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Pathways to Teenage Female Depression: the Role Testosterone Plays in the Association between Ecological Stress and Adolescent Depression Outcomes in Girls during Late Puberty

By

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M. A., Emory University, 2009

Thesis Committee Chair: Carol Hogue, PhD

An abstract of

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology

2014

Abstract

Pathways to Teenage Female Depression: the Role Testosterone Plays in the Association between Ecological Stress and Adolescent Depression Outcomes in Girls during Late Puberty

By **Tyralynn Frazier**

Purpose: This study examined free testosterone and the possible moderating effect of stress in the testosterone-depression association, in girls during late-puberty.

Methods: Participants included 267 girls who were recruited through the Student Information Management System of public school systems of 11 counties in western North Carolina. A total of 1104 biological and stress exposure measures were taken between the ages of 9 and 16. Due to the stratified nature of the sample, the Generalized Estimating Equation (GENMOD procedure) in SAS was used in the moderation model. Late-Puberty was assessed based on age defined as between 13-16, and blood spots were used to determine levels of free testosterone. Environmental stress was defined as a set of adolescent risk factors with known associations with negative behavioral and health outcomes. Birth weight (normal birth weight $\geq$ 2500g and low birth weight < 2500g), was measured as a potential confounder or effect modifier.

Results: Regression analysis revealed that in normal birth weight girls ages 13-16 (94% of the sample), stress does moderate the relationship between testosterone and depression. In the presence of little or no stress, there exists no significant relationship between testosterone levels and depression. Stress has a multiplicative effect such that the association between testosterone levels and probability of depression increase substantially with increases in exposure to environmental stress. For low birth weight girls, the odds of having depression was much higher than for normal weight girls (OR: 13.5, p-value: 0.002), but the low birth weight sample was too small to assess the effect of stress.

Conclusions: These findings suggest that the interactions between stress exposure and testosterone levels are important to consider when evaluating pathways towards elevations in depression among girls during late-adolescence.

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Chapter 1: Introduction

Human development is a cumulative process, and unpredictability or strife during one stage makes negotiating later stages more challenging. No period is more fraught with turmoil and potential challenge than the adolescent transition through puberty (Crain 2011). Social relationships are changing, the body is changing, and cognitive perceptions of the world are changing, all amidst a sea of hormonal fluctuations that Eric Erikson has referred to as the period of strife and storm (Douvan 1997). Pubertal bodies are on the precipice of adulthood. But increased risk-taking behaviors and risk for mental illness characterize this period. This introduction summarizes the biology of the adolescent transition and the impact stress can have on this transition. This is the background that motivates our study of selected physiological markers that might be contributing to the shift towards depression in pubertal girls.

Depression risk increases in late puberty for girls. It is one of the negative consequences of the brain and body's inability to adapt to acute and chronic stress. McEwen states that "brain circuits are plastic and remodeled by stress to change the balance between anxiety, mood control, memory, and decision making" (B. McEwen 2012). He and others suggest that the relationship between stress exposure and brain susceptibility to allostasis associated with depression is mediated by emotional self-regulation. We support our discussion of stress through the perspective of Porge's social engagement system. From this perspective, emotional self-regulation is controlled by the two subsystems of the autonomic nervous system. They are the parasympathetic (PNS) and sympathetic systems (SNS). We discuss how communication between the prefrontal cortex (PFC) and the limbic system control the level of policing the PNS can exert over the SNS, the significance of this policing, and what this has to do with adolescent depression. We also discuss how early stress exposure can disrupt the PFC-limbic

communication, and how puberty is a time when such disruptions are further exacerbated because of reorganizations and expansions taking place within the adolescent central nervous system. We end by discussing the stress-depression connection in girls during late puberty, and potential mediators in this relationship contributing to increased rates of depression during this time.

The Stress System

All organisms strive for safety. Social mammals have sophisticated systems of detection and response to environmental cues that signal when one is safe and when one is not safe. Not only does "safety" drive bio-behavioral activation but, according to some, trajectories of development towards adaptations or mal-adaptations in emotional regulatory responses are dependent on perceptions of "safety" (LeDoux 2012). Safety matters because we are hard wired to detect threat. We receive input through the primary senses (hearing, sight, taste, smell, touch). If that input is experienced as a potential threat then we must make a choice. In a fraction of a second, we either mobilize the body for fight, flight or freeze, or we engage in what Porges has described as the vagal brake (S. Porges 2003).

Initiating mobilization and social engagement begins, on a physiological level, with two subsystems of the autonomic nervous system; the parasympathetic (PNS) and sympathetic (SNS) nervous systems. The parasympathetic system is associated with growth and restoration. The sympathetic nervous system is associated with metabolic changes to deal with challenges from the external environment (Mai and Paxinos 2010). Sympathetic activation involves the withdrawal from social engagement and the conservation of processes in order to mobilize a response. Parasympathetic activation is characterized by processes that support social

engagement and extensions outward such as exploration and curiosity. Patterns of activation of these subsystems of the stress response have a direct effect on prefrontal cortical structures associated with patterns of appraisal and emotionality. What this means is that how the stress system is activated impacts how the prefrontal cortex, the executive controller, interprets and thus regulates the lower autonomic responses. This sounds circular, but recall that patterns of emotional regulation are being learned over the course of development.

The prefrontal cortex (PFC) controls how the general significance of a sensory experience is evaluated, or the personal meaning that experience creates (Mesulam 1998). This all happens extraordinarily fast.First, there is a sensory experience, then the perception of that experience, and then appraisal mechanisms that create the sense of what that experience means. An example through which to understand this prefrontal appraisal/emotional control is through the valence hypothesis. This hypothesis suggests that information processed through different sides of the PFC are experienced differently. Unpleasant emotions are processed on the right and pleasant ones on the left (Siegel, 2012). Why does this matter? Interpersonal engagement might be influenced by the location through which those engagements are processed. Siegel and others suggest that emotional styles in childhood might be a presentation of frontal lobe activation patterns. “Behaviorally inhibited (shy) children reveal a dominance in right frontal electrical activity at baseline; more adventurous children demonstrate left frontal activation.” (Siegel, 2012, p.178). Furthermore, if activation of left prefrontal lobe is absent then positive affect is absent and depression is expressed.

What influences right verse left activation? These emotional styles/prefrontal activation linkages are influenced by shared attunement between mother (or care giver) and child. Depressed mothers have fewer shared moments of positive affect with their infant. That infant in

turn has lowered left frontal activation and elevated right frontal activation. If sustained throughout the first year of life then that child will carry this pattern of activation into adulthood (Field and Diego 2008). What sounds circular is really a neurobiological learning process that happens during the formative years. This is one reason why these years are so important for learning patterns of emotional self-regulation.

This is a basic explanation of how the prefrontal cortex influences appraisal styles, but how does nervous system activation influence patterns of mobilization or engagement? We refer to SNS activation as mobilization because, in the face of a perceived external threat, the body is engaging metabolic changes that promote rapid response (Cannon, Wisdom of the Body 1932). Pupil dilation to improve sight, secretion of adrenaline and noradrenaline to accelerate heart rate and increase amount blood flow and blood vessel dilatation, inhibited intestinal peristalsis and secretions control the allocation of energetic resources, conversion of glycogen to glucose lead to increase blood sugar levels to give muscles a quick energy supply, and dilated bronchi allow increased oxygen to nourish the blood. In the short term, these changes enable a quick and effective response (fight or flight) via metabolic access to energy and conservation of resources directed away from processes not necessary for this quick response (Friedman and Silver 2007) (Sapolsky 1994).

Activation of the SNS leads to the release of corticosteroids. Continued high levels of these stress hormones can lead to high blood pressure and heart disease, damaged muscle tissues, inhibited growth, and immune system suppression. Prolonged higher blood sugar levels lead to decreased ability of the cells to respond appropriately to insulin produced by the pancreas and decreased ability for the pancreas to produce enough insulin to control blood sugar levels. Ultimately this results in the type of insulin resistance characterized by type-II diabetes. Long-

term exposure to epinephrine and norepinephrine, associated with the mobilization response, leads to structural changes in the brain that weaken working memory (via impact on hippocampus and medial prefrontal cortex). The net result is physiological degradation, decreased ability for effective mental processing, and increased risk for both physical and mental disease formation. SNS tone activation is associated with markers of stress we often associate with allostatic load (Padgett and Glaser 2003) (Djuric, et al. 2008). Because SNS is responsive to environmental stimuli via somatic input (see, hear, smell, taste), and that input is associated with the activation markers used to measure allostaic load (blood pressure, HPV, C-reactive protein, etc.), we can say that allostatic load is a marker of prolonged activation caused by externally stressful experiences (McEwen and Gianaros 2011). This is the conventional way of understanding allostatasis. But really allostatic load is not a measure of environmental experiences but a measure of mechanisms of appraisal and emotionality leading towards SNS activation. It is not the absolute event but the processing of the event that enables an environmental exposure to become physiologically stressful.

So how do experiences of stress lead to prolonged SNS activation? This depends on how an individual appraises an event, remembers that appraisal, and generalizes the meaning of that event (Ursin & Eriksen, 2004). This requires a brief discussion about memory. First, a few definitions:

- Working memory: This is the system of holding bits of short-lived information in the mind. Like a mental sketch pad, this information is placed in such a way that it can be manipulated through the coordination of images or verbal information. This is sometimes referred to a short term memory.

- Implicit memory: This type of memory does not involve the conscious manipulation of information. Here, past experiences influence the engagement with a task without conscious awareness of that experience.
- Explicit memory: Here recollection is conscious. An individual recalls a previous experience, for example, remembering to call your mother at 3:00 or pulling up the memory of a birthday party that happened last year (Cowan 2008) (Schacter 2011).

The emotionality of an event plays a strong role in whether something is remembered or not. It also plays a key role in type of memory engaged. Experiences linked with little emotional arousal are not often remembered. Experiences involving moderate emotional arousal are often remembered explicitly (explicit memory). Experiences that are overwhelmingly stressful or terrifying inhibit hippocampal processing in such a way that explicit memory formation is inhibited, and thus becomes difficult to retrieve. Now, that seems counterintuitive to the very drive of human evolution: stay safe and avoid danger for survival. Why then would we have difficulty remembering what really really scares us? It turns out that while elevated cortisol levels inhibit explicit memory formation, they promote implicit memory formation (Siegel, 2012). During trauma, the individual may focus on non-traumatic aspects of the environment. One's attention is divided, contributing to the event being encoded in implicit memory. This along with the negative impact high cortisol levels have on the hippocampus inhibit explicit memory production. Furthermore, implicit memory storage is associated with behavioral impulses, emotional reactions, and bodily sensations (Siegel, 2012). These experiences can be generalized and arouse emotions with no direct recollection of why arousal is occurring. We will discuss this below, but this provides an initial idea of why memory plays such an important role in emotional regulatory processes.

With respect to the autonomic nervous system, social engagement, engaging the vagal brake, and parasympathetic activation are key concepts. PNS activation is the feel good side of CNS processing. It is associated with proper digestive support functions ranging from the promotion of salivation to the promotion of healthy defecation. It promotes homeostatic or resting heart rate states not associated with cardiovascular disease. It also promotes tear production (lacrimation), urination, and basal bodily processes involved in healthy digestion, healthy immune function, relaxed breathing and heart rate, and a general feeling of calm where energetic resources are distributed as needed and not redirected for survival. Prolonged PNS activation means prolonged health and well-being. In essence, the PNS acts as an off switch for SNS activation enabling movement from mobilization to social engagement (Davidson and McEwen 2012) (Payer, et al. 2012) (Garland, et al. 2010) (Folkman 2010). This health promotion can be extended into interpersonal interaction. PNS activation promotes engagement with the world, curiosity and interest in exploration verses disengagement and inward focus promoted by SNS activation (McCracken and Gutierrez-Martinez 2012) (Siegel 2012). This policing or control of SNS happens via the engagement of the vagal brake.

In order to understand vagal brake we must first understand the concept of vagal tone, i.e., the tension of the vagus nerve fibers innervating the SA node (pace maker of the heart). This influences the vagus nerve's ability to decrease the heart rate. PNS tone withdrawal is coordinated with increases in SNS tone excitation (Essential Clinical Anatomy 2006).

Journeyman, air traffic controller, and grand wonderer- the vagus nerve has been personified in such impressive ways because it is the longest cranial nerve and is central to our notion of well-being. From the medulla oblongata of the brain stem, down the neck, the thorax, through the diaphragm, down through the abdomen, and into the pelvic region, vagal activity is

continuous and far reaching. It controls the resting states of the heart, lungs, eyes, adrenal glands, and digestive tract. This control is expressed via vagal tone or "tension" influencing heart rate. Emotional resilience is the gold mine of well-being. Characterized by the ability to move from an excited state to a relaxed one (S. W. Porges 2011), resilience can be measured by understanding vagal tone. High vagal tone means greater resilience to stress, a greater ability to return to homeostatic, or resting states after arousal, or the ability to turn on the PNS response in the face of stress. Low vagal tone implies difficulty with emotional self-regulation associated with difficulty engaging this return to homeostasis. In summary, high inhibitory control of the vagus nerve, or ability to access the vagal brake, means the vagus nerve's ability to put the brakes on the revving heart rate is strong. Engaging the vagal brake engages the PNS. Problems engaging the vagal brake creates prolonged elevations in heart rate which lead to the activation of the long-term stress response characterized by HPA-axis activation and corticosteroid release (S. W. Porges 2011).

Many nuances to this relationship make this characterization a bit of an over-simplification, but it is an accurate approach to understanding in order to gain a broad picture of how the autonomic stress system (ANS) works. Patterns of tone engagement depend on choices that are made instantaneously. In the face of a perceived threat, can I turn down the mobilization-for-defense response and engage self-soothing and calming behaviors, or do I remain in a sustained state of disengagement and defense? How that decision is made is dependent on the environment in which an individual develops. To summarize, threats to safety in an unsafe environment lead down the path to allostasis. Threats to safety in a safe environment lead to pro-social and engaging behaviors. How one determines what is safe involves a number of complex factors. Genetic predisposition, epigenetic changes, the *in utero* environment, childhood trauma,

and early attachment styles all play a central role in how processing responses develop within an individual. Below we will discuss how the relationship between these internal processes and external conditions determine an individual's subjective experience, or how they identify what is safe and what is a threat.

Identifying Safety

In the next few sections we are going to break down the process of determining if environments are safe, layer by layer. We begin by discussing how early stress and trauma might impact perceptions of safety. We then go through physiological events defining the polyvagal system. Finally, we go through how chronic override of PFC's top-down control due to life stress or trauma can make it difficult to access the vagal brake associated with PNS activation.

Perceptions and Survival

It has been long observed that early life and adolescent exposure to stress are associated with increased risk of depression, heart disease, obesity (Dallman 2009) and a number of other stress related illnesses later in life (Djuric, et al. 2008). How do early events have such far reaching influences? Life history theory (Kaplan, et al. 2000), theories of allostatic load (McEwen and Gianaros 2011), and other theories that point towards the physical accumulation of stress through events over the life course focus on external stressors that push internal processes towards disease states (Geronimus, et al. 2006). This model focuses on activation of SNS tone leading to all the things discussed in the previous section. But PNS tone is a marker of internal homeostasis. From this perspective, there is room for judgment as to whether a threat is really threatening or not. This moment of choice is mediated by the top down control the prefrontal cortex has on limbic activation.

Regions of the brain are often referred to as lower and higher. This hierarchy of function comes partly from physical position but mostly from evolutionary theory and a sort of personified status these different regions hold. “Lower” refers to parts of the brain that showed up very early in our evolutionary past as far back as reptiles (often referred to as the reptilian brain). “Higher” often refers to regions of the brain that have evolved more recently. Areas associated with executive type functions, found in modern human brain, usually fit this description. Lower limbic areas receive input from the bodily senses, but it is the higher areas that integrate that information into meaning. Meanings are the complex thoughts and actions we plan as a result of the input from those lower regions. It is the prefrontal cortex that controls our ability to integrate this information. Dan Siegal put it nicely when he said that

> Meaning making, emotional regulation, empathy, and compassion all come from the integrating ability of regions within the prefrontal cortex. Attentional and emotional modulations occur here that allow one to reflect on a sensory experience rather than just react to it (Siegel, 2012, p.167).

This idea of prefrontal control of meaning making is physiologically represented in what is called neural integration. The greater the integration the greater the capacity for positive emotional arousal because the individual has a broader perspective and increased ability to access differential, linked information. This means an increased ability for interpersonal integration (easier time with group collaborations, for example) (Siegel, 2012). Further, increased interpersonal integration is associated with increased intelligence. Practically, if we want children to be intelligent we need to help them learn how to feel good and engage others with awareness, empathy and meaning in a positive way. The details of neural integration are complex. We will briefly go through 3 types of integration to give an understanding why this is important for emotional self-regulation and identifying safety.

Vertical integration Somatic regulation is controlled by the brain stem. General feelings of warmth, pain, and proprioception are all involved in this regulation. Vertical integration involves the physical formation of fibers that connect somatic input with other vertically distributed neural structures including structures of the limbic region associated with emotional processing. This integration happens through the middle prefrontal cortex (insular cortex). Integrating somatic regulation and limbic circuits via the middle PFC is necessary for self-reflexive observation. This means being aware of input coming from the body senses and recognizing the range of thoughts and ideas that are present with this body awareness. In early childhood, this integration is a product of secure attachment arising from the mental attunement received by a caregiver (Siegel, Mindfulness, Psychotherapy and the Brain n.d.).

Bilateral Integration: We have all heard the right-brain/left-brain dominance discussion in popular science. It is true that the different hemispheres of the brain have general differences in function. Ambiguity, analogues thinking, big picture (gestalt) formations, and non-verbal autobiographical memory characterize the right brain. Interestingly, this is also the place of non-verbal empathy and stress response activity (Siegel, 2012). The left is linear, logical, linguistic, and literal. One thing following the next in a logical cause-effect manner, using words, and a binary or clear sense of things (right or wrong, yes or no, on or off etc. . .) characterize the left hemisphere.

This brings up cross dominance and mixed dominance. Integration is necessary for efficient function. Primarily, people develop unilateral cerebral dominance (or lateralization). This means that dominance is found on one side of the body, from eyes all the way down to the legs. If this is not the case then messaging between the two hemispheres of the brain must be well integrated because information is coming from two perceptually different areas. Mixed

dominance comes with perceptual and organisational differences that could potentially be problematic. It has been proposed that children with learning disabilities might be experiencing cross dominance. This means that they might be, for example, right handed but left eye dominant, thus having two competing perceptual modes. This creates a range of challenges from reading problems to impulsivity issues (Hannaford 2005).

This is why understanding bilateral integration is important. To understand bilateral integration the example of storytelling is often used (Siegel, 2012). The logic, sequence, linearity and facts of the story are key, but a great story is compelling, engages non-verbal imagery and emotion, and reflects an emotionality with which people can connect. A truly great story teller has a truly well-established bilateral integration.

From a developmental perspective, the right hemisphere is the first to develop. During the first 3 years of life the activity here is most vigorous. This is one aspect of why psychologists talk about this time as foundational for establishing emotional affect throughout life.

Dorsal-Ventral integration: Dorsal-Ventral integration supports bilateral integration (Knyazeva, Fornari and Meuli 2009). According to Treverthen, "each hemisphere has a dominant pathway: right with dorsal and left with ventral. Each circuit or 'stream' mediates differential forms of motivational processes and motor control, and creates different representational processes on either side of the brain . . . Dorsal-ventral integration would allow for less lateralization of the more complex representational processes originating from each side of the brain." (Siegel, 2012, p.340).

A well-integrated brain is one that enables an individual to feel good, engage others with awareness, and engage empathically and with meaning while being able to think logically. These

are functions associated with PNS activation. A well-integrated brain is one that perceives the world as more likely being safe. This discussion of integration is really the physiological explanation of what is going on when individuals are securely attached to their caregiver, have not experienced significant early life trauma, and basically have had a chance to explore the world emotionally and physically in a developmentally appropriate way with lots of support and safety. But what happens when trauma is experienced survival circuits develop that affect the vagal brake (LeDaux, 2012).

Survival circuits begin with the experience of an unconditioned threat coupled with auditory, visual or other sensory cues. These biologically insignificant or nonthreatening stimuli acquire threat status because they occur in conjunction with a biologically significant threat. For example, a child is physically abused while a parent yells at her. The unconditioned stimulus "yelling" becomes associated with pain and physical harm. When that child hears yelling, fear centers of the brain are stimulated and the innate defense behavior associated with physical abuse becomes a response associated with yelling. Appeasing, submitting, disappearing all might be a response the child engages to "survive" the abuse. Later in life, that person has an argument with her partner and yelling ensues. Immediately, that person's brain is telling her that her life is in danger, and allostatic pathways are activated followed by innate defense behaviors of appeasing, submitting, disappearing or disengaging the behaviors that kept her safe as a child (LeDoux 2012).

This person is also not as adept at engaging the vagal brake, and might tend towards mobilization and disengagement rather that the social engagement associated with meaning making, emotional regulation, empathy, and compassion. This response may have protected her in childhood, but now it is creating a context in which she cannot engage in a way that actually

enables the safety she so desperately is seeking. The threat circuit no longer needs the yelling parent and has become generalized. In this girl's moment of choice, the threat to survival overrides the PFC control necessary to increase PNS tone and feel good.

The stressed brain perceives stress even when it is not there (generalization). When this occurs, efficient processing controlled by top-down function is cut off and lower brain regions are engaged. This engagement leads to SNS activation that can become prolonged. Mental sketch pad dysfunction (i.e. working memory) is associated with cumulative stress activation. Cumulative stress is associated with long term disease. Survival circuits shape how we perceive the world, and they determine if we engage the world as a safe place or a not safe place. The more consistently stressed we are during development, the more likely we will be to perceive the world as an unsafe place. One interesting line of future inquiry is understanding how stress inhibits neural integration leading to increased hemispheric lateralization and decreased integration.

We know is that when the vagal brake is not engaged easily then the world is a not a safe place. We cannot get what we need. We engage mobilization and sympathetic activation to defend ourselves from the ever-present perception of threat. When the vagal brake is actively engaged easily then the world is a safe place. We can get what we need by engaging prefrontal regulation and more substantive understandings of threat in the face of sensory stimulation. We have access to flexible and well integrated internal thinking and more integrated interpersonal interactions. Parasympathetic activation enables engagement, outward exploration, compassion, connection, and a return to feeling safe and secure.

Stress and the Adolescent Transition

Many bio-physiological changes during adolescence distinguish this period from both childhood and adulthood. The choice of lenses through which we understand these distinctions is vast, including stress in the environment, changes in endocrine regulation and production, and CNS regulation associated with cognition. In this summary we take a global perspective of how all of these systems engage each other during this transition. The challenge with this approach is glossing over essential details in trying to make complex linkages, but the benefit is providing a more dimensional understanding of stress in the context of the adolescent transition.

The Brain

Humans have really big brains for their body size, but cognition is not all about absolute size. Connectivity, or the physical link between different parts of the brain that activate together during a task and/or share common developmental trajectories, is a primary factor in how the brain functions. How regions within the brain communicate underlay complex processing associated with what we call "maturity." The presence and speed of connectivity is controlled by the processes of pruning and myelination.

Myelination is a process of significant developmental importance. Myelin are the fatty insulating sheaths wrapped around axons, the white matter (Berlucchi 1981) (Shanks, Rockel and Powel 1975) . Our neuronal ability to process information hinges on myelin. These deposits act as insulation material that promotes 100% increase in speed and 30% increase in recovery processing, leading to a 3,000 fold increase in amount of information transmitted per second. Unmyelinated neurons are called grey matter. During fetal and infant development, grey matter rapidly increases. Pruning follows this expansion whereby nerve connections (synapses) and neurons that are not worked die away. This overgrowth and elimination process also occurs

during early puberty. Through this process, patterns of connectivity among neurons are being established. Adolescence is a time of general cortical thinning ultimately caused by pruning and myelination, thus evidence that adolescence is a critical time in the development of the architecture of the brain.

When we look more closely at cortical structures associated with connectivity and expansion we see that adolescents suffer from an "out of sync" brain. Limbic structures associated with affect (amygdala) and memory (hippocampus) increase in volume. While the consolidation of tracks linking regions that facilitate complex coordination associated with executive functions, such as the implementation of rational analyses, planned intentions, managing countervailing feelings and impulse control are not fully developed. White matter organization during adolescence correlates in specific brain regions with improvements in language, reading, ability to inhibit a response, and memory (Deutsch, et al. 2005) (Liston, et al. 2006). White matter volumes increase and grey matter volumes follow an inverted U developmental trajectory with peaks in high-association areas such as the dorsolateral prefrontal cortex (Nagy, Westerberg and Klingberg 2004).

The developmental time course for the prefrontal cortex plateaus between mid-adolescence and early adulthood. These findings demonstrate that although the development of gray matter has largely leveled off after a period of neural pruning between early childhood and adolescence, white matter fiber tracks (myelin sheaths) that form the thick insulation around axons connecting various regions of cortex continue to develop into adulthood (Paus 2005). Late maturation of the dorsal-lateral PFC which is important in judgment, decision making, and impulse control has prominently entered discourse affecting the social, legislative, judicial, parenting, and education realms. This is consistent with a growing body of literature indicating a

changing balance between earlier-maturing limbic system networks, the seat of emotion, and later-maturing frontal systems (Somerville, Jones and Casey 2010).

The Body

"When I grow up, I want to have boobies!" As the Pussy Cat Dolls will attest, the pubertal transition is often associated with morphological changes associated with Tanner stages of development (Marshall and Tanner 1969). Secondary sex characteristics such and breast development and pubic hair growth belay internal endocrine dynamics between the brain/CNS and bodily organs and tissues that are, arguably, the real drivers of the psycho-behavioral (and possibly psychological illnesses) associated with the later stages of puberty. Endocrine cascades function in a stepwise process from the brain to the body and back to the brain. Hormones released from the hypothalamus (brain) activate hormonal secretions from the pituitary (still in the brain). These secretions stimulate production in targeted bodily organs. Stress hormones such as cortisol are produced from the adrenals. Reproductive organs are also targeted for the production of testosterone and estradiol. As circulating levels of these steroid hormones increases they act as messengers to the brain sending information about what is going on in the body. The brain, in turn, exerts regulatory control over the process up-regulating or down-regulating the initiating hormones. These closed loops of control are referred to as axes. Stress is experienced via the Hypothalamic-Pituitary-Adrenal axis (HPA) and gonadal hormones are controlled via the Hypothalamic-Pituitary-Gonadal axis (HPG).

During adolescence, HPA-axis activity is up. HPG activity increases dramatically during the pubertal transition. Cortisol, a glucocorticoid, can suppress both gonadal and brain level HPG activity. Sexual dimorphism in HPA regulation is present at birth. The Adrenocorticotropic

hormone (ACTH): cortisol ratio is bigger in infant boys. Gender differences in stress response seem to persist beyond childhood. Stress, both acute and chronic, has also been shown to affect the reproductive axis in girls, but the extent to which gonadal steroid hormones and glucocorticoid contribute to vulnerabilities to depression during adolescence has not been fully investigated (Gaillard and Spinedi 1998). Increased testosterone is associated with increasing depression during the adolescent transition in girls. In this study, we ask if stress may play a role in the testosterone-depression association. We hypothesize that stress is either mediating or moderating the testosterone-depression link.

Chapter 2: Manuscript Chapter

Background

Adolescence is a time of developmentally synchronized reorganization in mood regulation and sexual differentiation (Reyna , Chapman and Dougherty, et al. 2012). Driven by activation and organizational effects of gonadal hormones, the pubertal transition is an interplay between gonadal steroid hormone timing and the timing of adolescent brain development, and both contribute to variations in vulnerabilities to psychopathologies such as depression (Culbert, et al. 2013). Developmental divergence in sexual differentiation parallels a divergence in rates of depression during puberty.

Depression is the leading cause of disability in the U.S. (The World Health Organization 2008). However, depression effects women more than me, beginning in late puberty. During late puberty, rates of DSM-IV uni-polar depression in girls first begin to deviate from boys, increasing twofold (Angold, Costello, et al. 1999) to about 4% in girls with no raise in boys during this time. Later, in early adulthood, rates begin to rise for both men and women. At any given moment, between 10%-25% of adult women and 5%-12% of adult men are suffering from depression (National Institute of Mental Health 2014). This initial gender split during puberty suggests that depression might have developmental origins, and understanding the mechanisms underlying female increases might involve developmentally relevant hormones that drive mood regulation and sexual differentiation such as estrogen and testosterone.

Estrogen has been associated with depression but does not explain the pubertal shift in depression rates in girls (Andersen and Teicher 2008). Testosterone levels have been associated with depression in late adolescent girls. An early study by Angold et al found that higher

testosterone levels were directly correlated with higher rates of depression in girls during late adolescence (Angold, Costello, et al. 1999). A later study by Granger et al also observed a positive association between depressive symptoms and testosterone levels in girls (Granger, et al. 2003). Associations between pubertal hormones and psychopathologies such as depression are complicated to understand. They tend towards nonlinearity and often involve various interactions (Hyde, Mezulis and Abramson 2008). For example, in men testosterone levels and depression are characterized by a U-shaped relationship where men with the highest and lowest levels reported the greatest number of depressive symptoms (Seidman, et al. 2001). Testosterone levels are also dependent on environmental conditions such that, for example, testosterone levels interacted with the quality of the parent–child relationships for both girls and boys (Booth, et al. 2003).

According to McEwen, stress changes the balance between "anxiety, mood control, memory, and decision making" such that individuals exposed to high levels of stress experience a higher susceptibility to depressive disorders (Karatoreos and McEwen 2013). Stressful life events have long been associated with onset of depression (Kendler, Karkowski and Prescott 1999), and this is particuallry true during adolecence (K. Grant, B. Compas, et al. 2003) (K. Grant, B. E. Compas, et al. 2004) (Tram and Cole 2000). One of the physiological paths to depression via stress exposure may be through the developing endocrine axes. HPA-axis hormone activation and reorganization during adolescence is generally believed to impact mental health via signals sent by the hippocampus (Romeo 2013). The adolescent brain, particularly in the limbic regions, goes through a maturation process that under certain conditions such as stress exposure, might promote depressive disorders. This is not the only proposed path towards adolescent depression.

Observations about the hippocampo-hypothalamic system of adults born low birth weight, for example, has gained increasing attention because of its observed role in the relationship between excessive adrenal steroid exposure and the neurobiological changes that can subsequently increase risk for disorders such as depression (Romeo 2013). Such observations lead towards two interesting pathways in the production of female depression: the birth weight pathway and the environmental stress exposure pathway. We hypothesize, in this work, two possible roles for testosterone in these pathway associations. 1) Testosterone is either mediating or moderating the association between birth weight and adolescent depression, and 2) testosterone is either mediating or moderating the stress-depression association in adolescent girls. We use logistic regression analysis to model the association of testosterone with low birth weight and pubertal elevations in depression rates among girls. We test for mediating or moderating effect of testosterone on the association of low birth weight with depression. We use maturational status, defined by age, as a point whereby we compare differences in hormonal levels by a dichotomized birth weight measure. We also evaluate the role early life stress factors play in this relationship

Methods

Study Design

Data come from the Great Smoky Mountains Study (GSMS) of children and adolescents. A detailed description of the study design and the stress measurement tool used can be found in an earlier publication (Costello, et al. 1996). We present a summary here.

Sampling

In the early nineties, a representative sample of 4500 9, 11, and 13 year-olds was recruited through the Student Information Management System (SIMS) of public school systems of 11 counties in western North Carolina. Initially, a screening questionnaire was administered to parents by telephone or in person. This consisted of questions from the Child Behavior Checklist about the child's behavior (externalizing) problems, together with basic demographic information. All children scoring over a predetermined cut-off score of 20 on the behavioral questions, plus a 1-in-10 random sample of those scoring below the cut-off, were recruited for the longitudinal study. Both the eligible children and 1 parent were interviewed. They were both interviewed again 1, 2, and 3 years after the initial meeting. Table 1 illustrates the sampling framework used. The wave refers to the time in which data were collected, and the cohort refers to group included in that wave for data collection (Table 1). There are a total of 3733 observations on 1073 children in this analysis. The number of unique girls in the sample with biological, stress and birth weight data was 267. Total number of measures taken was 1104.

Psychiatric symptoms and disorders

Depression was assessed using The Children and Adolescent Psychiatric Assessment (CAPA). See Angold, Prendergast, et al. 1995 for a more detailed explanation of this assessment tool. Generally, this tool was used for psychiatric diagnoses and assessment of psychosocial adversities. Depression was defined as major depression, dysthymic disorder, or depression not otherwise specified. The time fame for depressive episode using the CAPA was within 3 months prior to the assessment interview.

Environmental Risk Factors

A large number of environmental risk factors for adversity during childhood and adolescents was included in the GSMS data set (Table 1, appendix). These risk factors were used in the stress exposure models to understand if cumulative exposure to these adversity measures is associated with depression (Costello, Worthman, et al. 2007). Environmental risk factors associated with psychosocial adversities were determined and assessed using the Child and Adolescent Psychiatric Assessment (CAPA). A risk factor was given a value of 1 if present and 0 if absent, and the total number of risk factors present was determined by the values (1,0) together.

Birth weight

Low birth weight was defined as < 2500g (<5.5lb). This is the standard cut off used in birth weight studies. For the purposes of consistency and comparability we maintained this practice. Birth weight was collected via maternal recall during one of the assessment interviews. The mothers presented this information along with other prenatal/perinatal information via a verbally administered questionnaire.

Hormonal Collection and Assay

Hormonal samples were obtained at the beginning of each interview session, as follows. Two finger-prick samples were collected at 20-min intervals, applied to specifically prepared paper, immediately refrigerated upon drying, and express shipped (without refrigeration) to the

laboratory within 2 weeks of collection. Samples were then stored at -23^{o}C until they were assayed. For more information about hormonal sample collection and measurement please see Worthman and Stallings 1997 (Worthman and Stallings 1997).

The blood spot testosterone (T) and estradiol (E2) assays are modifications of commercially available serum/plasma radioimmunoassay kits (Binax, South Portland, ME; Pantex, Santa Monica, CA; DSL, Webster, TX, respectively). Complete details of protocol, validation, performance, sample stability, and comparability to plasma or serum values for each blood spot assay are provided elsewhere (Worthman & Stallings, 1997*a*, *b*). To minimize effects of pulsatility, hormone values for each observation were taken as the average of the two blood spot samples. Formulae for conversion of blood spot assay values to serum/ plasma equivalents are given in Worthman & Stallings (1997*a*). All values in the present report represent plasma/serum equivalents and hence are comparable to the extant literature based on that medium.

Methods of analysis

The statistical test used in this study was the Generalized Estimating Equations (GENMOD procedure) in SAS software version 8 (SAS Institute 1994). This study consisted of multiple waves resulting in the presence of repeated measures. Due to this stratified sampling, a weighted analysis was required to adjust the standard errors of the parameter estimates (confidence intervals and P-values). Also a robust variance estimate of a ‘sandwich’ type was generated.

Mediation implies a mechanism through which the independent variable might be affecting the dependent variable. This is comparable to the concept of confounding in epidemiology. A mediation analysis was done to determine if testosterone was mediating the relationship between birth weight and depression. We also determined, via mediation analysis, whether testosterone was mediating the relationship between stress and depression. Next, we examined the moderating effect of testosterone in the birth weight -depression relationship. That is, we determined if the relationship between birth weight and depression is influenced by levels of testosterone. This is comparable to assessing whether testosterone is an effect modifier in epidemiological context. Next, we examine the moderating effect of testosterone in the stress-depression relationship. Finally, multiple regression analyses were conducted to examine the relationship between depression and the potential predictors, testosterone and stress.

Results

Depression Prevalence

The initial Great Smokey Mountain Study used a multi-wave, longitudinal design, whose participants ranged in age from 9 to 16 (Table 1). This is a cross sectional analysis of this population. In the current analysis we included only females, and we defined adolescence by age (13 years old or greater). The number of unique girls with no missing depression data or biological data totaled 267. The total number of times over the waves in which girls reported either having depression in the last three months or not having depression in the last three months was 1104. The details of these measures are broken down in Table 2.

Of the 267 girls, 17 were born low birth weight (6.37%). Five (29.41%) had at least one reported experience of depression in the 3 months prior to an interview. This is 4.08 (95% CI=1.41-9.47) times the odds of ever reported depression among girls with normal birth weight. In addition, frequency of depressive episodes was also greater among girls who had low birth weight. Adolescent girls in this population who were born low birth weight had 11.2 (95% CI= 6.29-19.51) times the odds of having an incident of depression in the three months prior to an interview compared with normal birth weight adolescent girls in this population.

The three-month prevalence of depressive disorder among all adolescent girls in this population is 4.12%. Among the population of normal weight girls, the prevalence of depression peaks at 2.9% at age 16 (Figure 1). Among the population of low birth weight girls, the prevalence of depression peaks at 31.7 % at age 15 (Figure 1). For both groups there was a marked increase in depression starting at age 13 (NBW 0-2.0% and LBW 0-28%).

Depression and Testosterone

A. Test of Mediation

Birth weight had no association with time-of-day corrected testosterone levels during adolescence (Path A, OR: 1.0, p-value: 0.449) (Figure 2). However, there was a substantial association of testosterone with depression (Path B, girls, 13-16, OR: 1.04, p-value: 0.0039). Thus for a 1unit ng/dl increase in testosterone there was a 4% increase in probability of depression. In path C, birth weight in girls age 13-16 was associated with depression (OR: 13.5, p-value: 0.0002). Testosterone does not seem to be mediating the relationship between birth

weight and depression. Birth weight does not seem to be working through testosterone to impact depression outcomes.

B. Test of Moderation

The interaction term "Testosterone*birth weight" is not significantly associated with depression when included in a logistic regression interaction model (Tesscor: OR: 1.04. p-value: 0.0016, Birth weight: OR: 11.55, p-value: 0.2572, Tesscor*Birth Weight: OR: 1.02, p-value: 0.766) (Figure 3). Testosterone does not seem to be moderating the relationship between birth weight and depression.

Depression and Stress

A. Testosterone Mediation

We determined the incidence of depression for girls age 13-16 both in the presence and absence of stress, by birth weight (Table 3). At this point, incidence of low birth weight becomes so small that we can no longer continue to include birth weight in the analysis, because the number of low birth weight girls contributing to incidents of depression in the absence of stress exposure was less than 5 (with 22 reports). Such a small number of incidents would not be significantly informative (Table 4). From here on we look at the role of testosterone in the stress-depression relationship in only normal birth weight girls.

In Figure 4, Path A illustrates that stress exposure had no association with time-of-day corrected testosterone levels during adolescence (OR: 1.003, p-value: 0.425). Path B suggests that testosterone was associated with depression (girls, 13-16, OR: 1.04, p-value: 0.0039). Path C

shows that stress in girls age 13-16 was associated with depression (OR: 18.6, p-value: 0.0002). Testosterone does not seem to be mediating the relationship between stress and depression in girls who were normal weight at birth.

The interaction term "Testosterone*Stress" was significantly associated with depression when included in a logistic regression interaction model (Tesscor: OR: 0.919. p-value: 0.0324, Stress: OR: 0.279, p-value: 0.3379, Tesscor*Stress: OR: 1.129, p-value: 0.0034). In NBW girls ages 13-16, testosterone did seem to be moderating the relationship between stress and depression (Figure 5).

The Depression Model

Table 5 summarizes the analysis results. In model 1 both testosterone and stress are positively and significantly correlated with depression (Testosterone: OR: 0.099, p-value: 0.004, Stress: OR: 7.51, p-value: 0.0006), indicating that the presence of higher levels of either predictor was associated with increased incidence of depression. In model 2 the interaction term was included to determine if this model of moderation fit our data better then model 1.

When the interaction term is added the effect of testosterone on the log odds of depression is not the same depending on the number of stressors the adolescent girl is exposed to. In model 1 we forced the slope of testosterone to be the same regardless of the number of stressors exposed. We assume that the effect is the same at every level of stress exposure. The intercept increased with each added stress exposure, but the relationship between depression and testosterone stayed constant. In the second model we allowed the slope to change by including the interaction term. When we relaxed this slope criterion we observed that the beta for stress is

different in intercept with each added stressor. In this case, the intercept decreases by 1.27. The interaction beta is the difference in slope for each stressor. The slope gets steeper with the addition of each stressor by 0.12. This gets slightly more positive with one stressor. With 2 stressors the intercept goes down to -6.23 and the slope gets even steeper (0.16). By the time we get to 6 stressors the slope = 0.64 and intercept = 11.31. QIC estimates suggest that including the interaction term (model 2) is a significantly better fit with our data (Table 5). The relationship between testosterone levels and probability of depression is exponentially greater in the presence of stress, and virtually absent in the absence of stress (Figure 5).

Discussion

Adolescence is a time of increased vulnerability to depression. We found that large disparities in depression risk by birth weight do exist. Birth weight has no association with testosterone levels and, when entered in to a regression model, birth weight does not weaken the relationship between testosterone and depression. This suggests that birth weight is not working through testosterone levels in increasing depression risk. The path towards depression is unique and independent for both testosterone and birth weight (Figure 2).

Stress exposure has repeatedly been associated with increases in depression risk. When we look at stress exposure by birth weight, the incidence of low birth weight becomes so small that we can no longer continue to include birth weight in the analysis (Table 4). Stress exposure and androgen levels among normal birth weight girls is of great interest because most girls were of normal birth weight. Among normal birth weight girls, the risk of depression is about 17 times higher when exposed to at least 1 stressor than for girls not exposed to stress at all (Andersen and

Teicher 2008). What is most interesting is that stress is not working through testosterone to impact depression, but in NBW girls ages 13-16, testosterone does significantly moderate the stress-depression relationship (Table 4).

The effect of stress on testosterone levels is such that in the absence of stress the probability of depression has little or no relationship with the level of testosterone. With each additional stressor the relationship between depression and testosterone increases. Interestingly, it is two or more stressors that have the greatest impact on the testosterone –depression association (Figure 5). The implications of these observations are that testosterone may have a functional role in female development outside of the sexual differentiation mechanisms. It is unclear if this is organizational or activational, and the extent to which the timing of stress exposure and type of stress exposure is important is unclear. These observations would need to be repeated in order to verify these findings. More work is also needed to understand the functional role of testosterone in girls, not in comparison to boys necessarily, but as having variations in effect within the developing female body.

There are two primary limitations to this study. First, the model was designed such that each environmental stressor had equal impact. This may not be an accurate representation of variation in physiological impact different stressors might have. Also, we do not account for severity of exposure of a given stressor. Second, there might be a time dependent component to stress exposure. In this study design, I was not able to incorporate when the stress exposure occurred with the testosterone-depression story. These are both limitations worth addressing in future studies.

Chapter III: Summary, Public Health Implications and Possible Future Directions

Emotional arousal is readily accessible during adolescence, but emotional self-regulation is a little more difficult because of how the brain is developing. The development of mature emotional self-regulation is a process of repeated environmental experiences that promote positive self-reflection, empathy and SNS down regulation. The work presented here suggests that stress can have a major impact on a girl's biological production of depression. What is even more interesting about this work is the suggestion that HPA and HPG-axes interact in the production of mental health during a particularly salient developmental transition. Further inquiry into the nature of these interactions over various developmental time courses could prove very informative in understanding the differential impact of stress on maturation.

From a public health perspective, timing and type of stress exposure and its relationship with testosterone levels could help determine which stressors would have the greatest public health impact if interventions targeted their reduction.

Changes in the coordination between HPA and HPG activation during the pubertal transition also put into question how and when experiences in early childhood set the stage for controlling emotional arousal during this adolescent shift. How do environments in which this transition occurs shape how emotional arousal will be regulated when that person reaches adulthood? Does testosterone driven depression lead to increased episodes of depression in adulthood? Does testosterone driven depression have a more pronounced physiological impact on neurobiological development? Or is testosterone driven depression more transient, in that it does not relate to later incidence?

In the introduction we discussed the importance of emotional self-regulation in development. The study presented here contributes to a broader conversation about the developmentally significant impact stress can have on health. From birth to adolescence, how stress regulation is understood and the communication happening between upper and lower regions of the brain, and the physiology of the brain-body interaction shifts as the developing brain changes. Earlier events will influence later emotionality. This study contributes to understanding physiological mechanisms underlying changes in emotionality from a developmental perspective. Such mechanistic understandings are beneficial because they open the possibility of finding ways to disrupt these mechanisms and prevent the production of mental illnesses such as depression.

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Figures and Tables

Table 1: Waves of data from the Great Smoky Mountain Study used in the present analyses.

	Wave	2	3	4	5	6	7	8
Cohort	Age	1994	1995	1996	1997	1998	1999	2000
A	10	**A2**						
	11		**A3**					
B	12	**B2**		**A4**				
	13		**B3**					
C	14	**C2**		**B4**		**A5**		
	15		**C3**		**B5**		**A6**	
	16			**C4**		**B6**		**A7**

Table 2: Characteristics of the study population.

Depression*

Unique Girls in the Sample

Low Birth Weight

	Yes	**No**	**Total**
Yes	5	12	17
No	18	232	250
	23	244	267

RR=4.083 (95% CI=1.41-9.47)

OR=5.37 (95% CI=1.46-19.0)

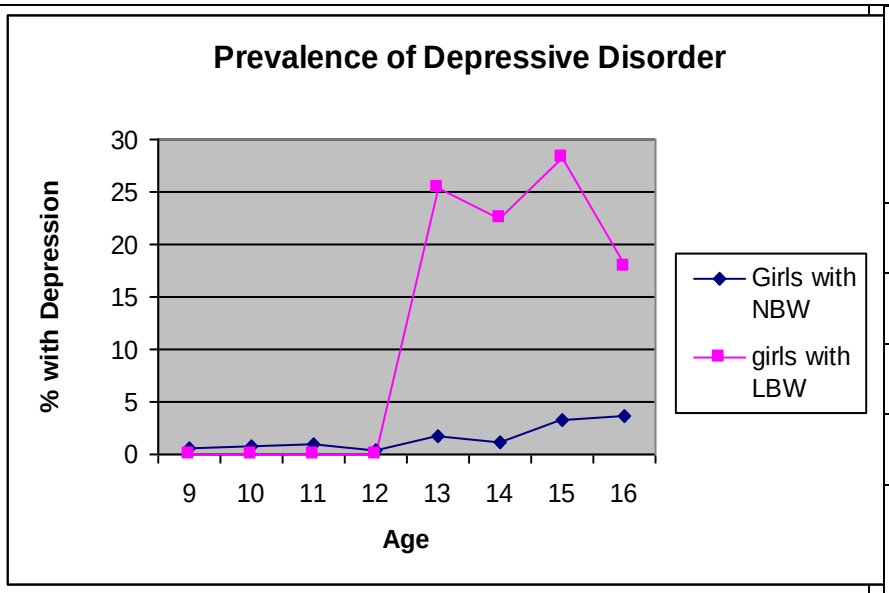
Incidents Based on the Unique Girls

	Yes	**No**	**Total**
Yes	20	51	71
No	26	1007	1033
	46	1058	1104

RR=11.19 (95% CI=6.29-19.51)

OR=15.19(95% CI=7.57-30.43)

*Report of depression in the 3 months prior to the interview.

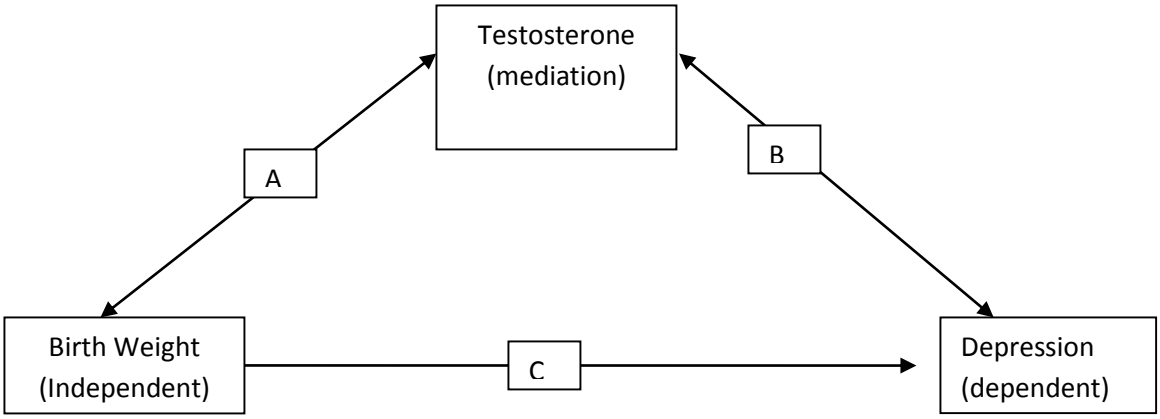


age	# of obs NBW (%dep)	# of obs LBW (%dep)
9	89 (0.1)	2 (0)
10	128 (1.1)	9 (0)
11	269 (1.0)	18 (0)
12	252 (0)	15 (0)
13	273 (2.0)	16 (27.9)
14	289 (2.3)	15 (25.9)
15	274 (2.7)	24 (31.7)
16	239 (2.9)	16 (24.7)

Figure 1. Prevalence of Depression. When comparing the prevalence of depression by age between low birth weight and normal birth weight girls, increased depression prevalence among low birth weight girls is higher.

Test of Mediation

Is testosterone mediating the birth weight-depression story?



Mediation
Path A: Birth Weight =tesscor (path A) Tesscor: OR: 1.0, p-value: 0.449
Path B: Dep = tess (path B) Tesscor: OR: 1.04, p-value: 0.0039
Path C: Dep =Birth Weight (path C) Birth Weight: OR: 13.5, p-value: 0.0002
Mediation model:
Tesscor: OR: 1.04, p-value: 0.0011
Birth Weight: OR: 22.01, p-value: < 0.0001

Figure 2. Mediation with Birth Weight. Testosterone does not seem to be mediating the relationship between birth weight and depression. Mediation implies a mechanism through which the independent variable might be affecting the dependent variable. Birth weight does not seem to be working through testosterone to impact depression outcomes. Next, we examine the moderating effect of testosterone in the Birth weight -depression relationship. This means that we will determine if the relationship between Birth weight and depression is influenced by levels of testosterone.

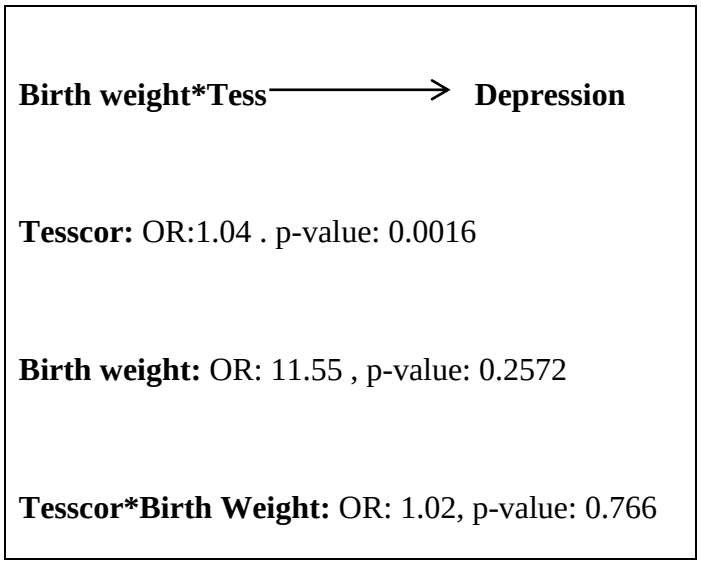
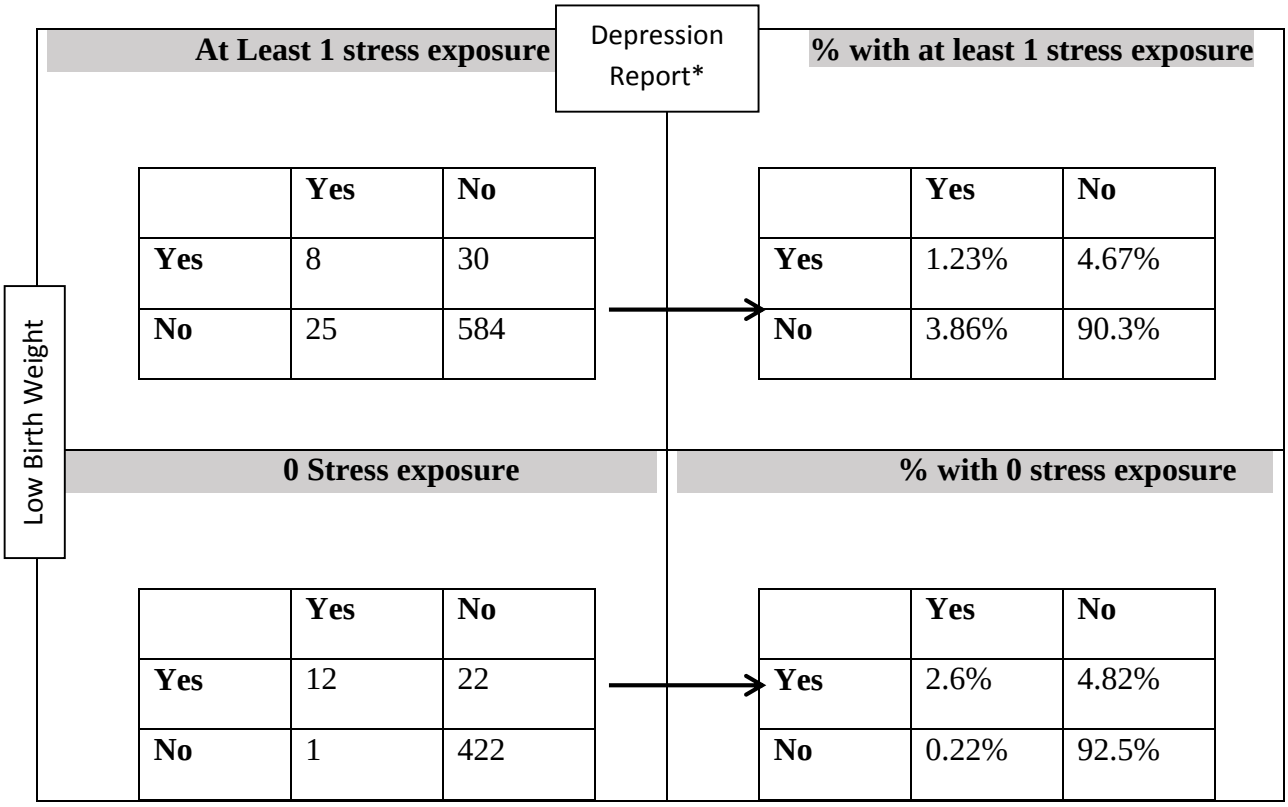


Figure 3. Moderation with Birth Weight. Testosterone does not seem to be moderating the relationship between birth weight and depression.

Table 3. **What is the role of stress in the birth weight-depression association?** The incidence of depression for girls age 13-16 in the presence or absence of stress.



	Yes	No
Yes	8	30
No	25	584

	Yes	No
Yes	1.23%	4.67%
No	3.86%	90.3%

	Yes	No
Yes	12	22
No	1	422

	Yes	No
Yes	2.6%	4.82%
No	0.22%	92.5%

*The number of times depression was reported in the 3 months prior to an interview.

Table 4: Stress Exposure Raw Data. When we look at stress exposure by birth weight the incidence of low birth weight becomes so small that we can no longer continue to include birth weight in the analysis. From here on we will look at the role of testosterone in the stress-depression relationship in only normal birth weight girls.

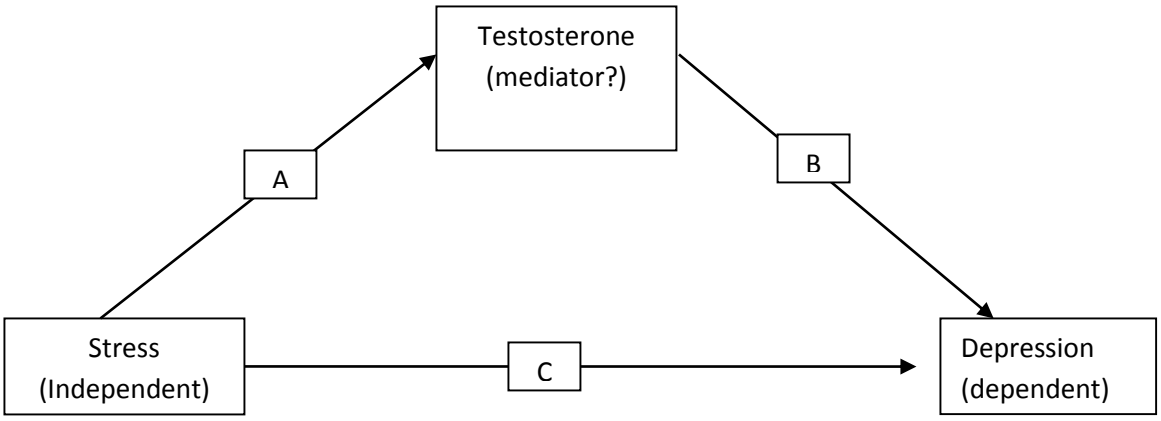
Stress	Depression Reports*	
	No	**Yes**
At least 1	584	25
None	422	1

RR: 17.3645 CI 95%: 2.3619, 127.6627

OR: 18.0651 CI 95%: 2.4382, 133.8493

*This adds to 1,032, not 1104 because those girls with missing biological data were not included here.

Testosterone's role in the Stress-Depression Story



Normal Birth Weight
Path A: Stress=tesscor (path A)
Tesscor: OR: 1.003, p-value: 0.425
Path B: Dep = tess (path B)
Tesscor: OR: 1.04, p-value: 0.0020
Path C: dep =Stress (path C)
Stress: OR: 18.6, p-value: 0.0002
Mediation model:
Tesscor: OR: 1.04, p-value: 0.0006
Stress: OR: 15.84, p-value: 0.0043

Figure 4. Mediation with Stress. Testosterone does not seem to be mediating the relationship between stress and depression. Next, we examine the moderating effect of testosterone in the stress-depression relationship. This means that we will determine if the relationship between stress and depression is influenced by levels of testosterone.

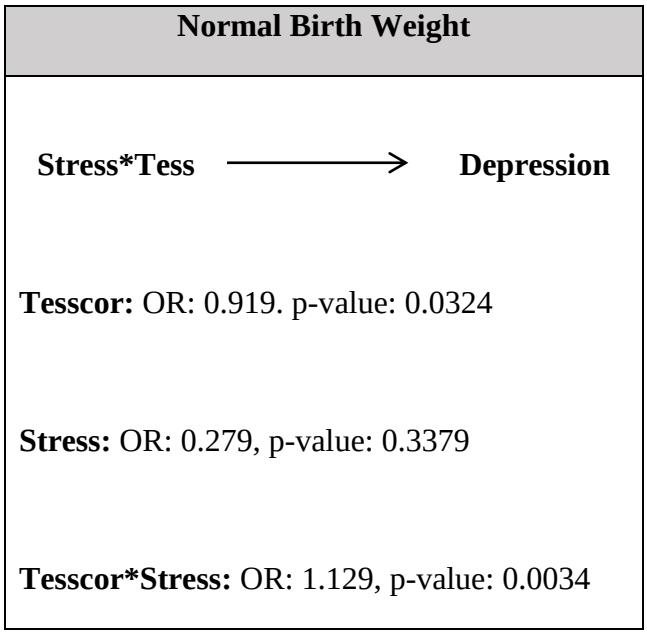
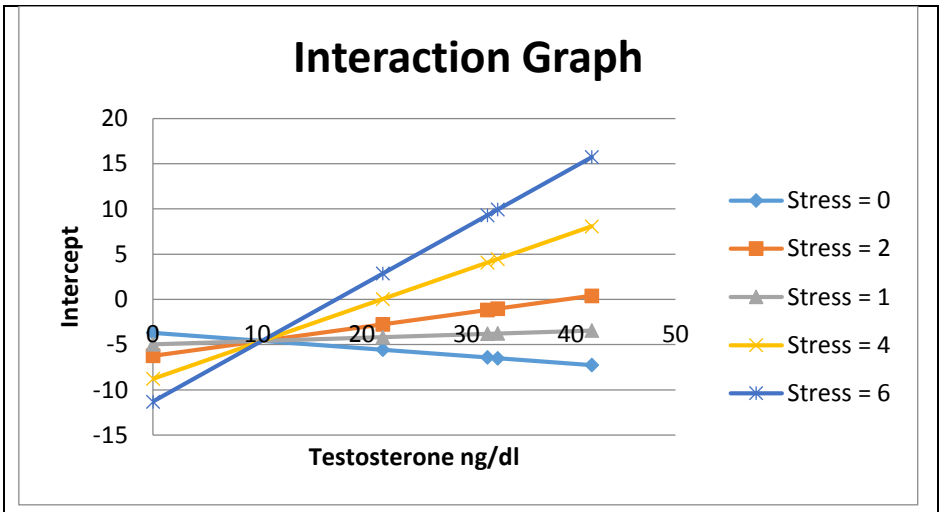


Figure 5. Moderation with Stress. In NBW girls ages 13-16, testosterone does seem to be moderating the relationship between stress and depression. Next, we compare models with and without the interaction term to determine which better fits our data.

Table 5. The Depression Models: This is modeling depression in normal birth weight girls ages 13-16 in our sample.

	Model 1 β (OR)	p-value	CI (95%)	Model 2 β (OR)	p-value	CI (95%)
Intercept	-7.6961 (-4.978)	<0.0001	-9.68—5.709	-3.6907 (-0.972)	0.0007	-5.8181—1.5634
Testosterone	0.0363 (0.099)	0.0043	0.0114-0.0613	-0.0845 (2.634)	0.0324	-0.1620—0.0071
Stress	2.7624 (7.51)	0.0006	1.186-4.339	-1.2749 (1.44)	0.3379	-3.8821-1.3323
Stress X Testosterone	----------	----------	----------	0.1215 (0.3302)	0.0034	0.0402-0.2029
QIC	209			206		



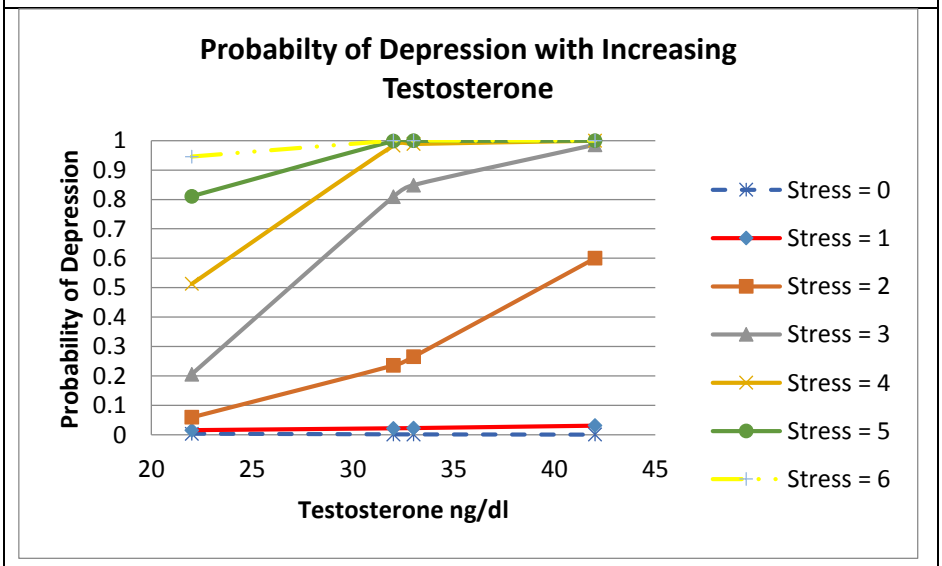


Figure 5. Probability of Depression graphs. In NBW girls ages 13-16, testosterone did seem to be moderating the relationship between stress and depression based on the relationship between probability of depression and testosterone levels with increasing stress exposure.

Appendices 1

Table 2. Childhood and Adolescent Effects on Female Adolescent Depression*

Risk Factor Present During ≥1 y	During Childhood†					During Adolescence‡				
	Prevalence of Risk Factor, %	Prevalence of Risk Factor With and Without Associated Depression, %		OR (95% CI)	*P* Value	Prevalence of Risk Factor, %	Prevalence of Risk Factor With and Without Associated Depression, %		OR (95% CI)	*P* Value
		With	Without				With	Without		
Family below federal poverty line	19.3	18.5	28.3	...	...	21.8	21.3	27.3	...	...
Parent unemployed	17.9	18.3	12.5	...	...	15.2	15.3	13.9	...	...
≥4 Children in home	3.2	3.4	0.0	...	...	7.6	8.1	2.2	...	...
Stepparent	11.1	11.0	11.6	...	...	18.0	18.2	15.7	...	...
Single parent	16.0	16.2	13.8	...	...	31.3	28.7	59.3	3.7 (1.5-9.2)	.004
Time in foster care	2.3	2.5	0.3	...	...	3.0	2.2	17.0	9.0 (2.3-35.3)	.002
Moved ≥4 times in 5 y	14.1	13.2	23.3	...	...	11.1	9.6	28.7	3.9 (1.3-11.0)	.01
Dangerous neighborhood	0.7	0.6	1.2	...	...	1.5	0.9	7.5	...	...
Dangerous school	0.1	0.1	0.0	...	...	3.6	3.3	7.3	...	...
Poor health	1.7	1.8	0.0	...	...	1.5	0.9	8.6	20.9 (3.2-137.7)	.002
Obesity	18.3	18.5	16.5	...	...	32.8	32.9	32.4	...	...
Lax parental supervision	5.5	5.8	2.9	...	...	18.1	16.3	38.1	...	...
Harsh parental discipline	4.4	4.6	1.9	...	...	2.4	1.5	11.6	9.3 (2.7-32.2)	<.001
Overprotective or intrusive parenting	1.5	1.7	0.0	...	...	8.3	7.7	49.0	...	...
Child treated as scapegoat	1.5	1.6	0.0	...	...	1.5	0.8	8.9	23.7 (4.9-114.8)	<.001
Violence between parents	13.8	13.0	21.5	...	...	11.5	10.8	19.0	...	...
Deviant peers	11.1	10.4	18.9	...	...	15.6	13.8	36.8	3.6 (1.4-9.3)	.006
Family structure change	8.6	8.5	9.2	...	...	10.5	9.7	18.3	...	...
Parental drug abuse	10.6	10.7	10.0	...	...	14.0	13.1	24.1	...	...
Parental crime	18.0	18.5	12.3	...	...	11.3	10.9	15.6	...	...
Parental mental illness	21.8	20.4	36.8	2.3 (0.9-5.7)	.09	25.4	21.9	64.0	6.3 (2.5-15.9)	<.001
Maternal depression	8.4	8.2	11.2	...	...	9.9	8.1	29.5	4.7 (1.7-13.2)	.003
Physical abuse	3.4	3.3	3.8	...	...	5.0	3.1	25.7	10.5 (3.2-36.3)	<.001
Sexual abuse	9.5	8.5	19.7	2.6 (0.9-8.1)	.09	6.5	5.5	17.3	3.6 (1.2-10.7)	.02
Neglect	1.4	1.3	2.4	...	...	5.4	3.0	32.1	15.4 (5.2-45.8)	<.001
Extreme stressor	39.1	36.9	63.7	3.0 (1.2-7.5)	.02	51.9	50.0	71.9	2.9 (1.1-7.8)	.04
Other life event	36.3	33.6	66.1	3.8 (1.5-9.5)	.004	56.0	52.7	91.8	10.1 (2.4-43.7)	.002
Anxiety disorders	4.2	3.8	8.6	...	...	7.2	3.8	44.6	20.2 (6.9-59.3)	<.001
Disruptive behavior disorders	3.5	2.7	12.5	5.2 (1.5-10.9)	.001	8.0	4.3	49.1	21.5 (7.9-58.2)	<.001
Posttraumatic stress disorder	13.2	12.1	25.0	14.1 (2.4-83.7)	.004	16.0	13.7	41.6	13.7 (3.4-55.1)	<.001
Drug abuse or dependence	NA	NA	NA	...	...	8.1	6.5	26.0	5.0 (1.6-15.4)	.005
School dropout	NA	NA	NA	...	...	1.8	1.1	8.4	...	...
Expulsion	NA	NA	NA	...	...	1.2	0.5	9.4	22.7 (4.1-125.7)	<.001
Criminal conviction	NA	NA	NA	...	...	2.6	2.3	6.1	...	...

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio.
*Ellipses indicate variables dropped from the model because of nonsignificance.
†Age range, 9 to 12 years.
‡Age range, 13 to 16 years.

List of Terms and Abbreviations

Amygdala	A ganglion of the limbic system adjoining the temporal lobe of the brain and involved in emotions of fear and aggression
Autonomic nervous system	(ANS) The part of the nervous system responsible for control of the bodily functions not consciously directed, such as breathing, the heartbeat, and digestive processes.
Hippocampus	The elongated ridges on the floor of each lateral ventricle of the brain, thought to be the center of emotion, memory, and the autonomic nervous system.
Hypothalamic - Pituitary-Adrenal axis	(HPA-axis) The hypothalamic-pituitary-adrenal axis is a complex set of interactions between the hypothalamus (a part of the brain), the pituitary gland (also part of the brain) and the adrenal or suprarenal glands (at the top of each kidney.) It is a major part of the system that controls your reaction to stress, trauma and injury.
Hypothalamic - Pituitary-Gonadal axis	(HPG-axis) The hypothalamic–pituitary–gonadal axis is a critical part in the development and regulation of a number of the body's systems, such as the reproductive and immune systems. Fluctuations in the hormones cause changes in the hormones produced by each gland and have various widespread and local effects on the body.
Myelination	Myelination is the process by which a fatty layer, called myelin, accumulates around nerve cells (neurons).
Pre-frontal cortex	(PFC) The prefrontal cortex, also called PFC, refers to the anterior part of the frontal lobes of the brain.
Stress	A state of mental or emotional strain or tension resulting from adverse or very demanding circumstances.
Tanner stages	Dr. Tanner and others in his research group reviewed the collected data and compartmentalized what is a continuous process of development into five stages. The Tanner staging system evaluates both breast development and pubic hair.
Tesscor	Testosterone levels that were corrected for the time of day in which the samples were taken