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The Role of Acculturative Stress on Maternal Anxiety and Cortisol Levels During Pregnancy

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Abstract

Fifty-two percent of women report experiencing anxiety symptoms while pregnant. Experiencing anxiety during pregnancy is associated with adverse prenatal and birth outcomes for mothers and children. Mexican American women experience unique psychosocial factors, such as lack of culturally competent mental health care, language barriers, and discrimination, which could place them at risk for experiencing higher anxiety levels in the perinatal period compared to other women. Mexican American women also experience acculturative stress that results from the psychological and cultural challenges when interacting between cultures that could impact anxiety levels during pregnancy; however, the association between acculturative stress and anxiety during pregnancy has not yet been established empirically. In addition to the possible impact of cultural stressors on anxiety, stressors can also have physiological effects that may place pregnant Mexican American women at risk for developing mental health symptoms. The measurement of stress-related biomarkers, such as long-term hair cortisol, may contribute to the understanding of the role of cultural stressors in perinatal anxiety. A sample of 151 Mexican American pregnant women completed five assessments across their pregnancy to assess state anxiety, acculturative stress, and hair cortisol levels. The study aimed to explore the role of acculturative stress on the trajectory of cortisol levels and anxiety symptoms in Mexican American pregnant women. The study addressed three hypotheses: 1) participants who reported high levels of acculturative stress would report high levels of state anxiety symptoms across pregnancy; 2) participants who reported high levels of acculturative stress would exhibit increasing levels of cortisol across pregnancy as measured in hair; 3) high levels of maternal cortisol would mediate the relationship between high acculturative stress and elevated maternal anxiety symptoms. A multilevel model analysis showed that acculturative stress was associated

with the trajectory of anxiety symptoms (b = -0.047, t (153) = -2.03, p = 0.044); high levels of acculturative stress were associated with an increase early in pregnancy and steady decline of anxiety symptoms across pregnancy. A multilevel model analysis showed no significant relationship between acculturative stress and the trajectory of maternal hair cortisol levels. Maternal hair cortisol level was not a significant mediator for the relationship between acculturative stress and symptoms of anxiety. Findings suggest that Mexican American pregnant women who experience acculturative stress may experience higher symptoms of state anxiety early in pregnancy. Assessing acculturative stress in the perinatal period may help identify women who may be at risk for experiencing symptoms of anxiety and help promote cultural competence for future obstetric interventions.

The Role of Acculturative Stress on Maternal Anxiety and Cortisol Levels During Pregnancy

Experiencing anxiety during pregnancy is common. While 15% of pregnant women report experiencing anxiety symptoms early in pregnancy (Rubertsson, Hellström, Cross & Sydsjo, 2014), 50% of women report an increase in anxiety symptoms throughout pregnancy (Faisal-Cury & Rossi Menezes, 2007). Thus, it is important to identify the relevant predictors that contribute to anxiety symptoms during pregnancy. Women of Mexican descent may be particularly vulnerable to prenatal anxiety (Fleuriet & Sunil, 2014). Not only are they exposed to psychosocial stressors such as less access to prenatal services, low socioeconomic status, and language barriers (Korinek & Smith, 2011; Lara, Gamboa, Kahramanian, Morales, & Hayes Bautista, 2005; Sunil, Spears, Hook, Castillo, & Torres, 2010), but Mexican American women also face cultural stressors that may increase their likelihood of experiencing prenatal anxiety, such as acculturative stress, the stressors associated with the psychological and cultural changes that occur during the process of acculturation (Berry, 2005; 2006). In non-pregnant Mexican American samples, high levels of acculturative stress have been linked with symptoms of anxiety (Crockett et al., 2007; Hovey & Magaña, 2000; Revollo, Qureshi, Collazos, Valero, & Casas, 2011). However, the association between acculturative stress and symptoms of anxiety in pregnant Mexican American women is unknown.

Furthermore, objective measures are needed to identify the associations between cultural psychosocial stressors and risk for perinatal anxiety. Experiencing psychosocial stress and symptoms of anxiety is associated with dysfunctions of the stress response system, the hypothalamic-pituitary-adrenal axis (HPA) (McEwen, 2004; Holsboer, 2001; Vrekoussis et al., 2010). A common method to measure HPA axis activity is through cortisol output, the end product of the HPA axis (Miller, Chen, & Zhou, 2007). Cortisol can be measured retrospectively

in hair (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009) and is a novel way of measuring chronic stress. Therefore, maternal hair cortisol can be non-invasive biomarker for stress during the perinatal period. The current study will investigate whether acculturative stress, along with hair cortisol levels, contributes to maternal anxiety in Mexican American pregnant women.

Perinatal Anxiety

Anxiety is defined as a state of distress in preparation for possible future events (Brooks & Schweitzer, 2011). State anxiety refers to the level of current anxiety and is associated with feelings of tension, nervousness and worry, and physical changes such as sweating, trembling, and increase blood pressure (American Psychological Association, 2013). Investigating anxiety symptoms has become an important research topic for perinatal populations (Meades & Ayers, 2011) as 52% of women report an increase in symptoms of anxiety during pregnancy (Anxiety and Depression Association of America, 2009). It is typical to experience symptoms of anxiety given physical changes that occur and thoughts about the baby's health and possible complications of and preparation for childbirth (Geller, 2004). For example, in a sample of middle class, inpatient women in the postnatal period, 42.9% reported symptoms of anxiety (Reck et al., 2008). Other studies in minority populations have found up to 54% of women report anxiety symptoms across different stages of pregnancy (Lee et al., 2007).

Experiencing anxiety during pregnancy is associated with pregnancy complications (Kurki, Hiilesmaa, Raitasalo, Mattila, & Ylikorkala, 2000) and the development of mental health disorders, including postnatal depression (Coelho, Murray, Royal-Lawson, & Cooper, 2011). Not only does anxiety adversely affect the mother, but it also has consequences for the developing fetus as well. Offspring of mothers who experience anxiety have low birth weight and impaired fetal head and abdominal growth (Henrich et al., 2010). Moreover, these problems may extend

into childhood as experiencing prenatal anxiety might account for 10-15% of the risk for emotional and behavioral problems in offspring (Glover, O'connor, & O'Donnell, 2010). Mothers who reported anxiety symptoms during pregnancy were more likely to have children with behavioral problems, such as irritability and sleep and feeding problems, than non-anxious mothers (De Weerth, Hees, & Buitelaar, 2003). Thus, anxiety is a serious issue that needs to be addressed in the perinatal period. Because of the deleterious effects of anxiety symptoms during pregnancy on pregnant women and offspring, it is important to determine the factors that contribute to mental health and to investigate vulnerable populations.

Prenatal Psychosocial Stress and Mental Health in the Mexican American Women

Stress during pregnancy may lead to experiencing anxiety symptoms. The term stress is used to describe a challenging event that disrupts stability (McEwen & Wingfield, 2003). According to the diathesis-stress theory, experiencing stress is related to the development of psychological disorders (Hinkle, 1974). Having a predisposition, diathesis, and experiencing environmental challenges, stressors, may increase an individual's likelihood to develop mental health problems. Predispositions may include psychological factors, such as personality, dysfunctional thinking and coping that have a biological basis and stressors could be psychosocial stressors (Cohen, Janicki-Deverts, & Miller, 2007; Leach, Poyser, & Fairweather-Schmidt, 2015; Pagel, Smilkstein, Regen, & Montano, 1990). One type of stress that pregnant women may face is psychosocial stress, which occurs when an individual encounters environmental demands that exceed one's capacity for coping (Cohen, Janicki-Deverts & Miller, 2007). Several psychosocial stressors and correlates of prenatal anxiety have been identified in the literature, including low socioeconomic status, low income, and low social support (Leach, Pyser & Fairweather-Schmidt, 2015; Pagel, Smilkstein, Regen & Montano, 1990). In a sample of

Latina and Black pregnant women, experiencing psychosocial stressors, including discrimination, predicted an increase in anxiety symptoms throughout pregnancy (Rosenthal et al., 2015). Vulnerable minority populations may experience culturally specific stressors that can increase their likelihood of experiencing anxiety symptoms compared to other populations.

Mexican American women are vulnerable to psychosocial stress because they are more likely to experience language barriers, discrimination, and separation from family, friends and social resources than their white counterparts (Harley & Eskenazi, 2006). Pregnant women who report low levels of education, low self-esteem, low maternal age, and low partner satisfaction are more likely to develop an anxiety disorder compared to those who do not report psychosocial stressors (Martini, Petzoldt, Einsle, Beesdo-Baum, Hofler & Wittchen, 2015). Furthermore, the lack of mental health services may contribute to the likelihood of developing an anxiety disorder. Ethnic minority groups who report low socioeconomic status also report not being treated or not receiving treatment for mental health problems (Wang et al., 2005). When compared to other ethnic/racial minorities, Latina groups including Mexicans receive fewer mental health services (Mulvaney-Day, Alegria, & Sribney, 2007). Mexican American women may also lack access to prenatal care. Rates of first trimester prenatal care for Hispanic women without pre-pregnancy Medicaid coverage were lower compared to those with coverage (Rosenberg, Handler, Rankin, Zimbeck & Adams, 2007). Given that stress often precedes anxiety symptoms (Hovey, 2000; Littleton, Breitkopf & Berenson, 2006), Mexican American women may be at a higher risk for developing an anxiety disorder due to experiencing psychosocial stressors.

Acculturative Stress and Mental Health in Mexican American Pregnant Women

The minority stress theory states that experiencing stress due to marginalized status is associated with psychological distress (Meyer, 2003). Minority stressors are unique and may

play an additive role to general stressors. Thus, individuals who are part of a minority group may be required to greater adaptation effort to unique cultural stressors when compared to individuals who are not part of minority groups (Carr & Umberson, 2013; Pearlin & Bierman, 2013). Ethnicity related stressors, such as prejudice and discrimination, result in heightened stress responses that may influence mental health outcomes such as psychological stress, anxiety, and overall mental wellbeing (Williams, Neighbors, & Jackson, 2008; Paradies, 2006). Not only are Mexican American women exposed to ethnicity related stressors, such as discrimination and language barriers, but some may also experience acculturative stress. Acculturative stress is defined as the stressors that results from the cultural and psychological changes that occur when interacting between two or more cultures (Berry, 2006). Acculturative stress involves discord in cultural values, practices, customs, and language (Gil, Vega, & Dimas, 1994). When an individual encounters a threatening situation, such as incongruence in cultural experiences, he or she may perceive the situation as being stressful (Barlow, 2002). For example, a study found that, for individuals of Mexican descent living in the United States, acculturative stress was a predictor of psychological distress even when controlling for other sources of stress (e.g., income, education, and years in the U.S; Rodriguez, Myers, Morris, & Cardoza, 2000). Thus, acculturative stress could be a psychosocial factor that can have detrimental effects on mental health.

Acculturative stress has been linked with mental health problems in Mexican-Americans. Amongst Mexican American farmworkers, experiencing high levels of acculturative stress was associated with an increased risk of experiencing high levels of anxiety (Hovey & Magaña, 2000). Similar patterns were seen in adolescents in that acculturative stress was also associated with higher anxiety symptoms in this age group (Crockett et al., 2007). Pregnant women who

experience acculturative stress may be particularly susceptible to experiencing mental health symptoms. Early in pregnancy, Mexican-American women who report high levels of acculturative stress also report increased levels of depressive symptoms (D'Anna-Hernandez, Aleman & Flores, 2015). This association may extend into the postpartum period as Mexican American adolescent mothers who experienced high acculturative stress during late pregnancy and postpartum period reported depressive symptoms (Zeiders, Umaña-Taylor, Updegraff, & Jahromi, 2015). However, the role of acculturative stress on maternal anxiety is not known. Further research is needed to explore the effects of acculturative stress on anxiety for pregnant Mexican American women given the relationship with adverse perinatal outcomes in both mothers and children.

Stress Response System During Pregnancy

Experiencing psychosocial stressors may be associated with physiological changes that can have deleterious effects on mental health. The HPA axis is known as the primary neuroendocrine system that controls the body's reaction to stress (McEwen, 2004). The HPA axis also involves an automatic negative feedback circuit to regulate homeostasis, the ability of one's body to maintain a balanced equilibrium (Lefmann & Combs-Orme, 2014). The stress reaction begins when cells in the hypothalamus produce corticotropin-releasing hormone (CRH). CRH then binds to receptors in the anterior pituitary, which produce adrenocorticotropic hormone (ACTH). ACTH is transported to the adrenal glands where adrenal hormones, including cortisol, are secreted into the bloodstream (Chrousos, 1998). The negative feedback process results in the secretion of cortisol from the adrenal glands. Then cortisol binds with receptors in the hypothalamus and anterior pituitary to inhibit the release of CRH and ACTH. The negative

feedback process brings the body back to stability in order to maintain normal functioning (Weinstock, 1997).

During pregnancy, the HPA axis is altered dramatically by the influence of the placenta (Christian, 2012). The placenta produces large amounts of corticotropin releasing hormone (CRH) as gestational age increases (Lindsay & Nieman, 2005). Plasma CRH is associated with the placental clock that determines the timing of delivery (McLean & Smith, 2001). Placental CRH is buffered by CRH-binding-globulin (CBG), which drops before timing of labor (Christian, 2012). Increases of placental CRH could bind to receptors in the anterior pituitary, and in turn the anterior pituitary could secrete adrenocorticotropic hormones (ACTH). Consequently, the production of maternal cortisol could increase and result in hypercortisolism (Hobel, Dunkel-Schetter, Roesch, Castro, & Arora, 1999; Lindsay & Nieman, 2005). Hypercortisolism is associated with physiological changes, such as an increase in dopaminergic neurotransmissions, that may account for psychological symptoms, including depression (Holsber, 2001). As pregnancy progresses, women undergo physiological changes that lead to an increase in cortisol levels (Mastorakos & Illias, 2000). An increase of cortisol is typical across pregnancy, as it supports the maturation of fetal organs (Majzoub & Karalis, 1999; Waffarn & Poggy Davis, 2012) and prepares the body for labor (Carr, Parker, Madden, MacDonald, & Porter,1981; McLean & Smith., 2001). However, an excess in cortisol levels may lead to a dysregulation of the HPA axis (Miller, Chen, & Cole, 2009). Psychosocial stress is also associated with cortisol levels (Obel et al., 2005). Experiencing stressors may challenge the state of homeostasis (Chrousos & Gold, 1992) and thus dysregulate the stress response system (Tsigos & Chrousos, 2002; Van Den Berg et al., 2008). Minority populations experience greater psychosocial stressors when compared to their white counter parts (Korinek & Smith, 2011; Lara

et al., 2005; Sunil et al., 2010). These psychosocial stressors include unique stressors such as acculturative stress (Berry, 2006; Hovey & Magaña, 2000; D'Anna-Hernandez, Aleman & Flores, 2015). However, the effects of acculturative stress on the HPA axis is unknown.

Previous findings suggest that dysregulation of the HPA axis has been reported in individuals who experience anxiety symptoms (Hughes et al., 2012; McEwen, 2004; Holsboer, 2001). When an individual experiences anxiety, the anxiety stimulates the autonomic nervous system which provides sensory information to the amygdala. However, the amygdala is also sensitive to glucocorticoid signals (Sapolsky, 2004). Thus, experiencing stress along with an increase in glucocorticoid output from the HPA axis is associated with an increase in amygdala function and in turn higher sympathetic nervous system activation (Risbrough & Stein, 2006), perhaps exacerbating symptoms of anxiety. The minority stress theory also suggests that experiencing stress due to minority status results in psychological and physiological responses (Meyer, 2003). Experiencing stress due to marginalized status may influence the stress response in the HPA axis and in turn mental health symptoms. Acculturative stress may also influence physiological mechanisms that may be related to symptoms of anxiety, however, this is unknown.

As psychosocial stressors may impact physiological mechanisms that can place an individual at risk for mental health problems, it is important to use objective measures that assess the biological changes that occur when facing a stressor. Cortisol levels have been utilized to measure HPA activity (Ito et al., 2005; Raul, Cirimele, Ludes, & Kintz, 2004) and as a potential biomarker of maternal stress (Field & Diego, 2008). Pregnant women who experience high levels of psychosocial stressors have higher cortisol levels than those who experience low psychosocial stress (McLean & Smith, 2001). Although some literature indicates that

dysregulation of HPA axis paired with high cortisol levels are related to experiencing psychosocial stressors (Diego et al., 2006), there are inconsistencies in the findings (Ehlert, Patalla, Kirschbaum, Piedmont, & Hellhammer, 1990; Nierop, Bratsikas, Klinkenberg, Nater, Zimmermann, & Ehlert, 2006). In Mexican American pregnant women, higher reports of acculturation were associated with a flatter diurnal salivary cortisol slope in late pregnancy (D'Anna, Hoffman, Zerbe, Coussons-Read, Ross, & Laudenslager, 2012). Because acculturative stress is associated with mental health problems, including depression, in Mexican American pregnant women, (D'Anna-Hernandez, Aleman, & Flores, 2015), it is important to determine if pregnant Mexican American women are experiencing acculturative stress along with elevated cortisol levels to measure its association with anxiety symptoms.

Hair Cortisol as a Marker of Long-Term Stress Exposure

There are many measures that can be used to assess cortisol, including saliva, serum, and urine; each measure requires multiple samples to accurately assess cortisol levels (Russell, Koren, Rieder, & Van Uum, 2012). However, these are instantaneous measures that are useful for acute, but not chronic measurement of stress. Cortisol hair may provide a useful non-invasive technique to measure long-term cortisol exposure. Physiological concentrations of cortisol are found reliably in human hair (Raul, Cirimele, Ludes, & Kintz, 2004). The use of hair cortisol is a novel, noninvasive technique for biological sample collections (Balikova, 2005) that can be collected once to capture a retrospective calendar of cortisol activity (Wennig, 2000). Hair cortisol is a valid measure of elevated cortisol production for up to six months retrospectively (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009). In addition, given that follicles in human hair respond to CRH stimulation similarly to cortisol secretion that is seen in HPA axis activity (Ito et al., 2005), hair cortisol can be used to measure long-term HPA activity. Hair, unlike other

cortisol measures, can be adjusted for time of day or week collected as it can be cut retrospectively to represent previous cortisol production. Hair cortisol may be a particularly useful tool to capture HPA axis activity during the prenatal period. The first trimester can be a difficult time to access women as they visit clinics for their first prenatal appointments at varying times. However, time can be adjusted by using hair cortisol, as it can be measured retrospectively. For example, the first hair sample that is collected from the root includes cortisol levels three months prior to the time of collection.

Previous literature suggests hair cortisol levels are a valid measure for cortisol output. In a sample of women who were between 2-4 days postpartum, hair samples were collected to measure cortisol levels retrospectively. Results showed a consistent decrease in cortisol levels from hair segments from the root down, demonstrating an increase in cortisol levels from the beginning of pregnancy towards the end of pregnancy (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009). In a sample of pregnant Mexican American women, salivary and hair cortisol levels were correlated and both increased during pregnancy (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011). Thus, hair cortisol levels appear to be a valid measure for prenatal cortisol activity. In a sample of healthy pregnant women, high reports of perceived stress were correlated with high levels of maternal hair cortisol (Karla, Einarson, Karaskov, Uum, & Koren, 2007). However, there is a need to investigate physiological effects and cultural stressors in Mexican American pregnant women. Thus, the use of hair cortisol may shed light on the physiological effects of acculturative stress on maternal anxiety throughout pregnancy.

Current Study

Because acculturative stress is associated with mental health problems in Mexican American pregnant women, it is important to determine if pregnant Mexican American women

are experiencing acculturative stress along with elevated cortisol levels and assess associations with anxiety symptoms. The current study investigated the role of acculturative stress on anxiety and cortisol in Mexican pregnant women. The following study had three hypotheses: 1) It is hypothesized that pregnant women who reported high levels of acculturative stress would report high levels of state anxiety symptoms across pregnancy. 2) Pregnant women who reported high levels of acculturative stress would exhibit increased levels of cortisol, as measured in hair, across pregnancy. 3) Lastly, maternal cortisol levels would mediate the relationship between acculturative stress and anxiety.

Method

Participants

A group of 232 Mexican American pregnant women were asked to participate in the study. Sixty refused to participate. A sample of 172 were recruited at a local community clinic. Twenty-one participants were not included in the study (12 withdrew from the study, 4 moved/transferred clinics, 3 miscarried, 1 did not return the clinic, and 1 withdrew due to health complications). A final sample of 151 women were included in the analysis. The attrition rate of the current study (14%) is similar that of previous studies that report ~15-20% attrition in a similar pregnant population (D'Anna-Hernandez, Aleman, & Flores, 2015; Lee et al., 2007). To be eligible to participate in the study, women must be at least 18 years old, of Mexican descent, have a singleton pregnancy, be non-smoking, and have no current tobacco, illicit, or prescription drug use (prenatal vitamins are acceptable).

Procedure

A longitudinal study design was conducted across pregnancy. Participants completed assessments before or after their prenatal clinic appointments. The assessments were conducted

in the waiting room area or in the medical provider's offices when available. Participants completed a total of five assessments for the study (see Figure 1). The first contact with the participants began when they were less than 15 weeks pregnant. The first assessment consisted of the consent process, demographic questionnaires, acculturative stress questionnaire, and nicotine and illicit drug use urine tests. Acculturative stress was only measured once as acculturation levels are associated with personality factors and change after major life events (Berry, 2005). The three trimester assessments occurred when participants were between 15-17, 25-27, and 33-35 weeks gestation. During the first trimester (15-17 weeks gestation), participants completed state anxiety measures, and the first hair sample was collected. During the second trimester (25-27 weeks gestation), participants completed state anxiety measures, and the second hair sample was collected from the same previous head location. During the third trimester (33-35 weeks gestation), participants completed state anxiety measures. The last assessment was completed at the participants' home after birth and consisted of the third hair sample collection from the same previous location. All measures were offered to participants in both Spanish and English. Participants were compensated $25.00 at the end of every assessment they completed; a total of $125 was provided as compensation for completion of each part of the study.

Recruitment Process

Potential participants were approached in the clinic before or after their appointments. They were asked if they wanted to participate in a study to see how stress impacts mothers and babies in Mexican-Americans. Women who were pregnant and Mexican-American, were presented with a laminated paper timeline that depicted the study procedures. The research assistant described the assessments and time that is required to participate in the study. Women who wanted to participate started their first assessment the same day or at their next appointment.

Women who wanted to think about participating were given a flyer with the study information and phone number and were asked if research staff can follow up with them in their next appointment. All research subjects provided written and informed consent and all procedures were approved by the Institutional Review Board and the clinic's board of directors.

Consent Process

All interested participants provided written consent before beginning the study. During the consent process, participants were told that the researcher planned to learn more about the relationship between factors that relate to a pregnant woman's stress levels. Also, participants were told that hair samples would be collected three times during the study at the same location. Potential discomfort with the questionnaires and hair collection were discussed. Participants were reassured that they may choose to not answer certain questions or choose to provide hair samples themselves if they did not feel comfortable with staff collecting the hair sample. Participants were told that the study is confidential and that participation would not impact their services with the clinic. Also, participants were advised that study is voluntary and they may choose not to take part of the study at any time. Participants who chose to participate signed the consent form and a copy of the consent form was given to all participants.

Demographics Questionnaires

Participants were asked to provide their contact information including name, address, phone number, and alternative contact information. Demographic questionnaires were also used to collect information about participants' place of birth, years in the U.S., primary language, language fluency, and marital status. Previous pregnancy information questions were also asked including gravidity (total number of pregnancies, including current), number of term and preterm deliveries, and history of a miscarriage, number of living children, and pre-pregnancy weight and

height. Participants were also asked to report their socioeconomic information including household annual income, educational years, and employment status. Medical history of any medical problems including chronic hypertension, diabetes, major surgery, lupus, thyroid, asthma, obesity, or other problems were also assessed (see *Appendix A)*.

Acculturative Stress

To measure participants' acculturative stress, the Social Attitudinal Familial and Environmental Stress Scale (SAFE) was used (Padilla, Wagatsuma, & Lindholm, 1985). The SAFE was administered during the first trimester. The SAFE is a 24-item scale with responses that range from 0 "Does not apply" to 5 "Extremely stressful." For instance, one of the items states "I feel uncomfortable when others make jokes about or put down people of my ethnic background." The SAFE has been translated to Spanish and used to determine the relationship between acculturative stress and mental health in both Mexican American immigrant populations (Hovey & Magana, 2000) and Mexican American pregnant women (D'Anna-Hernandez, Aleman & Flores, 2015). The SAFE has shown good reliability scores with a Cronbach's alpha of .90 among Mexican Americans (Hovey, 2000). The current study also showed good reliability scores with a Cronbach's alpha of 0.92.

State Anxiety

Participants were asked to complete the state portion from the State Trait Anxiety Inventory (STAI) once every trimester. The STAI measures participants' current anxiety feelings such as tension, nervousness, and unease at the current moment (Spielberger, Gorsuch, & Lushene, 1970). The STAI contains a 20-item scale that ranges from 1 "Never" to 5 "Very much so." Items include statements such as "I am presently worrying over possible misfortune." The STAI is one of the most commonly validated self-report measures for perinatal state anxiety

(Dennis, Coghlan, & Vigod, 2013; Meades & Ayers, 2011) and has been used in Mexican American populations (Britton, 2008; Engle, Scrimshaw, Zambrana, & Dunkel Schetter, 1990). The STAI Spanish translation has been validated (Novy, Nelson, Smith, Rogers, & Rowzee, 1995). The STAI has shown good reliability scores with a Cronbach's alpha of 0.95 for prenatal state anxiety (Grant, McMahon, & Austin, 2008). The current study also showed good reliability Cronbach's alphas of 0.88 for trimester one, 0.87 for trimester two, and 0.87 for trimester three.

General Stress

To investigate acculturative stress as a unique stressor in Mexican American women, a perceived stress measure was used to control for general stress. Participants completed the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein,1983). The PSS measures participants' degree of stress-related feelings and thoughts in the last month. The PSS is a 14-item scale with a 5 point Likert scale that ranges from 0 "Never/Rarely" to 4 "Very Often." The PSS includes items such as "How often have you felt that you were unable to control the important things in your life?." The PSS has been used to compare general perceived stress and mental health symptoms in Mexican American pregnant women (D'Anna-Hernandez, Aleman, & Flores, 2015). The PSS scale Spanish version has been validated (Sanz-Carrillo, Garcıa-Campayo, Rubio, Santed, & Montoro, 2002). The PSS has good reliability scores with a Cronbach's alpha of .81 for pregnant Latina women (Mann et al., 2010). For the current study, Cronbach's alpha was 0.72.

Hair Cortisol Levels

Hair cortisol levels have been found to be a valid measure and a practical biomarker for maternal HPA function (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Kalra, Einarson, Karaskov, Van Uum, & Koren, 2007). Hair samples were collected at 15-17 weeks,

25-27 weeks, and the home birth assessment from the same head location in both assessments. Hair cutting scissors were used to collect the samples. To obtain the hair sample, the scissors were positioned as close to the scalp as possible and a patch of one centimeter was collected. Hair growth rate is approximately 1 cm per month (Russell, Koren, Rieder & Van Uum, 2012). Previous studies using hair cortisol in pregnant women determined 1.1 cm growth per month (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011). Hair from the same location was collected at all three times to measure the approximate last three months' cortisol production which equates to a trimester. Hair samples were taped and stored in labeled aluminum foil. Hair samples were then washed twice in isopropanol and dried for four days. Next, hair samples were grounded using a Retsch ball mill for approximately 10 minutes at 25 Hz. The powdered hair was weighed in glass tubes and extracted in 1ml of high-performance liquid chromatography (HPLC) grade methanol at room temperature for 24 hours. After samples were extracted, they were transferred to a microcentrifuge tube and spun for 120 seconds. Supernatant was removed and placed into another microcentrifuge tube and left to dry at 38 °C overnight. Samples were then reconstituted with assay buffer and a commercial high sensitivity enzyme immunoassay (EIA) kit (Salimetrics, LLC) was used to determine cortisol levels. To measure the reliability of immunoassay test results, the coefficient of variation (CV) is reported. The CV is defined as the standard deviation of a group of numbers divided by the mean of the group of numbers. The CV is reported in two measures, the inter-assay CV and the intra-assay CV (Salimetrics, LLC). When many samples are tested, it is necessary to use multiple assay plates, the plates where tubes that contain the cortisol samples are placed to be assessed. The inter-assay CV is an expression for the consistency between assay plates. The intra-assay CV is used to calculate the deviation within the samples in the assay plate. To have acceptable consistency between plates, it

is recommended to have an inter-assay CV under 15 and intra-assay CV under 10. For the current study, the inter-assay coefficient was 15.67 and intra-assay coefficient was 16.78. Hair cortisol levels were obtained as pictogram/milligram (pg/mg).

Analytic Approach

Pearson's correlations were computed to determine the relationship between demographic variables, including years of education, income, and age, and state anxiety symptoms. Analyses of variance (ANOVAs) were conducted to investigate the relationship between categorical demographic variables such as employment and marital status with state anxiety. Demographic variables with significant relations to study variables were included as covariates when conducting analyses to test the hypotheses. Hair cortisol values were natural log (LN) transformed to standardize the data as it has been used in previous research (Hoffman et al., 2016).

Because the within-person data obtained likely violate the assumption of independent observations, a multilevel model analysis was used. The intraclass correlation (ICC) coefficient, a statistic that is used to calculate the degree of relatedness between individuals who share a fixed characteristic (Graves & Frohwerk, 2009), was calculated to determine whether there was a clustering effect in the data. The ICC of the current study was 0.542. Therefore, 54.2% of the variance in maternal anxiety scores is associated with the pregnancy trimesters. Thus, a multilevel model is suggested. To test the first hypothesis that pregnant women who reported high levels of acculturative stress would report high levels of state anxiety symptoms across pregnancy, a multi-level model was conducted. In the model, acculturative stress was entered as an independent variable (IV) along with time to predict the trajectory of maternal anxiety throughout pregnancy. To assess significance, a multilevel model analysis provides an

independent variable and time interaction. Thus, if the interaction between acculturative stress and the trajectory of maternal anxiety is significant, then we can determine the influence of acculturative stress on maternal anxiety at trimester one, two and three.

To test the second hypothesis that pregnant women who reported high levels of acculturative stress would exhibit increasing levels of cortisol, as measured in hair, across pregnancy, a multi-level model was conducted. In the model, acculturative stress was entered as an independent variable (IV) along with time to predict the trajectory of hair cortisol levels throughout pregnancy. To assess significance, a multilevel model analysis provides an independent variable and time interaction. Thus, if the interaction of acculturative stress and the trajectory of hair cortisol levels is significant, then we can determine acculturative stress influences hair cortisol levels at trimester one, two and three.

To test the third hypothesis that maternal cortisol levels would mediate the relationship between acculturative stress and maternal anxiety, a mediation analysis was performed using PROCESS in SPSS. Acculturative stress was entered as the IV, hair cortisol levels from the trimester three was entered as the mediator variable, and maternal anxiety symptoms from trimester three was entered as the outcome variable. Previous maternal anxiety, perceived stress and income (see below) were entered as covariates in the model. The indirect effect of acculturative stress on maternal anxiety was computed to assess significance.

Power Analyses

A statistical power analysis was performed using G*Power 3.1 to estimate the required sample size. Because power analysis for multilevel modeling is still in its infancy statistically, power analysis for repeated measures (RM) ANOVA is commonly used in its place (Hayes, 2006). However, no previous studies were found that conducted a repeated measures ANOVA

similar to the proposed study's variables. Thus, as in the case with missing effect sizes, a small to medium standardized effect size was used (Cohen, 1992). For hypothesis one and two, a power analysis was conducted using a small to medium effect size for repeated measures within factors ANOVA of 0.175 (Aberson, 2011). Results show that at 80% power and alpha 0.05, a sample of 68 participants is needed. For hypothesis three, a power analysis was conducted using a medium effect size for a mediation analysis. The effect size for cortisol as a mediator of maternal anxiety and depressive symptoms was 0.08 (Van Den Berg et al., 2008). Power analysis for hypothesis one indicated that for a small to medium effect size of 0.08, at 80% power and alpha of 0.05, a sample of 130 participants is needed. Previous studies have reported approximate 20% for attrition rates (D'Anna-Hernandez, Aleman, & Flores, 2015; Lee et al., 2007). Therefore, 26 participants were added to the calculation to account for attrition rates. In order to achieve statistical power, a sample of 156 participants is needed. The sample size for the current study was $N = 151$.

Results

Descriptives

Income was negatively associated with prenatal anxiety in the second ($r = -0.20$, $p = 0.017$) and third trimester ($r = -0.17$, $p = 0.046$) and included in the analyses as a covariate. All other variables in Table 1 were unrelated to prenatal anxiety. Maternal anxiety symptoms did not differ across pregnancy (RM ANOVA, $F(2, 149) = 2.360$, $p = 0.100$). Anxiety scores in the first trimester ranged from 20-59, with a mean of 36.12 and standard deviation of 9.71. Anxiety scores in the second trimester ranged from 20-63 with a mean of 34.18 and standard deviation of 9.16. Anxiety scores in the third trimester ranged from 20-60 with a mean of 35.17 and standard deviation of 9.45. Although there is no clinical cut-off value for the STAI, previous work in

pregnant women populations have used the scores above 45 as an indicator for high state anxiety (Austin, Tully, & Parker, 2007; Podvornik, Globevnik, & Praper, 2015). In the first, second and third trimesters, respectively, 19.4%, 11.1%, and 15.2 % of women scored above 45. However, all the women reported at least one anxiety symptom across their pregnancy.

Ln-transformed maternal hair cortisol significantly differed across pregnancy (RM ANOVA, F (2, 144) = 7.87, p = 0.001). A Fisher's least significant difference (LSD) test was conducted to detect differences in hair cortisol between trimesters. Hair cortisol levels in the first trimester (M = 1.57, SE = 0.103) were significantly lower than the second trimester (M = 1.90, SE = 0.099; p < 0.001) and third trimester (M = 1.86, SE = 0.097; p = 0.003). However, there were no significant differences in hair cortisol levels between the second and third trimesters (p = 0.751; *Figure 3*). Hair cortisol levels in the first trimester ranged from 0.06-4.35, with a mean of 1.50 and standard deviation of 0.88 natural log (LN) transformed pg/mg. Hair cortisol levels in the second trimester ranged from 0.18-4.56, with a mean of 1.88 and standard deviation of 0.89 LN pg/mg. Hair cortisol levels in the third trimester ranged from 0.18-4.44, with a mean of 1.84 and standard deviation of 0.93 LN pg/mg. Ln-transformed maternal hair cortisol did not significantly differ by season of delivery (RM ANOVA, F (4, 68) = 0.636, p = 0.638), not by body mass index (RM ANOVA, F (3, 64) = 0.51, p = 0.677), nor by pregnancy complications such as premature labor (RM ANOVA, F (2, 64) = 0.742, p = 0.48), or delivery type (RM ANOVA, F (2, 64) = 0.062, p = 0.940).

Acculturative Stress and Symptoms of Anxiety Throughout Pregnancy

It was hypothesized that pregnant women who reported high levels of acculturative stress would report high levels of state anxiety symptoms across pregnancy. A multilevel model analysis showed that for prenatal anxiety, there was no effect of time (b = 1.009, t (153) = 1.390,

$p = 0.160$). However, acculturative stress was significantly correlated with anxiety, such that those who reported higher acculturative stress also reported higher trimester anxiety level on average ($b = 0.164$, t (153) = 2.85, $p = 0.005$). Acculturative stress was associated with the trajectory of anxiety symptoms ($b = -0.047$, t (153) = -2.03, $p = 0.044$), indicating that high levels of acculturative stress were associated with high symptom levels early in pregnancy and steady decline of anxiety symptoms across pregnancy (*Figure 4*).

Acculturative Stress and Hair Cortisol Levels Throughout Pregnancy

It was hypothesized that pregnant women who reported high levels of acculturative stress would exhibit increasing levels of cortisol, as measured in hair, across pregnancy. A multilevel model analysis showed that for prenatal anxiety, there was no effect of time ($b = 1.78$, t (47) = 1.25, $p = 0.21$). Acculturative stress was not associated with hair cortisol levels ($b = -0.04$, t (60) = -0.39, $p = 0.69$). Acculturative stress was also not associated with the trajectory of prenatal anxiety hair cortisol levels ($b = 0.018$, t (46) = 0.42, $p = 0.67$; *Figure 5*).

Hair Cortisol as Potential Mediator Between Acculturative Stress and Symptoms of Anxiety

It was hypothesized that maternal cortisol levels would mediate the relationship between of acculturative stress and maternal anxiety. For the third trimester, five thousand bootstrap samples were run. Results showed no evidence of a significant indirect effect of acculturative stress on anxiety via hair cortisol levels for the third trimester while controlling for previous anxiety and income ($\beta = 0.0018$, SE = 0.0043, 95% CI [-0.0024, 0.0192]). Acculturative stress was also not associated with hair cortisol levels (a path: $b = 0.05$, $p = 0.58$). Furthermore, hair cortisol levels were not associated with prenatal anxiety (b path: $b = 0.03$, $p = 0.55$). Acculturative stress was not associated with prenatal anxiety (c path: $b = 0.01$, $p = 0.85$). The

direct effect of acculturative stress after controlling for the mediating influence of hair cortisol levels on prenatal anxiety was not significant (*c'* path; $b = 0.01$, $p = 0.87$; *Figure 6*).

For the second trimester, five thousand bootstrap samples were run. Results showed no evidence of a significant indirect effect of acculturative stress on anxiety via hair cortisol levels for the second trimester, while controlling for previous anxiety and income ($\beta = -0.0025$, SE = 0.0054, 95% CI [-0.0183, 0.0043]). Acculturative stress was not associated with hair cortisol levels (*a* path: $b = -0.04$, $p = 0.62$). Furthermore, hair cortisol levels were not associated with prenatal anxiety (*b* path: $b = 0.05$, $p = 0.32$). Acculturative stress was not associated with prenatal anxiety (*c* path: $b = 0.01$, $p = 0.69$). The direct effect of acculturative stress after controlling for the mediating influence of hair cortisol levels on prenatal anxiety was not significant (*c'* path: $b = 0.02$, $p = 0.65$; *Figure 7*).

Discussion

This study examined the role of acculturative stress on hair cortisol levels and symptoms of anxiety in Mexican American pregnant women. It was hypothesized that pregnant women who reported high levels of acculturative stress would report high levels of symptoms of state anxiety across pregnancy. It was also hypothesized that pregnant women who reported high levels of acculturative stress would exhibit increased levels of cortisol, as measured in hair, across pregnancy. Lastly, it was hypothesized that maternal cortisol levels would mediate the relationship between acculturative stress and symptoms of state anxiety. Acculturative stress was associated with elevated levels of symptoms of state anxiety early in pregnancy and a slight decline in symptoms in later pregnancy, even when controlling for perceived stress. However, levels of acculturative stress were not associated with the trajectory of hair cortisol levels across

pregnancy. Moreover, maternal hair cortisol was not a significant mediator of the relationship between acculturative stress and symptoms of state anxiety.

Maternal Anxiety

Maternal symptoms of state anxiety were associated with acculturative stress during pregnancy in the current study even while controlling for perceived stress. This finding suggests a unique role of acculturative stress in predicting maternal symptoms of anxiety. These results are consistent with previous work in other populations demonstrating an association between increased acculturative stress and symptoms of state anxiety in groups such as farmworkers, adolescents, college students, and other immigrants (Crockett et al., 2007; Hovey & Magaña, 2000; Revollo et al., 2011; Suarez-Morales & Lopez, 2009). These results are also consistent with the minority stress theory as ethnicity-related stressors are associated in heightened stress responses that are related to anxiety (Williams, Neighbors, & Jackson, 2008; Paradies, 2006). However, the mechanism by which acculturative stress leads to symptoms of anxiety is unclear. General models of stress suggest that perceiving a situation as threatening or exceeding one's ability to cope results in stress and negative emotional experiences (Barlow, 2004; Lazarus & Folkman, 1984) and thus may be associated with anxiety symptoms. Likewise, acculturative stress is associated with negative emotional states, including anxiety (Williams & Berry, 1991). As pregnant Mexican American women go through the acculturation process, it is possible that they feel pressured to assimilate to mainstream culture or experience cultural loss, which both are associated with subjective perception of stress and negative emotions (Crockett et al., 2007; Kartal & Kiropoulos, 2016). The current study's findings also support the stress diathesis theory, which posits that having a predisposition to negative emotional experiences (e.g. dysfunctional thinking and coping) and experiencing a stressor could lead to adverse mental health outcomes

(Cohen, Janicki-Deverts, & Miller, 2007; Leach, Poyser, & Fairweather-Schmidt, 2015; Pagel, Smilkstein, Regen, & Montano, 1990). Individuals who experience unique psychosocial cultural stressors may be at risk for mental health issues due to relatively greater amount of adaptive resources that may be required to cope with these particular types of stressors (Carr & Umberson, 2013; Pearlin & Bierman, 2013). Mexican American women may not have the necessary adaptive resources that can help lessen stress responses associated with cultural adaptation. For example, in Mexican American college students, social support moderated the effects of acculturative stress on anxiety symptoms such that participants who reported high acculturative stress and high social support also reported low anxiety symptoms (Crockett et al., 2007). As decreases in social support are associated with cultural adaptation (Oppedal, Roysamb, & Sam, 2004), acculturative stress may tax social support resources. For example, in Latino immigrants, low levels of acculturative stress were associated with high levels of social support networks (Lueck & Wilson, 2011). Acculturative stress is also associated with psychological and cultural changes that may result in a discord in cultural identification between the native and mainstream culture (Berry, 2006) that may result in stress (Lazarus & Folkman, 1984; Dohrenwend, 2000). It is possible that the distress experienced by incongruence in cultural values, practices, and language (Gil, Vega, & Dimas, 1994) may surpass one's ability to cope. Mexican American pregnant women who experience acculturative stress may perceive it as a threat and not have the ability to cope with such a threat, and thus are at risk for adverse mental health experiences. Moreover, weakening ties with Mexico and having poorly established ties in the U.S. may lead to marginalization within communities or families (Sundquist & Winkleby, 1999). Women who are marginalized may be at risk for experiencing poorer health outcomes because they often lose social support and positive influences of their native culture (Harley &

Eskenazi, 2006). Having strong ties with communities or families is important during pregnancy as it is a vulnerable time for adverse mental health outcomes (Canals, Esparo, & Fernandez-Ballart, 2001). Because of the unique role acculturative stress may have to general stressors experienced during pregnancy, the overall stress could become excessive and in turn influence symptoms of anxiety.

However, acculturative stress was also associated with a slight decline in state anxiety symptoms in late pregnancy. Other studies addressing maternal mental health, depression specifically, have seen a decline in symptoms late in the perinatal period in Mexican-American mothers (Beeghly et al., 2002; Zeiders, Umaña-Taylor, Updegraff, & Jahromi, 2015) or it is possible that during late pregnancy women learn how to cope with cultural stressors and thus experience less anxiety. Given that fetal exposure to maternal mental health has been shown to program infant outcomes early in pregnancy (Davis et al., 2007), it is important to understand why acculturative stress is associated with early perinatal anxiety. To our knowledge, this study was the first to uniquely associate acculturative stress with early perinatal anxiety symptoms, regardless of perceived stress. Stressors associated with cultural adaptation early in pregnancy may place Mexican American women at risk for experiencing anxiety symptoms and place them at risk for adverse perinatal experiences.

Hair Cortisol Levels

Acculturative stress was not associated with the trajectory of hair cortisol levels across pregnancy. Inconsistent with this finding, previous work has identified associations between psychosocial stressors and cortisol levels during pregnancy (McLean & Smith, 2001). In addition, previous work in Mexican-American pregnant women suggests that levels of acculturation are related to a flatter diurnal salivary cortisol slope in late pregnancy (D'Anna,

Hoffman, Zerbe, Coussons-Read, Ross, & Laudenslager, 2012). However, there are differences between the aforementioned and current studies. Results of the current study may not support previous research because of the use of different assessments of cortisol response, including salivary cortisol (Nierop et al., 2006; Obel et al., 2005; Parcells, 2010). The assessment of cortisol is complicated by the diurnal cortisol cycle, which consists of an increase in secretion of cortisol followed by a decline over the remainder of the day (Edwards, Clow, Evans, & Hucklebridge, 2001). Salivary cortisol samples provide acute measurements of stress and are influenced by the time of collection (Russell et al., 2012). Another possible explanation for the difference in findings could be the how different measures of cortisol are driven by different mechanisms that influence the stress response system (McEwen & Gianaros, 2010). For example, impaired immune system function is associated with stress processes in the context of socioeconomic status (Dowd & Goldman, 2006; Wright & Steptoe, 2005). Also, the cardiovascular system is influenced by the stress response system as high wakening cortisol levels are associated with cardiovascular disease (Reynolds et al., 2010). Therefore, looking at different systems may shed light to the physiological response of stressors during pregnancy. Other variables found to alter cortisol levels in pregnancy, such as season of delivery, body mass index, and pregnancy complications (Braig et al., 2015; Hoffman et al., 2016) were not associated with hair cortisol levels in this study. However, frequent hair washing is associated with lower hair cortisol levels (Hoffman et al., 2013) and the current study did not include participants' reports of hair washing. Thus, it is unclear whether physiological stress systems are associated with anxiety during pregnancy in Mexican-American women.

In addition, ethnic differences in patterns of prenatal cortisol have been identified and may be associated with disparities in mental health outcomes in vulnerable populations. For

example, African American pregnant women show a blunting of cortisol increase in the third trimester relative to Hispanic and White women (Glynn et al., 2007). As maternal cortisol levels typically increase across the three trimesters (Kalra et al., 2007; D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011), the blunting of cortisol is atypical. This pattern may reflect discriminatory experiences or allostatic load (cumulative exposure to stressors; McEwen & Gianaros, 2010), which may more clearly mirror the experience of Mexican-Americans in the current study and account for blunted cortisol late in pregnancy. Most of the previous literature is conducted in non-Hispanic White samples and cross sectional studies and our results are representative of a lower income community-based racial/ethnic minority sample. To our knowledge, this study was the first study to investigate acculturative stress and the trajectory of hair cortisol values during pregnancy. More work is needed on ethnic differences in cortisol during pregnancy to understand these different patterns.

Maternal hair cortisol was not a significant mediator of the relationship between acculturative stress and maternal anxiety. Cortisol has been found as a physiological mediator for the relationship between psychosocial stressors and mental health in some (Field & Diego, 2008; Obel et al., 2005), but not all studies (Ehlert, Patalla, Kirschbaum, Piedmont, & Hellhammer, 1990; Nierop, Bratsikas, Klinkenberg, Nater, Zimmermann, & Ehlert, 2006). Elevated maternal stress is associated with dysregulations in cortisol production (Miller, Chen, & Cole, 2009). Dysregulation of cortisol levels has been associated with adverse mental health outcomes, including anxiety (Hughes et al., 2012; McEwen, 2004; Holsboer, 2001). In non-pregnant samples, blunted diurnal patterns of cortisol have been associated with generalized anxiety in elderly individuals (Mantella et al., 2008) and trait anxiety in pre-pubertal adolescents (Van Den Bergh, et al., 2008). In pregnant women, the results are mixed. Work that has looked at single

cortisol samples during pregnancy found no significant associations between salivary cortisol levels and maternal anxiety (Harville et al., 2009) while other work suggests depressed anxious women demonstrate a higher cortisol awakening response (Shea et al., 2007). In addition, in late pregnancy, maternal anxiety has been associated with a flatter diurnal response over the day (Kivlighan, DiPietro, Costigan, & Laudenslager, 2008). The current study only collected state and not trait levels of anxiety to measure the fluctuation of anxiety throughout pregnancy. As hair provides a retrospective measure of the average of area under the curve for cortisol exposure (Wennig, 2000), it may be that associations between trait anxiety and a long-term average measure of diurnal cortisol patterns over the past three months could not be determined. It may be that a moment-to-moment account of HPA function in saliva may have provided a more ideal measurement. Furthermore, cortisol levels are the end product of the HPA axis (Weinstock, 1997). It is possible that other components of the HPA axis, such as glucocorticoid receptors, may better depict the physiological responses when experiencing stress (Webster et al., 2002).

Strengths and Limitations

This study has several strengths. First the current study is a prospective longitudinal study that collected anxiety measures at multiple time points throughout pregnancy to detect any change or stability in symptoms. Second, the study collected multiple hair cortisol samples to investigate long-term physiological mechanisms. In addition, the study was conducted in the vulnerable, fast-growing Mexican population with all women showing anxiety symptoms and over half showing an increase in symptoms during pregnancy. In addition, rates of anxiety (15.2% of women during pregnancy scored >45 on the STAI) were similar to that of the general population (Strine et al., 2015) with all women showing at least some anxiety symptoms. Lastly, the current study accounted for important demographic variables such as income. The study also

accounted for variables that influence hair cortisol levels such as season of delivery, body mass index, and pregnancy complications.

However, there are limitations. First, as this was a longitudinal study, there was attrition. It could be that the most anxious women did not continue in the study, skewing the results; however, rates of attrition in the current study are similar to those in other studies with the same population (D'Anna-Hernandez, Aleman, & Flores, 2015; Lee et al., 2007). Women who did not complete the study reported more symptoms in the first (M = 40.92, SD = 9.63) and second trimester (M = 38.57, SD = 8.38) when compared to those who did complete the study. Also, the study did not include individual experiences that may have influenced cortisol levels such as social support, previous occurrence of a stressful life event (though perceived stress was accounted for), and hair washing frequency, which has been found to be associated with lower hair cortisol levels (Hoffman et al., 2013). Lastly, the study was correlational; therefore, there no causal link between acculturative stress and anxiety symptoms was established.

Conclusion

The present study is one of the first studies to examine the associations between acculturative stress and hair cortisol levels on symptoms of state anxiety in pregnant Mexican American women. Results suggest that Mexican American pregnant women who experience acculturative stress may experience higher symptoms of state anxiety early in pregnancy. Future studies should Include measures of individual experiences (e.g. social support, previous occurrence of a stressful life event). Also, variables that influence hair cortisol levels, including hair washing, should be collected. Lastly, future studies should compare acute measures (e.g. salivary cortisol) and chronic measures (e.g. hair cortisol) of cortisol output. The current study had several implications. First, identifying acculturative stress during early obstetric visits may

help identify factors relevant for symptoms of anxiety early in pregnancy. Second, adding coping mechanisms related to acculturative stress into culturally-based interventions could optimize mother/child perinatal health outcomes. Lastly, findings may promote cultural competence in health care settings. Cultural competence in the health care setting is critical to the health care experience (Englander et al., 2013). Cultural competence may also lead to positive cross-cultural interactions (Harris, 2010) to help health care workers respond with cultural sensitivity to patients' values, customs, and beliefs (Betancourt, Green, & Carrillo, 2002). Thus, these changes could influence decision making and interventions to improve obstetric care.

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Figure 1
Timeline of Experimental Procedure

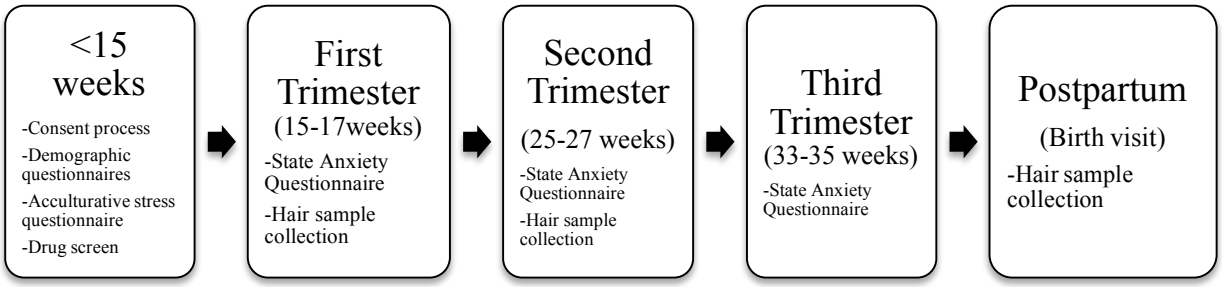

Figure 2
Mediation Model

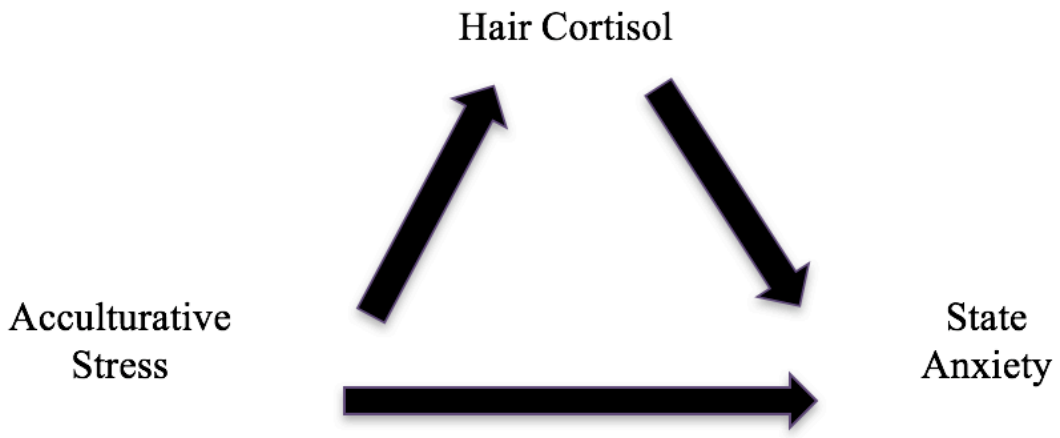

Table 1 Participant characteristics

Characteristics	N	%
Age		
18-20	17	11.3
21-29	88	58.2
30-39	45	29.8
>40	1	0.7
Employment status		
Unemployed	84	55.6
Part-time	37	24.5
Full-time	29	19.2
Unknown	1	0.7
Years of education		
<12	63	36.6
12	56	32.6
>12	32	18.6
Unknown	1	0.7
Marital status		
Married	58	38.4
Living together, not married	67	44.4
Separated	5	3.3
Never married	21	13.9
Family income		
<$10,000	14	9.6
$11,000-$20,000	52	34.4
$21,000-$30,000	50	33.1
$31,000-$40,000	18	11.9
>$41,000	14	9.3
Unknown	3	2.0
Place of birth		
U.S.	50	33.1
Mexico	101	66.9
Primary language		
English	17	11.3
Spanish	109	72.2
Both	25	16.6

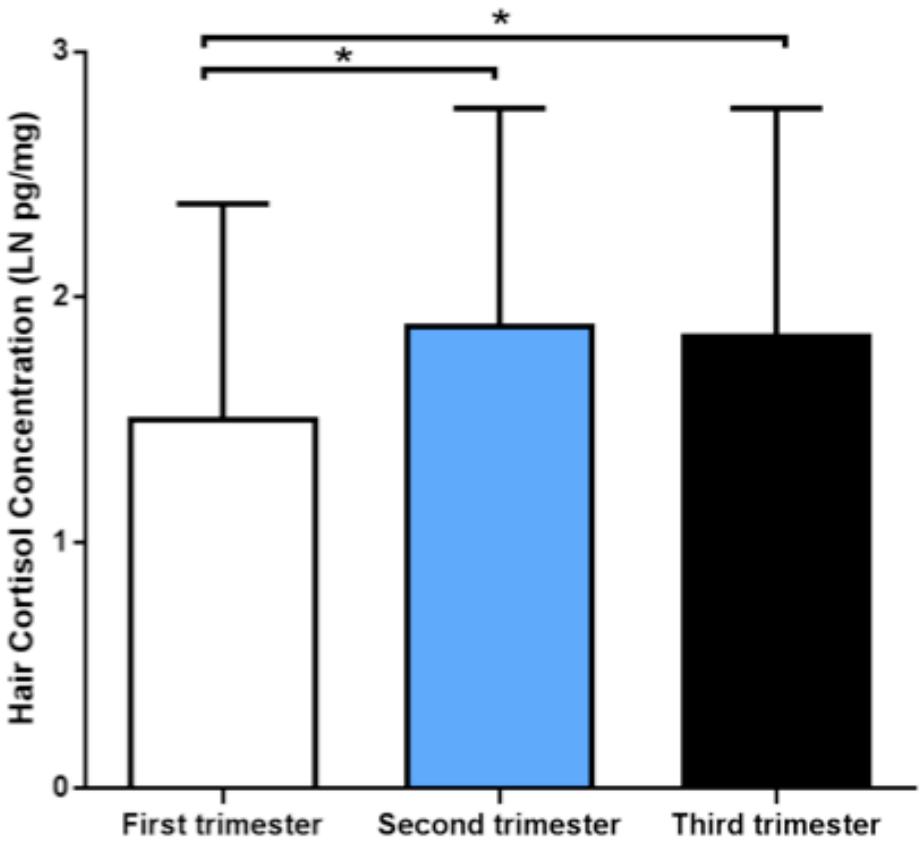

Figure 3. Maternal hair cortisol concentration (natural log [LN] transformed pg/mg) in the first, second, and third trimester of pregnancy. Data represent mean and standard deviations. Brackets represent Fisher's least significant difference (LSD). Hair cortisol levels in the first trimester were significantly lower than the second ($p < 0.001$) and third trimester ($p = 0.003$). There were no significant differences in hair cortisol levels between the second and third trimesters ($p = 0.759$).

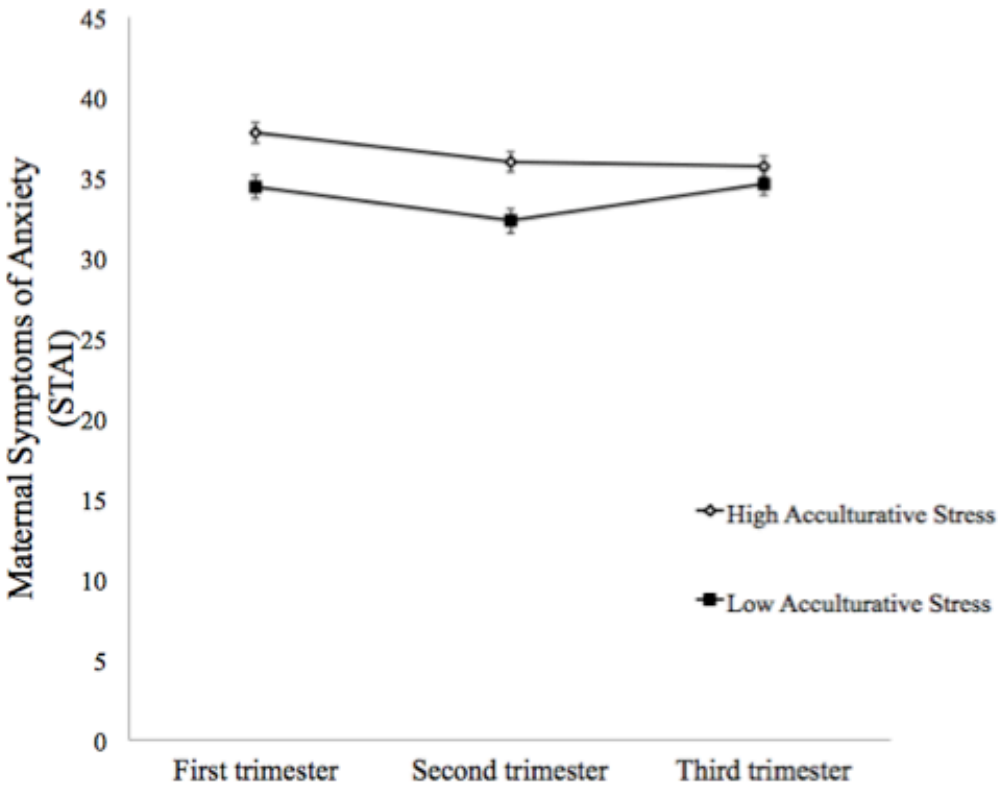

Figure 4. The trajectory of the relationship between acculturative stress and maternal symptoms of anxiety (STAI). For prenatal anxiety, there was no effect of time (b = 1.009, p = 0.16). Pregnant Mexican American women who reported higher acculturative stress also reported high maternal symptoms of anxiety (b = -0.047, p = 0.044). Thus, high levels of acculturative stress were associated with an increase early in pregnancy and steady decline of anxiety symptoms across pregnancy. The figure shows dichotomous categories by using median splits of acculturative stress for graphical representation.

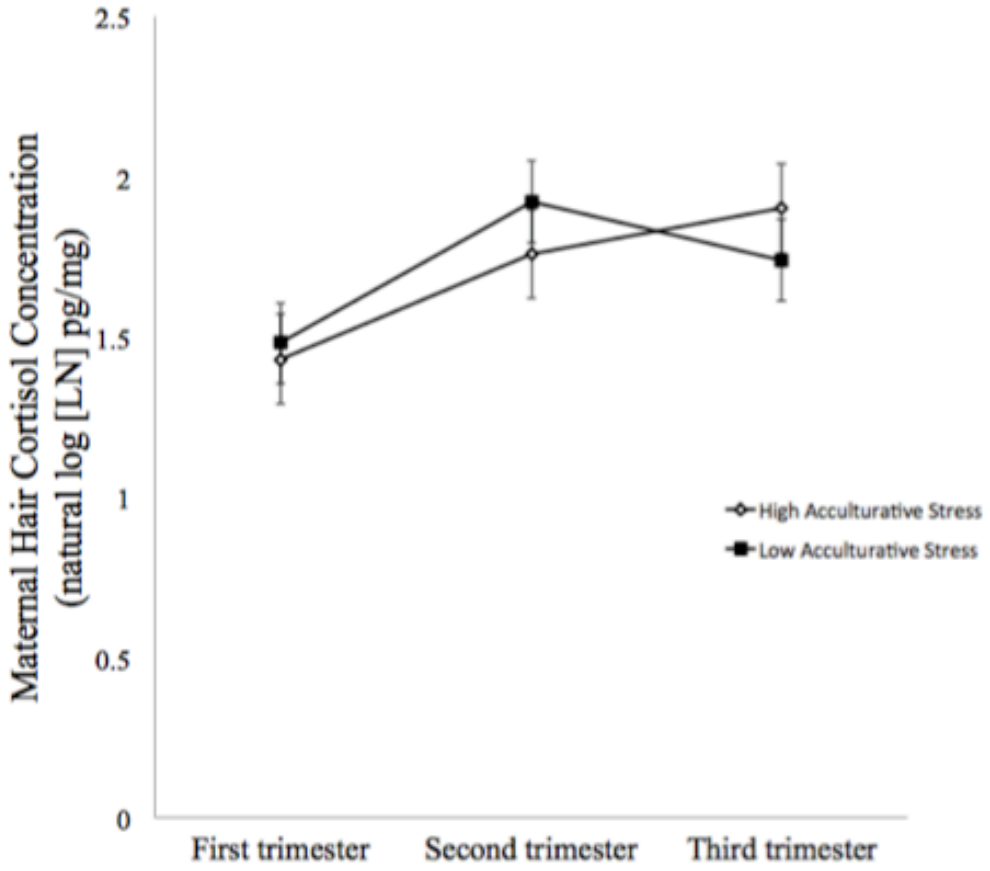

Figure 5. No significant associations were found trajectory of the relationship between acculturative stress and maternal hair cortisol concentration (natural log [LN] transformed pg/mg). The figure shows dichotomous categories by using median splits of acculturative stress for graphical representation.

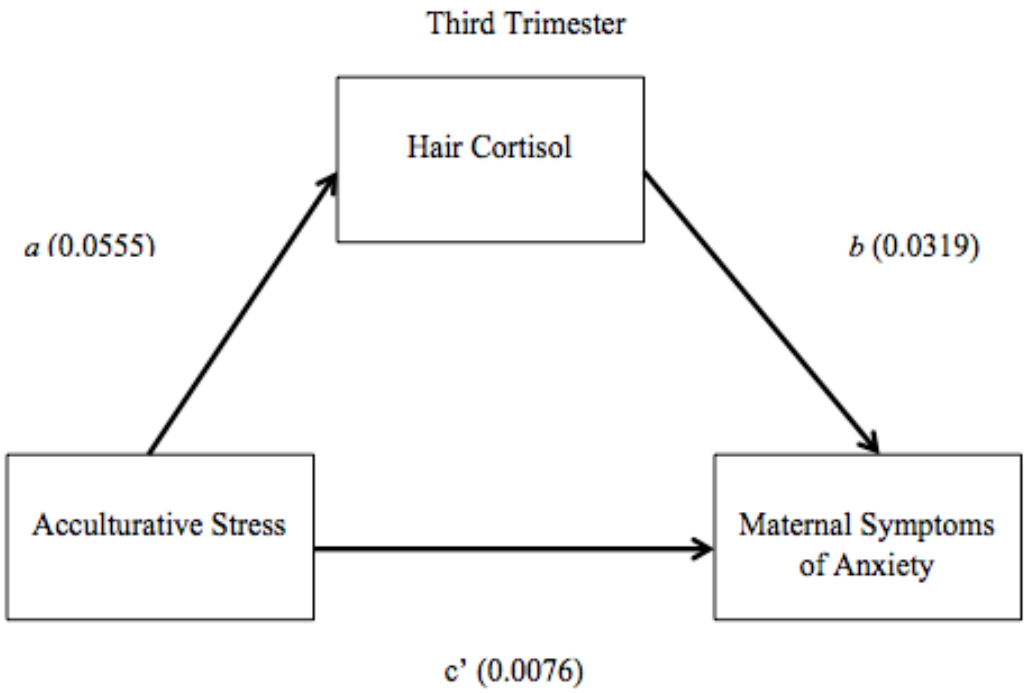

Figure 6. Unstandardized regression coefficients of the relationship between acculturative stress and maternal symptoms of anxiety during the third trimester as mediated by hair cortisol. Hair cortisol is regressed on acculturation to produce *a* (0.0555) and maternal symptoms of anxiety is regressed on hair cortisol and acculturative stress, which yields *b* (0.0319) and c' (0.0076). The indirect effect *ab* = -0.0004 is not statistically different from zero as realized by a 95% confidence interval that includes zero, (-0.0024-0.0192).

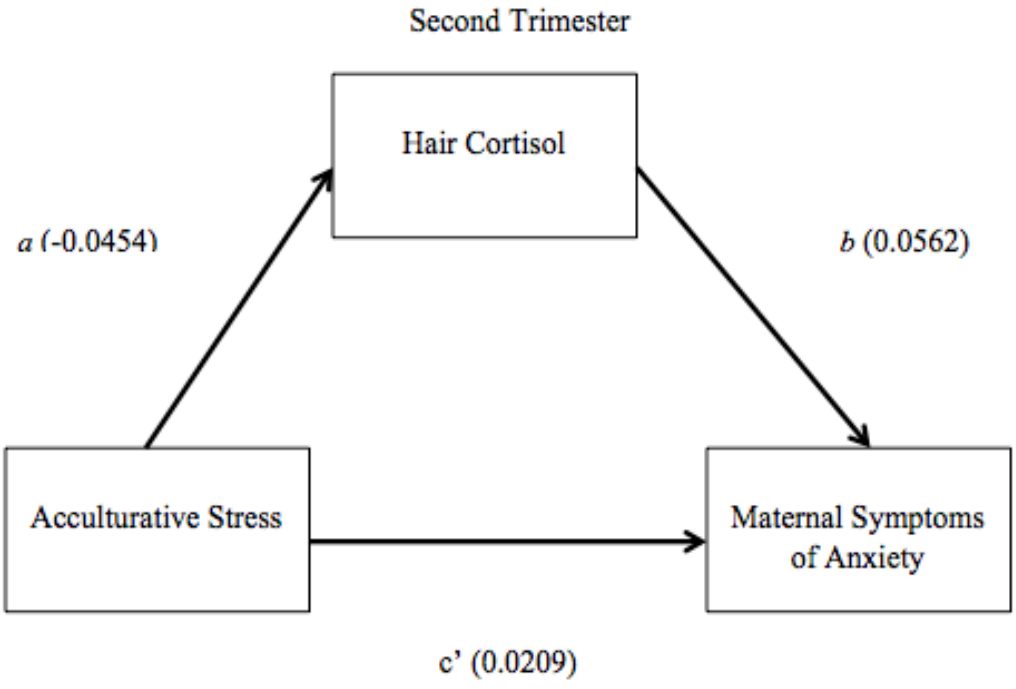

Figure 7. Unstandardized regression coefficients of the relationship between acculturative stress and maternal symptoms of anxiety during the second trimester as mediated by hair cortisol. Hair cortisol is regressed on acculturation to produce *a* (-0.0454) and maternal symptoms of anxiety is regressed on hair cortisol and acculturative stress, which yields *b* (0.0562) and c' (0.0209). The indirect effect *ab* = -0.0004 is not statistically different from zero as realized by a 95% confidence interval that includes zero, (-0.0183-0.0043).