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PRESENCE OF MENARCHE IS ASSOCIATED WITH HIGH DEPRESSIVE SYMPTOMS AND CORTISOL LEVELS IN ADOLESCENT GIRLS

par

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Ce mémoire intitulé : Presence of menarche at the time of high school transition is associated with high depressive symptoms and cortisol levels in adolescent girls

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Résumé

Plusieurs études antérieures ont proposé que la ménarche pouvait représenter une vulnérabilité accrue au développement de la dépression en augmentant la réactivité au stress chez les filles ayant atteint leur cycle menstruel. Dans la présente étude, les symptômes dépressifs et les niveaux de cortisol salivaire ont été mesurés chez 198 garçons et 142 filles (11 - 13 ans), et ce, à quatre reprises au cours de leur première année de transition vers l'école secondaire, une période de stress chez les adolescents. Les résultats ont montré que les filles qui avaient atteint la ménarche au moment de la transition vers le secondaire avait des niveaux significativement plus élevés de symptômes dépressifs et de cortisol salivaire entre l'automne et le printemps, comparativement aux filles qui n'avaient pas encore atteint la ménarche. Ces dernières présentaient des niveaux plus élevés de symptômes dépressifs que les filles sans et les garçons. Les filles sans ménarche présentaient d'avantages des niveaux de symptômes dépressives plus élevés que les garçons. En utilisant l'âge de ménarche comme variable catégorique, les résultats démontrent que les filles ayant eu leur ménarche plus jeunes présentent des symptômes dépressifs plus élevés tout au long de l'année scolaire, alors que les filles qui ont commencé leur cycle menstruel à l'âge dit 'normal' présentent des symptômes dépressifs transitoires. Globalement, ces résultats suggèrent que la ménarche est un indice significatif d'une vulnérabilité accrue pour les symptômes dépressifs et les niveaux de cortisol plus élevés chez les adolescentes qui font leur entrée au secondaire. Également, ces résultats suggèrent qu'un âge précoce de ménarche peut exposer à long-terme le cerveau en développement à des niveaux élévés de cortisol, rendant ainsi ce groupe d'adolescentes plus vulnérables à la dépression.

Mots-clés : adolescence, ménarche, dépression, cortisol, cerveaux

Abstract

It has been proposed that the onset and/or earlier age at menarche confer greater vulnerability to depressive symptoms by increasing the reactivity of menarcheal girls to stressors associated with adolescence. In the present study, we measured depressive symptoms and salivary cortisol levels in 198 boys and 142 girls (11 -13 years) tested four times during their first year of transition into high school, a period known to be associated with stress among adolescents. Results showed that girls who had reached menarche before the transition to high school transit presented significantly higher depressive symptoms and salivary cortisol levels across the school year, when compared to girls who had not reached menarche and boys. Girls who had reached menarche presented significantly higher depressive scores than girls who had not reached menarche and boys. Girls who did had not reached menarche also scored significantly higher on depressive symptoms when compared to boys. When we divided the menarcheal girls as a function of age of onset, we found that girls with early age at menarche presented consistently higher scores for depressive symptoms from the start of the school year to early spring. Girls with on-time menarche scored higher for symptoms of depression, but these were more transitory. Altogether, these results show that onset of menarche is associated with greater depressive symptoms and higher cortisol levels in adolescent girls going through the stress of high school transition. These findings also suggest that early menarche may confer greater vulnerability to depression due to long-term exposure of the developing brain to high cortisol levels.

Keywords : adolescence, menarche, depression, cortisol, brain

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List of Abbreviations:

ANS: Autonomic nervous system

CNS: Central nervous system

CRH: Corticotroping-releasing hormones

DHEA: Dehydroepiandrosterone

FSH: Follicle stimulation hormone

GC: Glucocorticoids

GnRH: Gonadotropic-releasing hormone

HPA: Hypothalamic-Pituitary-Adrenal axis

HPG axis: Hypothalamic-pituitary-gonadal axis

LH: Lutinizing hormone

MC: Mineralocorticoids

PPD: Postpartum depression

PMDD: Premenstrual dysphoric disorder

PMS: Premenstrual syndrome

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1. Introduction

1.1. Global Depression Rates

Major depression is a common psychiatric illness associated with negative and prolonged alterations in mood, behavior and self-appraisal (Lewinsohn, Rohde, & Seeley, 1998). It has a debilitating impact on social, emotional, and psychological functioning, leading to impairments in the interpersonal, academic and professional domains (Wells et al., 1989). Major depression is part of a group of mood disorders classified in the Diagnostic and Statistical Manual IV (DSM-IV-TR) of the American Psychiatric Association, as an Axis-1 Major Mental Health Disorder (APA, 2000). Other Axis-I mood disorders include bipolar disorder I and II, dysthymic disorder and cyclothymic disorder, all of which involve depressed affect (APA, 2000).

Depression is a global problem. A worldwide epidemiological study entitled 'The Global Burden of Disease Study for 2001', analyzed data gathered by the World Health Organization (WHO) (Lopez, Mathers, Ezzati, Jamison, & Murray, 2006). From this data, Lopez and others (2006) found that depression was the leading cause of 'years of life lived with disability' (YLD) for both men and women. Affective disorders have no racial, regional, or social boundaries, although the prevalence may vary in each sphere (WHO, 2004). The WHO's World Health Report (2001) states that approximately 450 million people suffer from a mental health disorder and that one in four will develop a mental disorder in their lifetime. It is estimated that 340 million people suffer from depression (WHO, 2004).

The economic impact of major depression is far-reaching and difficult to quantify. Depression is one of the 10 leading causes of disability with a projected estimate of 5.7% of the 'Disability Adjusted Life Years' (one year of productive life) lost by 2020 (C. J. Murray &

Lopez, 1996). The economic burden of major depression effects many domains such as healthcare, drug costs, crime, lost productivity and wages, often with a negative personal and economic hardship on family and social relationships (WHO, 2001). Depression is therefore a pervasive health and socioeconomic problem that extends globally. Although there are some regional differences in the prevalence of depression, overall the same pattern emerges across countries.

Turning our attention towards Canada, we find that the prevalence of depression in the general population closely matches those found globally. A prospective longitudinal Canadian study entitled the National Population Health Survey, which followed a nationally representative community sample, was conducted between 1994 and 2006 (Patten, 2009). Follow-up assessments were completed every two years to identify new and recurring cases of major depression. Patten (2009) found that the point prevalence (annual prevalence in the year studied) of major depression ranged between 4% and 5% of the general Canadian population. However upon closer inspection, another local and global phenomenon emerged revealing an uneven distribution within populations disfavoring women.

1.2. Statistics on Women and Depression

A higher prevalence of depression among women has been found throughout the world using a variety of diagnostic schemes and design methods (Wolk, 1995). Globally, the rates of depression are 50% higher for females (Lopez et al., 2006; for a review, see Kessler, 2003). Lopez and colleagues (2006) showed that women were more vulnerable to psychiatric conditions related to internalizing disorders such as anxiety and depression. Men tended to suffer more from externalizing disorders such as aggression and substance abuse (Lopez et al., 2006).

Further, females were more likely to have other co-occurring psychiatric and neurodegenerative disorders such as anxiety and senile dementia, known to be highly comorbid with female depression (Lopez et al., 2006). This report shows that sex-based differences in the modes of expression of depressed affect, occur cross-culturally.

Interestingly, when the Canadian sample was split by sex, a similar pattern to the Global Burden of Disease Study emerged (Patten, 2009). Canadian men who reported at least one episode of major depression represented 3.2% of the population (Patten, 2009). The same study showed that more than two times the number of Canadian women reported receiving a clinical diagnosis of major depression, with a first episode of depression in the year reported, and representing 7% of the population (Patten, 2009). Of the men and women who had at least one depressive episode, 19.7% suffered from subsequent episodes in a follow-up interview. Like the annual prevalence rate, there were sex differences for *recurrent depression*. The rate of recurrence for major depression in women was 24.2% whereas only 14.2% of the male population reported a recurrence of depression. Therefore, this Canadian population study mirrors the global data, and shows that having a single occurrence of major depression places a person at greater risk for a subsequent depressive episode, and the likelihood of both first and recurrent episodes are significantly higher in women than in men.

2. Theories of why women are more prone to depression

There are a number of theories to explain why women are more prone to depression than men and these theories cover three domains; psychological, biological, and socio/cultural.

***2.1. Cognitive Theory**:*

First developed by Beck (1963) Cognitive Theory has been extensively researched (Haaga, Dyck, & Ernst, 1991). Beck (1963) proposed that early experiences lead to the development of core beliefs he called schemas. A schema is a mental framework from which pre-conceived ideas become organizing patterns of thought, behavior, and social information (Hammen, Marks, Mayol, & deMayo, 1985). A self-schema is a framework built upon cognitive representations of the self, and placed into memory (Beck, 1963; Hammen et al., 1985). A negative self-schemas is associated with the vulnerability to depression (Hammen et al., 1985). Negative self-schemas that are accompanied by prolonged feelings of helplessness and hopelessness (Hammen, et al., 1985) as well as dysfunctional attitudes, that often lead to major depression (Abramson, Metalsky, & Alloy, 1989). No study to our knowledge has provided evidence for *sex-based* differences for depression solely on the basis of cognitive styles. However, studies have shown that certain cognitive styles associated to depression, such as rumination, are more frequent in women than in men (Hankin & Abramson, 2002; Nolen-Hoeksema & Gigus, 1994). Hyde and colleagues propose an integrative model to explain the emergence of sex differences for depression ought to comprise of cognitive, affective, and biological mechanisms (Hyde, Mezulis, & Abramson, 2008). The authors assert that negative cognitive styles (ie: objectified body consciousness and rumination) increase the vulnerability to depression in young women (Hyde, Mezulis, & Abramson, 2008). Therefore, certain negative cognitive styles, which are more common in females, appear to be an important factor when considering sex specific factors in the vulnerability to depression.

2.2. Biological theories:

There are theories which support a biological origin for the increased vulnerability to depression in women. The premenstrual period, which occurs two to five days before menstruation, is associated with hormonal changes that may affect a woman's psychological and emotional equilibrium, cause discomfort, and is commonly referred to as premenstrual syndrome (PMS) (Endicott, 2000). PMS is associated with bloating, fatigue, and emotional reactivity, experienced two to five days prior to menstruations, thus during the late luteal phase of a woman's cycle (Endicott, 2000). If PMS is deemed by a clinician to significantly interfere with a client's daily functioning and interpersonal relations, during the late luteal phase of her cycle, a woman may receive a psychiatric diagnosis of Depressive Disorder-Not Otherwise Specified according to the DSM-IV-TR (APA,2000). The DSM-IV-TR (2000) is presently being revised to include Premenstrual Dysphoric Disorder (PMDD) as an official diagnosis (APA, 2000). The menstrual cycle occurs throughout a healthy woman's childbearing years with one exception -- pregnancy.

Pregnancy is a time when women undergo new and rapid hormonal changes which support the growing fetus inside her womb. During the post-postpartum period, there is another rapid decline in prenatal hormones to be replaced with post-natal hormones for the purpose of helping the mother return to her formal physical state and to support lactation (Ancelin, Scali, & Ritchie, 2007). Recent studies have shown that this is a particularly risky time for a mother to develop a particular type of depression labeled post-partum depression (PPD). During gestation, there is a sudden rise is progesterone, a hormone that signals the body to support a growing fetus, as well as a steady drop in estrogen. Progesterone is believed to buffer the effects of stress and help the mother feel more relaxed (Lupien, et al, 2009; Steiner, Dunn, & Born, 2001). The stress

buffering effects may be lost immediately after childbirth as progesterone drop dramatically (Steiner, Dunn, & Born, 2001), making a person more vulnerable to stress when in a stressful environment. PPD can be of short duration beginning within 48 hours post-partum and last up to eight to 12 weeks, or until first post-partum menstruation (Ancelin et al., 2007). PDD is reported to have debilitating effects on the mother and negatively impact the mother-infant bond (Zajicek-Farber, 2009).

2.3. Socio-Economic theories:

Other theories which may explain why women are at a higher risk for depression than men are based on difficulties experienced in the social, cultural and economic domains. For instance, social roles can be a source of strain for women who aspire to attain the ideals of the modern day woman (Chonody & Siebert, 2008). Women must negotiate the demands of family, children, spouse, work, and community. Women who adhere to exceedingly high performance standards for themselves may put their effectiveness and personal worth into question making them more vulnerable to depression. Unequal power status may also be a contributing factor to the onset of depression (Chonody & Siebert, 2008). Unequal power status can include being passed over for a job promotion due to extra responsibilities outside of the work arena that must be fulfilled. These include household chores and childcare duties, which reduce career enhancing behavior such as working longer hours, obtaining higher education (Chonody & Siebert, 2008). Women are more likely than men to be economically disadvantaged (Chonody & Siebert, 2008). More women live at or below the poverty line than men, and are often single mothers. Women suffer higher rates of physical and sexual abuse and men, and are at higher risk for depression as well (Maniglio, 2010).

Although the above is not an exhaustive list of potential psychological, biological and socio-cultural theories, these examples propose explanations as to why women are more prone to depression than men. Further, it is important to note that most people with a history of depression, reported their first onset to have occurred in childhood or adolescence (Kesseler & Wang 2008). Consequently, in order to better understand the risk factors associated with the sex gap ratio in depression, it is important to study first onset of depression in childhood and adolescence.

3. Prevalence of depression during childhood and adolescence

In an epidemiological survey, Kesseler and Wang (2008) reported that childhood presentations of mood disorders often resulted in the most debilitating psychopathologies in adulthood with high comorbidity with other disorders. The seriousness of this finding becomes most evident when other reports state that one in eight teenagers under the age of 18 suffer from a mental disorder (WHO, 2004). This statistic increases to one in five for economically disadvantaged children (WHO, 2004). Adolescent onset of major depression is strongly associated with chronic and recurrent depression in adulthood (Pine, Cohen, Cohen & Brooks, 1999; Romeo & McEwen, 2006). Despite the progress in the treatment of adolescent depression, only approximately 25% of depressed youth receive treatmenthttp://jama.ama-assn.org/content/301/21/2215.full - ref-6 (Kennard et al., 2006) while 20% develop recurrent and chronic treatment resistant depression (Birmaher et al., 2000).

3.1. Emergence of the Sex Gap Ratio of Depression during Adolescence:

There is strong research evidence to support a sex effect for depressive disorders. A dramatic shift in the sex gap ratio has been observed at adolescence. Girls aged 15 to 18 are reported to be twice as likely to suffer from depression, as boys (Hankin, et al., 1998; Nolen-Hoesksema & Girgus, 1994; Patton et al., 2008). In fact, emerging sex differences in internalizing behaviors, such as depressive affect or feelings of ineffectiveness, have been shown to favor girls somewhere between the ages of 11 and 15 (Cyranowski et al., 2000). In contrast, research on pre-pubertal children has either yielded no sex difference in depression (Angold & Rutter, 1992), or showed that young boys are slightly more likely than girls to become depressed before the age of 11 (Andersen, Williams, McGee & Silva, 1987; Hankin et al., 1989). The observation of the emergence of this sex gap in depression rates at puberty raised the question about the role of important pubertal milestones in increasing vulnerability to depression in girls/women (Young & Altemus, 2004). There are three pubertal milestones that occurs during adolescence that are important in here: the growth spurt, the Tanner Stage, and the activation of the hypothalamic-pituitary-gonadal (HPG) axis.

3.1.1. Adolescence and the growth spurt:

Adolescence is a time of rapid physical growth and is considered to be a reliable indication of pubertal onset (Cameron, 2002; p.45). The onset of puberty is determined by combining two growth measurements; the age of minimum velocity (the age at the start of the growth spurt) and the age of maximum velocity (the age at the end of the growth spurt). The age of minimum velocity occurs at the first appearance of a growth spurt, and it is considered to be the moment of pubertal onset. The age of maximum velocity occurs approximately 3 years after the age of minimum velocity and represents the maximum rate of growth in this period (Cameron, 2002;

p.46). The age of minimum velocity may vary as much as one year between populations and sexes, and boys undergo their adolescent growth spurts, on average, two years later than girls (Cameron, 2002; p. 46). Peak growth velocity rapidly decreases around 16 or 17 years of age for girls, and around 18 to 19 years of age for boys, in Western populations (Cameron, 2002; p. 47). There is some variability among individuals, populations, and sexes, as to the onset, velocity and timing (age) of the growth spurt (Cameron, 2002; p.51).

Children undergo a slow and steady increase in body weight until the start of puberty when there is a sudden and rapid increase in body mass (Cameron, 2002). This spike in weight gain between childhood and puberty usually occurs at 11.5 years of age (Cameron, 2002); Sizonenko, 1978). Early maturers who reach final size earlier than their same sex peers are taller and heavier than the average and late maturers (Tanner, 1962). Tanner (1962) also noted that early maturers had a shorter growth cycle, which was offset by the slightly greater than average growth velocity in childhood and a more intense growth spurt at puberty. The opposite was observed in late maturers (Tanner, 1962). The tempo of growth, also known as the rate of maturation, is correlated with other markers of physical maturation, such as bone age (density) and secondary sexual characteristics.

3.1.2. Tanner Stage:

Somatic growth and development researcher James Tanner published the classic text, *Growth at Adolescence* in 1962 (Tanner, 1962). Today it remains a classic text and continues to be well cited in the literature (Angold & Costello, 2006; Giedd et al., 2006). Tanner is most well-known for systematizing and codifying morphological changes from childhood to adulthood in both sexes. The Tanner Staging is a widely used five-point scale ranging from 1 (pre-pubertal) to 5 (post-pubertal) and used in the assessment of morphological changes expressed at

secondary sexual characteristics which are related to pubertal status (Angold & Costello, 2006) *(see Tanner, 1962)*. This instrument is commonly administered as a self-report (Giedd et al., 2006), parental report (Rosemalen et al., 2005) or as a physician reported measure (Paikoff et al., 1991) of primary and secondary sex characteristics.

The Tanner staging remains a popular research tool for identifying and codifying the development of secondary sexual characteristics for depression studies with teenagers (Angold & Costello, 2006; Rosemalen et al., 2005). This system has been particularly useful for classifying the precise pubertal stage at which the rates of depression may increase in a given sample. Angold and Costello (2006), known for their Great Smokey Mountain Study which looked at risk of depression in relationship to puberty in a large sample of teens, state that it is essential to put staging into context relative to the peer group under study, given the wide variability among populations. The authors note the importance of making the distinction between pubertal level, pubertal timing, and final status. *Pubertal level* is the stage or progress of puberty. P*ubertal timing* is the age at which a specific level is achieved relative to peers. *Final status* marks these end of puberty (Angold & Costello, 2006). Combining the Tanner Staging with pubertal level and timing information in depression studies on adolescents, offers a richer understanding of the phenomena.

The value in specifying this information becomes apparent when studying the sex differences in depression at puberty. Angold, Costello and Worthman (1998) studied 1073 boys and girls between the ages of 9 and 13 with the aim of identifying whether depression was predicted by pubertal status or timing. Parents and children were interviewed using the Child and Adolescent Psychiatric Assessment (Angold et al., 1995), and then children were classified by Tanner staging. Three annual follow-up interviews were conducted to re-assess pubertal development

and depressive symptomatology. The study showed that boys had higher rates of depression than girls at Tanner stage I (pre-pubertal), but not after, and girls were found to have higher rates of depression than boys by Tanner stage III, but not before (Angold et al., 1998). This study supports previous research that puberty is a key period for understanding the sex difference in the risk for depression.

However, simply matching morphological changes to the stage at which the risk for depression is the greatest has its limitations. For instance, the identification and classification of pubertal status is determined simply by visual inspection, which does not inform the researcher about the participant's hormonal status, shown to be an important consideration when trying to identify risk factors for affective disorders (Lupien et al., 2009). Other studies show that the causal explanation for this sex difference is more closely associated with changes in androgen and estrogen levels that may accompany developing secondary sex characteristics (Angold, Costello, Erkanlin & Worthman, 1999; Patton & Viner, 2007). These changes in sex steroids hormones occur at the onset of adolescence and are due to the activation of the HPG axis.

3.1.3. Activation of the Hypothalamic-Pituitary-Gonadal Axis**:**

The HPG axis is comprised of the hypothalamus, the pituitary and the gonads (testes and ovaries). The HPG axis regulates reproduction and the development of secondary sex characteristics, and is up-regulated at puberty. Priming for the activation of the HPG axis occurs at adrenarche (Cutler, Loriaux, 1980; Sizoeneko, 1978). Adrenarche is the earliest stage of the development of secondary sexual characteristics. It precedes observable physical development of secondary sex characteristics, which do not occur until the activation of the HPG axis, one or two years after adrenarche (6 to 8 year of age) (Cutler, Loriaux, 1980; Sizoeneko, 1978).

During this period, the adrenal cortex builds new tissue which becomes a third secretory zone called the zona reticularis. The zona reticularis produces high levels of C-19 steroid hormones, dehydroepiandrosterone (DHEA), and androstenedione (Cameron, 2002). These rising levels of circulating steroid hormones and sulfate conjugates (DHEA-S), cause non-detectible (non-observable) increases of sex hormones in the pre-pubertal child (Cameron, 2002). Adrenal androgens prime the HPG axis for activation and possibly desensitize the hypothalamus through increasing exposure to steroid levels outside its regulatory control (Sittery, 1990). An overproduction of adrenal androgens during this period can lead to precocious puberty through early HPG axis activation (Cameron, 2002). The relationship between adrenarche and the timing and tempo of puberty, is not yet clear. Nor are the factors that control the timing of adrenarche (Sittery, 1990).

Once adrenarche is complete, the HPG feedback systems begins maturation. The feedback relationships of the HPG axis, which was first expressed in the fetus then down-regulated by a negative feedback system after birth, becomes increasingly active (Winter, Faiman, Hobson, Prasad, Reyes, 1975). Heightened HPG axis activity first occurs with an increase of sleep-associated lutinizing hormone (LH) (Winter et al., 1975). The LH pulse frequency increases in girls at about eight years of age, and takes place in boys about one to two years later (Sizonenko, 1978).

In males, puberty occurs when the hypothalamic clock is activated releasing high levels of gonadotropic-releasing-hormone (GnRH) and gonadotropin (Ruben & Pfaff, 2009). Prior to the onset of puberty, LH and follicle stimulating hormones (FSH) are secreted in small amounts from the testes and are subject to negative feedback, the mechanism which terminates the secretions, once the required levels are reached (Ruben & Pfaff, 2009). A pulsatile pattern in the

hypothalamic-GnRH secretions initiates puberty (Ruben & Pfaff, 2009). Over time, the hypothalamus and the pituitary gland become less sensitive to circulating steroids, thus allowing for increasing concentrations of gonadal steroids and gonadotropins to circulate (Ruben & Pfaff, 2009). Increasing concentration of testicular testosterone and circulation FSH stimulate the growth of sperm cells (Ruben & Pfaff, 2009).

In girls, ovarian growth and maturation occurs throughout childhood (Peters, Himelstein-Braw, & Faber, 1976). Increased LH stimulation at the onset of puberty leads to increased levels of ovarian steroid production (Ruben & Pfaff, 2009). At puberty, both estrogen and testosterone levels rise to near adult female levels, which temporarily results in higher testosterone to estrogen ratio during puberty relative to adult females (Cameron, 2002). These hormonal changes result in breast enlargement, pubic and auxiliary hair, height increases, and adiposity accumulation, that occur as a consequence of greater steroid production (Cameron, 2002). Menarche, the first appearance of menstrual bleeding, is a reflection of increasing estrogen levels reaching sufficient levels for endometrial shedding (Cameron, 2002). Menarche also signals the beginning of menstrual cycles.

The menstrual cycle last between 24 and 32 days and consists of three phases: follicular (including menses), ovulation, and the luteal phase (Cameron, 2002). This monthly pattern is characterized by the cyclical precipitation of monthly hormones. The follicular phase corresponds to the first 14 days of the cycle, leading up to ovulation (Sizonenko, 1978). The first two to three days correspond to the menstrual period where both the estrogens and the progesterones levels are low (Sizonenko, 1978). In the early follicular phase, estrogen levels are low and insufficient to initiate a negative feedback involving LH release. By approximately day five, estrogens and progesterones increase as a result of follicular production (Sizonenko, 1978).

By the middle of the follicular phase, the previously suppressed LH becomes stimulated following a rise in estrogen levels (Sizonenko, 1978). Estrogens peak during the late follicular phase, and is quickly followed by ovulation (Sizonenko, 1978). Ovulation occurs as the LH surge leads to the release of the mature ovum from its follicular matrices, while progesterone levels rise. Estrogen levels then dip as LH peaks. Progesterone levels reach a peak approximately seven days after the LH surge, suppressing new follicular growth (Sizonenko, 1978). If conception does not occur, the corpus luteum will degenerate (Sizonenko, 1978). A rise in estrogen occurs during the early luteal phase prompting reduced circulating levels of progesterone mid-luteal as it begins a new monthly cycle in the early follicular phase (Sizonenko, 1978).

The complex relationships between the sex steroids associated with activation of the HPG axis also has concomitant effects that have been associated with onset of adolescence.

4. Factors explaining the emergence of the sex gap radio in depression during adolescence

Although the existence of a sex effect in depression that occurs during adolescence is well established, the reasons for it remain unclear. Recently, studies have tried to determine what could trigger this sex difference in the vulnerability to depression beginning in adolescence. Researchers have turned their attention toward identifying two closely related factors, namely stress levels during adolescence, and age at menarche.

4.1. *Stress, the Hypothalamic-Pituitary-Adrenal (HPA) axis and depression during adolescence*

Adolescents begin to face the added stress of rapid psychological and social changes which

may help to explain the rising rates of depression among early-adolescent females (Brooks-Gunn & Warren, 1989). These changes add new potential stressors to the life of teenagers. Such stressors are experienced as personal and social pressures. Young adolescents must adapt to new and increasing peer, dating, academic, parental, and societal pressures, often while transitioning to a new high school.

In a previous study performed with 406 children aged between 6 and 16 years, Lupien and collaborators (2001) have shown that the transition from elementary to high school leads to a significant increase in stress hormone levels in adolescents (Lupien, King, Meaney, & McEwen, 2001), suggesting that the transition to high school at the beginning of adolescence acts as a significant stressor. The commencement of high school marks the first major change in status for a teenager. The teenager then moves from a relatively small, community-based school to a larger impersonal unit, and also moves from being the oldest of the school, to being the youngest. Although for many adolescents, high school is a long-awaited change in status, accompanied by new freedoms and new challenges, the transition to high school is often associated with negative outcomes including poorer attendance, declines in grades, newly emerging discipline problems, and new feelings of alienation or social rejection (Moyer, 1982). As well, studies have shown that the transition to high school is often accompanied by a decline in a sense of school belonging and an increase in depressive symptoms (Newman, Newman, Griffen, O'Connor, & Spas, 2007).

During the same period, young teenaged girls and boys must also begin to establish an evolving social and sexual identity, as independence from parents increases. Girls have the additional challenge of negotiating their self-concept with new social roles and greater social expectations associated with the development of secondary sex characteristics. Their visibly

maturing sexuality made obvious by breast growth, for instance, which has the potential to make girls feel more sexualized whether it is desired or not (Brooks-Gunn & Warren, 1989; Ruble & Brooks-Gunn, 1982). These new psychosocial demands begin in early-adolescence potentially causing greater stress levels which may additively contribute to the sexual dimorphism of depression.

Exposure to stress leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamic-pituitary-adrenal (HPA) axis, is the main mammalian stress response system. The brain is part of the central nervous system (CNS). It takes in environmental information. It then treats the information and produces a 'fight or flight' response. This is what our ancestors have used as a survival mechanism.

The CNS is connected to the involuntary autonomic nervous system (ANS) and the voluntary somatic nervous system. It organizes the information in terms of input and output. The information must reach the brain though input of information sensory receptors such as the eyes and ears, and create an appropriate output response. Heightened stress leads to the activation of the HPA axis leading to the secretion of cortisol, one of the major stress hormone in humans.

The main components of the stress system are the corticotropin-releasing hormones (CRH), the locus coeruleus/norepinephrine autonomic system, pituitary-adrenal axis, and the peripheral limbs (arms and legs) which are connected to the autonomic nervous system (Ruben & Pfaff, 2009). ACTH hormone is a peptide hormone produced by the cells of the anterior pituitary gland and carried through the blood stream to its effector organs, and finally the adrenal cortex. Glucocorticoid (GC) zona fasciculate cells are where the adrenal cortex synthesizes and secretes CG in response to ACTH secreted by the anterior pituitary, which stimulate cortisol release. Cortisol is the end product of the HPA axis (Ruben & Pfaff, 2009). These hormones regulate

HPA axis activity as well as the termination of the stress response via negative-feedback loops on the hypothalamus, the hippocampus, the frontal lobes, and the pituitary gland (Chrousos, 2000).

There are two types of receptors, mineralocorticoids (MCs) receptors and GC receptors. Both influence the body's metabolism during stress, but GCs are especially important as they have anti-inflammatory, immuno-suppressive functions, and release the body's stored glucose energy. During chronic stress, elevating cortisol has been shown to produce damage to body systems resulting in various pathologies including depression (Juster et al., 2011).

Both basal and stress-induced HPA activity have been shown to be significantly heightened during adolescence (McCormick, et al., 2004; Netherton C., Goodyer, E., Tamplin, A, & Herbert 2004). Moreover, numerous studies have found that chronic exposure to high levels of cortisol plays a role in the precipitation of major depressive disorders in adolescence (Cyranowski, Frank, Young, & Shear, 2000; Goodyer et al., 1996; Halligan, Herbert, Goodyer, & Murray, 2007) and adulthood (Brown, 1978). High levels of stress may be especially significant during this important period of brain maturation for heightened risk of depressive disorders (Born, et al., 2002; Steiner, et al., 2003).

One study reported that adolescents diagnosed with major depression had higher evening cortisol, which predicted disappointing life events leading to more mood disorders (Angold, 2003). In a study on salivary cortisol in relation to puberty and sex, morning samples of cortisol in mid-postpubertal girls was greater than in mid-postpubertal boys, but no difference was found in pre-pubertal boys and girls (Netherton, Goodyer, Tampling & Herbert, 2004). The authors reported that the sex difference in morning cortisol levels were observed at Tanner stage III, indicating a possible change in HPA axis activity and regulation associated with increasing

levels of gonadal hormones at this developmental stage (Netherton, et al., 2004). These hormonal changes, in combination with the HPA axis, may be an important risk factor for depression in adolescent females, as proposed by Lupien and others (2009). Higher cortisol *alone* does not explain why females become more susceptible to depression in adolescence, however the influence of a second endocrine system entering into interaction with the HPA axis – namely the HPG axis – is likely.

The hormones secreted by the HPA and HPG axes engage in complex cross-talk at puberty allowing for greater physical and physiological sexual differentiation to occur (Born, et al., 2003; McCormick & Mathews, 2007). Specifically, estrogens have been shown to increase HPA-axis activity through a complex process resulting in the inhibition of its own negative feedback, thereby enhancing stress hormone secretion (Handa et al., 1994; Viau & Meany, 1991). Therefore the rising estrogen levels in pubescent females are more likely to cause an increased sensitivity to stress, resulting in higher cortisol levels and more depression (Young & Altemus, 2004), than their male counterparts.

Steiner and others (2003) reported that it is the *sudden appearance* of higher levels of estrogens at puberty that causes an increase in the sensitivity of some mood regulating neurotransmitters. This increased sensitivity translates into greater moodiness and irritability putting teenaged girls at greater risk for interpersonal conflict (Steiner et al., 2003). In a review of neuroendocrine effects on mood, Spinelli (2005) reported that both estrogen and progesterone have an effect on neurotransmitter systems that regulate mood. Serotonin is a key neurotransmitter involved in mood regulation (Steiner, Dunn, & Born, 2003). The serotonergic system is particularly sensitive to the presence or absence on estrogen and progesterone, which in turn has an impact on cognition and behavior (Spinelli, 2005).

The simultaneous impact of psychosocial and hormonal changes may have an additive effect putting those who are more vulnerable to stress, at risk for chronically high levels of circulating cortisol, a known antecedent often leading to depressive disorders (Dorn & Chrousos, 1997; Goodyer, Herbert & Altham, 1998; Lupien, McEwen, Gunnar & Heim, 2009). Although there is strong evidence for the hormonal contribution of depression in pubescent females, stress may predict the age at which the HPG-axis is activated, leading to an increased vulnerability to depression.

4.2 Onset of Menarche and Risk for Depression in Girls

Many studies have shown that menarche marks a transition in the risk for depression (Graber, Brooks-Gunn & Warren, 2006; Patton, et al., 1996; Riittakerttu et al., 2003). Menarche occurs toward the end of puberty and is defined as the occurrence of first menses. It coincides with Tanner staging IV, the last stage before the adult status (Tanner staging V).

Menarche is considered to be an important milestone in measuring pubertal maturation (Patton et al., 1996) as it is the result of rapid hormonal changes (Sizonenko, 1978). In 1996, Patton and colleagues found that early age at menarche was associated with an increased risk of depression, even after controlling for other important factors such as parental divorce. From these results, it has been suggested that the relation of depression to menarche could be due in part to the rapid hormonal changes associated with onset of menarche.

In the literature pertaining to the association between menarche and depression during adolescence, there are three types of measures that have been used. First, studies used ‘age at menarche’. In these studies, the authors use self-report or objective measures of age of onset of menarche, to determine whether age at menarche is associated with presence of depression in

adolescent girls. Other studies used 'menarcheal timing' and test its association to occurrence of depression in adolescent girls. In these studies, adolescent girls who reach early menarche (typically at 11 years or below based on mean age at menarche in large population studies (Joinson, Heron, Lewis, Croudace, & Araya, 2011; Stice, Presnell, & Bearman, 2001); 'early menarche group' are compared to adolescents girls who reach menarche at a normal timing (between 12 and 15) or who reach menarche at a later time (above 16 years of age). Finally other studies used 'menarcheal status' whereby girls are grouped as a function of presence or absence of menarche at the time of the study.

4.2.1. Association between Age at Menarche and risk of depression in adolescent girls

Girls with early age at menarche have been shown to have higher rates of lifetime history of a mood disorder compared to those who did not have early menarche (Graber, Seeley, Brooks-Gunn, Lewinsohn, 2004). Born, Shea and Steiner (2003) reported that hormonal changes at menarche coincided with an increase in the rate of depression – suggesting that estrogen plays a significant role. Others have reported that early maturing girls secrete more estradiol (an estrogen), and have higher levels of serum estradiol than late maturers, a difference that persisted into their early 30s (Vihko & Apter, 1984; Apter, Reinila, & Vihko, 1989). Kaltiala-Heino and colleagues (2003) reported that age at menarche was significantly associated with elevated depression and anxiety and externalizing behaviours such as aggression. When those with externalizing symptoms were excluded from the analyses, the association of internalizing symptoms with early puberty persisted among girls (Kaltiala-Heino, et al., 2003).

In another study, it has been shown that early age at menarche predicts increased depressive disorders when women are in their late 40's (Harlow, Cohen, Otto, Spiegelman, & Cramer,

2004) . However, discrepant data have been obtained as other studies showed that women who reached menarche after the age of 16 had *greater* levels of depressive symptoms at age 31, showing that later (instead of earlier) age at menarche is associated with depression in adulthood. Finally, other studies showed no association between age at menarche and depressive symptoms at age 18 and age 30 (Boden, Fergusson, & Horwood, 2011).

4.2.2. Association between menarcheal timing and risk of depression in adolescent girls:

Given the discrepancies reported in studies measuring the association between age at menarche and presence of depressive symptomatology in adolescence and adulthood, scientists have compared groups of adolescent girls based on menarcheal timing. Three studies have been performed using menarcheal timing as a measure of the association between menarche and risk for depression in adolescents. These studies have shown that girls with early menarche present increased depressive symptomatology when compared to girls with on-time or late menarche (Deng et al., 2011; Joinson et al., 2011; Stice et al., 2001).

4.2.3. Association between menarcheal status and risk for depression in adolescent girls:

More recent studies are assessing the acute association between menarcheal status (girls who started their menses or not) and presence of depressive symptomatology during adolescence. Using this measure, it has been shown that girls who have started their menses report significantly greater depressive scores than girls who did not start their menses at the time of the study (Capron, Therond, & Duyme, 2007; Kutcher et al., 2004) . In a sample of 2525 boys and girls grouped as a function of age (12-13 years versus 14-15 years versus 16-17 years), Patton and colleagues (Patton et al., 1996) found that in girls of all age groups, presence of menarche

was associated with increased depressive symptomatology.

5. Models to explain the association between onset of menarche and depression

The results of studies measuring the association between onset of menarche and depression in adolescent girls have led to two contrasting hypotheses. The first hypothesis, called the 'stressful change hypothesis' (Joinson et al., 2011) proposes that menarche itself is a stressful event and that all girls will eventually experience some level of distress, regardless of their timing of maturation. According to the stressful change hypothesis, the highest level of depressive symptoms should occur around the time of onset of menarche and symptoms are likely to be transient.

The second hypothesis is called the 'early timing hypothesis'. This hypothesis suggests that girls who mature earlier than their peers are more vulnerable to psychological distress (Caspi & Moffitt, 1991; Ge, Conger, & Elder, 1996). As we have summarized previously in our introduction, the transition into puberty is a critical developmental period that is often associated with increased conflicts with parents, development of romantic relationships, and changes in body image (Caspi & Moffitt, 1991; Conger, McCarty, Yang, Lahey, & Kropp, 1984). The early timing hypothesis proposes that these changes may have more severe effects on girls who mature at an earlier age than on girls who mature later, because of the additive effects that these environmental stressors may have over time in girls who reach early menarche (Joinson et al., 2011).

6. Position of the problem and hypotheses

The timing hypothesis and the stressful change hypotheses propose that the stress associated with either early maturation (early timing hypothesis) or with the appearance of first menses (stressful change hypothesis) will be key at predicting the association between menarche and

depressive symptomatology in adolescent girls exposed to an environmental stressor.

However, to this day, no studies to our knowledge have looked at a large sample of girls in a small age range, testing specifically for the effects of menarcheal status in adolescents exposed to a similar psychosocial stressor. Our review of the literature revealed that most studies on the effects of menarcheal onset and depression are retrospective, which rely on human memory which increases the likelihood for error. Thus, the following is an investigation of age at menarche and salivary cortisol in relationship to depression in both a retrospective and prospective manner. We investigated whether menarcheal status at the time of transition to high school would impact depressive symptomatology using scores from the Children's Depression Inventory (CDI) and if girls who started menarche before the study would have higher cortisol levels than both boys and girls who have not reached menarche at the time of the study.

In the present study, we assessed depressive symptomatology and salivary cortisol levels in 504 boys and girls aged between 11 and 13 years at 4 occasions during the school year. This protocol allowed us to compare depressive symptomatology and cortisol levels in girls who had reached menarche at the start of the study, to those who had not reached menarche at the end of the study. Based on the literature, we hypothesize that girls aged between 10 and 13 years who reached menarche before the start of the study should have significantly greater depressive symptomatology and cortisol levels than both boys and girls who have not reached menarche independently of Body Mass Index (BMI) otherwise known as a the height to weight ratio and age. If onset of menarche acts as a significant physiological and psychological trigger on the HPA axis, we also hypothesized that girls who reached menarche during the study should present depressive symptomatology and cortisol intermediate to that of girls who reached menarche and those who did not.

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ONSET OF MENARCHE BEFORE HIGH SCHOOL TRANSITION IS ASSOCIATED WITH HIGH DEPRESSIVE SYMPTOMS AND CORTISOL LEVELS IN ADOLESCENT GIRLS

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Short title: Menarche, depression and cortisol levels

Abstract

It has been proposed that onset and/or earlier age at menarche confer greater vulnerability to depressive symptoms by increasing reactivity of menarcheal girls to stressors associated with adolescence. In the present study, we measured depressive symptoms and salivary cortisol levels in 198 boys and 142 girls (11 -13 years) tested four times during their first year of transition into high school, a period known to be associated with stress among adolescents. Results showed that girls who had reached menarche before transition to high school presented significantly higher depressive symptoms and salivary cortisol levels across the school year when compared to girls who had not reached menarche, who presented higher depressive scores than boys. When we divided menarcheal girls as a function of menarcheal timing, we found that girls with early menarche presented constant depressive symptoms across the school year while girls with on-time menarche presented transient depressive symptoms. Altogether, these results show that onset of menarche is associated with high depressive symptoms and cortisol levels in adolescent girls going through the stress of high school transition and they suggest that early menarche may confer greater vulnerability to depression due to long-term exposure of the developing brain to high cortisol levels.

Keywords : adolescence, menarche, depression, cortisol, brain

Introduction

Women are about twice as likely as men to experience a lifetime episode of major depression (for a review, see (Kessler, 2003). Of great contemporary interest in psychiatric epidemiology, the higher prevalence of depression among women has been found throughout the world using a variety of diagnostic schemes and design methods (Wolk, 1995). Before adolescence, the rates of depression are similar in girls and boys (with a slightly higher rate in boys (Cyranowski et al., 2000), while at the onset of puberty (in the age range of 11-14), the sex proportion of depression dramatically shifts to a 2:1 (girls:boys) ratio (Kessler, 2003). This emerging sex gap in depression rates at puberty has raised questions about the role pubertal milestones exact in increasing vulnerability to depression in girls/women (Young & Altemus, 2004). To this day, the most consistent finding in the literature related to the higher prevalence of depression in women is that the risk of depression in adolescent girls or in adult women increases significantly with earlier age at menarche (Harlow et al., 2004; Kutcher et al., 2004).

Kaltiala-Heino and colleagues (Kaltiala-Heino, Kosunen, & Rimpela, 2003; Kaltiala-Heino, Marttunen, Rantanen, & Rimpela, 2003) measured the association between age at menarche and depressive symptoms in a large sample of 33,000 Finnish girls and boys (included in the study for comparison) aged 14 to 16 years. The results showed that among girls, self-reported depression was associated with early age at menarche. Early age at menarche has also been shown to be associated with presence of depressive symptoms in adulthood, although the results are less consistent. Harlow and colleagues (Harlow et al., 2004) studied depressed and non-depressed adult women (aged 36 to 45 years) and reported that in the depressed group, early age at menarche predicted increased depressive symptomatology in adulthood. However, the association between age at menarche and depressive symptoms is less clear when measuring

non-depressed samples of adult women. Herva and colleagues (Herva et al., 2004) studied 3952 Finnish women aged 31 years and found that women who reached menarche after the age of 16 had greater levels of depressive symptoms at age 31, showing that later (instead of earlier) age at menarche is associated with depression in adulthood. In contrast, Boden and colleagues (Boden et al., 2011) reported no association between age at menarche and depressive symptoms at age 18 and age 30 in a sample of 497 women followed longitudinally.

Given the discrepancies reported in studies measuring the association between age at menarche and presence of depressive symptomatology in adolescence and adulthood, scientists have compared groups of adolescent girls based on menarcheal timing. In these studies, adolescent girls who reached early menarche (typically at 11 years or below based on mean age at menarche in large population studies (Joinson et al., 2011; Stice et al., 2001); the 'early menarche group') are compared to adolescents girls who reached menarche at a normal timing (between 12 and 15; 'on-time menarche group') or who reach menarche at a later time (above 16 years of age; 'late menarche group'). Using this methodology, studies have shown that girls with early menarche present with increased depressive symptomatology when tested between the ages 13 and 15 (Joinson et al., 2011; Stice et al., 2001), or when tested during high school or college years (Deng et al., 2011). Altogether, these results show that girls who reach early menarche have an increased risk of experiencing depressive symptomatology during adolescence when compared to girls who reach menarche on-time.

The results of studies grouping girls as a function of menarcheal timing have led to the 'early timing hypothesis', which suggests that girls who mature earlier than their peers are more vulnerable to psychological distress (Caspi & Moffitt, 1991; Ge et al., 1996). The transition into puberty is a critical developmental period that is often associated with increased conflicts with

parents, development of romantic relationships, and changes in body image (Caspi & Moffitt, 1991; Conger et al., 1984). The early timing hypothesis proposes that these changes may have more severe effects on girls who mature at an earlier age than on girls who mature later, because of the additive effects that these environmental stressors may have over time in girls who reach early menarche (Joinson et al., 2011).

Although studies assessing girls as a function of menarcheal timing have led to important results on the long-term consequences of early menarche on adolescent depressive symptoms, these studies do not address the potential acute associations between presence of menarche and depressive symptomatology as a function of various psychosocial stressors known to be salient during adolescence. For example, various studies have shown that presence of family discord predicts early menarche and depressive symptoms in girls (Ellis & Essex, 2007; Ellis & Garber, 2000). In order to measure the acute associations between psychosocial stressors, menarche and depressive symptoms among adolescent girls, scientists are using 'menarcheal status' as a grouping variable, whereby girls are grouped as a function of presence or absence of menarche, at the time of the study.

Using menarcheal status as a grouping variable, Capron and colleagues (2007) found that in non-intact families (loss of a parent through divorce or death), girls who have their menses report significantly greater depressive scores than girls who had not yet started their menses at the time of the study. The authors hypothesized that non-intact families provided a less stable and emotionally supportive environment for children than intact-families. In another study, Kutcher and colleagues (Kutcher et al., 2004) measured depressive symptoms as a function of menarcheal status in adolescent girls (12-15 years) with depressed mothers (high-risk group) or non-depressed mothers (low-risk group). The results showed that significantly more girls from the

high-risk group (93%) had started their menses at the start of the study when compared to girls from the low-risk group (77.5%). In a sample of 2525 boys and girls grouped as a function of age (12-13 years versus 14-15 years versus 16-17 years), Patton and colleagues (Patton et al., 1996) found that in girls of all age groups, presence of menarche was associated with increased depressive symptomatology.

The results of the studies grouping girls as a function of menarcheal status have led to the 'stressful change hypothesis' (Joinson et al., 2011) which proposes that menarche itself is a stressful event and that all girls will eventually experience some level of distress, regardless of their timing of maturation. According to the 'stressful change hypothesis,' the highest level of depressive symptoms should occur around the time of onset of menarche and symptoms are likely to be transient. Both the timing hypothesis and the stressful change hypotheses propose that the stress associated with either early maturation (early timing hypothesis) or with the apparition of first menses (stressful change hypothesis) will be key at predicting the association between menarche and depressive symptomatology in adolescent girls exposed to an environmental stressor.

While these two hypotheses are important for conceptualizing the factors that may lead to increased depressive symptomatology as a function of menarcheal timing/status in adolescent girls, there are various factors that have not been taken into consideration in studies measuring the association between menarche and depressive symptoms during adolescence. The first is the exclusion of boys when assessing depressive symptoms in adolescent girls as a function of menarcheal status. The inclusion of boys into the study population is important because although the majority of previous studies performed on menarcheal status and depressive symptoms in girls showed that non-menarcheal girls have lower depressive symptoms than menarcheal girls, it

is not clear at this point whether non-menarcheal girls present depressive scores that are in the range of those observed in boys or whether these scores are higher than those of boys. Higher depressive symptoms in non-menarcheal girls when compared to boys would suggest that sex is an important factor for predicting the sex-gap ratio appearing during adolescence.

The second problem relates to the fact that many studies do not assess the presence of important confounding variables that may explain the association between presence of menarche and increased depressive symptomatology in adolescent girls. While menarche itself may act as a stressor in the life of adolescent girls as suggested by the stressful change hypothesis (Joinson et al., 2011), there are other factors related to menarche that may also be at play in the association between depressive symptoms and presence of menarche in adolescent girls. For instance, body mass index (BMI) increases significantly with the onset of puberty and it has been shown to be associated with both levels of depressive symptoms (Cortese et al., 2009; Needham & Crosnoe, 2005) and timing of menarche (Lee et al., 2007; Wouters, Larsen, Dubas, & Geenen, 2010). Second, sex identity and the stress that may be associated with it could be a significant predictor of the occurrence of depressive symptoms after menarche. Sex identity relates to how one sees him/herself on a continuum of femininity and masculinity (for a review, see(Johnson, 2007)). Both boys and girls develop their sex identity in the context of strong societal messages about what is acceptable in any given sex as a function of biological sex (Knaak, 2004). Given that onset of menarche marks the biological entry into womanhood, in relation to the newly acquired ability to bare children, it is possible that individual differences in magnitude of feminine or masculine traits within girls who reached menarche might be a good predictor of depressive symptoms. Finally, increased social demands in conjunction with a heightened emphasis on interpersonal and romantic relations, have been shown to be significant predictors of occurrence

of depressive symptomatology for adolescents, at the time of school transition (Newman, Lohman, & Newman, 2007; Newman, Newman, et al., 2007). It may thus be possible that onset of menarche marks the presence of important changes in the number and/or type of friends that adolescent girls have. These changes may lead to increased stress levels that may predict increased depressive symptomatology in menarcheal girls.

The third problem relates to the absence of objective measures of stress in studies testing the early timing and/or stressful changes hypotheses. Both the timing hypothesis and the stressful change hypothesis propose that the stress associated with either early maturation 'early timing hypothesis' or with the apparition of first menses 'stressful change hypothesis' will be key for predicting the association between menarche and depressive symptomatology in adolescent girls exposed to other environmental stressors. However, to this day, no study has measured biological markers of stress as a function of the presence of menarche in adolescent girls exposed to a similar stressor. It is not clear whether at this point whether the increased depressive symptoms reported in menarcheal girls are associated with a concomitant increase in biological markers of stress.

Heightened stress leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis and secretion of cortisol, one of the major stress hormones in humans. Both basal and stress-induced HPA activity have been shown to be significantly heightened at adolescence (McCormick, et al., 2004; Netherton C., Goodyer, E., Tamplin, A, & Herbert 2004), and numerous studies have found that chronic exposure to high levels of cortisol play a role in the precipitation of major depressive disorders in adolescence (Cyranowski et al., 2000; Goodyer et al., 1996; Halligan et al., 2007) and adulthood (Brown, 1978). It is thus possible that exposure to high levels of stress hormones in menarcheal girls is key in predicting increased depressive

symptoms.

Finally, although studies have assessed the impact of various stressful events such as parental conflicts (Ellis & Essex, 2007; Ellis & Garber, 2000), family structure (Capron et al., 2007), and maternal depressive symptomatology (Kutcher et al., 2004) in menarcheal and non-menarcheal girls, no study to our knowledge has assessed the association between menarcheal status and depressive symptoms in adolescent girls exposed to the *same* environmental stressor. In a previous study performed in 406 children aged between 6 and 16 years of age, we showed that transition from elementary to high school leads to a significant increase in cortisol in adolescents from both low and high socioeconomic status (Lupien et al., 2001). This finding suggests that transition to high school at the beginning of adolescence acts as a significant stressor as the transition to high school marks the first major change in status for a teenager. The teenager moves from a relatively small, community-based school to a larger impersonal unit, and also moves from being the oldest of the school, to being the youngest. Although high school is a long-awaited change in status accompanied by new freedoms and new challenges, the transition to high school is often associated with negative outcomes including poorer attendance, declines in grades, newly emerging disciplinary problems, and new feelings of alienation or social rejection (Moyer, 1982). Furthermore, studies have shown that the transition to high school is often accompanied by a decline in a sense of school belonging and an increase in depressive symptoms (Newman, Newman, et al., 2007).

Based on the analysis of the literature pertaining to the association between onset of menarche and depressive symptoms in adolescent girls, the goal of the present study was to test the early timing and stressful change hypotheses by measuring both depressive symptoms and salivary cortisol levels in a group of adolescent girls and boys who are simultaneously

experiencing the stress of a high school transition. In all participants, depressive symptoms and salivary cortisol levels were measured at four time-points during the school year. Testing began in September (first month of the school year) through February (mid-school year) in order to assess whether presence of depressive symptoms in a particular group would be transient or constant across the school year. We first predicted that if transition to high school is a significant stressor in the life of teenagers, then salivary cortisol levels should significantly increase across the school year in both boys and girls. Second, we predicted that if presence of menarche is key at explaining the appearance of the sex-gap ratio in depressive symptomatology above and beyond the effect of sex, than girls who started their menses prior to or at the beginning of the study should present significantly higher depressive scores than no-menarche girls and boys, and the two later groups should not differ from each other in terms of depressive symptomatology.

We then tested the predictions emerging from the stressful change and early timing hypotheses. If presence of menarche acts as a significant by temporary stressor in the life of adolescent girls as suggested by the stressful change hypothesis, then girls who have started their menses before transition to high school should present significantly higher depressive scores and salivary cortisol levels at the beginning of the study, when compared to girls who did not start their menses and boys. However, this difference should only be apparent at the beginning of the study and decrease with time. If the timing of menarche (early versus on-time menarche) is a better predictor of increased depressive symptoms in adolescent girls as suggested by the early timing hypothesis, then only girls who started menarche before the age of 11 should present increased depressive symptomatology when compared to girls with on-time menarche and this difference should be present across the four timepoints of the study.

Methods and Materials

Participants

A total of 504 adolescents (260 boys and 244 girls) ranging from 11 to 13 years (mean age : 12.02 ± 0.26 years) from two private high schools in Montreal, Canada, took part in the study. The study was approved by the Ethic Committee of the Douglas Mental Health Institute and the Mental Health Institute of l'Université de Montréal. All parents signed a consent form, while all teens signed an assent form. Throughout the school year, all teenagers received information on stress as part of an educational program developed for schools.

All teenagers were fluent in French and were tested during school hours. At both schools, Test 1 was administered during the last week of September, Test 2 in November, Test 3 in December and Test 4 in Mid-February. School 1 allowed testing at different times over the course of the day while School 2 allowed testing at the end of classes during students' mandatory study period. In order to control for the circadian cycle of cortisol throughout the day while maintaining the largest sample size possible, we selected from the original population those teenagers that were exclusively tested in the afternoon. This led to a sample size of 360 adolescents [198 boys and 162 girls; School 1 = 156 (77 boys and 79 girls); School 2 = 204 (120 boys and 84 girls) that are part of the analyses of this study. The mean age of the participants was 12.01 ± 0.22 years with a majority of teenagers being aged 12 at the time of the study (9 participants were 11 years, 335 participants were 12 years and 16 participants were 13 years). Adolescents were free of medication that may affect depressive symptoms or cortisol levels, and did not present neurological, psychiatric, drug use and general health problems.

Measures

Menarcheal status

Girls were asked at the beginning of the study (Time 1; September) and at the end of the study (Time 4; February) whether they had reached menarche ('have you started your menstruation?'), and if so to recall when to the nearest week (ie: "beginning of January") if they had begun menarche. Seventy girls had reached menarche before the beginning of the study (thereafter called the 'menarche group'), while 72 had still not reached menarche by the end of the study (thereafter called the 'no menarche group'). There were 20 girls who reached menarche during the study but they were excluded from the analyses because the sample size was not large enough to allow appropriate comparisons with the menarche and non-menarche groups. Comparisons of girls from the menarche and no-menarche groups were performed in order to test the predictions emerging from the stressful change hypothesis.

In order to determine whether menarcheal timing would better discriminate girls on depressive scores and/or cortisol levels across the school year (early timing hypothesis), girls from the menarcheal group were further subdivided in secondary analyses into girls who reached early menarche (between 10 and 11 years of age; as previous studies have defined early menarche at 11 years and below based on large study samples; (Joinson et al., 2011; Stice et al., 2001) and girls who had on-time menarche (12-13 years; (Joinson et al., 2011; Stice et al., 2001). In this sample, there were 25 girls who reached early menarche (mean age: 11.9 ± 1.9 years) and 29 girls who reached on-time menarche (12.08 ± 0.28 years). For each girl, we calculated the number of months that elapsed between onset of menarche and actual age at the time of the study. As expected, the two groups differed significantly with regards to months since menarche

[$F(1,49) = 11.4$; $p < 0.001$]. For the girls who reached early menarche, there was a mean of 12.8 ± 1.9 months that had elapsed since onset of menarche and time of the study, while for the girls who reached on-time menarche, there was a mean of 5.5 ± 1.1 months that had elapsed between onset of menarche and time of the study. The two groups of girls did not differ with regards to age at the time of testing (see Table 2).

Measures

Depressive symptoms

In order to assess the presence and magnitude of depressive symptoms, we used the French-validated version (St-Laurent, 1999) of the 27-item Child Depression Inventory (CDI), which has been validated among children aged 7 to 17 years (Kovacs, 1981, 1991). The CDI consists of 27 items designed to detect depressive symptomatology. Each item contains three choices, ranging from 0 to 2, providing a possible raw score between 0–54. High raw scores of 19 and above are clinically meaningful. In addition to raw scores, the CDI provides standardized scores to calculate the total test scores as well as the subscale scores. The standardized scores (0-100) address reported sex and age differences found for the CDI (Kovacs, 1991). We report the standardized test scores to ensure that sex and age differences are better controlled for than by simply using the raw scores. The CDI was administered at each of the four timepoints of the study. Though only the participants who completed at least 2 of the 4 CDI questionnaires administered between T1 and T4 were included in the analysis.

Salivary cortisol levels

Cortisol levels were assessed in saliva samples obtained twice during each testing session. For each saliva sampling, participants provided 2mLs of pure saliva (no cotton swab) in a small tube (Salivette®; Sarstedt, Germany). The samples used for this study were all obtained between noon and 5 P.M. Approximately 45 minutes elapsed between retrieving Sample 1 and Sample 2 from all of the participants. The process was repeated for each testing session.

At the end of each testing session, saliva samples were stored in freezers at -20 degrees Celsius at the Centre for Studies on Human Stress (www.humanstress.ca) until assayed. Samples were thawed and spun at 3000rpm at 4°C for 20 min and cortisol concentrations were determined by radioimmunoassay using a kit from DSL (Diagnostic Systems Laboratories, Inc., Texas, USA). Salivary samples of cortisol were mixed with 500 µL of ^{125}I-labelled cortisol reagent and 500 µL of Cortisol Antiserum Complex reagent. Total binding and non-specific binding typically ranged between 47-63% and 0.5-1.5%, respectively. The separation of bound antigens was obtained by using a pre-reacted double antibody system. When using this technique, cross-reactivity of the antigen is less than 4% with 11-deoxycortisol and less than 1% with any other naturally occurring steroids. The intra- and inter-assay coefficients of variation were 4.6% and 5%, respectively. The limit of detection of the assay was 0.01 µg/dl. All samples were assayed in duplicates.

Potential confounders of the association between early menarche and depressive symptoms

Body mass index

Height and weight information was obtained from self-report. Body mass index (BMI) was then determined by dividing the weight (kg) by the square meter of the height. There were 121

cases of missing height or weight information, therefore BMI analysis included a reduced number of participants from each Group; Menarche Group *n*= 46; No Menarche Group *n*=47, and Boys Group *n*=146.

Sex identity

Sex identity was measured using the French version (Alain, 1987) of the Bem Sex Role Inventory (Bem, 1981). This scale provides independent assessments of masculinity and femininity in terms of the respondent's self-reported possession of socially desirable, stereo-typically masculine and feminine personality characteristics. The Bem Sex Role Inventory was completed once at the end of the study. Participant's total scores for the masculinity and femininity scales were included in the statistical analyses. There were 52 cases of missing data for the Bem questionnaire, therefore analysis included a reduced number of participants from each Group; Menarche Group *n*= 60; No Menarche Group *n*=69, and Boys Group *n*=179.

Number of best friends

Since not having any friends is a potential social stressor, social relation was measured in the present study by asking each adolescent to indicate the number of best friends they had at the time of the study. A questionnaire was completed by the participant at the end of the study, in order to have a better indication of the number of best friends each adolescent had 7 months after transition to high school. There were 46 cases of missing data for number of best friends, therefore analysis included a reduced number of participants from each Group; Menarche Group *n*= 69; No Menarche Group *n*=67, and Boys Group *n*=178.

Procedure

Participants were tested in groups, during class or a study period. At each testing session, participants provided a saliva sample in the salivette tubes at the start of testing. A demographics questionnaire, which was completed in the first testing session, provided information on sex, age, height, weight, medication and illnesses. This was followed by the completion of a series of cognitive tests (not discussed here) and psychological questionnaires which included the CDI. The Child Depression Inventory was completed at each testing session, while the completion of the Bem's Sex Role Inventory was completed at the last testing session. At the end of each testing session, a second saliva sample was obtained. The questionnaire on menstrual milestones in girls was administered at the beginning (Test session #1) and end (Test session #4) of the study.

Statistical analysis

Data were first examined for normal distributions and means and standard errors were calculated (descriptive study). Datapoints for the CDI and cortisol levels were removed if they were 3 SD above the mean of the group controlling for sex. The standard deviations were small, and only ten of the original 504 participants were removed from the data above 3 SDs (3 in the menarche group, 3 in the no menarche group and 4 boys). Preliminary analyses were performed for testing of each hypothesis compared groups on age, body mass index, sex identity and number of best friends (confounding variables). When variables were shown to be different across groups, they were included as covariates in subsequent analyses.

Total scores on the CDI served as the primary measure of depressive symptoms in the population. For salivary cortisol data, the average of the two cortisol samples taken at each

testing session was used as a measure of cortisol levels since this procedure takes into account intra- and inter-subjects variability in salivary cortisol sampling during group testing (Lupien et al., 2001). In order to assess the predictions emerging from the stressful change hypothesis, we performed one-way repeated-measure ANOVAs on total CDI scores and salivary cortisol levels with Group (menarche *versus* no menarche *versus* boys) as the between-subject factor and Time (time 1 through time 4) as the within-subject factor. In a secondary set of analyses, we tested the predictions emerging from the early timing hypothesis by performing a one-way repeated ANOVAs on total CDI scores and salivary cortisol levels in girls from the menarche group with Group (early *versus* on-time menarche) as the between-subject factor and Time (time 1 through time 4) as the within-subject factor. For all the repeated measure ANOVAs, Greenhouse-Geisser corrections were applied (Greenhouse, 1959) when the assumption of sphericity was violated. For all significant main and interaction effects, groups were compared using the Tuckey procedure. Given that data from the menarcheal group were used in two statistical analyses, we used a Bonferroni correction (0.05/2 = 0.025) for determination of significant effects across all analyses. All statistical analyses were performed using SPSS 16.0.0 software package.

Results

Analysis based on menarcheal status

Preliminary analyses on potential confounding variables : Table 1 presents the data on age, body mass index, sex identity and number of best friends in girls grouped as a function of menarcheal status (menarche *versus* no menarche) and in boys. Results of these preliminary analyses showed that groups did not differ with regards to age and number of best friends, although they did with regards to BMI [F (2, 238) = 4.29; $p < 0.01$], femininity [F (2, 307) = 18.05; $p < 0.0001$] and masculinity [F (2, 307) = 4.95; $p < 0.001$] scores. Group comparisons for BMI revealed that girls from the no menarche group presented significantly lower BMI than girls from the menarche group and boys ($p < 0.001$). Group comparisons on the femininity index showed that although boys presented a significantly lower femininity index than girls ($p < 0.01$), girls from the menarche and no menarche groups did not differ between each other. As well, for the masculinity index, boys presented a significantly higher index than girls ($p <0.01$), but girls did not differ on the masculinity index as a function of menarcheal status. Given that girls from the menarche and no menarche groups only differed between each other in terms of BMI, we entered BMI as a covariate in all subsequent analyses pertaining to menarcheal status.

Menarcheal status and depressive symptoms: Results of the one-way repeated measure ANOVA performed on depressive scores showed a main effect of Time [F (3, 753) = 12.67; $p < 0.0001$], a main effect of Group [F (2, 251) = 27.3; $p < 0.0001$], but no interaction between Time and Group (see Figure 1). Entering BMI as a covariate did not change these effects [F (2, 183) = 19.89; $p < 0.0001$; Group effect]. The main effect of Time showed that depressive scores decreased significantly with testing session across all groups. The main effect of Group average,

girls from the menarche group presented significantly higher depressive scores than girls from the no menarche group and boys. Moreover, girls from the no menarche group presented significantly higher depressive scores when compared to boys ($p < 0.01$).

Menarcheal status and salivary cortisol levels : Results of the one-way repeated measure ANOVA performed on salivary cortisol levels showed a main effect of Time [F (3, 846)= 17.49; $p < 0.0001$], a main effect of Group [F (2, 282) = 9.401; $p < 0.0001$], but no interaction between Time and Group (see Figure 2). Entering BMI as a covariate did not change these effects [F (2, 202) = 7.02; $p < 0.001$; Group effect]. The main effect of Time showed that salivary cortisol levels significantly increased throughout the school year across all groups. The main effect of Group showed that on average, girls from the menarche group presented significantly higher salivary cortisol levels when compared to girls from the no menarche group and boys. Girls from the no menarche group differed significantly from boys on salivary cortisol levels ($p < .01$).

In order to assess whether mean cortisol levels across the four testing sessions were associated with mean CDI scores, we performed Pearson's correlation between mean CDI scores and mean salivary cortisol levels in each group taken separately. We found no significant correlation between mean cortisol levels and mean CDI scores across groups.

Analysis based on menarcheal timing

Preliminary analyses on potential confounding variables : Table 2 presents the data on age, body mass index, sex identity and number of best friends in girls grouped as a function of menarcheal timing (early menarche *versus* on-time menarche). Results of these preliminary

analyses showed that groups did not differ with regards to age, BMI, femininity and masculinity scores, and number of best friends.

Menarcheal timing and depressive symptoms : Results of the one-way repeated measures ANOVA performed on depressive scores revealed a significant interaction between Group (early *versus* on-time menarche) and Time (time 1 through 4) on depressive scores [F (3, 99) = 4.41; p < 0.006]. Group comparisons at each timepoint showed that girls from the early and on-time menarche groups did not differ on depressive score for the first 3 timepoints of the study, although they did differ on depressive scores at the last timepoint of the study [F (1,60) = 8.41; p < 0.005]. At this timepoint, girls from the on-time menarche group showed significantly lower depressive scores, when compared to girls from the early menarche group (see Figure 3). A close look at Figure 1 and Figure 3 shows that at the fourth timepoint of the study, girls from the on-time menarche group presented depressive scores that were in the range of girls from the no-menarche group.

Menarcheal timing and salivary cortisol levels : Results of the one-way repeated ANOVA performed on salivary cortisol levels revealed no significant main effect nor interaction between Group and Time (see Figure 4).

Tables

Table 1. *Group differences for menarcheal status on potential confounding factors*

	Menarche (*N*=70)	No Menarche (*N*=72)	Boys (*N*=198)	*P* Value
Age	12.01 ± 2.46	12.01 ± 0.21	12.02 ± 0.26	n.s.
Body Mass Index	18.64 ± 2.46	17.36 ± 2.49	19.21 ± 4.39	$p < 0.01$ *
Feminity Index	5.7 ± 0.61	5.8 ± 0.67	5.29 ± 0.71	$p < 0.0001$ $^{\phi}$
Masculinity Index	4.6 ± 0.65	4.57 ± 0.71	4.86 ± 0.73	$p < 0.01$ $^{\pi}$
Number of best friends	3.2 ± 2.8	3.5 ± 2.07	3.07 ± 2.49	n.s.

Table 1. Group differences on potential confounding factors associated with depression and cortisol levels as a function of sex and menarcheal status in 360 adolescents (mean and standard deviation).

* = No menarche group significantly different from boys

ϕ = Girls (menarche and no menarche) significantly different from boys but no difference between the menarch and no menarche groups.

$^{\pi}$ = Girls from the no menarche group significantly different from boys

Table 2. *Group differences for menarcheal timing on potential confounding factors*

	Early menarche (*N*=25)	**On-time menarche (*N*=29)**	***P* Value**
Age	11.94 ± 0.34	12.08 ± 0.28	n.s.
Body Mass Index	18.85 ± 2.24	18.49 ± 2.64	n.s.
Femininity Index	5.78 ± 0.54	5.67 ± 0.67	n.s.
Masculinity Index	4.54 ± 0.71	4.68 ± 0.61	n.s.
Number of best friends	4.5 ± 3.7	3.9 ± 3.2	n.s.

Table 2. Group differences on potential confounding factors associated with depression and cortisol levels as a function of sex and menarcheal timing in girls from the menarche group (mean and standard deviation).

Figure Captions

Figure 1. Total CDI Scores (± standard error of the mean) at testing sessions 1 through 4 in girls from the menarche and no-menarche groups and boys. ** : Significantly different from the other two groups.

Figure 2. Salivary cortisol levels (micrograms/dl ± standard error of the mean) at testing sessions 1 through 4 in girls from the menarche and no-menarche groups and boys. ** : Significantly different from no-menarche girls and boys.

Figure 3. Total CDI Scores (± standard error of the mean) at testing sessions 1 through 4 in girls from the early menarche and on-time menarche groups. ** : Significant group difference.

Figure 4. Salivary cortisol levels (micrograms/dl ± standard error of the mean) at testing sessions 1 through 4 in girls from the early menarche and on-time menarche groups.

Figure 1. *Depressive symptoms as a function of menarcheal status*

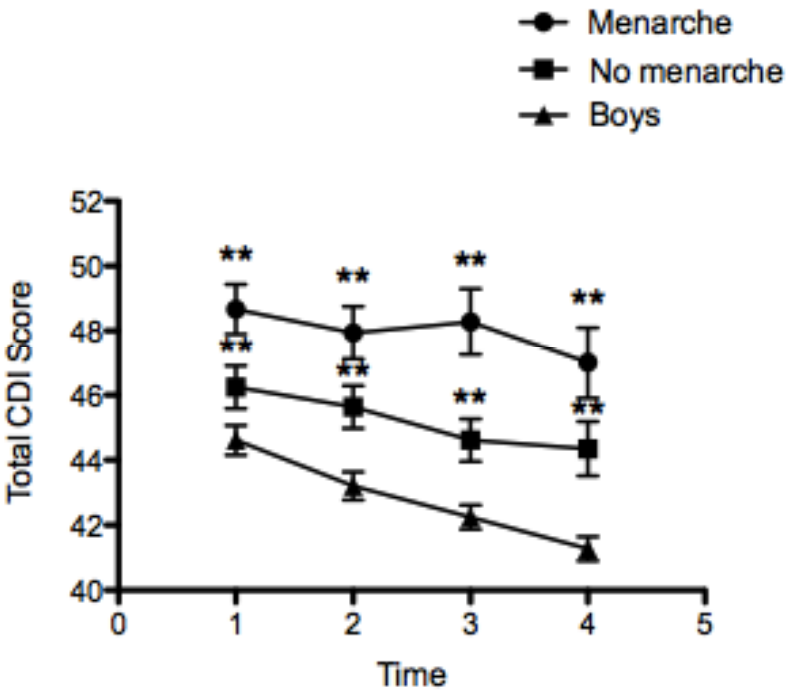

Figure 1. Total CDI Scores (± santard error of the mean) at testing sessions 1 through 4 in girls from the menarche and no-menarche groups and boys. ** : Significantly different from the other two groups.

Figure 2**.** *Salivary cortisol levels as a function of menarcheal status*

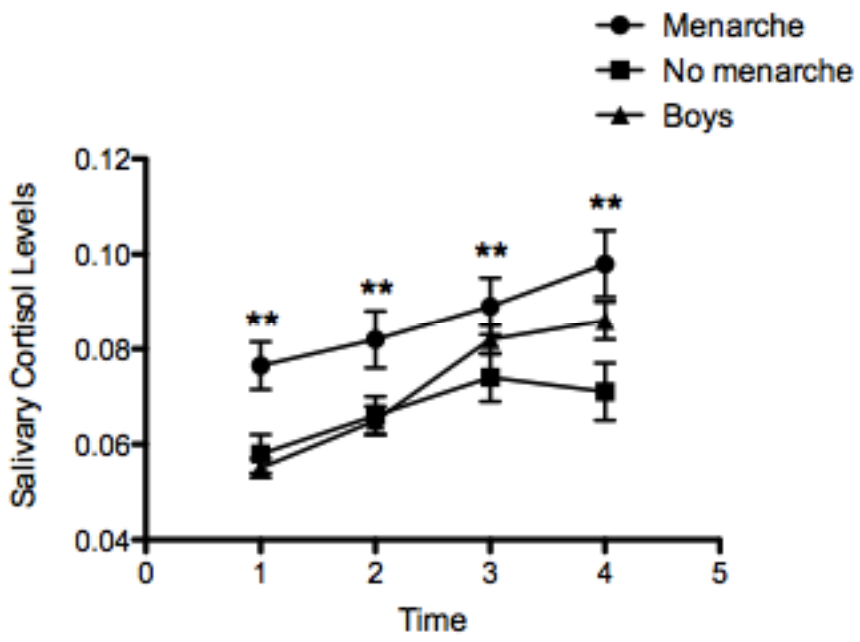

Figure 2. Salivary cortisol levels (micrograms/dl ± santard error of the mean) at testing sessions 1 through 4 in girls from the menarche and no-menarche groups and boys. ** : Significantly different from no-menarche girls and boys.

Figure 3**.** *Depressive symptoms as a function of menarcheal timing*

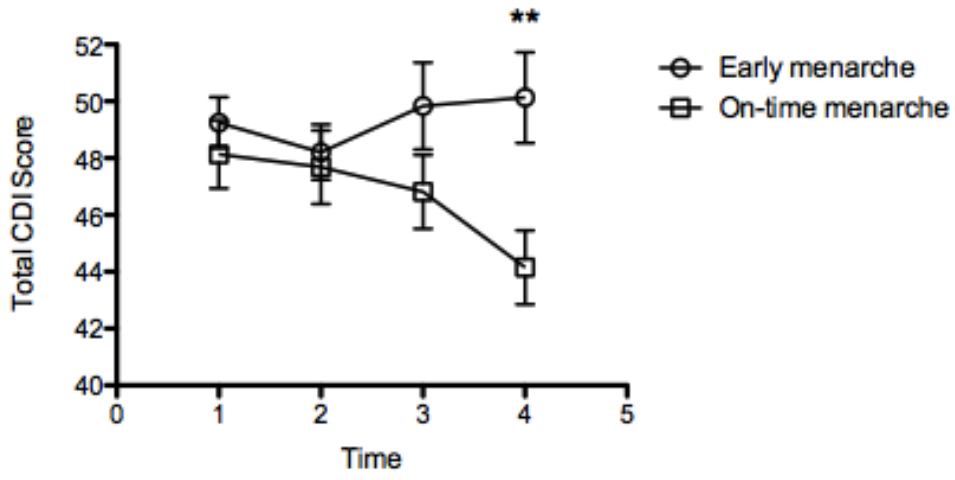

Figure 3. Total CDI Scores (± santard error of the mean) at testing sessions 1 through 4 in girls from the early menarche and on-time menarche groups. ** : Significant group difference.

Figure 4**.** *Salivary cortisol levels as a function of menarcheal timing*

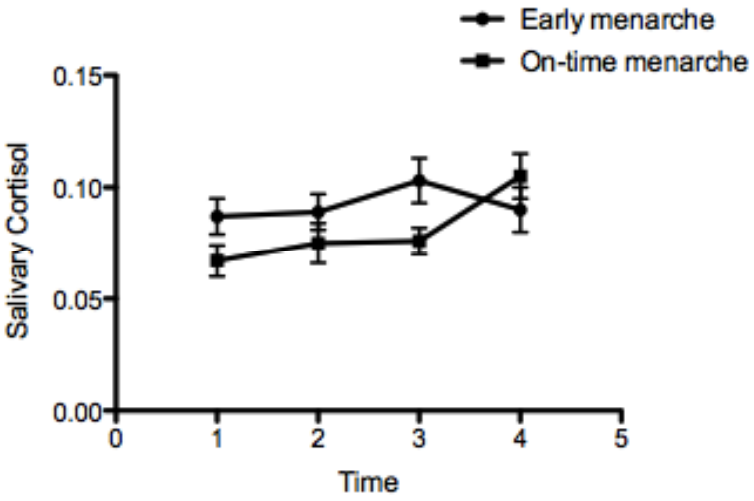

Figure 4. Salivary cortisol levels (micrograms/dl ± standard error of the mean) at testing sessions 1 through 4 in girls from the early menarche and on-time menarche groups.

Discussion

The results of this study provide three important sets of information. First, we found that girls from the menarche group presented significantly higher depressive scores than no-menarche girls and boys. Second, we expand our previous work by showing that transition to high school is associated with a significant increase in salivary cortisol levels in adolescent boys and girls, during the fall and winter months. Finally, we found that girls from the early menarche group showed consistently high levels of depressive symptoms during this study, while depressive symptoms in the on-time menarche group were more transient, with significantly lower depressive symptoms observed at the end of the study.

The significant increase in salivary cortisol levels observed across all groups confirms our first hypothesis that transition to high school is a stressful experience for adolescent boys and girls. However, it was unclear at this cross-sectional study whether the group differences were due to age and/or to high school transition. In the present study, we used a prospective longitudinal design in a large group of adolescents of a very narrow age range in their first year of transition to high school.

Given that all adolescents tested in the present study were assessed at the same time (months) of the year, it could be suggested that the significant increase of cortisol levels that we observed across the school year reflects natural seasonal variations in cortisol levels with higher levels observed in the Winter season (Time 3 and 4 of this study) when compared to the Fall season (Time 1 and 2 of this study). However, a recent study performed in 120 participants aged eight to 14, reported the presence of seasonal variations in salivary cortisol levels in adolescents, with the highest cortisol levels found during the Spring/Summer seasons (Matchock, Dorn, &

Susman, 2007). Winter is a period which Matchock and colleagues found was associated with the lowest levels of salivary cortisol levels in adolescents (Matchock, Dorn, & Susman, 2007). It is therefore likely that the stress of transition to high school was the cause of the significant increase in salivary cortisol levels reported in this population of adolescents.

The results of the analysis comparing boys and girls split as a function of menarcheal status (menarche *versus* no-menarche) revealed that although girls from the menarche group presented significantly higher depressive scores than girls from the no-menarche group and boys, girls from the no-menarche group still presented significantly higher depressive scores than boys. This result is not consistent with the hypothesis that menarche is the only factor behind the appearance of the sex difference in depressive symptoms at the time of puberty. Rather that sex is an important predictor of increased depressive symptoms observed in girls at the time of adolescence. In the present study, although we confirmed that boys report more masculine traits than girls, while girls report more feminine traits than boys, we did not find that sex roles differed across menarcheal groups. Moreover, while the no-menarche girls were not yet menstruating, changes relating to it were like in process. Finally, we did not find significant differences between groups on the number of best friends, suggesting that the sex difference in depressive scores cannot be attributed to sex roles or social relationships during transition to high school. Other factors related to sexually dimorphic behaviors thus have to be considered.

Sexual differentiation of the brain is a crucial developmental process that enables the lifelong expression of sexually dimorphic behaviors. Sexual differentiation of the brain occurs during a sensitive perinatal period during which gonadal hormones defeminize and masculinize the male brain, while a lack of gonadal steroids allows for feminization of the female brain (McCarthy, 2010). Various studies have shown that this hormonally-induced differentiation

alters neural structures in a permanent way, creating highly dimorphic brain regions (Arnold & Gorski, 1984; Nugent & McCarthy, 2011; Nugent, Schwarz, & McCarthy, 2011). Important sex differences have recently been reported in methylation, methyl-binding proteins, and chromatin modifications (Jessen & Auger, 2011) (Nugent & McCarthy, 2011), collectively suggesting that biological mechanisms are important for sexual differentiation of the brain. Though menarcheal status and/or timing are important factors that may explain the emergence of increased depressive symptomatology during adolescence in girls, other studies are needed in order to delineate whether these biological factors associated with sexual differentiation of the brain may contribute to the sex differences that exists for depressive symptomatology in adolescence.

The results of the present study support and extend both the stressful change and early timing hypotheses. The stressful change hypothesis predicted that if presence of menarche acts as a significant transient stressor (possibly due to the novelty, discomfort, adaption to sanitary needs etc.), then girls who started their menses before transitioning to high school should present significantly higher depressive scores and salivary cortisol levels at the beginning of the study, when compared to girls who did not start their menses and boys. According to this theory, increased depressive symptoms in menarche girls should be transient, ie., and therefore would be expected to be apparent at the beginning of the study. Indeed, from the menarche group presented significantly higher levels of depressive symptoms when compared to girls from the no-menarche group and boys, across the five month period.

Based on the early timing hypothesis we would expect that girls who started menarche before the age of 11 (early menarche group) should present increased depressive symptomatology when compared to girls with on-time menarche, and this difference should not be transient but be apparent at each time-point of the study. This was partially confirmed as we

have shown that girls from the early and on-time menarche group presented similar depressive scores at the first three time-points, while a group difference emerged showing, girls from the on-time menarche group had a significant decrease in depressive symptoms when compared to girls from the early-menarche group. Results nonetheless suggest that girls who mature earlier than their peers are more vulnerable to psychological distress (Caspi & Moffitt, 1991; Ge et al., 1996; Joinson et al., 2011), an effect that does not seem to be transient. However, based on this hypothesis, girls with on-time menarche should not have presented increased depressive symptoms in response to the stress of school transition. Yet, this particular group presented transient depressive symptoms that decreased over time. This result extends the stressful change hypothesis as it shows that onset of menarche in girls who reach on-time menarche may act as a transient stressor in girls, leading to transient depressive symptoms that may disappear with time.

Interestingly, although girls with early menarche differed from girls with on-time menarche on duration of depressive symptoms, both groups presented high levels of cortisol across the study when compared to girls who had not reached menarche and boys. There are two possibilities for the increased cortisol levels observed in menarcheal girls (early and on-time menarche). First, it suggest that the onset of menarche leads to increased secretion of cortisol in girls through the close interactions that exist between the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (Chrousos, Torpy, & Gold, 1998). Onset of menarche leads to increased secretion of gonadal steroids, and gonadal steroids are known to interact with the HPA axis under both basal and stressful conditions. Estrogens in particular have been shown to stimulate HPA axis activity (leading to increased secretion of stress hormones) and modify glucocorticoid and mineralocorticoids function in several animal species (Burgess & Handa, 1992; Handa, Burgess, Kerr, & O'Keefe, 1994; Viau & Meaney, 1991).

Although the effects of gonadal steroids on HPA axis activity in humans are less clear, studies show that estrogen replacement increases concentrations of free cortisol (Burgess & Handa, 1992; Chrousos et al., 1998). In a more recent study, Oskis et al., (Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009) measured diurnal (across the day) salivary cortisol levels in 61 girls aged 9 to 18 years as a function of menarcheal status. Results showed that adolescent girls who had reached menarche at the time of the study had higher levels of salivary cortisol 6 hours after awakening (approximately in the PM phase) when compared to adolescents who had not reached menarche. However, groups did not differ with regards to cortisol levels at later times of the day. If the onset of menarche acts by increasing cortisol levels through a neuroendocrine mechanism, then it is unclear why groups differ with respect to cortisol levels at some phases of the diurnal cycle and not at others.

The second possibility is that onset of menarche in girls leads to increased reactivity to environmental stressors. In the present study, we found that menarcheal girls presented significantly higher cortisol levels throughout the study when compared to non-menarcheal girls and boys. This would suggest that the stress of high school transition interacted with presence of menarche to increase cortisol levels across the school year. When we compared girls as a function of menarcheal timing, we found that girls with early and on-time menarche presented no difference in cortisol levels across testing sessions, which is consistent with the suggestion that presence of menarche more than its timing, may increase reactivity to environmental stressors. Given the close interactions existing between the HPA and HPG axes (Chrousos et al., 1998), it could be suggested that the increase in estrogen secretion associated with onset of menarche in adolescent girls plays a role in the regulation of the stress response. Further studies measuring

cortisol reactivity to psychosocial stressors before and after onset of menarche in adolescent girls would provide important data on this issue.

Constitutionally higher levels of cortisol associated with onset of menarche may enhance girls' vulnerability to depression in association with genetic and/or other environmental factors (Netherton, Goodyer, Tamplin, & Herbert, 2004). Yet, in the present study, we found no significant correlation between circulating levels of cortisol and depressive symptoms across groups. This result shows that actual circulating levels of cortisol, although they are significantly higher in menarcheal girls, may not be the cause of the increased depressive symptoms observed in this group.

Indeed previous studies performed in children (Ellis, Essex, & Boyce, 2005; Essex, Klein, Cho, & Kalin, 2002; Smider et al., 2002), adolescents (Goodyer et al., 1996; Halligan et al., 2007; Murray, Cooper, P.J., 2003) and adults (Burke, Davis, Otte, & Mohr, 2005; Gold, Drevets, & Charney, 2002; Heim et al., 2002) suggests that it is not the actual levels of cortisol that seems to predict onset of depression, but rather the long-term exposure of the brain to high levels of cortisol. If onset of menarche leads to increased cortisol levels through an endocrine mechanism and/or through increased reactivity to environmental stress, then it implies that girls who reach early menarche are exposed to high levels of cortisol at a significantly younger age and for a significantly longer period of time than girls who reach on-time menarche. The longer time of exposure to high levels of cortisol at a younger age may be the key at explaining the presence of consistent elevations in depressive symptoms in girls with early menarche.

Because they are liposoluble steroids, stress hormones have the capacity to rapidly access the brain, where they bind to glucocorticoid receptors in the hippocampus, amygdala, and frontal cortex (Lupien, McEwen, Gunnar, & Heim, 2009), three brain regions that have been involved in

the development of depressive disorders (for recent reviews, see (Bellani, Baiano, & Brambilla, 2011; Koenigs & Grafman, 2009; MacQueen & Frodl, 2011). Interestingly, these three brain regions do not develop at the same rate in humans (for a review, see (Lupien et al., 2009). Total hippocampal volume remains relatively stable between ages 4 and 25, suggesting that it is fully developed before the age of 4 although it continues to show neuronal proliferation, myelination, and pruning (Gogtay et al., 2006; Teicher, Andersen, Polcari, Anderson, & Navalta, 2002). The amygdala exhibits a continuous period of development extending from year 1 through late childhood (Tottenham, 2009), while white and gray matter volumes follow an inverted U developmental trajectory with the latest peaks occurring in high association areas such as the dorsolateral prefrontal cortex. In humans, the frontal lobe gray matter reaches its maximal volume at 11.0 years in girls and 12.1 years in boys (Giedd & Rapoport, 2010).

Early menarche in girls has been described as occurring at 11 years or earlier, which is the exact age at which the frontal lobes are in the process of reaching their maximal volumes in girls (Giedd & Rapoport, 2010). Chronic exposure of the still developing frontal brain regions to high levels of cortisol could induce a heterotypic reorganization of synaptic development, programming of neurotrophic factors, or changes in gene expression in fontal brain regions that could modify the developmental trajectories of these girls.

In contrast, for girls who reach on-time menarche (ie. between 12 and 15 years of age), increased cortisol levels associated with onset of menarche would occur at a time at which the frontal brain regions have reached their maximal volume. This may offer greater protection against high levels of cortisol in these girls when compared to girls who reach early menarche. According to this hypothesis (that we propose to call the 'brain vulnerability to early menarche hypothesis') early menarche should be associated with significant differences in the development

of glucocorticoid-sensitive brain regions, with a particular effect on frontal regions. Future studies measuring frontal lobe volumes and/or function in relation to menarcheal status and/or timing will be important at testing this developmental hypothesis of the effect of early menarche on depressive symptoms in adolescent girls.

Strengths and Limitations

The current study has a number of strengths, including a large sample size, repeated measures of self-reported depressive symptoms and cortisol levels, a narrow age range, and availability of data on potential confounding factors. Although the present results provide important data with regard to the effects of menarcheal status and timing on depressive symptoms and cortisol levels in adolescent girls, there are limitations to our study that merit consideration. First, we only tested adolescents from private high schools who were from medium to high socioeconomic status (SES). Although this method allowed us to control for the confounding factor of SES on depressive scores and cortisol levels in adolescents, our findings may not be generally applicable to adolescents from low SES. In previous studies, our group has shown that children from low SES present significantly higher cortisol levels than children from high SES, although the effects of SES was not observed at the time of adolescence (Lupien et al., 2001), suggesting the presence of an equalization process of SES on cortisol levels during adolescence (West, 1997). Still, it is unclear at this point whether low SES may interact with high cortisol levels to confer greater vulnerability to depressive symptomatology in menarcheal girls.

Second, we have not controlled for the menstrual cycle at the time of each testing session in the menarcheal group. Differences in circulating levels of cortisol have been reported as a function of the menstrual cycle, with highest levels of basal and reactive cortisol levels being

observed during the luteal phase when compared to the follicular phase (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). It is thus possible that more girls provided cortisol samples while in the luteal phase, at each testing session, which would account for the increased cortisol levels observed in this group. Although this is possible, it is highly improbable given that our sample size was large enough to allow for random distribution of testing throughout the menstrual cycle and allowed for meaningful inferences about cortisol levels that were independent of the menstrual cycle of menarche girls. Yet, future studies measuring salivary levels of gonadal steroids in girls and boys should provide important data on the association between menstrual cycle and presence of depressive symptoms in adolescent girls.

Third, we did not control for the presence of discordant family relationships on the association between menarche and depressive symptoms in this population of adolescent girls. Studies by Ellis (Ellis & Essex, 2007; Ellis & Garber, 2000) showed that discordant family relationships predict early pubertal timing in girls. As well, Capron and colleagues (Capron et al., 2007) found that in non-intact families, menarcheal girls report significantly greater depressive symptoms than no-menarcheal girls. Consequently, it is possible that the effect of menarcheal status and/or timing on depressive symptoms and cortisol levels in adolescent girls may be modulated by the presence of family discord.

Finally, in the present study, we did not measure whether presence of depressive affect *during* childhood might predict early age at menarche and increased reactivity to stress during adolescence. In one of the first study ever performed on the association between age at menarche and depressive affect, Graber and colleagues (Graber, Brooks-Gunn, & Warren, 1995) measured the longitudinal associations between breast development, weight, family relations and depressive affect in 75 pre-menarcheal girls aged 10 to 14 years. Results showed that breast

development, weight, family relations and depressive affect were predictive of age at menarche. In another study, Rierdan and colleagues (Rierdan & Koff, 1990) tested 92 girls who changed from pre- to post-menarcheal status between 2 test occasions 6 months apart. Results showed that depressive symptoms during the pre-menarcheal status significantly predicted negative emotional response to menarche. Given that girls from the menarche group, who presented with high depressive scores at the four time-points in this study, had already started their menses before the onset of the study, it may be that presence of depressive affect early in childhood leads to early menarcheal timing and increased cortisol response to environmental stressors during adolescence. Clearly, further longitudinal studies performed before and after onset of menarche will be necessary to assess whether presence of depressive symptoms in childhood predicts early menarche and increased reactivity to stress during adolescence.

Implications and future directions

Altogether, the results of our study extend the early timing and stressful change hypothesis by showing that presence of menarche at the time of high school transition is associated with significantly higher depressive symptoms and cortisol levels, although this effect is more transient in girls who reach on-time menarche when compared to girls who reach early menarche. Given that presence of menarche is associated with significantly higher cortisol levels, we propose that longer-term exposure to high levels of cortisol in adolescent girls with early menarche may have negative impact on the developing brain, leading to increased vulnerability to depression. Previous studies performed in children have shown that cortisol levels can be significantly decreased by targeted interventions (Dozier, 2006; Fisher, Stoolmiller, Gunnar, & Burraston, 2007). The present results are important for they suggest that

interventions designed toward adolescent girls going through menarche at the time of high school transition could potentially reduce the occurrence of depressive disorders in menarcheal girls (Adam, Sutton, Doane, & Mineka, 2008).

7. General Discussion

There were three main findings in this study. First, we found that salivary cortisol rose consistently from the start of the school year (September) at Time 1, to the last testing session at Time 4 (February), across groups. The second finding revealed that menarche is an important risk factor in the vulnerability to depression. Specifically, girls from the menarche group presented significantly higher scores on the CDI when compared to girls who had not reached menarche (no-menarche), and boys. A significant sex effect was also found. Like the menarche group, no-menarche girls had significantly higher scores on the CDI than boys. Third, the age at which menarche was attained was a key factor for more sustained depressive symptoms. Girls who reached menarche at an 'early' age (before age 11) were more likely to have higher depression scores that persisted throughout the study than girls who reached menarche 'on time', these girls having more transient elevated depression scores. We conducted preliminary analysis on possible confounding variables and found that groups did not differ significantly on age, BMI, femininity and masculinity scores, nor did they differ on the number of "best friends", taken in the present study as a proxy measure of social support in this population of adolescents making the transition to high school.

In the following discussion of our results, we will discuss each of these important findings and put them into perspective with new data in the field of adolescent stress and depression.

8. Stress, cortisol, and transition to high school at puberty

In the present study, salivary cortisol was collected within the same months, weekdays and time of day, to neutralize diurnal and seasonal variations in cortisol levels between groups. Salivary cortisol levels were found to increase between Fall (Time 1 and Time 2) and Winter (Time 3 and Time 4) across all groups. The main effect of group showed that for each testing session, girls from the menarche group presented with significantly higher salivary cortisol levels when compared to girls from the no-menarche group and boys. This result confirms the 'stressful change' hypothesis which proposes that menarche itself is a stressful event and that all girls will eventually experience some level of distress, regardless of their timing of maturation. However, the no-menarche group did not differ significantly from the boys in terms of cortisol secretion across the year. This finding is therefore indicative of a general rise in cortisol levels at puberty, with menstruating girls presenting with higher than average cortisol levels. The main causes for increased cortisol levels among young teenagers making the transition to high school, and girls who have reached menarche in particular, may be found in the environmental, biological or genetic domains, or a combination thereof.

8.1 The Environment: Stress and change during developmentally sensitive periods

In this study, we used a prospective longitudinal design of a large group of 11 to 13 year olds. We predicted that the transition from primary school to high school would be a significant factor of stress and result in higher mean salivary cortisol levels across groups in our sample. Our results supported this prediction. At first glance, it appears adequate to conclude that the first year of high school, especially the first few months, is a critical period of psychological and social adjustment in pubertal girls and boys. Our study suggests that this is an especially stressful period, although it is not clear whether this effect is due to age/and or high school

transition, or to a group effect that may be particular to the higher homogeneous socioeconomic status of the adolescents who were included in our sample.

These findings are in contrast with a previous study that reported that cortisol levels, taken from a large sample of preadolescents and adolescents, decreased over the Fall and Winter seasons (Matchock, Dorn, & Susman, 2007). However, in this study, the adolescents were only tested as a function of seasons and not with regard to exposure to an important life transition such as high school transition. In our study, we found that cortisol rose from the Fall to the Winter. The difference in our findings may be due to methodology differences between our study and that of Matchock and colleagues (2007). For instance, Matchock and colleagues (2007) study included children aged 8 to 14 years. It may be argued that an 8 year old has vastly different physiological changes and social-psychological pressures than a 14 year old teenager.

The present study on the other hand, offered a unique opportunity to study a large sample of teenagers who are at a particular phase in their social and biological development. This isolates our sample from certain extraneous variables such as the process of adrenarche which may occur simultaneously but independently of gonadarche, generally occurring in 6 to 8 year olds (Ellis & Essex, 2007). Gonadarche and not adrenarche is associated with a change in diurnal rhythms (Ellis & Essex, 2007). Therefore there are known distinct physiological differences between the pre-pubertal and pubertal children that must be taken into consideration when grouping children with wider age ranges. One factor that is both unique and common to the group that we tested, is that every student in our study experienced the same environmental stressors simultaneously – the transition from primary school to high school. We believe that this particular environmental stressor is the key factor for the rise in cortisol over time that we observed in the present study.

8.2 Biology: Stress and the HPA Axis from Adolescence to Adulthood

Adolescence is a period that is associated with heightened basal HPA activity (Lupien et al. 2009). It is also a time when certain brain regions continue to develop, such as the amygdala and the prefrontal cortex (Lupien et al., 2009). An important time in which the HPA system has a significant impact on the brain is at puberty. At this time the HPA axis can effect important changes on the brain, if it is activated too soon (Giedd & Rapoport, 2010). The frontal lobe, responsible for organization, decision making, rational thinking and inhibition, does not complete its development until puberty. The frontal lobe gray matter reaches its maximum volume at 11years in girls and 12.1 years in boys (Giedd & Rapoport, 2010). This is a key factor when analyzing the impact of stress on the brain. The onset of menarche leads to a sudden increase in circulating basal cortisol, which when combined with greater environmental stress, possibly induced by such things as a new school environment, encourage greater amounts of stress hormones to easily access the brain. If the brain has not finished developing before the onslaught of HPA axis hormones (cortisol), which rise rapidly at menarche, there may be a risk of permanently establishing a weakened capacity for effectively managing the influx of stress hormones in the brain, thus increasing the vulnerability to stress-related onset of depression. In other words, when the stress system does not function properly as a result of, or in conjunction with, physiological, psychological, and behavioral changes associated with this important period of brain development, it may lead to increased vulnerability to various mental health disorders, including depressive symptomatology (Dorn & Chrousos, 1997).

Our study support previous findings which show that menarche may be a particularly critical period for deregulations in the HPA system (Dorn & Chrousos, 1997). Other biologically critical periods include the intrauterine and neonatal periods, though more studies are needed (Dorn &

Chrousos, 1997). The other crucial periods in the life of a woman that may lead to increased reactivity to stress due to interactions between the HPA and HPG axis are adrenarche, gonadarche (Ellis & Essex, 2007), the post-partum period, and the menopausal period (Dorn & Chrousos, 1997).

One example of gonadal vulnerability factors interacting with stress hormones, to confer greater vulnerability to depression, is postpartum depression (PPD). Postpartum depression is considered to be a transient depression lasting from the first week postpartum to several months. (Steiner, Dunn, & Born, 2001). Statistically, women of child bearing years are at the greatest risk for developing major depression and this condition affects approximately 15% of women and is highest in the postpartum period (Brummelte & Galea, 2009). Brummelte and Galea (2009) conducted a review on the contribution of hormones during pregnancy and postpartum in the development of major depression. The authors report that there is a convergence of studies that point to a dysregulation of the HPA axis as the most prominent endocrine change associated to depression, and is also the targeted system for therapies (Brummelt & Galea, 2009).

One major hypothesis for PPD is the 'ovarian-steroid-withdrawal hypothesis' which states that when estrogen (estradiol and progesterone) levels rise rapidly after conception to over 1000x their pre-pregnancy levels and then see a dramatic drop after birth with the release of the placenta from the mother (Hendrick et al., 1998), this drop in gonadal steroids leads to increased vulnerability to depression in postpartum mothers. Others suggest that a major factor for PPD is mainly due to the HPA axis and the main factor for increased risk of depression. Magiakou and others (1996) point to the hyperactive HPA axis found in the third trimester, as they found that blunting of ACTH was significantly more severe and long lasting in the PDD group. The

researcher proposed that it is the suppressed ACTH response to CRH which might serve as marker of PPD (Magiakou, et al. 1996)

Harlow and colleagues (2003) studied the influence of depression on reproductive endocrine and menstrual markers associated with pre-menopause and found that women who had a history of depression had earlier and more pronounced pre-menopausal symptoms at enrollment in the study, than women with no history of depression (Harlow et al., 2004). The study also found that women who presented with a lifetime history of depression also had higher follicle-stimulating hormone and luteinizing hormone levels and lower estradiol levels (Harlow et al., 2003). Therefore a lifetime history of depression has a reciprocal effect on hormonal function in later life (Avis, Brambilla, McKinlay, & Vass, 1994; Harlow et al., 2004); Harlow et al., 2003), suggesting that the hormonal dysregulations which may occur with early menarche in adolescent girls may have long-lasting effects. To this day, no study has assessed whether early menarche in adolescent girls leads to increased vulnerability to postpartum depression in adulthood or to increased depressive symptoms during menopause in older women. Such studies would provide very important data on the complex interplay that may exist between the HPA and HPG axes in conferring greater vulnerability to depressive symptomatology in some women as compared to others.

8.3 Genetics: Potential Heritability of Stress Reactivity

Bartels and colleagues (2003) conducted a study to estimate the genetic and environmental influences in basal salivary cortisol levels for a group of 180 12-year-old twin pairs. The authors found that there was a high correlation between pairs in the morning and afternoon samples, but not for the evening samples (Bartels, de Geus, Kirschbaum, Sluyter, & Boomsma, 2003).

Heritability of the cortisol levels was highest at 45 minutes after awakening (60% heritability) and then decreased throughout the day, with no sex difference (Bartels et al., 2003). These results suggest that genetic factors are important at predicting awakening/morning cortisol levels, but as the day goes by and environmental factors get involved, there is less genetic effects on cortisol levels and more environmental factors explaining inter-individual variability in cortisol levels. Given that our study was performed in the afternoon hours, we can conclude based on these genetic/environmental data that the group differences we observed in cortisol levels are due to both genetic and environmental factors that may differ as a function of sex and menarcheal status in adolescent girls.

9. Depression, menarcheal, status and sex

The second important finding of our study was the presence of a significant effect of group for depressive scores across the study. Our results revealed that menarche is an important risk factor in the vulnerability to high depressive symptomatology. Specifically, girls in the menarche group scored as significantly more depressed on the Child Depression Inventory (CDI) than girls who had not reached menarche (no-menarche), and boys. A significant sex effect was also found. Not only were girls who reached menarche more depressed than the no-menarche girls and boys, but the no-menarche girls also scored significantly higher on the depression scale than the boys. Our study shows that although menarche is a clear risk factor for the onset of depression, there is also a definite sex effect emerging. In the next section, we discuss the importance of taking this additional sex effect (over that of menarche) in predicting increased vulnerability to depression during adolescence.

9.1 Sex Differences in Depression and the Importance of the Measures We Use

There are several possible causes for a sex effect to appear in this study. Part of the reason for sexually dimorphic behaviors as we discussed in the introduction of this paper, has much to do with sex socialization. Apart from sex socialization, another reason is perhaps biological influences. For instance, sexual dimorphism of the brain must also account for some of the social, behavioral, as well as the hormonal sex differences that become even more pronounced at puberty. Sexual differentiation first occurs during a perinatal sensitive period in the brain (McCarthy, 2010). At this time, gonadal steroid hormones defeminize and masculinize the male brain, while a lack of these steroids allow for feminization of the female brain (McCarthy, 2010). Various studies have shown that this hormonally-induced differentiation during the perinatal period permanently alters neural structures, thus producing highly dimorphic brain regions (Nugent & McCarthy, 2001; Nugent, Schwarz, & McCarthy, 2001).

Although there may be a biological basis for the sex difference observed in depressive symptomatology during adolescence, it may also be possible that the male brain expresses depression in a different way than the female brain. If this is the case, then it might be possible that the screening procedures that we used to assess depressive symptomatology in boys and girls are biased toward one sex, leading to increased rates of depression in one sex when compared to the other sex, a finding that is a potential artifact and due to sex-biased measures.

Indeed some studies have shown that most depression inventories given to children, including the CDI, do not capture the emotional and cognitive styles of depressed boys (Nolen-Hoeksema, Girgus, & Seligman, 1991). These adolescent depression questionnaires are skewed to identify depression using questions that probe for "internalizing" type behaviors. For instance a depressed boy might answer '*no'* to the statement "*I cry often*," while a sad or

depressed girl is more likely to respond '*yes'* to that question . Yet, this difference may be due to difference in sex socialization, with boys having learned throughout development that '*boys don't cry'*. Consequently, given that sex socialization patterns in girls are more favorable to crying when having sad emotions, this will lead to girls receiving higher scores on depression scales that are more in line with their feelings indicating that they have a sad affect.

A boy is more likely to expresses his sadness externally (through anger and aggression for instance) and may therefore answer 'no' to a question on crying. If the questionnaire does not capture externalizing behaviors such as expressing anger through kicking, aggressive behaviors etc., this will lead to a false low score of boys on a depression scale geared toward internalizing behaviors (Twenge & Nolen-Hoeksema, 2002). Therefore the questionnaires used in many studies may not be sensitive enough, or taxonomically designed, to identify depressive behaviors that are more commonly expressed by boys (Twenge & Nolen-Hoeksema, 2002). The behaviors generally classified as externalizing behavior include; violence (physical aggression), smoking, drug use and alcohol abuse – behaviors which may later be classified as conduct disorders. The CDI does not probe for this type of behavior and this may explain why we found significantly lower scores on the CDI in boys when compared to girls from the menarche and no menarche groups.

There are very few studies that examine depression in boys as the main objective. However, there is one longitudinal Finnish research report that studied the factors associated with childhood depressive symptoms in boys between the ages of 8 and a follow-up at age 18 that revealed very interesting results. Ronning et al. (2011) studied the conditions associated with depressive symptoms in 2,348 boys aged 8 to 18. Using the CDI as the measure for depressive symptoms (with all its limitations described above) at age 8 and the Beck Depression Inventory

at age 18 to determine depressive symptomatology, researchers also gathered information on family factors, life events, adaptive functioning, and substance abuse (Ronning, et al., 2011). The results showed that a stressful childhood, which included parental divorce, predicted childhood and adolescent depression in this population of boys (Ronning et al., 2011). The findings also pointed to depressive symptoms being highly correlated with poor adaptive functioning at age 18 as well as drug use (Ronning et al., 2011).

Other studies have shown that poor adaptive functioning and drug use perpetuate and have a comorbid relationship to depression (Archie, Kazemi, & Akhtar-Danesh, 2011). Of equal importance to the accurate scoring of the CDI is the accurate reporting of the results. Previous reviews have cited the importance of employing caution in the administration and interpretation of results obtained using the CDI (Fristad, Emery & Beck, 1997). Though the CDI is a well validated and frequently cited psychological self-assessment tool for children and teenagers, researchers caution about the language used when presenting the results. For instance, the use of the CDI as a single instrument is not sufficient to make a clinical diagnosis of major depression. Nonetheless, there is much evidence to support the CDI as valid instrument for single measure reliably detecting depressive symptomatology in children (Fristad, Emery & Beck, 1997; Kamphaus & Frick, 2005; Matthey & Petrovski, 2002). In our study, we judiciously employed wording that would not mislead the reader into believing that the adolescents in our study were clinically diagnosed or confirmed has having depression. It is for this reason that we described the results of our study in terms of "depressive symptomatology," or "depressed affect" to describe our measures instead of 'clinical depression' or 'depression'.

10. Depression and menarcheal timing in adolescent girls

The third important finding of our study is the interaction we reported between the early-menarche group and the on-time menarche group for depressive symptoms across the school year. The early menarche group presented with significantly higher CDI scores than the on-time menarche group, although this difference was only significant at Time 4 (last time point). What is important to note here is that although the girls who reached early and on-time menarche were scoring as significantly more depressed than the boys, this difference only persisted with the early menarche group. That is, the effect of depression on the on-time menarche group was only transient. Therefore, our findings concur with previous studies showing that early menarche may be a risk factor for current and onset of depression in adulthood (Kaltiala-Heino, Kosunen, et al., 2003; Stice et al., 2001). These studies have described various factors that may explain why early menarche may confer greater vulnerability to depressive disorders in adolescent girls and we analyze them in the next section.

10.1 Environmental stress factors and early menarche

There are many factors which contribute to the increased risk of mood disorders among girls, one of which is a stressful home and family environment. In a study on the psychological antecedents of variations in girl's pubertal timing, Ellis and Graber (2000) measured mother's history of mood disorders, mother's age at menarche, romantic relationships and mother's perception of the overall quality of family relationships with daughters' pubertal development. As well, the psychiatric histories of the mothers were assessed with the Structured Clinical Interview (Ellis & Graber, 2000). The results showed that the mother's history of a mood disorder significantly predicted earlier pubertal timing in the daughters, mediated by mother's perceived stress and the absence of the biological father (Ellis & Graber, 2000). Early teenage

pregnancy in the mother also predicted earlier pubertal timing in daughters as well as more family dysfunction (Ellis & Graber, 2000). This study shows that the emotional and psychological stress induced by environmental factors can be significant enough to affect stress hormone and gonadal hormone changes in adolescent girls.

In addition to the hormonal changes that can occur in young girls due to stressful environments, at a time when the brain is less equipped to handle these stresses (Giebb & Rapoport, 2010), stress sensitivity can lead to internalizing symptoms which may increase later vulnerability to depression. Natsuaki et al (2009) examined pubertal timing and salivary cortisol reactivity to interpersonal stress tasks in evenly matched boys and girls of 11 to 16 year of age. The authors found that early maturing adolescents had greater sensitivity to stress, this sensitivity in turn increased the risk of internalizing problems (Natsuaki et al, 2009). Cortisol reactivity was higher for girls in the interpersonal challenge than for boys, and more strongly associated to internalizing problems (Natsuaki, 2009).

10.2 Genetic Background, Early Menarche and Depression

Just as for cortisol levels, it is possible that genetic effects may be involved in the greater vulnerability to depressive symptoms reported in girls with early menarche. Silberg and others (1999) investigated the shared and unique genetic and environmental variation for depression among pre-pubertal and pubertal, male and female, twin pairs from the Virginia Twin Study of Adolescent Behavioral Development (Silberg et al., 1999). They compared the trajectory of depressive symptoms among each group split by sex and stage of development, and incorporated an index of environmental stress experiences in the previous 12 months. The authors found that the impact of life events on depression was especially significant among the pubertal females

(Silberg et al., 1999). Heritability factors for depression and the persistence of depression among the pubertal females was strongly mediated by genetic factors (Silberg et al., 1999). The authors also report that part of the tendency toward depression were linked to a common set of genes in the adolescent girls, but that there is an increase in genetic variance for life events (Silberg et al., 1999).

10.3. Gene/Environment Interactions : Epigenetic Influences on Menarche and Depression

Although a person's genetic make-up is just one of several risk factors associated with early menarche and greater vulnerability to depression, new research in the area of epigenetics is increasing our understanding of the importance and complexity of environmental influences on associated mechanisms for gene expression. Epigenetic mechanisms involve heritable and lasting changes in the control of gene expression, without altering the genetic code (Tsankova, Renthal, Kumar, & Nestler, 2007).

Studies have identified a specific set of enzymatic modifications to chromatin structures that affect the regulation of gene expression in a manner that is transmissible to new cells (Tsankova et al., 2007). With even greater implications for psychiatric disorders, these epigenetic mechanisms also effect neurons that do not divide but instead maintain the alterations within the effected cells (Tsankova et al., 2007). These modifications are part of a dynamic process that involves modifications to the chromatin, modulating DNA-protein interactions and histone methylation, without effecting the DNA (Tsankova et al., 2007). This chromatin remodeling is involved in the birth and regulation of new neurons which occur in the hippocampus and the sub-ventricular zone adjacent to the striatum (Tsankova et al., 2007). It is well established that the hippocampus is vulnerable to the effects of stress and depression (Lupien, et al., 2009).

Chronic stress has been shown to leave lasting epigenetic alterations in the hippocampus in animal research. Tsankova and colleagues (2004) conducted a study which demonstrated the lasting effects of electroconvulsive stimulation (also known as electroshock therapy) on histone modification in the hippocampus of rats. In brief, the theoretical justification for inducing electroconvulsive seizure as a treatment for severe and recurrent depression is based on the moderate success rate of multiple treatments of electroshock treatment (ECT) in treatment resistant patients (Medda, Perugi, Zanello, Ciuffa, & Cassano, 2009). Tsankova and colleagues (2004) suggested that the repeated administration of chronic electroconvulsive seizures would force a long-term adaption at the level of gene-expression (Tsankova, Kumar, & Nestler, 2004). The authors found that *chronic* electroconvulsive seizures produced chromatin remodeling that differed from the changes that occurred after the *acute* administration of electroconvulsive seizures and acetylation of specific gene promoter regions (Tsankova et al., 2004). This demonstrates that the type and duration of exogenous stimuli can effect specific modifications at the gene level, leading to an altered physiological process which, in certain cases, can translate into greater vulnerability to stress or depression.

Another interesting study by Berton and others (2006) used chronic social defeat stress in mice, the animal model believed to mimic human symptoms of depression, to demonstrate changes in gene expression. The study showed that socially avoidant behavior will appear after prolonged exposure to an aggressor (reversed by chronic treatments of anti-depressants), with co-occurring alterations to chromatin regulation of specific genes located in the hippocampus (Berton et al., 2006). This study shows that modifications made at the gene level, induced by environmental stimuli, coincides with the expression of adaptive social behaviors.

The gene-environment interaction is by far one of the most complex scientific approaches researching the cause and treatment of major depression and stress-induced illnesses. Epigenetic studies are a new timely approach to scientific research requiring the collaborative efforts of different branches of research which include; socioeconomic, biological, social-psychological, and neuropharmacological branches, joining forces with genetic research to find the causes and potential cures for depressive disorders. As research from these different branches begin to converge, the benefits of a more inclusive approach to the study of gene expression and behavior research will become quite evident.

11. A new hypothesis: Brain vulnerability to early menarche

In the introduction of this M.Sc. thesis, we have described the two hypotheses that have been proposed to explain the association between menarche and presence of depressive symptomatology in adolescent girls. The first hypothesis, called the 'stressful change hypothesis' (Joinson et al., 2011) proposes that menarche itself is a stressful event and that all girls will eventually experience some level of distress, regardless of their timing of maturation. According to the stressful change hypothesis, the highest level of depressive symptoms should occur around the time of onset of menarche and symptoms are likely to be transient.

The second hypothesis is called the 'early timing hypothesis'. This hypothesis suggests that girls who mature earlier than their peers are more vulnerable to psychological distress (Caspi & Moffitt, 1991; Ge et al., 1996). The transition into puberty is a critical developmental period that is often associated with increased conflicts with parents, development of romantic relationships, and changes in body image (Caspi & Moffitt, 1991; Conger et al., 1984). The early timing hypothesis proposes that these changes may have more severe effects on girls who mature at an

earlier age than on girls who mature later, because of the additive effects that these environmental stressors may have over time in girls who reach early menarche (Joinson et al., 2011).

The stressful change hypothesis predicted that if presence of menarche acts as a significant transient stressor in the life of adolescent girls, then girls who have started their menses before transition to high school should present significantly higher depressive scores and salivary cortisol levels at the beginning of the study, when compared to girls who did not start their menses and boys. However, this difference should only be apparent at the beginning of the study and decrease with time. In contrast, the early timing hypothesis predicted that menarcheal timing would be a greater predictor of depressive symptoms in adolescent girls than menarcheal onset, so that only girls who started menarche before the age of 11 should present increased depressive symptomatology when compared to girls with on-time menarche and this difference should be present across the four time-points of the study.

The results of the present study confirm and extend both the stressful change and early timing hypotheses. First, we showed that girls from the menarche group presented significantly higher levels of depressive symptoms when compared to girls from the no-menarche group and boys, although this effect was not transient, as predicted by the stressful change hypothesis. Second, we found that girls from the early and on-time menarche group presented similar depressive scores at the first three time-points of the study, while a group difference emerged at the end of the study, when girls from the on-time menarche group presented a significant decrease of depressive symptoms when compared to girls from the early menarche group who continued to present high depressive symptoms.

Interestingly, in the present study, it is the measurement of cortisol levels in the two groups

of girls based on menarcheal timing (early *versus* on-time menarcheal groups) that helped us better understand the potential mechanism by which early menarche may confer greater vulnerability to depressive symptomatology in adolescent girls. Indeed, although girls with early menarche differed from girls with on-time menarche on duration of depressive symptoms, both groups (early and on-time menarche) presented high levels of cortisol across the study when compared to girls who had not reached menarche and boys. Previous studies have clearly shown that it is the chronic exposure of the brain to high levels of cortisol that may be detrimental for an organism, more than acute levels at one point of an individual's life (Burke et al., 2005; Ellis et al., 2005; Essex et al., 2002; Gold et al., 2002; Goodyer et al., 1996; Halligan et al., 2007; Heim et al., 2002; Murray, Cooper, P.J., 2003; Smider et al., 2002).

The significantly increased levels of cortisol in the early and on-time menarcheal girls suggest that a common hormonal mechanism, induced by the complex interactions between the HPA and HPG axes, may explain the effect of menarche on depressive symptoms in girls. If onset of menarche leads to increased cortisol levels through an endocrine mechanism and/or through increased reactivity to environmental stress, then it implies that girls who reach early menarche are exposed to high levels of cortisol for a significantly longer period of time than girls who reach on-time menarche. The longer time of exposure to high levels of cortisol may be the key at explaining the presence of consistently high depressive symptoms in girls with early menarche.

This process is then further complicated by the ongoing maturation of the adolescent brain. In reference to animal studies, Romeo and McEwen (2006) reported that the HPA system is plastic during puberty and experience-dependent. The frontal lobe, the hippocampus, the prefrontal cortex, the amygdala (Romeo & McEwen, 2006) as well as the ventral striatum

(Andersen & Teicher, 2008), are not fully developed until the end of adolescence and these same brain regions contain large numbers of glucocorticoid receptors (McEwen & Alves, 1999; Romeo and McEwen, 2006). These regions govern executive function, hormonal production signals and emotional memories among others. Given the intricate relationships between the HPA and HPG system and the prolonged developmental period of the brain regions that are highly susceptible to glucocorticoids' influence, it might be possible that in addition to being exposed for longer periods to high levels of cortisol, girls with early menarche are exposed to high levels of cortisol at a younger age, ie. at an age at which the brain is not still fully developed. Exposure of these key brain regions to high levels of cortisol could lead to changes in developmental trajectories that could increase vulnerability to depression in girls with early menarche. Figure 1 below presents a schematic representation of the 'Brain Vulnerability to Early Menarche Hypothesis'.

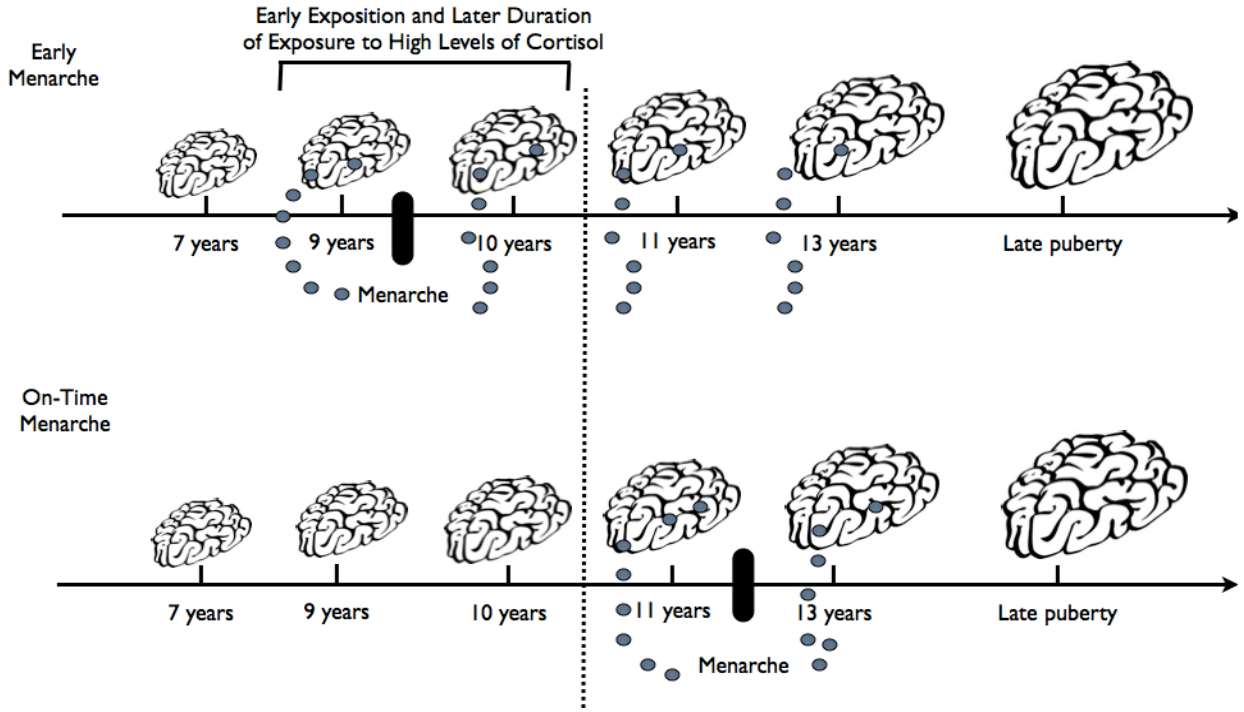

Figure 1. Schematic representation of the Brain Vulnerability to Early Menarche Hypothesis. Early menarche leads the developing brain to be exposed to high levels of cortisol at a young age and for a longer period of time than on-time menarche. In this Figure, the black bars represent onset of menarche and the grey circles represent increased cortisol levels due to menarche reaching the brain at different developmental periods.
Reference: (Joinson et al., 2011).

This hypothesis, that we have called the 'Brain vulnerability hypothesis to early menarche' is of great interest for the field of psychoneuroendocrinology because it offers for the first time a potential mechanism by which early menarche may confer greater vulnerability to depressive symptomatology in adolescent girls. Clearly, further studies will be needed in order to study the impact of menarcheal status and/or timing on brain structures and/or functions.

12. Directions for future research

The results of this study show that the transition to high school is a stressful period for adolescents. The onset of menarche has been shown to be significantly associated to higher depressive symptomatology and higher cortisol levels, than in age-matched girls with no menarche and boys, during this period. In the following section, we wish to develop more on future directions that would lead to the development of useful interventions in order to help adolescents deal with the stress of school transition, with interventions that may be personalized toward boys and girls and toward menarcheal and non-menarcheal girls.

The DeStress for Success program *for Teenagers Making the Transition to High School*

It would be an extremely difficult undertaking to turn the transition to high school into a relaxing, non-stressful experience for most new students. A more realistic solution would be to help teenagers identify potential stressors and provide coping skills. For this reason, our

laboratory, led by Sonia Lupien, Research Director of the Centre for Studies on Human Stress (CSHS), seized the opportunity to disseminate the information gathered from years of stress research. As part of my Master's research, I joined and collaborated with this large team of researchers and graduate students. This was a large project entitled *DeStress for Success* Program which comprised a series of stress management workshops, offered at the two high schools that participated in this study. The *DeStress for Success* program provided five weekly 45 minute workshops. The goal of the workshops was to educate students about the personal (subjective) nature of potential stressors, the physiological mechanisms involved in the stress response, and healthy coping skills to help them better manage their stress, delivered in an age-appropriate and relevant way.

At the end of the workshop series, we asked students to rate the workshops and tell us if they found them helpful enough to recommend them to a friend. The results of this survey were published in the 7th issue of Mammoth Magazine, an educational newsletter published by the CSHS (you can download the Magazine and results of the survey at : http://www.humanstress.ca/mammoth-magazine.html). The feedback was very positive, and the vast majority of the students provided their feedback. Of the students who responded, 80% said that they found the *DeStress for Success* workshops very useful, 90% said that they considered their understanding of the nature and sources of stress have improved, and 80% would recommend the program to a friend (Mammouth Magazine, No 7. Oct. 2009).

Clearly, based on the results of the analyses described in the present M.Sc. thesis, it will be of great importance to compare menarcheal and non-menarcheal girls in their response to the *DeStress for Success Program*. With these additional analyses, we may have found that both groups did not benefit in the same way or to the same extent from the program, which would

help scientists develop personalized educational program on stress as a function of sex and/or menarcheal status.

Although the experimental data on the efficacy of the Program is still being analyzed, it appears to meet a definite need among young adolescents. The implementation of such a program in our children's school curriculum would offer young people practical information in a fun environment while providing tools to help them cope with stress in a healthy and constructive manner. Based on the results of the present study showing that transition to high school play a role in increasing cortisol levels and depressive symptoms in teens, the present program leads to important opportunities to help teenagers deal with the stress of high school transition, with special emphasis on adolescent girls who make this life transition at the same time as they experience entry into womanhood. Such preventive programs may eventually help these girls better control the additive effects of menarche and school transition on their stress level, and potentially prevent the development of depressive symptomatology at a time when girls should enjoy the new challenges of adolescence.

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Appendix A

Child Depression Inventory

Child Depression Inventory (French Version)

1 QUESTIONNAIRES:

Pour chaque groupe de trois phrases, choisis la phrase qui te décrit le ***mieux*** depuis les **deux dernières semaines**.

Fais un signe comme ceci ☑ à côté de ta réponse.

1. ☐ Je suis triste de temps en temps.
   ☐ Je suis souvent triste.
   ☐ Je suis toujours triste.

2. ☐ Rien ne fonctionnera jamais pour moi.
   ☐ Je ne sais pas si les choses fonctionneront pour moi.
   ☐ Les choses fonctionneront pour moi.

3. ☐ Je réussis bien la plupart des choses.
   ☐ Je ne réussis pas plusieurs choses.
   ☐ Je ne réussis rien.

4. ☐ J'ai beaucoup de plaisir.
   ☐ J'ai parfois du plaisir.
   ☐ Je n'ai jamais de plaisir.

5. ☐ Je suis toujours méchant(e).
   ☐ Je suis souvent méchant(e).
   ☐ Je suis méchant(e) à l'occasion.

6. ☐ De temps en temps, je pense à des mauvaises choses qui pourraient m'arriver.
   ☐ J'ai peur que des mauvaises choses m'arrivent.
   ☐ Je suis certain(e) que des choses terribles vont m'arriver.

7. ☐ Je me déteste.
   ☐ Je ne m'aime pas.
   ☐ Je m'aime bien.

8. ☐ Toutes les mauvaises choses sont de ma faute.
   ☐ Plusieurs mauvaises choses sont de ma faute.
   ☐ Les mauvaises choses ne sont habituellement pas de me faute.

9. ☐ Je ne pense jamais à me tuer.
   ☐ Je pense à me tuer, mais je ne le ferais pas.
   ☐ Je veux me tuer.

10. ☐ J'ai le goût de pleurer à chaque jour.
    ☐ J'ai souvent le goût de pleurer.
    ☐ J'ai parfois le goût de pleurer.

11. ☐ Il y a toujours des choses qui me dérangent.
    ☐ Il y a souvent des choses qui me dérangent.
    ☐ Il y a parfois des choses qui me dérangent.

12. ☐ J'aime être avec des gens.
    ☐ Je n'aime pas souvent être avec des gens.
    ☐ Je ne veux être avec personne.

13. ☐ Je n'arrive jamais à me faire une idée sur quelque chose.
    ☐ Il est difficile pour moi de me faire une idée sur quelque chose.
    ☐ Je me fais une idée très facilement sur quelque chose.

14. ☐ Mon apparence est bien.
    ☐ Il y a quelques points négatifs par rapport à mon apparence.
    ☐ Je suis laid(e).

15. ☐ Je dois toujours me forcer pour faire mes travaux scolaires.
    ☐ Je dois souvent me forcer pour faire mes travaux scolaires.
    ☐ Je n'ai pas de difficulté à faire mes travaux scolaires.

16. ☐ J'ai de la difficulté à dormir chaque soir.
    ☐ J'ai souvent de la difficulté à dormir.
    ☐ Je dors plutôt bien.

17. ☐ Je suis parfois fatigué(e).
    ☐ Je suis souvent fatigué(e).
    ☐ Je suis toujours fatigué(e).

18. ☐ Je n'ai jamais le goût de manger.
    ☐ Souvent, je n'ai pas le goût de manger.
    ☐ Je mange plutôt bien.

19. ☐ En général, je ne pense pas à la souffrance (au mal) et à la douleur.
    ☐ Je pense souvent à la souffrance (au mal) et à la douleur.
    ☐ Je pense toujours à la souffrance (au mal) et à la douleur.

20. ☐ Je ne me sens pas seul(e).
    ☐ Je me sens souvent seul(e).
    ☐ Je me sens toujours seul(e).

21. ☐ Je n'ai jamais de plaisir à l'école.
    ☐ J'ai parfois du plaisir à l'école.
    ☐ J'ai souvent du plaisir à l'école.

22. ☐ J'ai beaucoup d'amis.
    ☐ J'ai quelques amis, mais j'aimerais en avoir plus.
    ☐ Je n'ai pas d'amis.

23. ☐ Mon travail à l'école est bon.
    ☐ Mon travail à l'école n'est pas aussi bon qu'avant.
    ☐ Je ne réussis plus dans les matières scolaires dans lesquelles j'avais du succès auparavant.

24. ☐ Je ne peux jamais être aussi bon(ne) que les autres jeunes.
    ☐ Je peux être aussi bon(ne) que les autres jeunes si je le veux.
    ☐ Je suis aussi bon(ne) que les autres jeunes.

25. ☐ Personne ne m'aime vraiment.
    ☐ Je ne sais pas si quelqu'un m'aime.
    ☐ Je suis certain(e) que quelqu'un m'aime.

26. ☐ Habituellement, je fais ce qu'on me dit de faire.
    ☐ La plupart du temps, je ne fais pas ce qu'on me dit de faire.
    ☐ Je ne fais jamais ce qu'on me dit de faire.

27. ☐ Je m'entends bien avec les gens.
    ☐ Je me chicane souvent.
    ☐ Je me chicane tout le temps.

Appendix B

BEM Sex Role Inventory – Short Form (French Version)

Bem Sex Role Inventory (French version)

INSTRUCTIONS:

À la page suivante, vous trouverez une liste de traits de personnalité. Nous aimerions que vous vous décriviez à partir de ces caractéristiques. C'est-à-dire que vous indiquiez, sur une échelle de 1 à 7 jusqu'à quel point ces traits de personnalité sont vrais pour vous.

Example: *Amical(e)*

Indiquez **1** Si vous n'êtes jamais ou presque jamais *amical(e).*

Indiquez **2** Si vous n'êtes habituellement pas *amical(e).*

Indiquez **3** Si vous êtes quelquefois mais rarement *amical(e).*

Indiquez **4** Si vous êtes occasionnellement *amical(e).*

Indiquez **5** Si vous êtes souvent *amical(e).*

Indiquez **6** Si vous êtes habituellement *amical(e).*

Indiquez **7** Si vous êtes toujours ou presque toujours *amical(e).*

Ainsi, si vous trouvez que vous êtes *quelquefois mais rarement* « amical (e)», *jamais ou presque jamais* « peureux (se)», *toujours ou presque toujours* « responsable, » et *souvent* « méchant(e), » alors vous évalueriez ces caractéristiques comme suit :

Amical(e)	6	Responsable	7
Peureux (se)	1	Méchant (e)	5

S.V.P. **Ne laissez aucune caractéristique non cotée**.

Je défends mes convictions			Je m'adapte facilement	
Je suis affectueux (se)			Je suis dominateur (trice)	
Je suis consciencieux (se)			Je suis sensible	
Je suis indépendant(e)			Je suis prétentieux (se)	
Je suis sympathique			J'aime prendre position	

Je suis d'humeur changeante			J'aime les enfants	
Je suis sûr(e) de moi			J'ai du tact	
Je suis sensible aux besoins des autres			Je suis agressif (ve)	
Je suis fiable			Je suis de nature doux (ce)	
J'ai une forte personnalité			Je suis conservateur (trice)	
Je suis compréhensif (ve)			J'agis en leader	
Je suis jaloux (se)			Je suis empressée d'apaiser les peines des autres	
Je suis énergique			Je suis renfermé(e)	
Je suis compatissant(e)			J'aime le risque	
Je suis franc(he)			Je suis chaleureux (se)	

Définitions des mots

Convictions : croyance ferme, certitude, ce dont on est convaincu
Affectueux : tendre, éprouvant de l'affection
Consciencieux : personne faisant preuve de sérieux, qui agit au mieux, effort sincère au travail
Indépendant : celui qui désire rester indépendant, qui veut être libre, ne dépendre de personne
Sympathique : accueillant, agréable, plaisant
D'humeur changeante : Avoir l'humeur (bonne ou mauvaise) qui change souvent
Sûr de moi : ayant confiance en soi
Sensible aux besoins des autres : en général, savoir quand agir pour aider ou réconforter les autres

Fiable : je suis une personne en qui on peut avoir confiance
Forte personnalité : avoir un caractère fort
Compréhensif : Qui comprend et excuse volontiers les gens, qui admet le point de vue des autres ; tolérant, indulgent : *Avoir des parents compréhensifs.*
Jaloux : qui envie la gloire, le succès, les talents, etc. d'autrui ; celui qui est tourmenté par la crainte de voir la personne aimée préférer être avec un autre que lui-même
Énergique : qui a de l'énergie, de la vigueur
Compatissant : qui éprouve un sentiment de pitié, qui a de la sympathie pour les problème d'un autre
Franc : parler ouvertement ; de façon honnête
Je m'adapte facilement : je réagis bien à des nouvelles situations
Dominateur : personne aimant dominer, exerçant un pouvoir autoritaire
Sensible : affecté avec facilité, émotif; qui réagit aux plus petites variations de son environnement
Prétentieux : celui qui se flatte de qualités qu'il n'a pas
Prendre position : défendre ses idées (ou opinion)
J'ai du tact : savoir parler aux gens sans jamais les blesser

Agressif : qui fait preuve d'agressivité, qui est provocant
De nature doux : gentil, délicat en vers les autres
Conservateur : Préférence pour la tradition, prendre peu de risques
Leader : (mot anglais) celui qui est à la tête d'un groupe et le dirige
Empressée d'apaiser les peines des autres : tendance à vouloir réconforter quelqu'un qui parait avoir de la peine
Renfermé : se dit d'un caractère peu communicatif
J'aime le risque : être quelqu'un qui aime prendre les risques – <u>en général</u>
Chaleureux : manifestant de la chaleur en vers les autres, de l'enthousiasme