediate use or long-term storage in adipose fat (Böttner et al., 2004; Emery Thompson & Knott, 2008). This evidence of changes in insulin secretion patterns, when taken together with observed increases in adiposity as girls mature, reflect critical changes in metabolic energy strategies to support HPGA maturation (Ellison, 2017; Kelsey & Zeitler, 2016; O'Keeffe et al., 2020; Reiches et al., 2013).

Changes in energy uptake and energy storage patterns are normally regulated by the hypothalamic-pituitary-adrenal axis (HPAA). Indeed, the HPAA mediates trade-offs in energy allocation among physiological processes that support maintenance, growth, and reproductive function (Chisholm et al., 2005). When an individual faces an urgent challenge, energy is diverted from lower priority tasks such as reproductive function to meet the demands of said challenge (Ellis, 2004; Nepomnaschy et al., 2004).

Importantly, while the role of the HPAA in modulating reproductive function in adult women has been previously explored (Nepomnaschy et al., 2007; Nepomnaschy et al., 2004; Nepomnaschy et al., 2006), to our knowledge, no previous longitudinal studies have evaluated its role in reproductive maturation as a metabolic energy regulation mechanism. Here, we hypothesize that the HPAA acts as a major regulator of metabolic energy strategies at different stages of maturation to meet the changing energy demands within girls across their adolescent transition. To contribute to this hypothesis, we test two predictions. We expect that HPAA activation (evaluated via changes in cortisol levels) will be associated with 1) energy uptake (evaluated via c-peptide, a marker of insulin) and 2) energy storage levels (evaluated via adiponectin, an inverse marker of visceral adipose fat mass) differently in pre-menarche compared to post-menarche stages (see variable definitions in Table 3-1).

# Methods

**Table 3-1:** Description of variables

| Variable name | Variable definition |
|---|---|
| Time relative to menarche | Number of months before and after a participant reached menarche relative to when they were in their pre-menarche and post-menarche stage, calculated as the difference between age at menarche and age at which participants provided their pre-menarche and post-menarche samples/data |
| Cortisol (ng/mL) pre-menarche | Log10 transformed measure of cortisol, which is a measure of HPAA activity; data collected every other day for 3 weeks during pre-menarche |
| C-Peptide (ng/mL) pre-menarche | Log10 transformed measure of c-peptide, which is a measure of energy uptake activity; data collected every other day for 3 weeks during pre-menarche |
| Adiponectin (ng/mL) pre-menarche | Log10 transformed measure of adiponectin, which is a measure of energy storage; data collected every other day for 3 weeks during pre-menarche |
| Cortisol (ng/mL) post-menarche | Log10 transformed measure of cortisol; data collected every other day for 4 months post-menarche |
| C-Peptide (ng/mL) post-menarche | Log10 transformed measure of c-peptide; data collected every other day for 4 months post-menarche |
| Adiponectin (ng/mL) post-menarche | Log10 transformed measure of adiponectin; data collected every other day for 4 months post-menarche |

## Study population

We used data from a longitudinal, naturalistic cohort study – the Consequences of Peri-conceptional Events (COPE) study (Barha et al., 2019; Nepomnaschy et al., 2004) – that began in 2000 and has followed Indigenous Kaqchikel Mayan girls since their conception. Participants live in rural communities in the highlands of Guatemala (Nepomnaschy et al., 2004). Data used in this study were collected during three field seasons: in 2013 from 21 Indigenous Kaqchikel girls (aged at the time 10-11 years old) who were conceived as part of the COPE study in 2000-2001; in 2017 for follow-up data from the original COPE participants (ages 14-15) and initial data for 25 additional girls (ages 12-15), some of whom are sisters of the original COPE participants; and in 2023 for follow-up data from all girls (ages 18-21 years old) who participated in 2013 and, or,

in 2017. Twenty-nine participants provided both pre- and post-menarche hormone data to evaluate within-individual changes across the transition. An additional 17 participants who only participated either during their pre-menarche stage (did not participate in follow-up field seasons in 2017 and, or, 2023) or post-menarche stage (began participating in COPE only after having gone through menarche) were also included in this study, to contribute additional information for inter-individual comparisons across the pre-post-menarche transition. Thus, a total sample of 46 girls was used in these analyses. Participants' reproductive maturity status was evaluated in each follow-up field season to monitor their transition to post-menarche. Data collected in 2023 were included in this analysis only for girls who had not reached menarche by 2017 (n = 9).

## Data and biospecimen collection

In 2013, 2017, and 2023, starting in January of each field season, participants self-collected first morning urine (FMU) samples in plastic, chemically inert urine collection containers. Collection frequency was daily for three weeks except Sundays in 2013 and every other day for four months in 2017 and 2023. The 46 adolescent girls provided an average of 16 FMU samples when they were pre-menarche and 29 FMU samples when they were post-menarche. On the same day as FMU sample collection, local women field assistants processed aliquots (2 mL) of the FMU samples, which were stored in cryo-vials at -10°C in the field laboratory. At the end of each field season, all samples were shipped on dry ice and stored at -80°C in the Maternal and Child Health Laboratory at Simon Fraser University (SFU).

Demographic questionnaires, which included questions regarding reproductive status and age at menarche, were conducted once with each participant in Kaqchikel or Spanish (the two local languages) during each field season. Self-reported age at menarche has been argued to be reliable; studies validating self-reports of age at menarche report consistent, high levels of accuracy in recall of age at menarche, though the accuracy of recall declines with older age (Cooper et al., 2006; Lundblad & Jacobsen, 2017).

## Hormone Assays

FMU samples provide information on biomarkers (hormone concentrations) of HPAA and HPGA activity and metabolic energy. FMU cortisol levels were used to evaluate participants' basal HPAA activity (measured as urinary-free cortisol levels) throughout the previous night, since the last urinary void (Table 3-1). FMU c-peptide levels provide information on metabolic energy uptake. C-peptide is a proxy for insulin levels; c-peptide is a segment of proinsulin that is released in 1:1 equimolar amounts with insulin during the conversion of proinsulin to insulin (Emery Thompson & Knott, 2008). Higher levels of c-peptide, thus higher levels of insulin, reflect more energy uptake as insulin facilitates glucose uptake from the bloodstream into cells. FMU adiponectin is used as a marker of an individual's stored energy reserves. Adiponectin, produced by white adipose tissue, is inversely related to the amount of accumulated visceral adipose fat mass (Brochu-Gaudreau et al., 2010). Higher adiponectin levels reflect lower levels of adipose fat storage, while lower levels of adiponectin reflect higher levels of adipose fat storage (Brochu-Gaudreau et al., 2010). Patterns of urinary alpha sub-unit of follicle-stimulating hormone (FSH) and urinary conjugates of estradiol and progesterone (estrone-3-glucuronide (E1G) and pregnanediol glucuronide (PdG), respectively) were used to assess and confirm self-reported reproductive status (i.e., whether girls were pre- or post-menarche during each data collection period) (Baird et al., 1991; Kassam et al., 1996; O'Connor et al., 2006).

Hormone analyses were conducted at the Maternal and Child Health Laboratory at SFU. Urinary biomarkers of HPAA physiology (cortisol), energetic status (c-peptide and adiponectin), and reproductive status (FSH, E1G, and PdG) were measured using enzyme-linked immunosorbent assays (ELISAs) (Quansys Biosciences, Utah, USA), which our lab has previously validated (lower limits of detection for cortisol= 0.343 ng/mL; c-peptide= 0.090 ng/mL; and adiponectin = 0.023 ng/mL) (Salvante et al., 2012). To adjust for variation in dilution levels for each urine sample due to daily variation in the hydration state of participants, all hormone concentrations were corrected for specific gravity using refractometry (Miller et al., 2004). To control for variation among assays, each of the samples from the same participant were run on the same plate. All intra-and inter-assay coefficients of variation were below 7% and 11%, respectively.

## Statistical Analysis

We first inspected the data for non-normal distributions and errors. Specifically, cortisol, adiponectin, and c-peptide, which were all right-skewed, were log10-transformed to be approximately normally distributed on the logarithmic scale (Osborne, 2019). This data transformation also aligns these variables on a comparable scale, to facilitate an evaluation of how they may change linearly in relation to each other (Osborne, 2019). Datapoints well outside established average biological ranges and those outside the interquartile range for each variable, within subjects, were examined. We checked if extreme values correlated with similarly extreme values of other hormones (suggesting an error in the sample) and, if so, those values were identified as outliers. All information about these possible outliers was evaluated and, if considered suspect, were removed from further statistical analysis. We identified and removed a total of 30 outliers from the dataset of 9660 data points.

To evaluate differences in the associations between cortisol and metabolic energy pre- to post-menarche, we fit two mixed-effects models with c-peptide and adiponectin as the response variables in each model, using the LME function in R (RStudio, 2023).

We fit the following linear mixed-effects models:

1) C-Peptide = Cortisol + Menarche status + Cortisol*Menarche status +Time relative to menarche + (1|subject);

2) Adiponectin = Cortisol + Menarche status + Cortisol*Menarche status+ Time relative to menarche + (1|subject).

Linear mixed-effects models take into account both fixed and random effects, the latter of which are included due to the repeated measures collected from individuals. This approach also accounts for, and handles, unbalanced and missing data, which are a feature of our dataset (Cnaan et al., 1997). They also allow the variance of c-peptide and adiponectin to change from pre- to post-menarche. For both the c-peptide and adiponectin models, we conducted a likelihood ratio test to compare the fit of the model to that of the model that assumes that the variance of the response is constant. Both models were adjusted for time relative to menarche (defined as the difference between the time when participants reached menarche and the time when data was collected pre-

and post-menarche), to adjust for variation in maturation status (time relative to menarche) of participants in each data collection period.

## Results

*Data description*

Trends in hormonal changes pre- to post- menarche within and among adolescent girls in our sample are presented in Figures 3-1 through 3-4. Figures 3-1 through 3-4 describe changes in summary statistics (3-1. means, 3-2. maximum values, 3-3. standard deviations, and 3-4. ranges) for cortisol, c-peptide, and adiponectin from pre- to post-menarche. Not all participants provided data from both pre- and post-menarche stages; these participants are represented by the single points in the figures (no line connecting to the other stage). While mean cortisol values do not show a consistent trend within all girls, cortisol maximum values and standard deviation do increase for most individuals from pre- to post-menarche. C-peptide mean and maximum values decrease for most girls from pre- to post-menarche, and adiponectin's mean and maximum values decrease while its standard deviation increases. The changes in range for adiponectin, c-peptide, and cortisol (difference between maximum and minimum values per individual) reflect the patterns we observed in standard deviations.

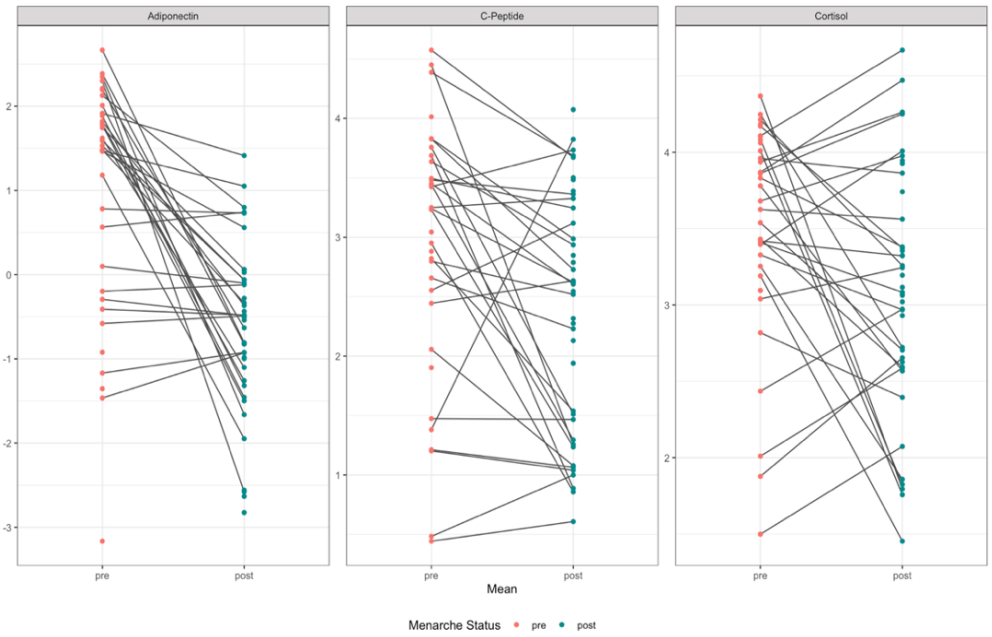


**Figure 3-1:** Change in mean hormone levels pre-post menarche within participants (log10-transformed biomarkers)

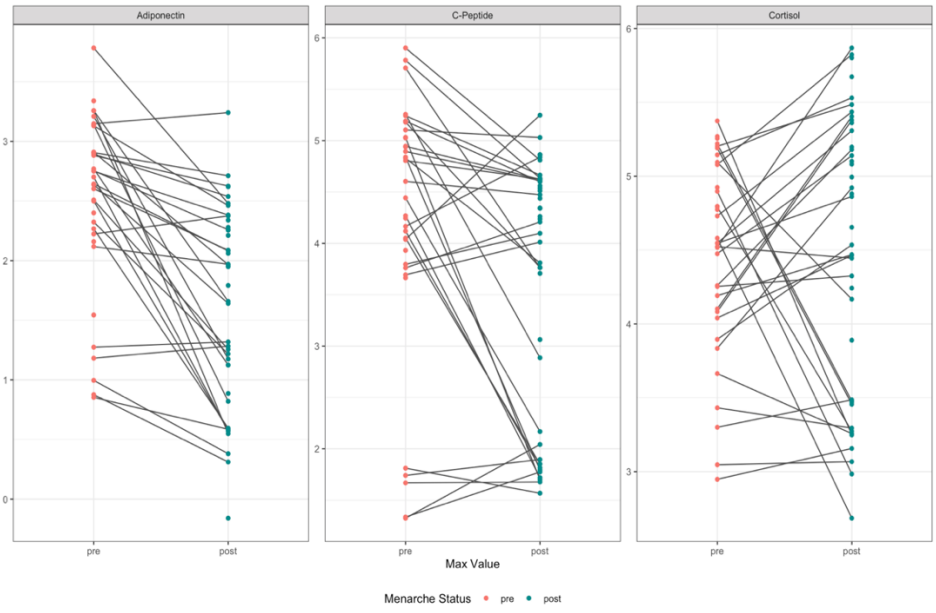


**Figure 3-2:** Change in maximum hormone levels pre-post menarche within participants (log10-transformed biomarkers)

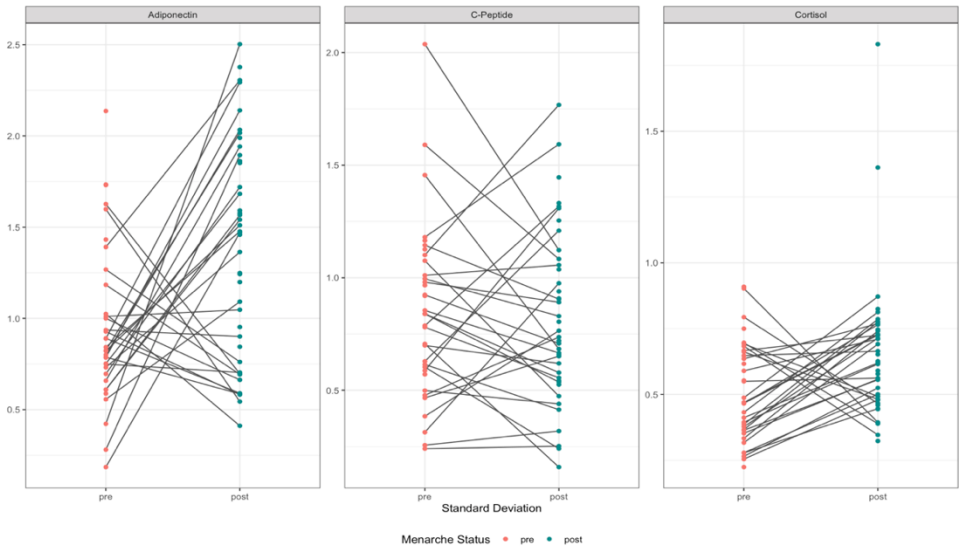


**Figure 3-3:** Change in standard deviation of hormone levels pre-post menarche within participants (log10-transformed biomarkers)

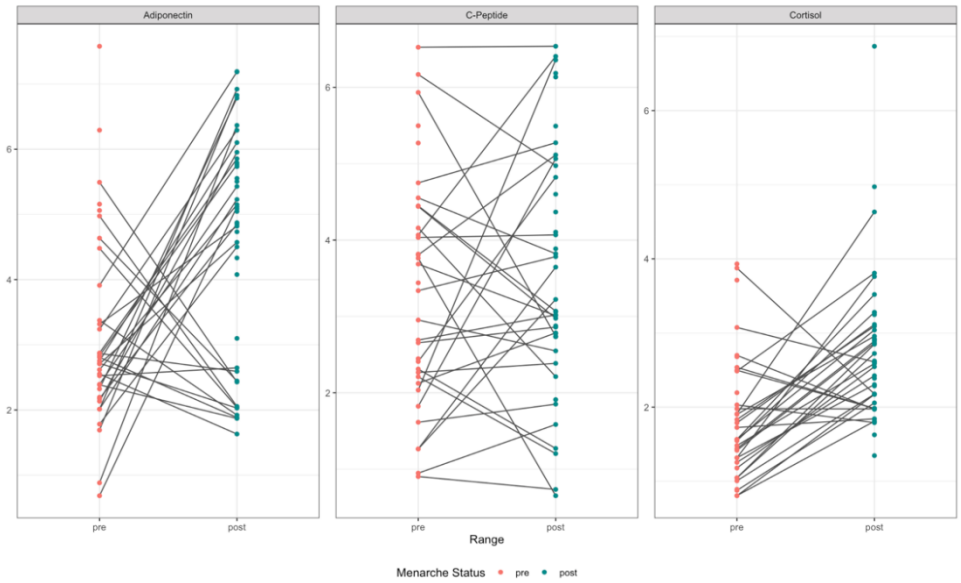


**Figure 3-4:** Change in range of hormone levels (minimum to maximum values) pre-post menarche within participants (log10-transformed biomarkers)

*HPAA and energy uptake across the transition*

The model allowing for a change in the variance of c-peptide post-menarche did not fit significantly differently from the model that assumes constant variance (likelihood ratio test p>0.05). As such, we report only results based on the latter model.

The cortisol by menarche stage interaction was not significantly associated with c-peptide (p>0.05), thus we removed the interaction term and re-fit the model. The refit model results are reported here. On average, c-peptide was significantly higher in pre-menarche compared to post-menarche stages (p<0.001), with all other variables held constant (Table 3-2, Figure 3-5). Cortisol was positively associated with c-peptide (p<0.001), with all other variables were held constant. For every unit (1 ng/mL) increase in log-transformed cortisol, we observed an increase in log-transformed c-peptide by 0.21 ng/mL (Table 3-2, Figure 3-5).

**Table 3-2:** Estimated effects of cortisol and menarche stage on c-peptide[1]

| | **Effect estimate** | **95% CI** |
|---|---|---|
| **(Intercept)** | 2.30 *** | 1.92, 2.67 |
| **Cortisol (ng/mL)** | 0.21 *** | 0.15, 0.26 |
| **Post-menarche** | -0.54 *** | -0.84, -0.23 |
| **Time relative to menarche** | -0.0 | -0.01, 0.00 |

*** p < 0.001; ** p < 0.01; * p < 0.05

[1]*log10-transformed c-peptide and cortisol*

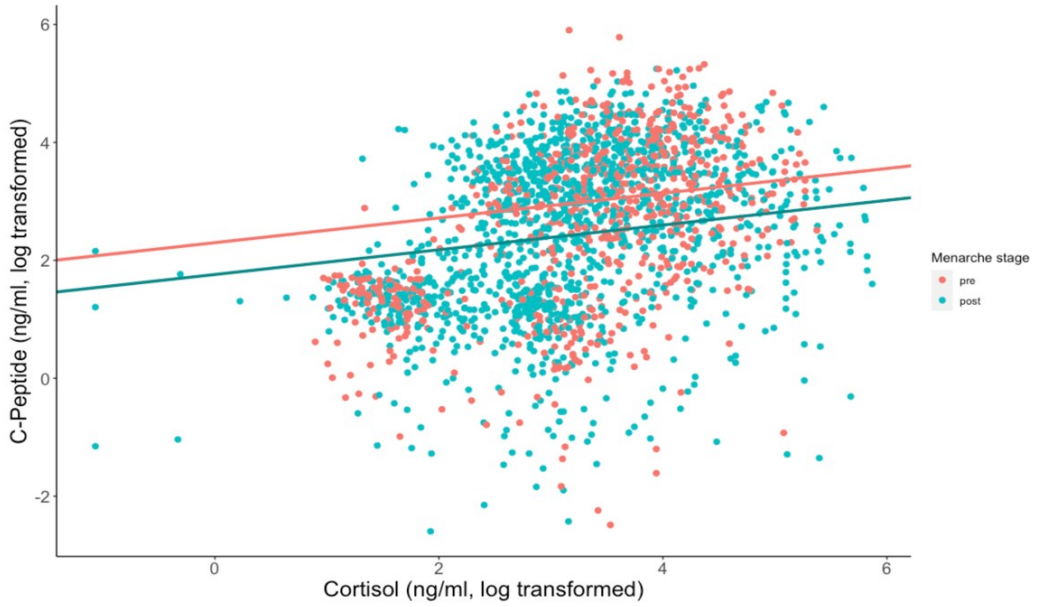


**Figure 3-5:** The fitted regression lines representing the estimated relationship between cortisol and c-peptide across the pre- to post-menarche transition

*HPAA and energy storage across the transition*

The model allowing for change in the variance of adiponectin post-menarche fit significantly better than the model that assumes constant variance (likelihood ratio test $p<0.0001$). Thus, we report the results based on the former, to allow for change in variance longitudinally.

The cortisol by menarche stage interaction was not significantly associated with adiponectin ($p>0.05$), thus we removed the interaction term and re-fit the model. The refit model results are reported here. On average, adiponectin was significantly higher in pre-menarche compared to post-menarche stages ($p<0.001$), with all other variables held constant (Table 3-3, Figure 3-6). Cortisol was positively associated with adiponectin ($p<0.001$), with all other variables held constant. For every unit (1 ng/mL) increase in log-transformed cortisol, we observed an increase in log-transformed adiponectin by 0.29 ng/mL (Table 3-3, Figure 3-6).

**Table 3-3:** Estimated effects of cortisol and menarche stage on adiponectin[1]

| | Effect estimate | 95% CI |
|---|---|---|
| **(Intercept)** | 2.15 *** | 1.26, 3.04 |
| **Cortisol (ng/mL)** | 0.29 *** | 0.21, 0.37 |
| **Post-menarche** | -4.94 *** | -5.50, -4.39 |
| **Time relative to menarche** | 0.06*** | 0.05, 0.07 |

*** p < 0.001; ** p < 0.01; * p < 0.05
[1]*log10-transformed adiponectin and cortisol*

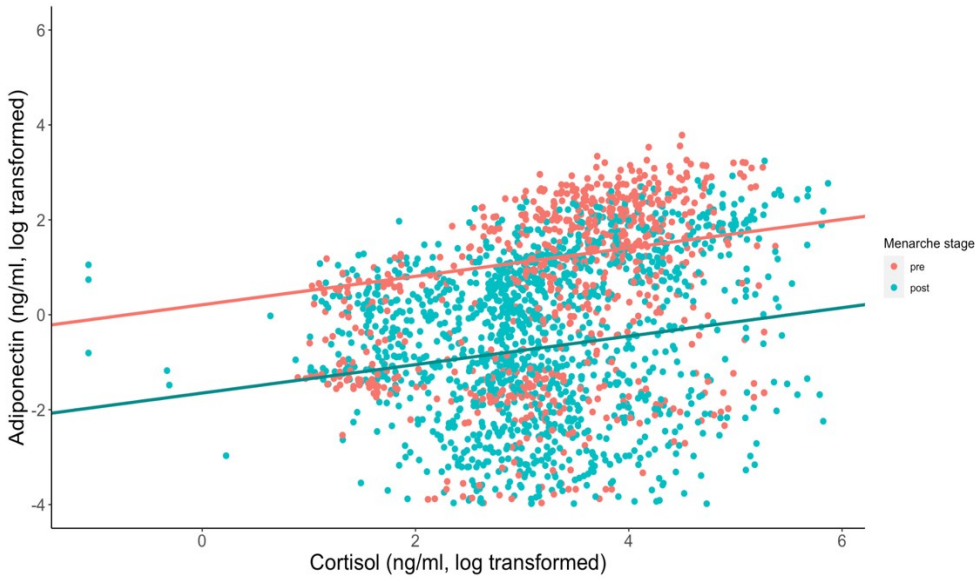


**Figure 3-6:** The fitted regression lines representing the estimated relationship between cortisol and adiponectin across the pre- to post-menarche transition

# Discussion

Our results showing statistically significant associations between cortisol and both c-peptide and adiponectin levels are consistent with our hypothesis that the HPAA plays a role in the mechanisms that regulate energetic strategies across the adolescent transition in girls. Specifically, cortisol was positively associated with c-peptide and with adiponectin during both pre- and post-menarche stages. Importantly, we observed that within girls' c-peptide levels were overall higher pre-menarche, reflecting higher insulin

levels, compared to their post-menarche stage, after adjusting for the effects of the other predictors. We also observed that within girls' adiponectin levels were overall lower, reflecting higher visceral fat storage, post-menarche, compared to their pre-menarche stage, after adjusting for the effects of the other predictors. Yet, contrary to our predictions, the relationship between cortisol and said markers of metabolic energy uptake and storage did not differ between pre- and post-menarche.

*Pre-menarche HPAA activity and energy strategies*

HPAA activation involves the release of glucocorticoids, including cortisol, to regulate energy allocation in response to energetic, immune, or psychosocial challenges (Di Dalmazi et al., 2012; Ellis, 2004). Here we evaluate HPAA as a possible modulator of insulin secretion (energy uptake) and adipose fat mass (energy storage) in response to energetic needs at different stages of adolescent maturation within individuals. While our results offer no support for our prediction that HPAA activity would modulate energy uptake and storage differently depending on maturation stage, we did find that c-peptide and adiponectin were both positively associated with cortisol, with no evidence of a difference in the magnitude of this relationship between pre- and post-menarche. These observations are consistent with the proposition that HPAA activity modulates energy uptake (via the regulation of insulin levels) and energy storage (via changes in adiponectin, with higher adiponectin reflecting less visceral adipose fat mass) across this reproductive transition (Table 3-2, Figure 3-5; Table 3-3, Figure 3-6).

The positive association we observed between cortisol and c-peptide (a proxy for insulin) is consistent with Huybrechts et al.'s (2014) findings from a cross-sectional analysis of post-menarche adolescent girls, wherein cortisol was positively associated with insulin secretion (Huybrechts et al., 2014). This cross-sectional analysis, however, did not include pre-transition participants. By comparing girls to themselves in both pre- and post-menarche stages, our longitudinal study expands on Huybrechts et al.'s (2014) observations and presents the first evidence suggesting that HPAA activity may be involved in energy uptake across the entire adolescent transition.

During early adolescence, there is a characteristic insulin resistant period, which is reported to trigger a compensatory increase in insulin secretion (Kelsey & Zeitler, 2016; Reiches & Ellison, 2022). The insulin resistant period commences prior to menarche,

before gonadotrophin (luteinizing hormone (LH) and follicular-stimulating hormone (FSH)) production increases as part of reproductive development (Jeffery et al., 2012). Evidence suggests that increased HPAA activity is associated with this insulin resistance among adolescent girls, leading to increased insulin secretion (Huybrechts et al., 2014). The positive association we observe between HPAA activation and insulin secretion when girls are still pre-menarche is, thus, consistent with the reported timing of this characteristic insulin resistant period beginning in early stages of reproductive maturation. This pattern of increased insulin secretion during earlier stages of reproductive maturation is also consistent with our findings that pre-menarche girls had overall higher insulin levels (higher c-peptide levels) compared to when they were post-menarche.

The positive association between HPAA activation and insulin levels in our sample may reflect a mechanism by which girls undergoing the adolescent transition can meet the high energy needs of growth and reproduction. The compensatory increase in insulin secretion occurring in response to insulin resistance has been proposed as a mechanism to increase energy uptake to stimulate HPGA maturation (Kelsey & Zeitler, 2016; Reiches & Ellison, 2022). Insulin is also involved in potentiating estrogen and progesterone production by granulosa and theca cells in the ovaries and further helps enhance LH- and FSH-stimulated steroid production to establish regular ovulatory cycles (Greisen et al., 2001; Willis et al., 1996). As such, the compensatory increase in insulin secretion pre-menarche would support LH and FSH in initiating ovarian steroid production, including estradiol and progesterone synthesis. Increased estradiol also stimulates breast budding, skeletal growth, and other secondary sexual traits to develop simultaneously with HPGA maturation, prior to menarche (Lacroix et al., 2023; Sharma et al., 2016). Established ovulatory cycles, which indicate that girls are maturing reproductively, are associated with a return to normal insulin levels and regular insulin sensitivity (Kelsey & Zeitler, 2016).

The HPAA activity and insulin secretion patterns we observed have been reported in other reproductive life history transitions in women besides adolescence. During gestation, for example, increased cortisol secretion is associated with a reduction in insulin sensitivity, which prompts insulin resistance (Vejrazkova et al., 2014). This insulin resistant period triggers an increase in insulin secretion from pancreatic beta-cells, resulting in an increase in glucose uptake during later stages of gestation (Vejrazkova et

al., 2014). Following gestation, continued high insulin levels into the postpartum period have been proposed to stimulate the resumption of ovarian function (Valeggia & Ellison, 2009). Indeed, during the post-partum amenorrhea period that follows a birth, women also experience an insulin resistance period that precedes the resumption of regular ovulatory cycles (Valeggia & Ellison, 2009). Increases in insulin help enhance LH- and FSH-stimulated estrogen and progesterone production by ovarian cells to establish regular ovulatory cycles (Greisen et al., 2001; Willis et al., 1996). The resumption of ovarian function, in turn, resolves the insulin resistant period during the postpartum period – increasing estrogen levels restore normal insulin sensitivity (Valeggia & Ellison, 2009). These parallel insulin secretion patterns during adolescence, gestation and the postpartum period suggest that cortisol plays a role in the mechanism that mobilizes energy for uptake to support the energetic demands of (re-)establishing ovarian function during reproductive transitions with high energy needs.

Insulin resistance is associated with a decrease in glucose oxidation (using glucose for energy), and greater fat oxidation (mobilizing adipose fat mass as a source of energy) (Hannon et al., 2006). Fat oxidation yields more adenosine triphosphate (ATP) production per molecule compared to glucose oxidation, thus generating more energy per unit for use (Hannon et al., 2006). The increase in compensatory insulin secretion as a response to insulin resistance during adolescence may be a mechanism to shift towards using more fat storage as an energy source during earlier stages of maturation, prior to menarche. While insulin continues to facilitate glucose uptake to promote more overall available energy either for immediate use or storage, fat stores are prioritized as the main energy source for generating ATP for use.

The positive association we observe between cortisol and adiponectin may reflect the HPAA acting as a mechanism to mobilize energy from fat stores to support growth and reproductive maturation. Glucocorticoids, such as cortisol, mobilize free fatty acids from adipose fat stores into the bloodstream to provide energy to tissues (Di Dalmazi et al., 2012). As adiponectin levels are inversely related to visceral adipose fat mass, we interpret our findings to mean that pre-menarche girls in our sample have lower visceral adipose fat mass compared to when they are post-menarche. This result suggests that mechanisms may be in place to promote greater fat oxidation when girls are pre-menarche than post-menarche. Taken together, these results are consistent with HPAA activation playing a role in the shift towards fat oxidation providing the metabolic energy

source during earlier maturation stages to simultaneously fuel both somatic growth and maturation of the reproductive axis.

*Post-menarche HPAA activity and energy strategies*

In post-menarche girls, energy allocation shifts towards supporting reproductive maturation and the incipient ovarian cyclicity. We observed that girls' c-peptide levels were lower, reflecting lower insulin secretion levels, when they were post-menarche compared to when they were pre-menarche. Lower insulin levels indicate a resolution of the insulin resistant period, wherein normal insulin sensitivity resumes (Kelsey & Zeitler, 2016). This finding is consistent with the general trend that the adolescent insulin resistant period ends once ovarian cyclicity is established (Kelsey & Zeitler, 2016). Once the insulin resistance period is resolved post-menarche, glucose oxidation resumes as the predominant energy source (over fat oxidation) (Hannon et al., 2006). A shift back towards prioritizing glucose oxidation, and away from fat oxidation, would allow for greater fat accumulation; this shift coincides with our findings of greater fat accumulation observed among post-menarche girls.

Consistent with our predictions, girls' adiponectin levels were higher pre-menarche than post-menarche, which suggest that girls have higher visceral adipose fat mass once they are post-menarche compared to when they were pre-menarche. We hypothesize that once insulin resistance resolves and ovarian cycles are established, girls shift away from fat as the predominant energy source (Hannon et al., 2006; Moran et al., 1999), which allows them to store more metabolic energy in the form of fat tissue. These findings are consistent with those of other studies reporting that girls' adiponectin levels decline slightly across the transition (Böttner et al., 2004; Hannon et al., 2006; O'Keeffe et al., 2020). Our results are also consistent with studies that observe an increase in other measures of energy storage, including weight, BMI, and fat percentage, as adolescent girls reach menarche and continue to mature (Loomba-Albrecht & Styne, 2009; O'Keeffe et al., 2020). The observed increase in adiposity from pre- to post-menarche could be interpreted to support the increased energy requirements of being reproductively mature, including ovarian function. Additionally, girls who are more reproductively mature may be storing more energy in anticipation of future reproductive events such as pregnancies and maternal care, which are energetically costly. We propose that more energy being taken up is directed towards fat storage when girls are post-menarche as a mechanism

to increase total energy storage to meet the energetic needs of girls who are more reproductively mature.

## Limitations

Although our biomarkers of metabolic energy provide information about both energy uptake and energy storage, they do not provide information as to the specific tissues that are taking up and using this energy. Thus, we are unable to determine whether this energy was being mobilized and allocated towards tissues supporting somatic growth or reproductive development. We also do not know if girls were in their insulin resistant period. As such, we are unable to adjust for this information on insulin resistance, which influences insulin secretion levels (i.e. greater insulin secretion in response to insulin resistance). Additionally, we do not include measures of perceived stress or other socio-ecological challenges in our analyses, and thus cannot evaluate how environmental exposures, such as presence of infectious diseases, mortality risk, interpersonal conflicts, or psychosocial challenges, may also be using energy that is being mobilized by HPAA activation. As such, we should be cautious in our interpretations of how the HPAA may be meeting the energy requirements of growth versus reproductive maturation processes at different stages of maturation. Finally, despite the strength conferred to this study by the relative homogeneity of this study population, our sample size is relatively small, which may limit our ability to identify relevant associations beyond our findings, such as whether the HPAA's relationship with energy uptake and energy storage changes from pre- to post-menarche. Given our small study sample, and the relative homogeneity of our sample, the results from this study should be compared with those based on larger samples from a variety of populations to ascertain whether our results hold across different socio-ecological contexts.

## Significance and Implication of Findings

In sum, we propose that the observed relationships between HPAA activity, changes in insulin production, and increases in adiposity from pre- to post-menarche is part of a set of mechanisms that manages energy allocation to support girls' somatic and reproductive maturation pre-menarche, and energy storage, in the form of visceral fat, post-menarche. The increase in stored metabolic energy post-menarche would serve to afford the cost of future reproduction, such as gestation, and later lactation and maternal

care. To our knowledge, no previous studies have evaluated the role of the HPAA as a modulator of insulin secretion (energy uptake) and adipose fat mass (energy storage) in response to energetic needs at different stages of adolescent maturation within individuals.

To further evaluate how the HPAA plays a role in mediating variation in reproductive maturation outcomes, future studies should compare HPAA activity, and energy uptake and storage patterns with characteristics of reproductive function, such as the frequency and quality of ovulatory cycles, among post-menarche/cycling girls. Longitudinal study designs are ideal for evaluating how metabolic energy strategies are associated with the quality of ovarian function after girls reach menarche, as they would follow participants over the remainder of their transition to observe whether these associations change, within individuals, as girls continue to mature reproductively. Additionally, said studies should further investigate the role of the HPAA as a possible mediator in establishing ovarian function regularity vis-à-vis with energy availability. Examining factors associated with variation in ovarian cycle quality and regularity will be important in informing on how environmental contexts and socio-ecological challenges can lead to changes in reproductive health outcomes during adolescence and throughout adulthood.

# Chapter 4. Sleep, hypothalamic-pituitary-adrenal axis activity, and metabolic energy patterns across the adolescent transition in a group of Mayan girls

## Abstract

**Background:** During the adolescent transition, individuals face critical energetic trade-offs between growth and reproduction, modulated by the hypothalamic-pituitary-adrenal axis (HPAA). Sleep is another energy regulation mechanism - it lowers metabolic rate, reducing energy expenditure and prompting greater energy conservation. Given this function, sleep may play a critical role as an energy regulation and recovery mechanism during the adolescent transition.

**Hypothesis:** Changing energy needs imposed by somatic growth and reproductive maturation will influence changes in sleep quality.

**Methods:** We analyzed data from 20 Mayan adolescent girls (12 to 15 years) from Guatemala, collected over a 19-day period in 2017. We used first morning urine samples to quantify biomarkers of HPAA activity (cortisol) and metabolic energy (c-peptide, a proxy of insulin, and adiponectin, inversely related to adipose fat mass), and calculated sleep efficiency and total sleep time as measures of sleep quality and quantity, respectively. We conducted mixed effects models to evaluate both concurrent and time-lagged relationships among metabolic energy patterns, HPAA activity, and maturation stage with sleep patterns.

**Results:** Adiponectin was positively associated with sleep efficiency, and cortisol was negatively associated with total sleep time, when examined concurrently. While not significant, we observed a trend suggesting that girls within 1 year of reaching menarche had higher sleep efficiency compared to girls in the pre-menarche reference group. There was no evidence of any significant relationships with c-peptide.

**Discussion:** Our observations are consistent with our hypothesis that shifts in energy demands during adolescence are associated with changes in sleep quality. Adolescent girls with lower energy storage had higher sleep quality, suggesting that lower adiposity may be leading to increases in sleep quality to restore energy that was used for growth and reproductive maturation. We interpret this result as consistent with the proposition that improvements in sleep quality occur in response to the energetic demands of adolescent growth and maturation.

# Introduction

Adolescence is the critical transition from childhood to adulthood, which is characterized by changes in somatic growth patterns and the maturation of the reproductive system. These changes involve an increase in metabolic energy requirements (Belsky, 2012; Ellison, 2017; Ellison et al., 2012; Stearns & Koella, 1986). From a life history theory perspective, this transition involves energetic trade-offs between growth and reproduction (Ellis, 2004).

Young human females (hereafter referred to as 'girls') usually begin their adolescent transition between the ages of 8 and 13 years old (Moodie et al., 2020) (Chapter 1, Figure 1-1). The transition is preceded by adrenarche, the maturation of the adrenal glands, which begins between the ages of 6-8 years old (Ellison, 2017). The adrenal glands secrete androgen dehydroepiandrosterone (DHEA), which triggers the first true stage of adolescent development - thelarche. During thelarche, breast budding occurs, which is followed by pubarche - the development of pubic hair, changes in body odour, and other secondary sexual traits (Ellis & Essex, 2007; Parent et al., 2003).

Somatic growth rates, such as peak height velocity (the fastest rate of growth in height stature) and skeletal maturation (e.g., increases in bone mineral density and pelvic widening), increase towards the latter end of thelarche (Gomula et al., 2024; Sharma et al., 2016). As somatic growth progresses, a shift towards greater hypothalamic-pituitary-gonadal axis (HPGA) maturation occurs, characterized by increases in the production of reproductive hormones - gonadotrophins (luteinizing hormone (LH) and follicular-stimulating hormone (FSH)) and gonadal steroids (estrogen and progesterone) - triggering the onset of ovarian cyclicity (Huhmann, 2020; Martin & Valeggia, 2018; Sharma et al., 2016). Menarche, defined as the first occurrence of menses, typically occurs towards the latter end of the adolescent transition, often 2-2.5 years after the onset of thelarche (Cabrera et al., 2014), and once other developmental milestones have begun or been reached (e.g., skeletal maturation and pubarche) (Chapter 1, Figure 1-1) (Ellis, 2004).

Developmental changes in metabolic energy allocation are proposed to support a shift towards reproductive function. Absolute basal metabolic rate (BMR) and resting metabolic rate (RMR) are higher among adolescents who have begun thelarche,

compared to pre-thelarche adolescents, suggesting that more energy is being used during rest to support both somatic and reproductive maturation (Cheng et al., 2016). As girls approach their menarche transition, they are expected to gradually shift from investing metabolic energy in somatic growth to investing more energy towards the onset of ovarian function. Changes in adipose fat storage, simultaneously with changes in insulin secretion activity, appear to increase in girls as they become more reproductively mature (Kelsey & Zeitler, 2016; O'Keeffe et al., 2020; Reiches et al., 2013).

Metabolic energy allocation amongst concurrent somatic development and reproductive maturation is regulated, in part, by the hypothalamic-pituitary-adrenal axis (HPAA) (Harrell et al., 2016). HPAA activation involves the release of corticotrophin-releasing hormone (CRH) by the hypothalamus. CRH secretion triggers the release of adrenocorticotrophin hormone (ACTH) from the anterior pituitary, which, in turn, triggers the release of glucocorticoids, such as cortisol, by the adrenal glands (Joseph & Whirledge, 2017). Cortisol can trigger energy mobilization from adipose fat tissue and induce changes in insulin sensitivity to promote energy uptake by somatic and reproductive tissues to meet their energy demands (Di Dalmazi et al., 2012; Huybrechts et al., 2014). We have previously observed associations between HPAA activity, energy uptake, and energy storage in pre- and post-menarche girls, suggesting that the HPAA plays a role in regulating energy availability to different systems and tissues across the adolescent transition (Rowlands et al. in preparation).

Sleep is another important factor in adolescence whose role in energy allocation is yet to be fully characterized. Sleep functions as a critical energy restoration and conservation mechanism by reducing metabolic rate (energy utilization), especially during slow-wave sleep phases (deep sleep), which reduces energy expenditure and promotes greater energy conservation (Kayaba et al., 2017; Schmidt, 2014; Schmidt et al., 2017). Greater energy recuperation during sleep supports more efficient tissue repair and growth, immune system maintenance, and cognitive support, including memory consolidation, affective processing, and cognitive development (Sharma & Kavuru, 2010; Walker, 2009).

Total sleep needs have been hypothesized to increase in adolescents in response to increased energetic demands of this transition (e.g., to support cognitive development and somatic growth) (Carskadon et al., 2002; Ohayon et al., 2004). However, evidence

supporting this hypothesis is mixed. Some studies report that adolescents sleep less than younger children, while others report no significant changes at different stages within the transition (Ohayon et al., 2004; Sadeh et al., 2009; Shochat et al., 2014; Tarokh et al., 2016). A study evaluating sleep patterns among adolescents living in urban environments report that, while total night sleep time does not differ between different stages of adolescence, daytime sleepiness does increase among older adolescents (Carskadon et al., 1980). This is the only evidence consistent with the hypothesis that maturing adolescents have greater sleep needs. Yet, this evidence insinuates that achieving optimal sleep outcomes may be hindered by environmental factors (Carskadon et al., 1980; Ohayon et al., 2004). For example, school start times have been established as a key factor that prevents adolescents in Western industrialized settings from achieving adequate sleep, as they are discordant with the later sleep onset and later wake times that are characteristic of the adolescent transition (Santos & Louzada, 2022). Thus, it is important to consider both the socio-cultural and physical environment when evaluating sleep behaviours (choices or habits around sleep) and sleep patterns and outcomes (sleep quality, sleep quantity, and sleep architecture) among adolescents (Worthman & Melby, 2002).

Interestingly, adolescents from small-scale or non-industrial populations report overall poorer sleep quality and more variation in other sleep outcomes, compared to adolescents in industrialized settings (Ouyang et al., 2009; Silva-Caballero et al., 2023). These differences may be explained by other factors in the physical environment that affect entire communities, such as access to electricity or temperature-controlled environments, which can also affect sleep outcomes. Indeed, among non-Western and non-industrial populations with lower access to artificial light and technological developments, adults have poorer sleep quality compared to industrialized populations (McKinnon et al., 2022), which may also affect adolescents' sleep outcomes. Additionally, culturally enforced, social expectations impose various demands on adolescents in their transition to young adulthood (e.g., school attendance, academic performance, work, supporting the household, caring for younger siblings, and social activities). Said expectations may impact their sleep behaviours and ultimately their sleep patterns and outcomes (Worthman & Melby, 2002). Consistent with this tenet, among other factors, stress associated with culturally-imposed expectations can

exacerbate poor sleep quality and quantity (Buckley & Schatzberg, 2005; Carskadon et al., 2002; Worthman & Melby, 2002).

To our knowledge, no studies have evaluated how the HPAA and sleep may act synergistically to modulate metabolic energy management to meet the energetic demands of the adolescent transition. As such, it remains unclear whether energy needs are driving sleep activity to support maturation, and whether sleep acts as a mechanism to modulate metabolic energy restoration in response to the energy needs of the adolescent transition. To fill this gap in knowledge, we evaluate sleep's role as an energy restoration mechanism during reproductive maturation. We hypothesize that changing energy needs imposed by somatic growth and reproductive maturation will influence changes in sleep outcomes (i.e., sleep quality and sleep duration). Specifically, we predict that low energy availability (lower c-peptide, a marker of insulin, and higher adiponectin levels, an inverse marker of visceral adipose fat mass) will be associated with high sleep quality (measured by sleep efficiency) and long sleep duration (measured by total sleep time). See variable descriptions in Table 4-1.

# Methods

**Table 4-1:** Description of variables

| Variable name | Variable definition |
|---|---|
| Age | Chronological age of the participant at the start of data collection, in January 2017 |
| Maturation stage | Maturation stage organized in three categories: pre-menarche, +/-1 year relative to menarche, >1 year since menarche |
| Total Sleep Time | Total time spent asleep during one night of sleep, calculated as the sum of all the coded sleep periods during a given night |
| Sleep Efficiency (%) | Time spent asleep as a percentage of total time spent in bed during one night of sleep; sleep efficiency = total sleep time/total time spent in bed |
| Cortisol (ng/mL) | Log10 transformed measure of cortisol, a proxy for HPAA activity; quantified from the first morning urine sample that correlates to the previous night's sleep measurement |
| C-peptide (ng/mL) | Log10 transformed measure of c-peptide, a proxy for energy uptake activity; quantified from the first morning urine sample that correlates to the previous night's sleep measurement |
| Adiponectin (ng/mL) | Log10 transformed measure of adiponectin, negatively correlated with visceral adiposity, a proxy for energy storage; quantified from the first morning urine sample that correlates to the previous night's sleep measurement |

## Study population

To test the predictions that contribute to our hypotheses, we used data collected within the context of the Consequences of Periconceptional Events (COPE) study (Barha et al., 2019; Nepomnaschy et al., 2004). COPE is a longitudinal, naturalistic cohort that follows 52 girls throughout their development, in 21 cases from their conception in 2000-2001. COPE takes place in an Indigenous, Mayan Kaqchikel community in the highlands of Guatemala (Barha et al., 2019; Nepomnaschy et al., 2004). Complementing demographic and hormonal data collection as part of COPE, in a field season conducted in 2017, 20 COPE adolescent girls (aged 12-15) participated in the collection of sleep data.

## Data and biospecimen collection

Starting in January 2017, participants self-collected first morning urine (FMU) samples every other day over a four-month period in chemically inert plastic urine collection containers. During this data collection period, each participant provided an average of 36 FMU samples, representing a collection compliance rate of 88.25% (i.e., 36 of 41 possible samples per girl). Local female Kaqchikel and Spanish speaking field assistants collected and aliquoted (2 mL) the FMU samples each morning in the local laboratory and stored them at -10°C while in the field. At the end of the field season, all sample aliquots were shipped on dry ice to the Maternal and Child Health Laboratory at Simon Fraser University (SFU), where they are stored at -80°C.

Additionally, in January of 2017, 20 of the adolescent girls provided sleep data for 19 consecutive days at the start of the FMU sample collection period. Sleep data was collected via actigraphy watches (CamNtech Motionwatch8), which participants wore continuously to capture 24-hour active and rest periods. Actigraphy watches use a built-in accelerometer, which enables quantification of movement in one-minute time intervals – when little or no movement occurs, the time interval is quantified as a sleep period. Participants were instructed to press a button on the actigraphy watches when they went to bed and got up from bed, which provided more information for determining sleep periods (onset and awakening) and total time spent in bed to increase reliability of measures of sleep. To evaluate sleep patterns, we translated the actigraphy data into measures of binary sleep-wake periods (i.e., a one-minute period is coded as either an ‘awake’ period or a ‘sleep’ period), and evaluated these coded sleep-wake periods over 24-hour cycles to determine when sleep periods occurred. We used sleep efficiency, the percentage of total time spent asleep out of the total amount of time spent in bed per night, as our quantitative measure of sleep quality (Table 4-1). We used total sleep time, a measure of total sleep duration measured in minutes for each night of sleep, as our measure of sleep quantity (Table 4-1) (Samson, 2021). As our measures of sleep quality and quantity presented high levels of multicollinearity, they were included in separate models when considered as predictor variables.

Self-reported information regarding age at menarche was derived from demographic questionnaires administered once with each participant during the field season by the local field assistants. Self-reported age at menarche is considered a reliable technique,

as age at menarche presents high levels of recall accuracy (Cooper et al., 2006; Lundblad & Jacobsen, 2017). A subsequent field season conducted in 2023 included a demographic questionnaire in which follow-up questions related to age at menarche were asked. Information from these questions either confirmed the repeatability of self-reported age at menarche from 2017, or collected age at menarche information for the first time from girls who had yet to experience menarche during the 2017 field season.

## Hormone Assays

We used FMU samples to quantify biomarkers (hormone concentrations) of HPAA activity and metabolic energy. FMU free cortisol concentrations provide information on cortisol secretion, reflecting basal HPAA activity, throughout the night since the last urinary void (Table 4-1). FMU sampling reduces the confounding effects of food and caffeine consumption and physical activity that affect cortisol levels, as they do not normally occur during the sleeping period (Nepomnaschy et al., 2004). We used FMU adiponectin and c-peptide as markers of metabolic energy (Table 4-1). Adiponectin is produced by visceral adipose fat cells and, as such, it is used as a biomarker of energy storage. This hormone is inversely related to the amount of visceral adipose fat mass accumulated (Brochu-Gaudreau et al., 2010). Energy stored in adipose tissue can be mobilized and directed towards specific tissues. We used c-peptide as a proxy for insulin levels - c-peptide is released during the conversion of proinsulin to insulin, in 1:1 equimolar amounts with insulin (Emery Thompson & Knott, 2008). Higher c-peptide levels indicate that more insulin in being secreted by pancreatic beta cells (Petersen & Shulman, 2018). Insulin is an endocrine peptide hormone that facilitates glucose uptake in target tissues for either immediate use or for long-term storage (Petersen & Shulman, 2018).

We conducted all hormone analyses at the Maternal and Child Health Laboratory at SFU. To that aim, we used enzyme linked immunosorbent assays (ELISAs) (Quansys Biosciences, Utah, USA), which our lab has previously validated (lower limits of detection for cortisol= 0.343 ng/mL; adiponectin = 0.023 ng/mL; and c-peptide= 0.090 ng/mL) (Salvante et al., 2012). To adjust for urine dilution levels, we corrected all hormone concentrations for specific gravity using refractometry (Miller et al., 2004). To reduce the effects of variation among assays, we ran all the samples from the same

participant in the same assay. All intra-and inter-assay coefficients of variation were below 7% and 11%, respectively.

## Data Analysis

Hormone and sleep data alignment: We aligned FMU hormone and sleep data points so that a sleep night was matched to the same night's hormone levels, which corresponds to the following morning's FMU sample, in order to model metabolic energy levels, cortisol secretion, and sleep during the same night, longitudinally across our 19-day sleep sampling period. After aligning FMU and sleep data points, participants had an average of 7.4 matched data points.

Reproductive maturation status: We imputed participants' reproductive maturation stage based on information on age at menarche collected in the demographic questionnaire. Maturation stage was based on each participant's timing of when they reached menarche relative to when data was collected during the 2017 field season. To determine time relative to menarche, we subtracted age at menarche from age at the time of data collection in 2017. Age at menarche for participants who had not yet gone through menarche in 2017 was confirmed in the follow-up demographic questionnaire conducted in 2023. Significant differences in growth and maturation processes are observed among girls at various stages of maturation – those who are further from reaching menarche, close to reaching menarche, and in more advanced stages of maturation post-menarche (DiVall & Radovick, 2008; Mathur, 2022). These three stages reflect distinct, physiological differences with respect to growth and maturation, and shifting energy demands to support these developmental processes. As such, we categorized girls as being either "pre-menarche"; "+/- 1 year relative to menarche"; or ">1 year post-menarche" during the 2017 field season when sleep and hormone data were collected (Table 4-1) to ascertain whether sleep and energetics varied among maturation stages.

## Statistical Analysis

We first inspected the data for non-normal distribution and errors. Cortisol, adiponectin, and c-peptide, which were all right-skewed, were log10-transformed to be approximately normally distributed on the logarithmic scale (Osborne, 2019). Datapoints that fell

outside average biological ranges for each variable were examined. Those datapoints that were well outside the interquartile range, within and between participants, and correlated with extreme values of other hormones were identified as outliers with potential errors (e.g., sample or assay error) and removed from further statistical analysis. A total of 6 outliers out of 447 datapoints were identified and removed from the dataset.

We fit linear mixed effects-models using the LMER function in R (RStudio, 2023). Linear mixed-effects models take into account both fixed and random effects, which are likely present due to the nature of repeated measures collected on individuals. This longitudinal statistical modelling approach also accounts for unbalanced or missing data due to missing FMU samples (Cnaan et al., 1997).

To evaluate whether sleep activity for energy recuperation varies as a function of variation in metabolic energy needs, we fit the following linear mixed-effects models, where time t=0:

1) Sleep Efficiency = Adiponectin + C-peptide + Cortisol + Maturation stage + (1|subject)
2) Total sleep time = Adiponectin + C-peptide + Cortisol + Maturation stage + (1|subject)

To identify statistical significance of predictor variables in each model, we used a p-value with an alpha = 0.05, and 95% confidence intervals. The distribution of the residuals compared to the fitted models was assessed to evaluate normalcy.

# Results

*Description of the data*

The means, standard deviations, medians, and range, overall and stratified by maturation stage, for all predictor and outcome variables are described in Table 4-2. Trends in sleep and hormone patterns are presented in figures 4-1 through 4-5. Total sleep time did not present any trend across maturation stage. Sleep efficiency and adiponectin's patterns both followed an inverted U shape across the three maturation

stages. Cortisol's data pattern followed a U-shaped distribution across maturation stages. C-peptide increased very slightly across maturation stages (Figures 4-1 through 4-5).

**Table 4-2:** Data description of each variable[1] stratified by maturation stage

| | **Pre-Menarche (n=5)** | **+/- 1 Year (n=8)** | **> 1 Year (n=7)** | **Overall (n=20)** |
|---|---|---|---|---|
| **Age** | | | | |
| Median | 13 | 13.5 | 14 | 14 |
| **Total Sleep Time** | | | | |
| Mean (SD) | 6h 54m (41.5m) | 7h 7m (49.6m) | 7h 3m (44.2m) | 7h 2m (45.8m) |
| Median [Min, Max] | 6h 49m [5h 7m, 8h 41m] | 7h 11m [4h 50m, 8h 37m] | 6h 56m [5h 45m, 9h 8m] | 7h 1m [4h 50m, 9h 8m] |
| **Sleep Efficiency (%)** | | | | |
| Mean (SD) | 78.6 (4.37) | 81.8 (3.08) | 78.6 (2.17) | 79.9 (3.42) |
| **Cortisol (ng/mL)** | | | | |
| Mean (SD) | 3.58 (0.744) | 3.27 (0.697) | 3.31 (0.684) | 3.36 (0.679) |
| **C-Peptide (ng/mL)** | | | | |
| Mean (SD) | 1.09 (0.245) | 1.20 (0.335) | 1.31 (0.344) | 1.21 (0.313) |
| **Adiponectin (ng/mL)** | | | | |
| Mean (SD) | -0.393 (0.683) | -0.310 (0.461) | -0.381 (0.472) | -0.356 (0.498) |

[1]*log10-transformed cortisol, c-peptide, and adiponectin*

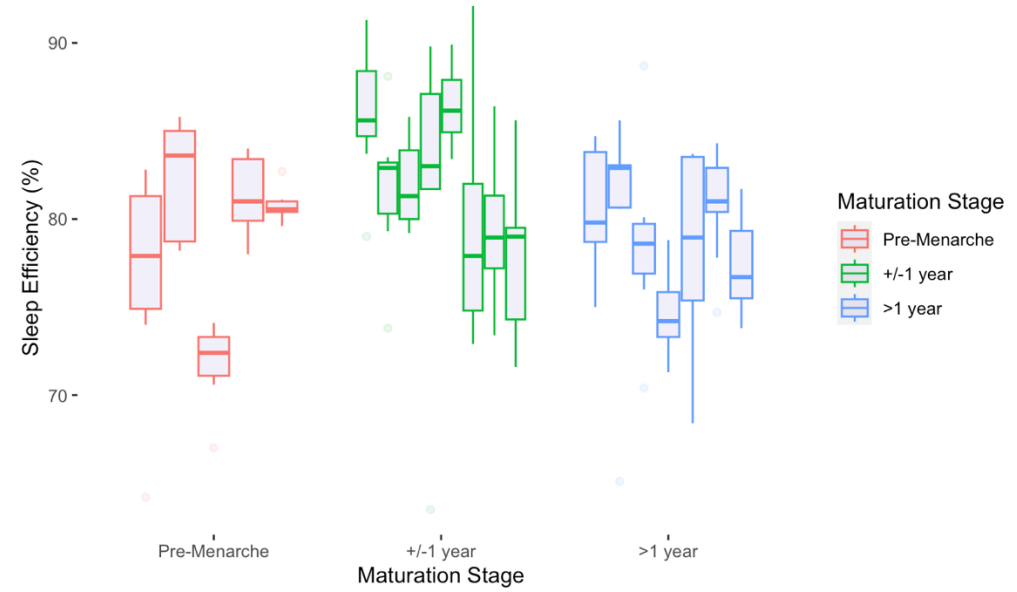


**Figure 4-1:** Distribution of sleep efficiency (%) within and among girls, across the transition, with participants ordered chronologically relative to menarche within their maturation stage

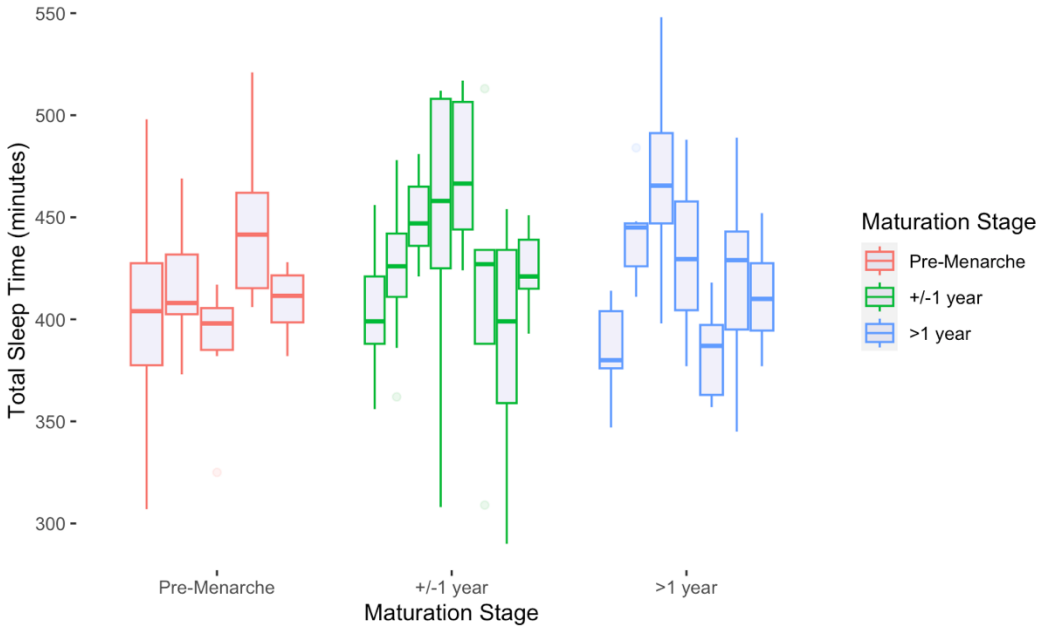


**Figure 4-2:** Distribution of total sleep time (in minutes) within and among girls, across the transition, with participants ordered chronologically relative to menarche within their maturation stage

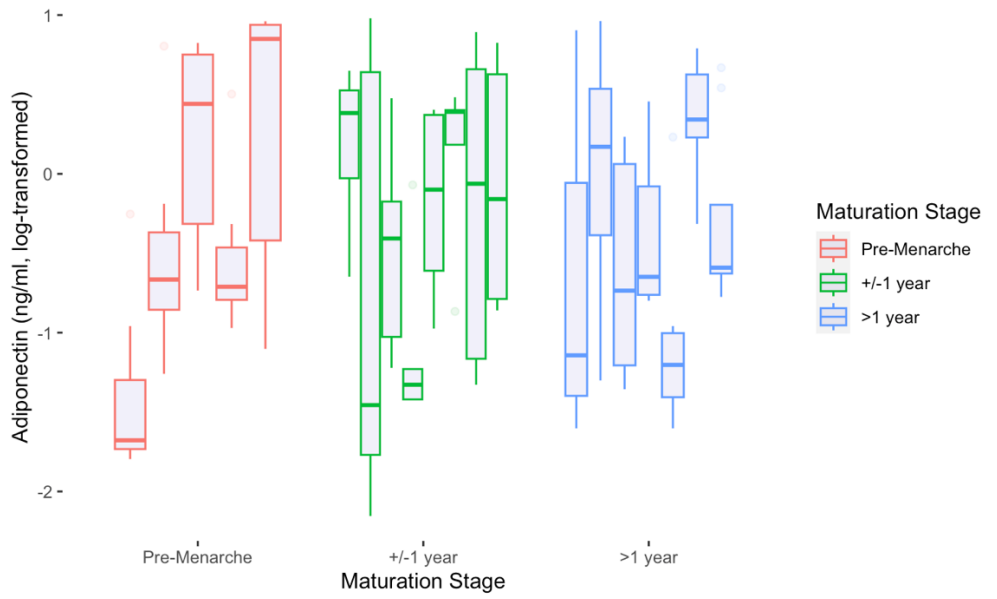


**Figure 4-3:** Distribution of log10-transformed adiponectin within and among girls, across the transition, with participants ordered chronologically relative to menarche within their maturation stage

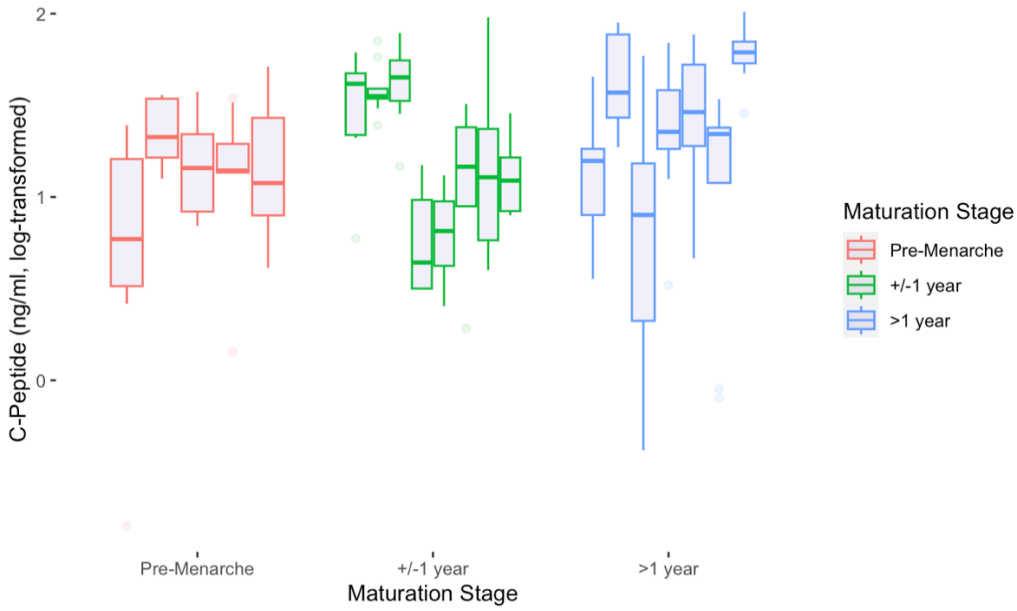


**Figure 4-4:** Distribution of log10-transformed c-peptide within and among girls, with participants ordered chronologically relative to menarche within their maturation stage

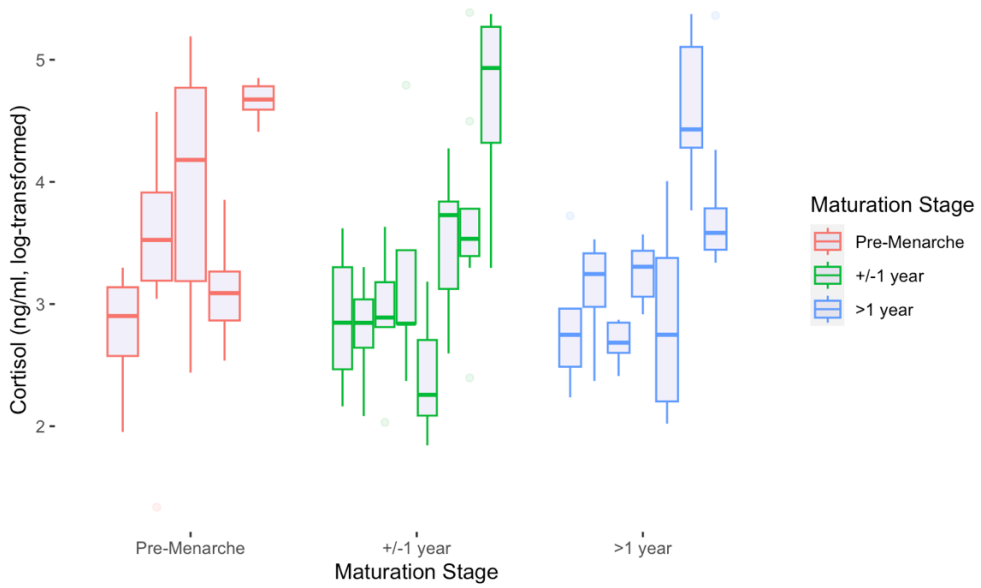


**Figure 4-5:** Distribution of log10-transformed cortisol within and among girls, across the transition, with participants ordered chronologically relative to menarche within their maturation stage

*Adiponectin and c-peptide relationships with concurrent sleep efficiency and total sleep time*

In our analysis evaluating factors associated with sleep efficiency, adiponectin was positively associated with sleep efficiency with an estimated effect of 1.35 (p-value < 0.01; 95%CI [0.318, 2.386]) (Table 4-3, Figure 4-6). Every one unit (1ng/ml) increase in log10-transformed adiponectin was associated with an estimated 1.35 percent increase in sleep efficiency, while all other variables were held constant. Neither c-peptide, nor any other variables were statistically associated with sleep efficiency, holding all other variables constant (all p values > 0.05, Table 4-3).

In our analysis evaluating factors associated with total sleep time, cortisol was the only predictor variable significantly associated with total sleep time, holding all other variables constant. Cortisol was negatively associated with total sleep time with an effect estimate of -10.89 (p<0.05; 95% CI [-21.308, -0.464]). For every unit (1 ng/ml) increase in log10-transformed cortisol, total sleep time decreased by 10.89 minutes. No other variables (including neither adiponectin nor c-peptide) were statistically associated with total sleep time (all p values >0.05, Table 4-3).

**Table 4-3:** Parameter estimates and confidence intervals for adiponectin c-peptide, and cortisol[1] effects on sleep efficiency (%) and total sleep time (minutes)

| | Sleep Efficiency | | Total Sleep Time | |
|---|---|---|---|---|
| | **Effect estimate** | **95% CI** | **Effect estimate** | **95% CI** |
| **(Intercept)** | 83.839*** | 78.593, 89.084 | 453.248*** | 405.556, 500.940 |
| **Adiponectin (ng/mL)** | 1.352* | 0.318, 2.386 | 7.458 | -2.213, 17.129 |
| **Cortisol (ng/mL)** | -0.877 | -2.010, 0.256 | -10.886* | -21.308, -0.464 |
| **C-Peptide (ng/mL)** | -1.297 | -3.045, 0.452 | 0.469 | -15.750, 16.688 |
| **Maturation stage +/-1 year** | 2.885 | -0.601, 6.371 | 11.705 | -18.924, 42.334 |
| **Maturation stage >1 year** | -0.067 | -3.644, 3.510 | 7.165 | -24.219, 38.548 |

*** p < 0.001; ** p < 0.01; * p < 0.05
Maturation stage reference group: pre-menarche
[1]*log10-transformed adiponectin, c-peptide, and cortisol*

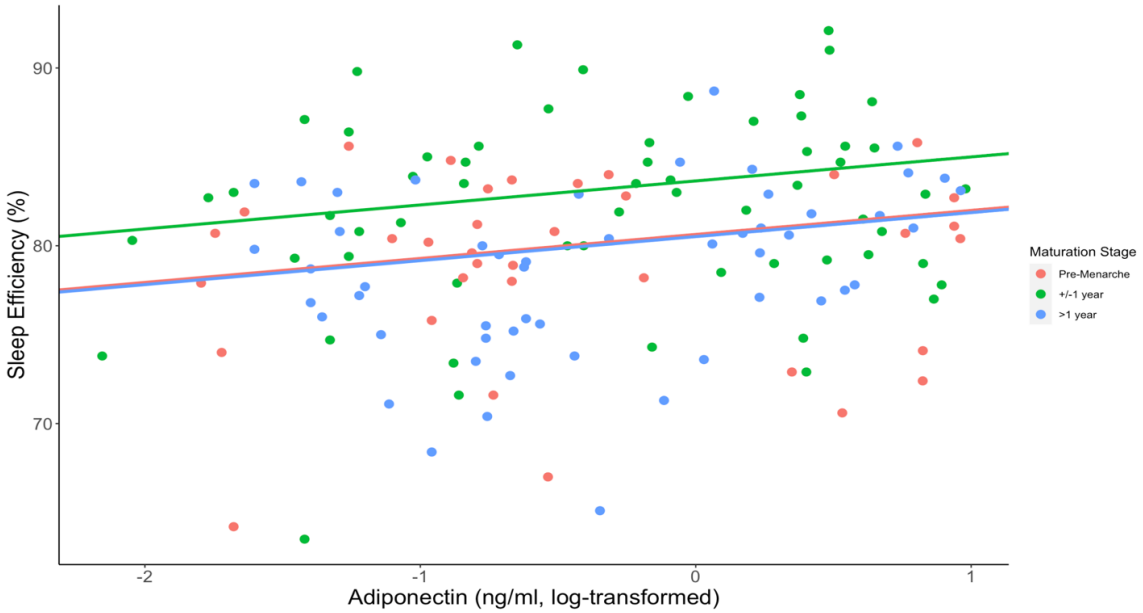


**Figure 4-6:** Estimated relationship between sleep efficiency (%) and log10-transformed adiponectin for girls, holding the mean of all other predictor variables constant (see means in Table 4-2)

# Discussion

Our analyses of data provided by a group of Kaqchikel Mayan adolescent girls during pre- and post-menarche stages suggest that only HPAA activity was associated with concurrently-measured sleep quantity, as we observed a negative association between cortisol and total sleep time. There was no evidence of any relationships between concurrently-measured energy storage or energy uptake and sleep quantity. In contrast, girls with lower levels of energy storage (indicated by high adiponectin) had higher concurrently-measured sleep quality (measured by sleep efficiency). Energy uptake, cortisol, and maturation stage were not associated with concurrently measured sleep quality. Given the significant associations observed between sleep quality and energy storage, here we focus on sleep quality and the process of energy restoration during the adolescent transition in conjunction with HPAA activity and maturation stage.

*Population differences in sleep quality and quantity*

We previously reported that sleep quality (measured by sleep efficiency) was lower among adult women in this Kaqchikel Mayan population than that of post-industrial populations from the Global North (McKinnon et al., 2022). On average, adult women in this population have an average sleep efficiency of 78.74%, while in a population from the United States, for example, adult men and women's average sleep efficiency was 83.26% (McKinnon et al., 2022). With an average of 79.9%, the adolescent girls' sleep efficiency in our sample is comparable to that of adult women in their community. Their sleep efficiency is relatively low compared to a cut-off value of 86% for healthy sleep efficiency for adolescents from post-industrial populations (Blunden & Galland, 2014). Yet, the sleep efficiency patterns among the Kaqchikel adolescent participants are similar to those reported for adolescents from a similar socio-ecological, Indigenous Mayan context. Silva-Caballero et al. (2023) report 84% and 78% sleep efficiency among adolescents from two agricultural Indigenous Mayan populations in Puebla and Campeche in Mexico, respectively, compared to 88% sleep efficiency among adolescents in post-industrial Mexico City (Silva-Caballero et al., 2023). Adolescents (48% female) from each population were between the ages of 11-16, and samples ranged from n=44 to n=50 (Silva-Caballero et al., 2023). Silva-Caballero and colleagues (2023) interpret the poorer sleep quality they observed in these populations as the result of social commitments and expectations rooted in traditional Mayan, agricultural settings,

as well as factors such as social sleeping practices combined with greater dependence on natural light cycles, compared to more urban settings (Silva-Caballero et al., 2023). Given potential similarities in culture and physical environments between the Indigenous Mayan populations in Mexico and the participants in our study, Silva-Caballero and colleagues' (2023) arguments could help explain the relatively low adolescent sleep quality we observe, compared to post-industrial populations.

Most studies evaluating sleep outcomes among other small-scale or non-industrial populations of adolescents focus on sleep onset times, and overall total nightly sleep duration, rather than sleep quality specifically. Those studies report, for example, that adolescents living in small-scale or subsistence populations in Argentina and China with no access to electricity have earlier sleep onsets and longer total sleep durations, compared to those with access to electricity who report later sleep times, but similar wake times thus resulting in shorter sleep durations (de la Iglesia et al., 2015; Ouyang et al., 2009). Total sleep duration in the sample of adolescent girls in our study (average of 7.02 hours/night) is lower than that of adolescents in rural China with poor access to artificial light (8.6 hours on weekdays and 9.4 hours on weekends) (Ouyang et al., 2009). Total sleep duration among adolescents in our study is also lower than sleep duration reported during the winter season among adolescents from an Indigenous Toba/Qom population in Argentina (8.47 hours) with no reported access to electricity (de la Iglesia et al., 2015). Interestingly, in the summer months the Indigenous Toba/Qom adolescents report 6.98 hours of sleep (de la Iglesia et al., 2015) which is similar to the 7.02 hours of sleep we recorded among the Kaqchikel adolescent participants. This similarity may be partially explained by the comparable sleep onset and wake times of the Toba/Qom adolescents during the summer season and those of our Kaqchikel participants, despite total daylight hours being different (14 hours during the summer in Argentina versus 11 hours during the dry season in Guatemala during which data for our study was collected). Similarities in electricity access, social activities, and other climatic conditions that may be associated with these two seasons (i.e., high temperatures) may also help explain similar sleep behaviours and subsequent sleep durations.

Given the variation in sleep patterns reported above in relation to environmental sleep conditions, it is important to consider the socio-cultural context in addition to the physical environment that may explain sleep patterns among adolescents (Worthman & Melby, 2002). The adolescent girls who participated in our study live in a small-scale,

agricultural community, where older daughters take on significant responsibility in caring for their family household as they mature. Adolescent girls play an important role in younger sibling care, and in food preparation, which is very involved and begins very early in the morning. Said responsibilities are examples of societal and cultural expectations among this population that impact adolescent girls' behaviour, and may ultimately impact sleep behaviours and sleep outcomes, e.g., earlier wake times and shorter sleeps (Worthman & Melby, 2002).

Among adolescents, stress associated with culturally imposed expectations, whether in non-industrial, or in modern-industrial contexts, may further exacerbate disrupted or poor sleep quality and quantity (Carskadon et al., 1980; Worthman & Melby, 2002; Worthman & Trang, 2018). This is consistent with our finding that higher levels of HPAA activity were associated with lower sleep quantity. HPAA activation reflects a stress response to challenges present in the environment; other studies evaluating the effects of the HPAA as an indicator of stress report that increased HPAA activation is associated with decreased sleep quality and quantity (Buckley & Schatzberg, 2005; Chrousos et al., 2000). As such, the association we observed could reflect how these external pressures negatively impact sleep outcomes. Further, these social pressures, and the larger context of socio-ecological challenges may impact the relationship between sleep and metabolic energy regulation.

*Sleep efficiency and energy storage*

Girls with lower visceral adipose fat storage (as indicated by higher adiponectin levels), had higher sleep quality (measured by sleep efficiency) which is consistent with our prediction that those with lower stored energy need to recuperate more energy. Our results are also consistent with those reported by other studies. Riso and colleagues (2018), for example, report that among young adolescents (aged 10-12) from Estonia, body fat percentage and waist to hip ratio were negatively associated with average sleep duration (less fat percentage being linked with longer sleep) (Riso et al., 2018). Similarly, studies conducted by Ogilvie and colleagues (2016) report that in a sample of 4,077 adults in the US, those with lower adipose fat mass showed higher sleep efficiency (Ogilvie et al., 2016). Our results are also consistent with those of Magnusdottir and colleagues (2021) who report that in a sample of adults, adiponectin was positively associated with sleep efficiency, reflecting a negative association between visceral

adipose tissue and sleep quality (Magnusdottir et al., 2021). Importantly, we should note that these studies were conducted to investigate links between sleep, energy reserves and cardiovascular health among adults, and these authors interpret their results to suggest that increased adiponectin levels, or lower body fat percentage, reduces the risk of high blood pressure, hypertension, and inflammation (Magnusdottir et al., 2021). We interpret the negative association we observe between energy storage and sleep quality to reflect a pathway by which adolescent girls may be recovering metabolic energy expended during reproductive maturation. As such, the sleep architecture among adolescent girls may reflect this energy restoration or conservation mechanism and may be different to the studies presented above, as it is specific and unique to this developmental period.

Relevant to the adolescent transition, sleep promotes greater energy recuperation and conservation by reducing metabolic rate, especially during slow-wave sleep phases (deep sleep), which reduces energy expenditure (Kayaba et al., 2017; Schmidt et al., 2017). Greater energy recuperation during sleep supports tissue repair and growth, and cognitive and immune system maintenance (Sharma & Kavuru, 2010; Walker, 2009). Accordingly, sleep triggers the secretion of growth hormone (Olarescu, 2019; Redwine et al., 2000). Growth hormone release is highest during slow-wave sleep periods and promotes tissue growth, and, specifically during adolescence, bone and cartilage growth (Kim et al., 2015). Childhood and adolescent developmental periods also represent a critical window for cognitive development. During this period, sleep duration and quality are strongly correlated to brain development, including associative memory processing, emotional regulation, affective brain processing, and motor development (Brand & Kirov, 2011; Walker, 2009). Cognitive development is an energetically demanding process. Indeed, poor energy availability is associated with disruptions in brain development and cognitive function (Benton et al., 1996; Prado & Dewey, 2014). Consistent with those observations, sleep disturbances and chronic poor sleep among adolescents are correlated with increased risk of disruption in cognitive processing and function, memory consolidation and increased risk of conditions such as ADHD (Brand & Kirov, 2011). Our analyses show that participants with lower energy stores presented higher sleep quality. Within the context just described, we interpret our results to mean that girls who are utilizing energy to support growth and maturation, including cognitive development, have

high energy needs, which are associated with higher sleep quality to recuperate these expended energy stores.

Greater sleep quality should reduce metabolic rate; thus less energy is being mobilized from storage, which should enable adolescent girls to recuperate sufficient energy to continue to support growth and maturation processes during their pre- to post-menarche transition (Schmidt, 2014; Schmidt et al., 2017). Future research should investigate bi-directional relationships between sleep and energy, to evaluate whether improvements in sleep may lead to subsequent changes in fat accumulation. Furthermore, future studies should evaluate how the HPAA interacts with sleep to possibly influence energy accumulation processes. Our previous findings suggest that the HPAA, in response to the energetic demands imposed by the adolescent transition, facilitates metabolic energy mobilization from fat stores (Rowlands et al., in preparation). Conducting bidirectional analyses would, thus, enable us to evaluate whether the HPAA and sleep are acting synergistically as energy regulation mechanisms to respond to the energetic demands of adolescent growth and maturation.

Girls who are closer to their menarche transition are expected to be investing in both growth and reproductive maturation. As such, girls within 1-year pre- or post-menarche may have higher energy demands than girls in very early stages of adolescence, before substantial investment in HPGA maturation has occurred, or girls in more advanced stages, when investment in somatic growth has slowed or ended (Cheng et al., 2016; Reiches & Ellison, 2022). Consequently, during the peri-menarche stage, girls may have greater energy needs and little excess energy to be allocated to fat storage. Although non-significant, our observation that girls within 1-year pre- or post-menarche had greater sleep quality is in line with the hypothesis that this period presents higher energy demands that precedes and follows menarche.

## Limitations

Our study design involves possible limitations to investigate associations between energy uptake and sleep quality. While we quantified c-peptide, our proxy for insulin, in first morning urine samples, which reflect nocturnal secretion, insulin secretion peaks in the mid-afternoon and falls during the night (Boden et al., 1996). Additionally, energy uptake patterns are susceptible to short term changes in energy intake, which are more

frequent during the day than during the night (Poggiogalle et al., 2018). As such we may not have adequate information on circadian insulin secretion patterns in our analyses to sufficiently identify their true associations with sleep patterns. Additionally, insulin resistance is characteristic of the early adolescent transition, prior to menarche, and triggers an increase in insulin secretion (Jeffery et al., 2012; Kelsey & Zeitler, 2016). However, we do not know which stage of insulin resistance participants are in. As such we were not able to adjust for insulin resistance, which may be introducing unaccounted variation in the analysis between insulin activity and sleep patterns. Additionally, we do not have accurate information on dietary intake or physical activity levels for participants. Thus, we cannot investigate their direct effects on metabolic energy activity and status, and their influence on participants' sleep behaviours and subsequent sleep patterns.

Maturation stage was based on self-reported age at menarche. Although studies validating self-reports of age at menarche report consistent, high levels of accuracy in recall of age at menarche (Cooper et al., 2006; Lundblad & Jacobsen, 2017), these validation studies are conducted primarily among Western, industrialized populations. The self-reported age at menarche among the population participating in this study may not be as reliable, thus introducing potential errors in the accuracy of a girl's maturation stage. Our sample size is also small, which may limit our statistical power to identify significant associations between energy status and sleep outcomes, or longer-term changes in energy storage in response to changes in sleep quality. However, the population in which this study was based has lower levels of heterogeneity, which is a strength of our study as it reduces the effects of potential confounding factors that may affect our results, thus improving our statistical power.

## Significance and implications of findings

Much of the scientific literature on sleep research focuses on overall sleep health, rather than maturation stage-specific patterns in sleep, and seldomly includes analyses of its associations with HPAA activity and metabolic energy in relation to the energetic demands of maturation. To contribute to this gap, our study investigates relationships between energy status, HPAA activity and sleep patterns in girls during their critical life history transition from pre- to post-menarche. We observe that during adolescence, low levels of stored metabolic energy, in the form of visceral fat, are linked with better sleep efficiency which should result in more effective energy restoration. These pathways

appear to be present across the transition from pre- to post-menarche, which provides evidence that sleep, in conjunction with the HPAA, plays an important role as a metabolic energy regulation mechanism to support healthy growth and development.

Previous research has identified socio-structural factors associated with the adolescent experience that negatively impact sleep, including school attendance and school start times, work, familial responsibilities, and increases in social activities and peer-influences (Worthman & Melby, 2002). Among the adolescents included in this study, cultural expectations such as household and familial responsibilities or variable access to electrical light may be negatively impacting sleep behaviour. Given these factors could be impacting sleep, our results should inform discussion around ways to better support adolescents, or to support local advocacy for infrastructure change, for positive impact on sleep behaviour. Targeting socio-structural factors that impact sleep during adolescence is necessary to support optimal growth and development, to support healthy life trajectories across the lifespan.

The overall rates of lower sleep quality we observed in our sample compared to post-industrial adolescent populations could introduce a compounding effect of poor sleep on metabolic energy availability in this population. Chronic poor sleep could limit the amount of energy that is recuperated, leading to delays in growth and variation in the pace of reproductive maturation. To further understand how sleep impacts growth and maturation trajectories, mediated by metabolic energy availability, future studies should compare adolescent sleep architecture in different socio-cultural contexts and its association with metabolic energy management. Understanding how differences in socio-cultural expectations, along with variation in access to electricity, home and school responsibilities, and peer networks, are associated with variation in maturation trajectories could improve our understanding of how sleep hinders or promotes optimal development.

Further research examining whether changes in adipose fat mass are linked to changes in sleep quality longitudinally, and whether these effects are dependent on growth and reproductive maturation stage, should also consider the longitudinal role that sleep plays in non-pathological variation in metabolic energy availability. To examine these relationships, studies should conduct long-term, within-participant, longitudinal analyses, to ascertain how sleep quality and quantity influence subsequent energy recuperation

and storage. These longitudinal studies should further evaluate how these sleep patterns act in conjunction with the HPAA to regulate metabolic energy availability and allocation, and how this activity relates to changes in growth and maturation across the transition. To generate more in-depth analyses of these sleep characteristics, and their associations with growth and maturation, within-participant analyses across the transition could also measure sleep architecture, to evaluate how slow-wave sleep and REM cycles (rapid eye movement) change across the adolescent transition. To investigate if metabolic energy storage levels, HPAA, and sleep patterns change as girls become more reproductively mature, future studies could also implement a sleep intervention to track subsequent changes in energy storage, increasing the frequency of biomarker and sleep data collection to capture temporal sleep-energy interactions. These approaches could use metabolic modeling to predict adiposity shifts based on improvements in sleep quality and incorporate physical activity data to assess its role as another mediator involved in the relationship between sleep and metabolic energy.

# Chapter 5. Discussion, significance, and implications for future research directions

## Overview of main findings

This dissertation investigated the hypothalamic-pituitary-adrenal axis (HPAA) and sleep as potential biological mechanisms that mediate metabolic energy availability across the adolescent transition among girls. I evaluated hypotheses on energetic trade-offs between growth and reproduction, and how the energetic requirements of reproductive maturation and function may change across the transition, prompting changes in metabolic energy management strategies. In the first study presented in this dissertation, I evaluated how factors such as somatic growth and metabolic energy regulation are associated with variation in the timing of menarche. In the second study, I evaluated whether the relationship between HPAA activation and metabolic energy changes within girls across their pre- to post-menarche transition. In the third study, I examined the role of sleep as another modulator of metabolic energy across the adolescent transition. This dissertation contributes knowledge on the biological mechanisms that mediate energy allocation across the pre- to post-menarche transition among adolescent girls, which can be used to understand how variation in metabolic energy can impact the onset, timing, and pace of the transition. The analyses in this dissertation were conducted using a mix of descriptive summary statistics, linear mixed-effect models, and linear regression models. I used cortisol as a biomarker of HPAA activity, c-peptide (a proxy of insulin secretion levels) as a biomarker of energy uptake, and adiponectin (a biomarker that is inversely related to visceral adipose fat mass) as a marker of energy storage levels. I used hormone concentrations of estrogen, progesterone, and follicle-stimulating hormone (FSH) to confirm reproductive status.

The first study evaluated predictions that test my hypothesis that the HPAA modulates metabolic energy availability between growth and reproductive maturation, leading to variation in energy available for maturation and thus variation in menarche timing. This study used data from a sub-set of a sample of Mayan adolescent girls from Guatemala who provided pre-menarche biospecimen samples and anthropometry data, and provided follow-up data on the age at which they reached menarche. After controlling for age at pre-menarche data collection, this study found that girls who had achieved greater proportions of final somatic growth had a shorter time remaining to menarche. This study also found that cortisol modulated the association between adiponectin and time remaining to menarche; longer time remaining to menarche was associated with

lower energy storage (adipose fat mass) as a function of greater HPAA activation. Taken together, these findings provide evidence in support of our hypothesis that the HPAA would regulate energy allocation strategies between growth and maturation leading up to menarche. However, there was no evidence to support our prediction that variation in energy uptake, specifically, would be associated with mean time remaining to menarche. These findings contribute novel information on the mechanisms involved in energetic trade-offs with challenges that require more immediate energetic investment, and the subsequent consequences on the pace of the adolescent transition.

The second study further explored the role of the HPAA as a major regulator of metabolic energy strategies across the transition. Specifically, I tested predictions that test my hypothesis that the HPAA will regulate metabolic energy differently at different stages of maturation, to meet the changing energy demands imposed by somatic growth and reproductive maturation within girls across their transition. This study used longitudinal, within participant data from a larger sample from the same cohort of Mayan adolescent girls from Guatemala. Findings from this study indicated that cortisol was positively associated with both c-peptide and adiponectin, with no evidence that the magnitude of these relationships was different depending on stage. These findings suggest that 1) the HPAA is involved in promoting energy uptake by prompting an increase in insulin secretion, to facilitate more glucose uptake to fuel the energy needs of tissues involved in physiological processes that support growth and reproduction; and 2) the HPAA is a mechanism that triggers energy mobilization from fat stores, to again support the concurrent energy needs of both growth and reproduction across the transition. While not consistent with our specific predictions, these findings do provide evidence that the HPAA is involved in metabolic energy allocation strategies across the pre-post menarche transition. We also found that insulin levels were higher among girls when they were pre-menarche compared to when they were post-menarche. We also observed that overall adiposity stores were higher among girls when they were post-menarche, compared to pre-menarche, which may suggest that fat accumulation occurs in anticipation of needing to support future reproductive efforts, such as pregnancy and lactation.

The third study examined sleep as an energy regulation mechanism that may act to restore or conserve energy to support somatic growth and reproductive maturation across the adolescent transition. I hypothesized that changing energy needs imposed by

somatic growth and reproductive maturation would drive changes in sleep quality. This study again used data from a subset of Mayan adolescent girls in Guatemala who provided sleep data along with biospecimen samples, enabling analyses that investigated the complex relationships between sleep and metabolic energy. Findings from this study indicated that low energy storage levels were associated with higher sleep quality, and that HPAA activity, but not metabolic energy, was associated with shorter sleep duration. Our finding that variation in energy storage was linked to improved sleep quality provides evidence in support of the hypothesis in this study. We also found that HPAA activity was associated with reduced total sleep time. These findings may suggest that we are observing the HPAA and sleep acting as synergistic mechanisms in responding to the energetic demands of adolescent growth and maturation. The relationships between the HPAA (measured using cortisol), energy uptake (measured using c-peptide, a proxy of insulin), energy storage (measured using adiponectin) and sleep outcomes identified across the pre- to post-menarche transition in each of the three papers are outlined in Figure 5-1.

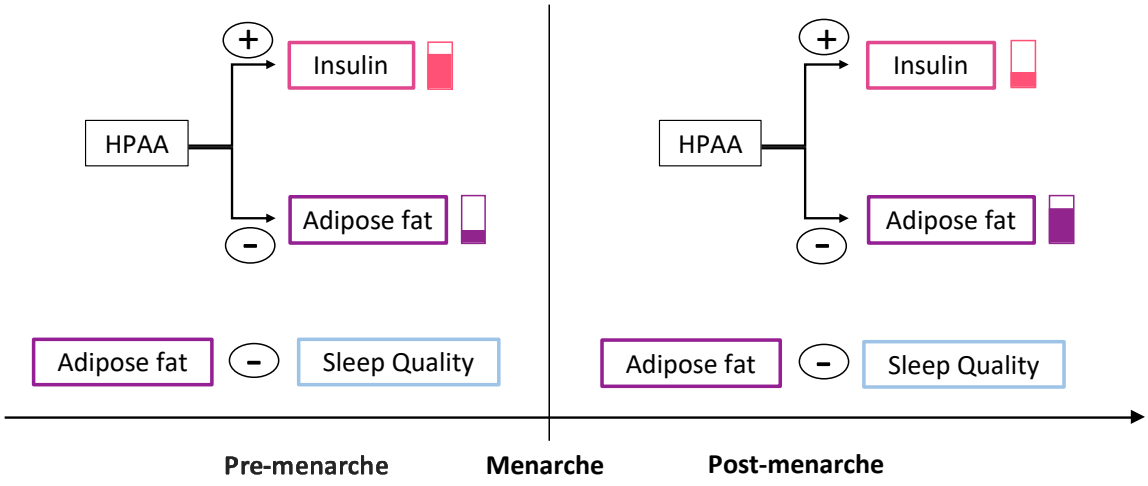


**Figure 5-1: Associations between the HPAA, energy uptake, energy storage, and sleep across the pre- to post-menarche transition**

## Energy regulation mechanisms across the adolescent transition

Taken together, my findings contribute knowledge on how the HPAA and sleep act as energy regulation mechanisms across the pre- to post-menarche transition.

*The HPAA and metabolic energy patterns*

My analyses in Chapter 2 found that the HPAA plays a critical role in energy allocation strategies during pre-menarche maturation stages. Specifically, the association between energy storage and menarche timing was a function of HPAA activity. With higher HPAA activation, lower energy stores were associated with a longer time remaining, while with lower HPAA activation, lower energy stores were association with a shorter time remaining to menarche. Cortisol, secreted as part of increases in HPAA activation, mobilizes free fatty acids from adipose fat stores into the bloodstream to provide energy to tissues (Di Dalmazi et al., 2012). The modulatory role of the HPAA on energy storage levels is also consistent with our findings from Chapter 3, in which HPAA activation was association with lower energy storage levels across the pre- to post-menarche transition. These findings contribute novel insights into the HPAA's mechanistic role in modulating energy allocation strategies between growth and reproductive maturation, leading up to menarche.

My analyses also found that greater HPAA activation was associated with higher insulin levels across the pre- to post-menarche transition within girls (Chapter 3). Insulin, secreted by pancreatic beta cells, promotes an increase in glucose uptake from the bloodstream into cells, which provides energy to cells either for immediate use, or for long-term storage in adipose fat tissue (Böttner et al., 2004; Emery Thompson & Knott, 2008). Pre-menarche girls exhibited overall higher insulin levels compared to post-menarche girls. These higher insulin levels correspond to a characteristic insulin resistant period that commences during early adolescence, in which a compensatory increase in insulin secretion occurs (Kelsey & Zeitler, 2016). This insulin resistant period is proposed as a mechanism to trigger greater insulin secretion to promote more glucose uptake by cells, supporting the energetically demanding processes of adolescence (i.e. somatic growth, cognitive development, and reproductive maturation and function) (Kelsey & Zeitler, 2016). Glucocorticoids, including cortisol, are also associated with

greater insulin resistance and decreased insulin sensitivity; however, this evidence is often presented in the context of investigating factors associated with cardiometabolic syndrome, including diabetes (Kamba et al., 2016; Mantero & Boscaro, 1992; Mino et al., 2002). In the context of adolescent development, we propose that the HPAA is involved in these mechanisms promoting greater insulin secretion, to promote greater energy availability to support the energetic demands of both growth and reproductive maturation requirements (Huybrechts et al., 2014).

Insulin resistance is also associated with shifts in metabolic energy sources for fuelling growth and maturation processes, suggesting another function of insulin during adolescence. Insulin resistance is associated with a decrease in glucose oxidation (using glucose for energy), and an increase in fat oxidation (mobilizing adipose fat mass as a source of energy) (Hannon et al., 2006). Fat oxidation yields more adenosine triphosphate (ATP) production per molecule compared to glucose oxidation, thus generating more energy per unit for use (Hannon et al., 2006). Insulin resistance, and the corresponding increase in insulin that occurs pre-menarche may facilitate a shift towards using more fat storage as an energy source during earlier stages of maturation. This finding is consistent with our results that adolescent girls had overall lower energy stores when they were pre-menarche, and higher energy storage levels when they were post-menarche. During pre-menarche stages, fat stores would be prioritized as the main energy source for generating ATP to simultaneously fuel both somatic growth and maturation of the reproductive axis.

Our findings that girls post-menarche and in more advanced stages of maturation had lower levels of insulin secretion coincides with a return to normal insulin sensitivity that occurs with the resolution of the insulin resistant period. Resolving insulin resistance shifts back towards prioritizing glucose oxidation as a predominate energy source over fat stores (Hannon et al., 2006). During post-menarche stages of maturation, fat accumulation is prioritized so that sufficient energy will be available in anticipation of future reproductive events such as pregnancy. This is consistent with our finding that among post-menarche girls, some of whom would be in more advanced stages of maturation and have established ovulatory cycling, adipose fat stores are higher. The relationships we observed in our analyses across all three studies in this dissertation contribute context to the proposed role that the HPAA plays in energy uptake and energy mobilization from fat stores during adolescence.

Although the associations we observed between HPAA activity and insulin levels were evidenced in Chapter 3, the prediction that HPAA activation would prompt increases in energy uptake via increased insulin secretion was not supported in Chapters 2 and 4. There are several reasons for the discrepancy of this finding across our studies. First, Chapter 3 conducts within-participant analyses, which introduces a more robust approach and increases power for identifying true associations, compared to the cross-sectional methodologies in Chapters 2 and 4. As well, the adolescent insulin resistant period is transient, and we were not able to identify whether participants were in their insulin resistant phase or not, which may have introduced unaccounted variation in insulin patterns in our analyses. Additionally, the nature of our sampling and analytic approach may not have been conducive to identifying any associations between HPAA activity and c-peptide levels. As insulin reflects a more immediate response to changes in blood glucose levels, and normally peaks in the afternoon, our sampling protocol of collecting first morning urine (FMU) may not have adequately captured true peaks in insulin levels. Thus, the timing of HPAA effects on insulin levels may not be compatible with the time parameters we set for our analyses. However, despite these limitations, this dissertation contributes to our understanding of the HPAA's relationship with insulin and provides evidence that the HPAA may be involved in instigating insulin resistance as a functional mechanism during adolescence.

*Sleep and the HPAA as synergistic metabolic energy regulation mechanisms*

In Chapter 4 I evaluated how sleep may also act to regulate metabolic energy availability across the pre- to post-menarche transition among adolescent girls. Given that sleep functions as an energy restoration and conservation mechanism, we predicted that the increased energetic demands of somatic growth and reproductive maturation would prompt higher quality and quantity sleep, optimizing the recuperation of energy expended during these processes. However, these biological processes are proposed to be bidirectional (Sharma & Kavuru, 2010; Short et al., 2018). As such, bi-directional models are needed to capture how sleep quality may affect subsequent, time-lagged energy storage levels facilitating energy recuperation, in response to energy needs and expenditure.

We observed that when evaluated concurrently, lower levels of energy storage (lower adipose fat mass) were associated with higher sleep quality, suggesting a need to

recuperate more energy to support continued growth and reproductive maturation. These findings may suggest that improved sleep quality is a mechanism to recuperate energy during adolescence. Sleep quality recuperates and conserves energy by reducing metabolic rate, especially during deep sleep phases (Schmidt et al., 2017). This reduction in metabolic rate means that less energy is being expended, thus less energy is being mobilized out of storage for use (Schmidt, 2014; Schmidt et al., 2017).

We only evaluated metabolic energy and sleep outcomes concurrently during one data collection period. Yet, there could be other processes involved in energy mobilization that occur simultaneously,  which could negatively affect energy storage , thus prompting increases in sleep quality. Within these same analyses that evaluated sleep and metabolic energy relationships, HPAA activity was included as a covariate. The HPAA plays a role in mobilizing energy from storage, which we identified from our findings in Chapters 2 and 3. As such, HPAA activity may be co-occurring with sleep, in which energy mobilization may be occurring simultaneously with energy that is being recuperated from sleep. These findings may reflect complex HPAA-sleep interactions, in which sleep is acting as a mechanism to restore sufficient energy to enable the HPAA to continue mobilizing energy towards growth and, or, reproductive maturation and function. There is already sufficient evidence of HPAA-sleep interactions - HPAA activation and sleep patterns interact on a bidirectional pathway (Buckley & Schatzberg, 2005; Chrousos et al., 2000; Nicolaides et al., 2000). However, these interactions are often discussed in the context of high stress exposure (Buckley & Schatzberg, 2005). Taken together, our findings elucidate how the HPAA and sleep may be acting synergistically as energy regulation mechanisms to respond to the energetic demands of adolescent growth and reproductive maturation.

## Contributions and implications

The three studies presented in this dissertation aimed to shed light on factors that contribute to variation in the adolescent transition among girls. Its main objectives were to evaluate metabolic energy mechanisms across the adolescent transition, how they may shift depending on maturation stage, and how they may impact variation in the timing, onset, and pace of maturation. It does so by testing two known energy regulation mechanisms – the hypothalamic-pituitary-adrenal axis and sleep. In evaluating factors that may modulate energy for supporting growth and reproduction across the transition, I

contribute knowledge on the biological pathways through which socio-ecological challenges may lead to variation in reproductive outcomes across the adolescent transition.

*Contributions to Life History Theory*

Analyses in this dissertation are contextualized within life history theory. From a life history theory framework, the adolescent transition involves energetic trade-offs between growth and reproduction, in addition to ongoing maintenance and responding to socio-ecological challenges (Belsky, 2012; Ellis, 2004). Variation in adolescent development, as a result of energetic trade-offs, reflects underlying life history strategies.

The evidence presented in this dissertation contributes new insights relevant to understanding how biological mechanisms function to mediate energetic trade-offs, and overall, the trajectory of the adolescent transition. The biological mechanisms evaluated here can be used to understand mechanistically how variation in life history strategies throughout adolescence occur. Specifically, it contributes information on the mechanisms that may be involved in modulating energetic trade-offs between lower priority tasks, such as reproduction, and more urgent socio-ecological challenges such as poor nutrition, high mortality, or infectious diseases, and how, mechanistically, this would lead to variation in adolescent development, including the onset, timing, and pace of reproductive maturation (Mishra et al., 2009; Stearns et al., 2000; Stearns & Koella, 1986). This body of work thus has important implications for advancing our understanding of adolescence as a life history transition.

*Contributions to understanding socio-ecological effects on health and development*

Moving beyond life history theory, the findings presented in this dissertation contribute important information for improving our understanding of the underlying mechanisms and pathways through which socio-ecological exposures could lead to variation in reproductive and health outcomes. Providing evidence for how environments can become embodied as physiological, developmental, or health outcomes is critical to inform and support intervention and policy change. Specifically, knowledge on how socio-ecological inequities can become biologically embedded and influence developmental processes, further leading to health inequities, provides concrete evidence demonstrating that changes in environmental contexts and availability of

supports, resources, etc., can have tangible effects in improving and optimizing developmental outcomes. In this dissertation I describe how some of these complex interactions reflect pathways that could mediate the effects of socio-structural-environmental exposures on health and development. These biological mechanisms are especially important to consider within the context of adolescence, as developmental outcomes during this period can have health and reproductive consequences across the lifespan. Variation in growth and maturation, such as very early or very late age at menarche, is associated with increased risk of subfecundity, cardiovascular disease and obesity, and mental health conditions such as depression, anxiety, and bipolar disorder, as examples (Elks et al., 2013; Herva et al., 2004; Lakshman et al., 2008; Remsberg et al., 2005; Rosso et al., 2020; Stice et al., 2001; Warp et al., 2024). Therefore, to mitigate poor reproductive and health outcomes across the lifespan, understanding how socio-ecological factors can influence altered growth and reproductive maturation is critical.

*Socio-cultural considerations*

Finally, to fully understand the implications of the biological mechanisms explored in this dissertation, it is important to consider them within the context of the social experience of the adolescent transition. While significant physiological changes occur with adolescence, dramatic psychosocial and socio-cultural changes are an intimate component of this transition. Adolescence is a formative transition from a socio-cultural perspective, in which important milestones that represent coming of age, which are often rooted in cultural traditions signifying the transition to adulthood, occur (Barry & Schlegel, 1980; Worthman & Trang, 2018). Social ceremony or rites of passage to mark maturation and the transition to adulthood are experienced almost universally, in both industrial and non-industrial populations, such as graduations, celebrations, or traditional ceremonies (Worthman & Trang, 2018). With this transition, new skill and knowledge acquisition, shifts in responsibilities, and a trend towards greater autonomy and independence occurs (Alethea, 2013). Adolescents also engage in new relationship and sexual experience initiation and relationship building. Complex social systems and social pressures among peers introduce new challenges to navigate independently (Alethea, 2013).

Given the formative intersecting physiological, neurological, psychological, emotional, behavioural and social transitions that occur during adolescence, it is imperative that

adolescence be studied and understood from a transdisciplinary lens. This dissertation advances our understandings of specific biological mechanisms that may be involved in mediating physiological processes during the transition. The knowledge contributions from this work can be linked to the socio-cultural perceptions and experiences of adolescence, to contextualize our findings and link them with other important changes that occur across the adolescent transition. For example, health and developmental outcomes occurring during adolescence, such as somatic changes or maturation of secondary sexual characteristics, are important in shaping social experiences, and can introduce social consequences to an already challenging transitional period. Further, shifting social-cultural and environmental challenges can impact sleep during adolescence, which, in interacting with other challenges or stressors at play, adds complexity to growth and development trajectories during adolescence. The biological mechanisms mediating certain developmental outcomes are shaped by these perceived social experiences, thus linking socio-environmental contexts to variation in development, such as accelerated or delayed transitions (Worthman & Trang, 2018). As such, the experience of the adolescent transition is represented by intersecting, bidirectional bio-socio-cultural relationships. These can influence how energy regulation mechanisms function to support energy allocation towards growth and maturation, while also responding to the many other challenges that are present during adolescence. This dissertation, while shedding light on aspects of biological pathways, points to several avenues for future inquiry.

## Future Directions

There are a number of avenues for future research that could stem from the research presented in this dissertation, which will help clarify or expand on the findings presented in these three studies. The first area pertains to addressing the constraints of conducting cross-sectional analyses in the first and third study. Specific to the first study, it would be beneficial to conduct longitudinal, within-individual analyses that follows up with girls repeatedly, every 1-2 months, to examine changes in growth (i.e., height stature) as well as HPAA and metabolic energy activity leading up to menarche. This would enable within-participant analyses for examining intra-individual variation in the role that the HPAA plays in modulating metabolic energy leading up to menarche, and the ensuing trade-offs between somatic growth and reproduction. Commencing data collection at an

established developmental stage would also strengthen analyses for the comparison of factors associated with inter-individual variation in menarche timing, and the pace of other developmental processes leading up to menarche. Future research could also expand on the third study by conducting longitudinal, within-participant analyses of the relationship between metabolic energy and sleep across maturation stages, to better identify nuanced variation in these relationships within individuals across the transition.

Our third study investigated the effects of energy demands on sleep. However, we were not able to evaluate whether any improvements in sleep would lead to subsequent changes in metabolic energy outcomes measured at a later time. To that aim, future studies should incorporate repeated measures across time for each participant and, in so doing, capture potential bidirectional, or feedback interactions, between sleep and metabolic energy. An important consideration for these analyses would thus be to further explore some of the bidirectional relationships between sleep and energy within the context of adolescent maturation. A model that can account for the bidirectional effects of sleep and energy, and in doing so, evaluate how these two factors influence each other temporally would contribute more information on the complex relationships that we attempted to identify in our models. Further analyses to better understand how variation in sleep and its interaction with metabolic energy strategies may lead to variation in maturation, such as menarche timing, is another important avenue to explore. This set of analyses would also be important to conduct within individuals, to examine longitudinally across the pre- to post-menarche transition how sleep supports maturation processes. These analyses would also provide more information on how poor, low-quality sleep may disrupt optimal developmental trajectories through its role as an energy regulation mechanism, thus providing more evidence to inform specific interventions for sleep optimization during adolescence.

Another set of future research possibilities pertains to the second study, in which we formulate next-step hypotheses. Stemming from our findings, we propose that the increase in compensatory insulin secretion as a response to insulin resistance during adolescence may be a mechanism to shift towards using more fat storage as an energy source during earlier stages of maturation, prior to menarche. Future research should examine whether changes in sources of energy occur across different maturation stages and are specific to the unique energy demands at each stage of the adolescent transition.

Given that we identify the HPAA and sleep as potential modulators of metabolic energy across the transition, further exploring their roles as energy regulation mechanisms would involve conducting causal mediation analyses. These analyses would enable us to test how exposures such as psychosocial stress, high mortality, endemic infectious diseases, or poor access to nutrition affect the timing, onset, pace, and quality of reproductive outcomes during development, through these biological pathways of HPAA, sleep, and metabolic energy strategies.

## Conclusion

In sum, my dissertation contributes knowledge on energy regulation mechanisms and patterns in metabolic energy among Kaqchikel Mayan girls during their adolescent transition. We find that the HPAA interacts with metabolic energy leading up to menarche, and across the pre- to post-menarche transition. The HPAA plays an important role in energy allocation strategies during critical growth and reproductive maturation periods. Further, we find that sleep may play a role in feedback interactions with metabolic energy during adolescence. Together, the HPAA and sleep may be acting synergistically to regulate metabolic energy strategies to meet the demands of growth and reproductive maturation. These findings suggest there are important biological mechanisms and pathways involved in girls' adolescent development, which can play a role in mediating the timing, onset, and pace of the adolescent transition. Our improved understanding of these biological mechanisms has important implications for understanding how socio-ecological factors can impact development, and can be used to inform on how to support optimal growth and reproductive maturation during adolescence.

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