ABSTRACT

Title of Document:	MEASURING ALLOSTATIC LOAD IN A NATIONALLY REPRESENTATIVE SAMPLE OF PREGNANT WOMEN
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Allostatic load (AL) is a measure of cumulative "wear and tear" on the body resulting from exposure to chronic stress. Recently, a potential link between AL and poor birth outcomes was proposed, although it is unknown whether AL can be measured in a meaningful way during pregnancy. To determine this, an AL index was created using data from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. The distribution of AL scores were significantly different in pregnant and non-pregnant women ($p<0.01$). AL scores were associated with race, age, income, and education level in the sample of non-pregnant women, but similar associations were not seen in pregnant women. Overall, the results of this study suggest that AL does not have the same attributes in pregnant women as it does in non-pregnant women. However, the findings suggest directions for future study of AL as a risk factor for poor birth outcomes.

MEASURING ALLOSTATIC LOAD IN A NATIONALLY REPRESENTATIVE SAMPLE OF PREGNANT WOMEN

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Public Health
2012

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List of Abbreviations

AL	Allostatic load
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
DBP	Diastolic blood pressure
HbA1C	Hemoglobin A1C
HCY	Homocysteine
HDL	High-density lipoprotein
HS	High school
MEC	Mobile examination center
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
SBP	Systolic blood pressure
SES	Socioeconomic status

Chapter 1: Introduction

Allostatic load (AL) is considered to be a measure of the cumulative "wear and tear" on the body that results from exposure to chronic psychosocial stress (McEwan and Stellar, 1993). Studies have suggested an association between AL and a variety of health conditions. Recently, a potential link between AL and adverse birth outcomes has been proposed in exploratory articles, although this relationship has been challenging to investigate because it is unknown whether AL can be measured in a meaningful way during pregnancy (Anderson, 2008; Shannon et al., 2007). Pregnant women are generally excluded from studies of AL because there is concern that the changing levels of AL-related biomarkers during pregnancy do not reflect a woman's true AL (Seeman et al., 2008). Indeed, pregnancy changes the levels of many AL-related biomarkers, including blood pressure, heart rate, cholesterol, and metabolic and inflammatory factors (Merck Manual, 2007). As a result, it is challenging to differentiate the effects of chronic stress on these biomarkers from the changes that occur as a normal part of pregnancy.

To our knowledge, this study is the first to determine the usefulness of measuring AL during pregnancy. Although the levels of AL-related biomarkers during pregnancy are different than their levels before and after pregnancy, the relative ranking of AL scores in pregnant women could provide meaningful information about the women's health risks related to chronic stress. A valid measure of AL could help identify pregnant women at risk of poor birth outcomes, such as low birthweight and premature birth, who do not have other known risk factors. Ultimately, this measure might help explain

differences in birth outcomes among women of different race/ethnic backgrounds and socioeconomic classes.

Chapter 2: Background and Literature Review

Chronic stress and risk of adverse birth outcomes

A variety of clinical and behavioral risk factors have been linked to an increased risk of adverse birth outcomes such as low birthweight and premature birth. The maternal risk factors most consistently associated with these outcomes include certain infections, a shortened cervix, a previous preterm birth, maternal age, underweight or overweight during pregnancy, diabetes mellitus, smoking or exposure to secondhand smoke, alcohol or drug abuse, multi-parity, and inadequate prenatal care (NICHD 2010; McCowan et al., 2009; March of Dimes, 2008; Ashdown-Lambert, 2005; Bibby & Stewart, 2004). However, established risk factors account for only a fraction of all poor birth outcomes (NICHD, 2010). Thus, a large proportion of low birthweight and premature infants are born to mothers who are not considered clinically to be "high-risk." Identifying other factors that may help explain these cases is an important challenge in maternal and child health.

Maternal stress has been studied as a potential contributor to adverse birth outcomes (Vrekoussis et al., 2010). Traditionally, stress has been considered in terms of acute events that activate the body's "fight-or-flight" response. Acute stress that occurs during pregnancy, for example exposure to a traumatic event such as the terrorist attacks on September 11, 2001, has been associated with increased incidence of both low birthweight and preterm birth in several studies (Ohlsson et al., 2010; Lauderdale, 2006; Smits et al., 2006). However, chronic stress before and during pregnancy may be an even more important contributor than acute stress to risk of adverse birth outcomes

(Vrekoussis et al., 2010). Chronic stressors are repetitive events or ongoing conditions that activate the physiologic "fight-or-flight" response but are not quickly resolved (Latendresse, 2009). Chronic stressors such as racial discrimination have been widely identified as a risk factors for adverse birth outcomes, including preterm birth, low birthweight, and miscarriage (Vrekoussis et al., 2010; Latendresse, 2009; Parker & Douglas, 2009; Hobel et al. 2008)

Disparities in birth outcomes

Disparities in birth outcomes among women of different race/ethnic backgrounds have been recognized for decades (Blumenshine et al., 2010; Parker Dominguez, 2008). These disparities are most pronounced in non-Hispanic Blacks compared to non-Hispanic Whites (Miranda et al., 2009). For example, in 2009 non-Hispanic Black mothers had more than 1.5 times the rate of preterm births and almost twice the rate of low birthweight infants than non-Hispanic White or Hispanic mothers in the United States (Martin et al., 2011). These disparities have been relatively constant over the past several decades and are correlated with socioeconomic status (SES), social factors such as marital status, and adverse environmental conditions such as exposure to environmental tobacco smoke (Blumenshine et al., 2010; Miranda et al., 2009). Perceived racial discrimination in various settings has also been associated with an increased risk of preterm birth and low birthweight in Black mothers (Giurgescu et al., 2011; Rankin et al., 2011; Collins et al., 2004).

Disparities in birth outcomes are also correlated with SES independent of race. Women with fewer years of education and lower income are more likely to have preterm

births or babies with low birthweight than are women of higher SES (Simmons et al., 2010; Bibby & Stewart, 2004). This link may be explained, in part, by differences in access to prenatal care and other services, and prevalence of behavioral risk factors such as cigarette smoking (Blumenshine et al., 2010; Villabi et al., 2007). Recent studies show that chronic stress is another factor contributing to racial and SES-related disparities in preterm birth and low birthweight (Parker Dominguez, 2008). In addition to individual-level SES, factors related to neighborhood-level SES have also been associated with risk of having a low birthweight or preterm infant (Metcalfe et al., 2011; Vinikoor-Imler et al., 2011). These neighborhood factors include median income, physical incivilities, crime rates, unemployment rates, and racial segregation (Metcalfe et al., 2011).

The weathering hypothesis

Although chronic stress appears to contribute to adverse birth outcomes, the factors that mediate this relationship have not been fully elucidated. To help explain racial and SES-related health disparities in birth outcomes, Geronimus (1992) proposed the concept of "weathering." This hypothesis suggests that the elevated risk of adverse birth outcomes among Black mothers compared to White mothers is due, in part, to premature aging of the body's physiologic systems. These differences affect multiple dimensions of health, including reproduction.

Among White women, there is little evidence of weathering (Geronimus et al., 1996). However, among Black women, the risk of having an infant with low birthweight increases with maternal age. The gap in risk of adverse birth outcomes between Black and White mothers increases as these women get older: Black mothers younger than 20

are about twice as likely as White mothers of the same age to have a low birthweight or very low birthweight infant. However, by age 34, Black mothers are about three times as likely to have a low birthweight infant and more than five times as likely to have a very low birthweight infants than White mothers of the same age (Geronimus et al., 1996). These disparities are especially pronounced among Black and White women of lower SES.

Geronimus (1992) speculated that Black women experience faster physiologic aging than White women for many reasons, among them increased stress due to family, work, and social obligations. Thus weathering could help explain the observed association between exposure to chronic stress and disparities in birth outcomes.

Allostatis and allostatic load

The biological mechanisms that underlie weathering remain unclear, although the concept of allostatic load (AL) has been proposed as one such mechanism (Geronimus et al., 2006). The foundation of AL is allostasis, or "stability through change" (Sterling & Eyer, 1988). Unlike homeostasis, which focuses on a predetermined set-point or a narrow range of physiologic functioning in which health can be maintained, allostasis suggests that there are optimal operating ranges and that physiologic systems work together in complex, nonlinear networks to maintain health (Carlson & Chamberlain, 2005). Allostasis emphasizes the need for dynamic, ongoing adjustments to adapt to external demands (Seeman et al., 1997). An organism achieves allostasis by matching the activity of multiple physiological systems to the ever-changing external environment (Juster et al., 2010).

AL was first introduced by McEwan and Stellar (1993). It is traditionally defined as a measure of the wear and tear the body experiences as it attempts to adapt to life's demands (Seeman et al., 2001). Systems involved in reacting to and coping with stress must be turned on at the appropriate times and then turned off when they are no longer needed. If these systems are turned on for too long or too often, or alternately if they cannot be mobilized adequately when needed, the resulting dysregulation contributes to wear and tear on the body (McEwan, 1998). Repeated cycles of allostasis in response to chronic stress can ultimately cause physiologic systems to operate inefficiently or outside their normal ranges. Consequently, AL tends to increase with age (Crimmins et al., 2003).

McEwan and Seeman (1999) expanded the definition of AL to include "a cascade of cause and effect" that begins with the release of primary mediators, specifically catecholamines and hormones such as cortisol, which comprise the body's "fight-or-flight" response to perceived stress. The release of these chemicals leads to changes in secondary mediators such as increased blood pressure and heart rate, which are measures of cardiovascular function, and changes in the levels of immune system factors such as C-reactive protein. It is these secondary mediators that are typically included in indexes of allostatic load, as they are most often measured clinically (Juster et al., 2010). It has been suggested that repeated and excessive activation of these secondary mediators can lead to tertiary outcomes such as chronic disease and adverse birth outcomes (Karlamangla et al., 2002).

Much of the current knowledge about the association of AL with particular health outcomes comes from the MacArthur Studies of Successful Aging (Karlamangla et al.,

2006; Karlamangla et al., 2002; Seeman et al., 2001; Seeman et al., 1997). These longitudinal studies, which followed a cohort of older American adults, found that higher AL scores were associated with poorer cognitive and physical performance (Karlamangla et al., 2002; Seeman et al., 2001; Seeman et al., 1997), increased all-cause mortality (Geronimus et al., 2006; Karlamangla et al., 2006; Karlamangla et al., 2002; Seeman et al., 2001), and elevated risk of cardiovascular events (Karlamangla et al., 2002). More recent research with other populations has led to similar findings; Borrell et al. (2010) found that AL was associated with an elevated risk of all-cause mortality in NHANES III participants. Mattei et al. (2010) discovered that AL was associated with several chronic diseases, including hypertension, diabetes, cardiovascular disease, and arthritis in participants of the Boston Puerto Rico Health Study.

Allostatic load and health disparities

AL appears to accumulate differently in people of different races, making it a promising mechanism to help explain racial health disparities. In a seminal study of AL and race, Geronimus et al. (2006) found that Blacks had higher AL scores than Whites at all ages, and the difference increased with age. Additionally, Black women overall had higher AL scores than Black men. The authors hypothesize that these differences may be a result of increased exposure to chronic stressors among Black women compared to other groups, stressors which require more "sustained and high-effort coping" that increases wear and tear on the body over time (Geronimus et al., 2006, p. 831). Chyu and Upchurch (2011) using data from NHANES and Peek et al. (2010) using a population-

based sample of adults in Texas also found that Black individuals had higher AL scores overall compared to people of other races.

Few studies have analyzed AL differences in people of race/ethnic backgrounds other than non-Hispanic Black and non-Hispanic White; however, several studies have shown that recent Mexican immigrants tend to have lower AL scores than immigrants who had been in the United States for longer or than U.S.-born Mexicans, Blacks, or Whites (Peek et al., 2010; Kaestner et al., 2010). Another recent study found that chronic work, financial, and caregiving stressors were associated with higher AL scores in middle-aged Mexican American women (Gallo et al., 2011).

Although studies to date have been limited, AL has been inversely correlated with SES independent of race (Gustafsson et al., 2011; Hawkley et al., 2011). Thus AL may also help explain SES-related health disparities. Individual-level SES factors that have been associated with higher AL scores have included lower household income and individual education levels (Chyu & Upchurch, 2011; Gustafsson et al., 2011; Seeman et al., 2008; Sabbah et al., 2008). Neighborhood-level SES factors associated with higher AL scores have included percentage of households with low education, percentage of households with low median income, percentage of households headed by only a female caregiver, and high unemployment rates (Bird et al., 2010; Merkin et al., 2009).

Allostatic load and reproductive health

Although AL has been associated with the risk of multiple health outcomes, only one study to date has examined AL in relation to women's reproductive health. This

study found that higher AL scores in adulthood were associated with self-reported earlier age at menarche (Allsworth et al., 2005).

Based on current knowledge of the effects of chronic stress during pregnancy, several exploratory articles have proposed that AL could help explain adverse birth outcomes (Anderson, 2008; Shannon et al., 2007). In their review, Shannon et al. (2007) assert that growing evidence supports a relationship between maternal stress and adverse pregnancy outcomes, and that AL may help explain these outcomes in women without other identified risk factors. Anderson (2008) adds that patterns seen in failure to progress during labor may be consistent with the AL model. Although these papers raise interesting questions, no studies have yet been undertaken to assess the relationship of AL and birth outcomes. In part, these studies have not been attempted because measuring the effects of stress during pregnancy is complicated by the normal changes in various AL-related biomarkers that occur while a woman is pregnant (Shannon et al., 2007). For this reason, most studies of AL to date have excluded pregnant women.

Public health significance

As of 2009, about 12% of all infants born in the U.S. were preterm (<37 weeks gestation) and 8.2% were low birthweight (<2,500 grams) (Martin et al., 2011). Preterm birth is among the leading causes of infant death (CDC, 2011a; NICHD 2010). Preterm birth and low birthweight are associated with an increased risk of respiratory problems, infections, cerebral palsy, vision and hearing abnormalities, and developmental disabilities (CDC, 2011a; March of Dimes, 2008). Additionally, these adverse birth outcomes have sequelae later in life, including an increased risk of coronary heart disease

and related outcomes such as stroke, hypertension, and non-insulin dependent diabetes in adulthood (Barker et al., 2002). For these reasons, reducing preterm births and low birthweight are among the Healthy People 2020 objectives (Healthypeople.gov, 2011).

Ultimately, finding a mechanism linking maternal chronic stress with risk of adverse birth outcomes could help identify more women at risk of having low birthweight or preterm infants. Determining whether AL can be measured in a meaningful way during pregnancy could set the stage for future studies of AL and birth outcomes.

Chapter 3: Research Questions and Specific Aims

The overall goal of this project was to determine whether AL can be measured in a meaningful way in pregnant women. To accomplish this, the characteristics of AL were examined to determine whether they are similar in pregnant women and non-pregnant women.

The six specific aims of this study were:

Aim 1: Establish norms for the distribution of each of the ten AL-related biomarkers among pregnant and non-pregnant women in the study sample.

Aim 2: Describe the levels of AL-related biomarkers at different stages of pregnancy, including by trimester and in early pregnancy (less than 5 months) vs. late pregnancy (5+ months).

Aim 3: Determine whether results from the study sample are consistent with ranges of AL-related biomarkers reported in clinical data for normal pregnant women.

Aim 4: Create an AL index and calculate AL scores in the sample of pregnant and non-pregnant women. If it is meaningful to measure AL during pregnancy, the mean and distribution of AL scores would be expected to be similar in pregnant and non-pregnant women.

Aim 5: Determine whether AL scores measured at different times during pregnancy are consistent with scores in pregnant women overall.

Aim 6: Determine whether AL has attributes in pregnant women similar to those in non-pregnant women. Specifically, examine AL with regard to race, age, education, and income—all factors known to be associated with AL in non-pregnant individuals.

Chapter 4: Methods

Data source and study population

This study assessed the levels of ten AL-related biomarkers in a nationally representative sample of pregnant and non-pregnant women aged 15-44. The sample came from the National Health and Nutrition Examination Survey (NHANES), an ongoing nationwide study from the National Center for Health Statistics (NCHS). Participants in NHANES are selected and weighted to represent the civilian, non-institutionalized U.S. population.

The NHANES study has two major parts: an in-home interview and a physical examination conducted in a mobile examination center (MEC). In this study, data about participants' demographic and socioeconomic characteristics, as well as medication use, came from the in-home interview. Laboratory test results and results of the physical examination came from the MEC. These data are freely available for download at http://www.cdc.gov/nchs/nhanes.htm. NHANES data are released in 2-year cycles. This study combined data from 1999-2006, an 8-year period (4 cycles) in which pregnant women were oversampled (Mirel et al., 2009).

As recommended in the NHANES documentation (CDC, 2011b), the variable RIDEXPRG was used to identify pregnant women. This variable represents pregnancy status at the time of the physical examination at the MEC and incorporates both urine pregnancy test results and self-reported pregnancy status (Table 1).

Table 1: NHANES variable RIDEXPRG and pregnancy status

Level of RIDEXPRG variable	**Label**	**Definition**
1	Pregnant at exam	All participants with a positive urine pregnancy test AND all participants who reported being pregnant but had a negative urine pregnancy test. (The latter situation accounts for less than 1% of all women in the sample used in this study who were coded as RIDEXPRG=1.)
2	Not pregnant at exam	All participants who reported not being pregnant and who had a negative urine pregnancy test.
3	Could not be determined	All participants who were interviewed but not examined, and who therefore did not have a urine pregnancy test result.

For the purposes of this study, women whose pregnancy status at exam was negative and who reported having given birth within the past year were not included in either the pregnant group or the non-pregnant group. These women were excluded because, although they are no longer pregnant, the levels of AL-related biomarkers are unlikely to return to non-pregnant levels soon after giving birth. The NHANES variable RHQ200, "Now breastfeeding a child?" was used to identify women who gave birth within the past year. Breastfeeding status was not of interest in this study; rather, this question was used because it is only asked of women who reported having had a live birth within one year of the survey. Women who answered either "yes" or "no" to this question were excluded from the sample, as either answer identifies the participant as having given birth within the past year.

In addition to comparing the levels of AL-related biomarkers in pregnant and non-pregnant women, this study considered them at different times during pregnancy: by

trimester and in early vs. late pregnancy. Women in NHANES who reported being pregnant were asked to report the month of their pregnancy. This information was used to determine the trimester of pregnancy: 1^{st} = 1, 2, or 3 months; 2^{nd} = 4, 5, or 6 months; 3^{rd} = 7+ months. It was also used to define early pregnancy (less than 5 months) and late pregnancy (5+ months). Pregnant women who did not report their month of pregnancy but who reported having their last menstrual period less than 2 months prior were considered to be in their first trimester and in early pregnancy. Pregnant women who did not report their month of pregnancy and who did not have their last menstrual period within the past 2 months were not included in this analysis.

Limiting the age range in this study to 15-44 years is consistent with the way the CDC reports natality data, including fertility rates and birth rates (Martin et al., 2011). Excluding women younger than 15 and older than 44 excluded only 6 pregnant women from the 8-year NHANES sample. Clearly, women outside this age range do not represent a significant proportion of all pregnant women, so restricting the ages to 15-44 years does not impact generalizability of the results.

A flow chart outlining how the samples of pregnant and non-pregnant women in this study were chosen from all NHANES participants is included in Appendix 1.

Choosing AL-related biomarkers

There is no single, "gold-standard" approach to operationalizing AL (Seeman et al., 2010). Juster et al. (2010) listed 25 biomarkers that have been used to create AL indices in previous studies. The biomarkers for this study were chosen from that list, based on the availability of each biomarker in the NHANES data set. Several available

biomarkers, including waist-to-hip ratio and body mass index, were excluded because they are unlikely to be meaningful for pregnant women. Glucose and insulin were excluded because they were measured only in fasting NHANES participants and therefore are available only in a small subset of NHANES participants. Additionally, fibrinogen was excluded because it was measured only in participants who were at least 40 years old and was not measured after 2002.

Of the remaining AL-related biomarkers, ten were included in each NHANES data cycle from 1999-2006 (Table 2). All of the biomarkers were measured as part of the physical and laboratory examination at the MEC. Because systolic and diastolic blood pressure were typically read several times over a period of minutes, these measures were averaged to create a single systolic and a single diastolic measurement for each participant. To be included in this study, each participant must have had values for at least 9 of the 10 available biomarkers.

Table 2: AL-related biomarkers chosen for this study

Biomarker	**Type**	**NHANES variable name/units**	**Function/significance** (Juster et al., 2010)
C-reactive protein (CRP)	Immune	LBXCRP (mg/dL)	Protein involved in activating the complement system. Levels rise in response to inflammation.
Albumin	Metabolic	LBXSAL (g/dL)	Water-soluble protein that helps regulate blood volume; the main protein in plasma.
Total cholesterol	Metabolic	LBXTC (mg/dL)	Lipoprotein used in the production of hormones and cell membranes.
High-density lipoprotein (HDL) cholesterol	Metabolic	LBDHDL/ LBDHDD (mg/dL)	AKA "good cholesterol;" a lipoprotein that transports cholesterol from tissues to the liver for removal.
Creatinine	Metabolic	LBDSCRSI (µmol/L)	A breakdown product of creatine phosphate in muscle. A marker of kidney function.
Hemoglobin A1C (HbA1C)	Metabolic	LBXGH (%)	An index of average plasma glucose concentration over a period of several months.
Homocysteine (HCY)	Metabolic	LBXHCY (µmol/L)	An amino acid synthesized from methionine; at high levels is considered a risk factor for cardiovascular disease.
Systolic blood pressure (SBP)	Cardio-vascular	BPXSY1-4 (mm Hg)	Maximum arterial blood pressure during each heartbeat.
Diastolic blood pressure (DBP)	Cardio-vascular	BPXDI1-4 (mm Hg)	Minimum arterial blood pressure during each heartbeat.
60-second pulse rate	Cardio-vascular	BPXPLS (30 sec. pulse * 2)	The number of heartbeats that occur in a 60-second period.

Establishing normal ranges for each AL-related biomarker

The major reason that AL is typically not measured during pregnancy is that the levels of AL-related biomarkers change dramatically during that time. However, research

suggests that these levels change in predictable ways. A literature review was performed to determine the normal ranges of the various AL-related biomarkers during pregnancy. It was then determined whether the level of each biomarker in the NHANES sample fell within the published ranges.

Studies undertaken to determine the reference ranges of specific biomarkers during pregnancy are relatively scarce and have varied methodology. Some studies report reference ranges for multiple biomarkers of interest, while others focus on a single biomarker. The following guidelines were developed to prioritize the available studies: 1) Reference ranges were reported from longitudinal studies, when available, of healthy pregnant women undertaken within the last two decades. 2) Studies with reference ranges provided for the entire pregnancy were preferred; the ranges are often broken down by week or by trimester in these studies. 3) Although the definition of a "healthy" pregnancy varied among the studies, all of the articles must have described their criteria for determining a healthy pregnancy. 4) In rare instances in which more than one study was available that met these guidelines, the reference range from the study with the largest sample size was reported.

In addition, clinical reference materials were used to identify the general trend or direction of change for each AL-related biomarker over the course of a pregnancy. (That is, whether the level of the biomarker increases, decreases, or fluctuates during pregnancy.) The mean level of each biomarker in the sample of pregnant women was then compared to the reference ranges established through previous studies and to the expected changes during pregnancy described in clinical reference materials.

Creating the AL index and reporting AL in pregnant and non-pregnant women

The AL index was created following the methods of several key AL studies (Seeman et al., 2008; Geronimus et al., 2006; Seeman et al., 2004; Seeman et al., 2001; Seeman et al., 1997). As in these studies, the current study used empirical cutoff points to establish "low-risk" and "high-risk" values for each biomarker. Those participants in the high-risk quartile for each biomarker were given a score of 1 for that biomarker; the others were considered low-risk and given a score of 0. For most AL-related biomarkers, high-risk values represent the top 25% of the distribution. However, for albumin and HDL cholesterol, high-risk values are those in the bottom 25% of the distribution.

The use of certain medications was taken into account when scoring several biomarkers, following Geronimus et al. (2006). Regardless of whether the value of total cholesterol and HDL cholesterol fell within the high-risk quartile, a participant was given a score of 1 for total and HDL cholesterol if she reported current use of medications to control cholesterol. Similarly, participants received a score of 1 for hemoglobin A1C if they reported current use of insulin or pills to lower blood sugar. Additionally, participants received a score of 1 for systolic and diastolic blood pressure if they reported current use of mediations to control blood pressure.

The argument for taking medication use into account when scoring AL is that these participants are considered to be clinically high-risk for conditions related to particular AL-related biomarkers. The use of these medications suggests that the individuals are at high enough risk of cardiovascular disease, diabetes, or other health conditions to require pharmacologic intervention, and thus should be counted as part of the "high-risk" group regardless of their current levels of the relevant biomarkers.

Scores for each biomarker were summed to create an AL score for each participant. Because each biomarker can be scored as either 0 or 1, AL scores range from 0 to 10.

Determining attributes of AL in pregnant women

To determine the attributes of AL in pregnant women, its characteristics in pregnant women were compared to those in non-pregnant women. Specifically, the association of AL with age, race, and SES were assessed. The two SES measures considered in this analysis were highest education level obtained and ratio of family income to poverty. These are among the few SES-related variables available in NHANES and were also used as measures of SES in several previous AL studies (Bird et al. 2010; Merkin et al. 2009; Kaestner et al. 2009; Seeman et al. 2008; Allsworth et al. 2005). Based on the results of previous research, it was hypothesized that pregnant women with higher AL scores would be more likely to be older, to be non-Hispanic Black vs. non-Hispanic White or Mexican American, to have lower income, and to have higher education levels compared with pregnant women with lower AL scores (Chyu & Upchurch, 2011; Seeman et al., 2008; Geronimus et al., 2006; Crimmins et al., 2003; Seeman et al., 1997).

There are numerous ways to define high and low AL. Most studies determine the threshold(s) for categorizing AL scores based either on previous studies or on the distribution of AL scores in the study sample (Juster et al., 2010). Previous research has suggested that AL is associated with significant differences in morbidity and mortality when AL scores reach 3 or 4 (Kaestner et al., 2009; Geronimus et al, 2006; Seeman et al.,

1997). Kaestner et al. (2009) performed analyses using both 3 and 4 as the cutoff for high AL, and found similar results in their study population with either cutoff. In the current study, high AL was defined as a score greater than or equal to 4, and low AL as a score less than 4. For completeness, the data were also analyzed using a high/low cutoff of 3.

Because having only two categories, high and low AL, is a relatively crude way to consider this construct, another analysis was performed with AL categorized into three groups: low (AL score of 0 to 1), moderate (AL score of 2 to 3), and high (AL score of 4 or greater). This categorization is based on approximate tertiles of the distribution of the pregnant and non-pregnant NHANES samples. Several past studies have also categorized AL using more than two groupings (Borrell et al., 2010; Peek et al., 2010; Seeman et al., 2004).

Statistical analyses

Because NHANES has a complex, non-random sampling design, the complex survey design procedures in SAS 9.2 were used for the statistical analysis. NHANES provides sample weights that account for non-response, stratification, and clustering (CDC, 2006). Variables to estimate variance estimation are also provided by NHANES for the primary sampling units (SDMVPSU) and strata (SDMVSTRA). Subsamples of NHANES data require special weighting; this study used the subsample weights included in the laboratory files. These subsample weights were combined for the four data cycles (8-year sample weights) as instructed in the NHANES Analytic and Reporting Guidelines (CDC, 2006).

All NHANES data for this study, including demographic information and laboratory data, were downloaded from the NHANES website (CDC, 2011b). The data files from the four data cycles 1999-2006 were appended and merged, recoding variables as appropriate.

For the first two research aims, which described the distribution of the ten AL-related biomarkers, two-sample independent t-tests were used to compare the mean levels of each biomarker in the samples of pregnant and non-pregnant women and for women in early vs. late pregnancy. ANOVA was used to compare the mean levels of each biomarker in women in the 1^{st}, 2^{nd}, and 3^{rd} trimesters of pregnancy. All tests performed in this study were two-sided and the alpha level was set to 0.05. Significant p values are designated with an asterisk in all tables and figures.

Research aims 4 and 5 involved calculating and then comparing AL scores in different groups of women. Because AL is an ordinal variable and is not normally distributed, non-parametric tests were employed to compare the distributions in different groups. The Wilcoxon rank sum test was used to compare the distributions of AL scores in the samples of pregnant and non-pregnant women and for women in early vs. late pregnancy. The Kruskal-Wallis test was used to compare the distributions of AL scores in women in the 1^{st}, 2^{nd}, and 3^{rd} trimesters of pregnancy. These tests are the non-parametric analogs of the independent two-sample t test and ANOVA, respectively.

Three types of statistical analyses were performed as part of research aim 6, which determined the attributes of AL in pregnant women with regard to race, age, income, and education level. The first was a univariate analysis to determine the relationship between AL category and each demographic variable. Chi-square and

ANOVA tests were used, as appropriate, to compare the proportions of high and low AL separately by race, age, income, and education level. The second analysis was a multivariate logistic regression to examine the contribution of each demographic variable (race, age, income, and education level) as a predictor of high AL when each of the other variables was held constant. The third was a cluster analysis, a process used to discover natural groupings and identify underlying patterns in a data set (Frades & Matthieson, 2010).

The present study appears to be only the second study to analyze AL using cluster analysis. Von Thiele et al. (2006) used cluster analysis to group variables related to recovery from work stress, and then used those groupings to predict AL scores in a group of adult women. The current study is the first to attempt a cluster analysis to help describe the characteristics of AL in a population.

The cluster analysis in this study employed the average linkage method to produce hierarchical clusters, which were then analyzed graphically in a dendrogram. The characteristics of the two resulting clusters were compared using independent two-sample t-tests (mean age and ratio of family income to poverty), chi-square tests (race and education level), and a two-sided Wilcoxon two-sample test (AL score). SAS 9.2 does not have a survey procedure for cluster analysis, so clusters were created and descriptive statistics calculated using the non-weighted observations. Because cluster analysis is an exploratory technique, this analysis still provided an overview of the characteristics of each cluster.

Human subject protections

All of the NHANES data used in study are de-identified and publicly available. People who choose to participate in the NHANES study give written informed consent, and NHANES data collection has been approved by an CDC institutional review board (CDC, 2006). The aims and methods of the current study were approved (as exempt) by the institutional review board at the University of Maryland, College Park on September 1, 2011.

Chapter 5: Results

Research Aim 1: Assessing AL-related biomarkers in pregnant and non-pregnant women

Demographic information

There were significant differences in age, race, and marital status among pregnant and non-pregnant women in the NHANES sample (Table 3). Pregnant women were younger and more likely to be married than non-pregnant women ($p<0.01$). This is to be expected, as a majority of children are born to younger, married women (Martin et al., 2011). Pregnant women were less likely to be non-Hispanic White and more likely to be non-Hispanic Black or Mexican American than non-pregnant women ($p<0.01$). This is also not surprising, as the birth rate for non-Hispanic Black and Mexican American women is higher than that for non-Hispanic White women in the United States (Martin et al., 2011). There was no significant difference in education level or ratio of family income to poverty between the pregnant and non-pregnant women ($p=0.16$ for education, $p=0.21$ for income).

Table 3: Demographics of pregnant and non-pregnant women

	Pregnant	**Non-pregnant**	**P value**
N	1138	4993	
Mean age	27.39	30.57	<0.01*
Race			<0.01*
Non-Hispanic White	56.43%	66.13%	
Non-Hispanic Black	14.22%	12.55%	
Mexican-American	16.14%	9.43%	
Other	13.20%	11.89%	
Education			0.16
Less than high school	22.44%	23.60%	
High school diploma	18.97%	22.98%	
More than high school	58.58%	53.42%	
Marital status			<0.01*
Married	64.27%	44.90%	
Widowed	<1%	<1%	
Divorced	<1%	7.28%	
Separated	1.84%	3.80%	
Never married	20.85%	35.45%	
Living with partner	12.08%	8.57%	
Ratio of family income to poverty	2.62	2.74	0.21

Distributions of AL-related biomarkers

The mean levels of each of the ten AL-related biomarkers were significantly different among pregnant and non-pregnant women in the NHANES sample ($p<0.01$; Table 4). Further discussion of the levels of each biomarker in pregnant and non-pregnant women is included with the results of research aim 3.

Table 4: Means and quartiles of AL-related biomarkers in pregnant and non-pregnant women

Measure	**Pregnant women**	**Non-pregnant women**	**Pregnant women**			**Non-pregnant women**		
	Mean (SE)	**Mean (SE)**	**25th centile**	**50th centile**	**75th centile**	**25th centile**	**50th centile**	**75th centile**
CRP (mg/dL)	0.78 (0.06)	0.41 (0.02)*	0.25	0.49	0.91	0.05	0.17	0.45
Albumin (g/dL)	3.60 (0.02)	4.27 (0.01)*	3.21	3.55	3.88	4.01	4.23	4.44
Total chol. (mg/dL)	213.24 (2.65)	186.70 (0.73)*	171.76	209.59	244.51	160.09	182.81	207.85
HDL chol. (mg/dL)	64.38 (0.99)	55.70 (0.38)*	51.48	62.95	73.79	44.15	53.60	64.15
Creatinine (umol/L)	48.22 (0.79)	63.49 (0.27)*	35.54	43.83	53.03	53.03	60.12	70.71
HbA1C (%)	4.95 (0.02)	5.18 (0.01)*	4.68	4.89	5.10	4.88	5.07	5.26
HCY (umol/dL)	4.44 (0.06)	6.90 (0.06)*	3.48	4.22	5.07	5.50	6.46	7.65
SBP (mm Hg)	108.34 (0.62)	111.00 (0.23)*	101.28	107.72	113.68	103.09	109.27	117.32
DBP (mm Hg)	59.60 (0.62)	68.50 (0.27)*	53.26	59.31	66.22	61.59	67.95	74.43
60-sec pulse	85.76 (0.50)	75.88 (0.24)*	76.11	83.57	92.90	66.65	74.00	82.21

Research Aim 2: AL-related biomarkers among pregnant women by stage of pregnancy

Demographic information

Based on the NHANES sample, there were no significant differences in mean age, race, marital status, education level, or ratio of family income to poverty in pregnant women in their 1st, 2nd, or 3rd trimesters, or in pregnant women in early vs. late pregnancy ($p>0.05$ for all; Tables 5 and 6). This result is as expected, as there is no social or biological reason to believe that women in different stages of pregnancy would differ in their demographic characteristics.

Table 5: Demographics of pregnant women by trimester

	1st trimester	**2nd trimester**	**3rd trimester**	**P value**
N	209	412	376	
Mean age	27.58	27.05	27.85	0.54
Race				0.18
Non-Hispanic White	64.44%	56.62%	52.80%	
Non-Hispanic Black	10.67%	14.95%	13.23%	
Mexican-American	18.00%	16.26%	15.12%	
Other	6.89%	12.17%	18.85%	
Education				0.18
Less than high school	26.59%	20.27%	15.44%	
High school diploma	12.75%	22.13%	15.36%	
More than high school	60.66%	57.60%	69.20%	
Marital status				0.69
Married	64.06%	65.37%	70.17%	
Widowed	<1%	<1%	<1%	
Divorced	<1%	<1%	<1%	
Separated	1.39%	1.64%	<1%	
Never married	21.50%	21.50%	14.89%	
Living with partner	12.47%	11.34%	14.89%	
Ratio of family income to poverty	2.50	2.56	2.87	0.12

Table 6: Demographics of pregnant women by early vs. late pregnancy

	Early pregnancy	**Late pregnancy**	**P value**
N	349	648	
Mean age	27.72	27.31	0.58
Race			0.16
Non-Hispanic White	60.39%	55.34%	
Non-Hispanic Black	12.27%	13.84%	
Mexican-American	18.49%	14.88%	
Other	8.85%	15.94%	
Education			0.09
Less than high school	24.72%	17.27%	
High school diploma	12.10%	20.91%	
More than high school	63.18%	61.82%	
Marital status			0.29
Married	66.78%	66.61%	
Widowed	<1%	<1%	
Divorced	<1%	<1%	
Separated	2.08%	<1%	
Never married	20.92%	18.04%	
Living with partner	9.81%	13.93%	
Ratio of family income to poverty	2.55	2.72	0.36

Distributions of AL-related biomarkers

Levels of all but one of the AL-related biomarkers (albumin) were significantly different among women in different trimesters of pregnancy in the NHANES sample ($p<0.05$; Table 7). Levels of all but two of the AL-related biomarkers (albumin and systolic blood pressure) were significantly different in women in early vs. late pregnancy ($p<0.05$; Table 8). Further discussion of the levels of each biomarker in different stages of pregnancy is included in the results of research aim 3.

Table 7: Means and quartiles of AL-related biomarkers in pregnant women by trimester

Measure	**Means by trimester**				**1st trimester quartiles (N=209)**			**2nd trimester quartiles (N=412)**			**3rd trimester quartiles (N=376)**		
	1st trimester	**2nd trimester**	**3rd trimester**	**P value for means**	**25th**	**50th**	**75th**	**25th**	**50th**	**75th**	**25th**	**50th**	**75th**
CRP (mg/dL)	0.82 (0.11)	0.71 (0.06)	0.73 (0.09)	0.69	0.18	0.49	1.02	0.26	0.50	0.86	0.27	0.50	0.84
Albumin (g/dL)	3.93 (0.03)	3.55 (0.04)	3.21 (0.03)	<0.01*	3.64	3.87	4.14	3.24	3.49	3.80	2.91	3.15	3.42
Tot. chol. (mg/dL)	172.00 (2.57)	218.72 (3.71)	248.10 (4.74)	<0.01*	151.42	165.78	188.22	191.56	212.94	243.77	219.40	243.57	273.40
HDL chol. (mg/dL)	59.33 (1.49)	68.40 (1.60)	67.61 (1.91)	<0.01*	50.07	56.93	67.33	54.04	68.47	80.18	54.31	66.98	79.23
Creatinine (umol/L)	53.78 (1.97)	44.53 (0.87)	45.88 (1.07)	<0.01*	40.53	53.01	53.74	35.38	40.33	53.02	35.39	41.94	53.03
HbA1C (%)	5.05 (0.03)	4.86 (0.02)	4.96 (0.02)	<0.01*	4.75	5.00	5.20	4.57	4.81	5.00	4.68	4.91	5.08
HCY (umol/dL)	4.90 (0.14)	3.96 (0.06)	4.06 (0.09)	<0.01*	4.01	4.55	5.55	3.32	3.79	4.51	3.41	3.90	4.73
SBP (mm Hg)	109.31 (1.16)	105.92 (0.81)	110.26 (0.87)	<0.01*	101.04	107.83	114.83	99.57	105.74	111.95	104.61	109.59	115.06
DBP (mm Hg)	62.14 (1.00)	56.09 (1.00)	59.80 (1.15)	<0.01*	55.07	61.59	68.81	49.99	56.85	61.97	53.47	59.43	66.57
60-sec pulse	82.36 (0.95)	85.54 (0.88)	90.17 (0.87)	<0.01*	72.58	80.46	90.53	76.94	83.00	91.91	80.84	88.37	99.64

Table 8: Means and quartiles of AL-related biomarkers in pregnant women by early vs. late pregnancy

Measure	**Means for early and late pregnancy (SE)**			**Early pregnancy (N=349)**			**Late pregnancy (N=648)**		
	Early	**Late**	**p value for means**	**25th centile**	**50th centile**	**75th centile**	**25th centile**	**50th centile**	**75th centile**
CRP (mg/dL)	0.82 (0.07)	0.69 (0.06)	0.17	0.22	0.58	0.99	0.26	0.49	0.85
Albumin (g/dL)	3.84 (0.03)	3.33 (0.03)	<0.01*	3.58	3.81	4.08	3.01	3.26	3.53
Total chol. (mg/dL)	184.00 (2.95)	238.31 (3.22)	<0.01*	160.31	173.87	200.79	209.53	235.60	261.99
HDL chol. (mg/dL)	61.74 (1.11)	68.37 (1.39)	<0.01*	50.61	60.33	71.35	54.04	68.19	80.95
Creatinine (umol/L)	50.66 (1.44)	45.31 (0.87)	<0.01*	37.69	53.00	53.03	35.38	41.32	53.02
HbA1C (%)	5.00 (0.02)	4.90 (0.02)	<0.01*	4.73	4.94	5.16	4.62	4.85	5.05
HCY (umol/dL)	4.60 (0.13)	4.01 (0.07)	<0.01*	3.62	4.34	5.20	3.34	3.88	4.53
SBP (mm Hg)	107.91 (0.93)	108.62 (0.65)	0.56	100.67	106.88	114.34	103.07	107.68	113.54
DBP (mm Hg)	60.86 (0.81)	57.73 (0.91)	0.01*	54.19	60.68	66.44	51.52	57.40	63.91
60-sec pulse	83.51 (0.83)	88.19 (0.68)	<0.01*	73.52	82.20	90.94	78.34	86.04	96.77

Research Aim 3: Establishing normal ranges for each AL-related biomarker

The existence of clinically established reference ranges for each AL-related biomarker in healthy pregnant women suggests that each of the biomarkers changes by a predicable amount during pregnancy (Table 9).

Table 9: Clinically established reference ranges for AL-related biomarkers in pregnant women

AL-related biomarker	**Expected change during pregnancy: Clinical reference materials**		**Reference ranges in healthy pregnant women: Results of clinical studies**			
	Expected change	*Source(s)*	*Reference range*	*Sample size*	*Criteria for determining a healthy pregnancy*	*Source*
C-reactive protein	Increases during pregnancy but typically remains within the normal range of non-pregnant women.	Gronowski, 2004	Serum CRP, mg/dL --Week 7-17: 0.32-11.91 --Week 17-24: 0.40-14.02 --Week 24-28: 0.43-20.28 --Week 28-31: 0.43-36.97 --Week 31-34: 0.33-11.92 --Week 34-38: 0.64-28.26	52	"Normal, spontaneous pregnancy at booking." Excluded women taking drugs other than iron and folic acid.	Larsson et al, 2008
Albumin	Begins to decline in early pregnancy, and decline continues throughout pregnancy. Decreases by 10-20% compared to pre-pregnancy levels. Overall fall of about 10 g/L.	Gronowski, 2004; Lockitch, 1993; Hytten & Lind, 1973	Blood albumin, g/L --Week 7-17: 32.2-43.2 --Week 17-24: 27.9-36.9 --Week 24-28: 27.0-34.6 --Week 28-31: 25.1-33.7 --Week 31-34: 24.4-33.7 --Week 34-38: 23.1-33.8	52	"Normal, spontaneous pregnancy at booking." Excluded women taking drugs other than iron and folic acid.	Larsson et al, 2008

Total cholesterol	First decreases, but increases by 2nd and 3rd trimesters. Overall increases 30-50% compared to pre-pregnancy levels.	Gronowski, 2004; Lockitch, 1993; Hytten & Lind, 1973	Cholesterol, mg/dL --1st tri: 177.9 +/- 33.4 (144.5-211.3) --2nd tri: 254.6 +/- 47.4 (207.2-302.0) --3rd tri: 282.6 +/- 72.3 (210.3-354.9)	23	Normal course and outcome of pregnancy, not taking any medication known to interfere with lipid metabolism.	Belo et al., 2004
HDL cholesterol	Increases by 12 weeks of pregnancy and remains increased through the rest of pregnancy. Maximum values at around 20 weeks. Total increase of 20-25%.	Gronowski, 2004; Lockitch, 1993	HDL-C, mg/dL --1st tri: 53.6 +/- 11.9 (41.7-65.5) --2nd tri: 63.3 +/- 8.4 (54.9-71.7) --3rd tri: 56.1 +/- 10.3 (45.8-66.4)	23	Normal course and outcome of pregnancy, not taking any medication known to interfere with lipid metabolism.	Belo et al., 2004
Creatinine	Significantly lower in pregnant women compared to non-pregnant women. Decreases 30% in pregnancy compared to pre-pregnancy levels.	Gronowski, 2004; Lockitch, 1993	Plasma creatinine, μmol/L --Week 7-17: 36-62 --Week 17-24: 34-58 --Week 24-28: 32-62 --Week 28-31: 32-56 --Week 31-34: 34-58 --Week 34-38: 33-60	52	"Normal, spontaneous pregnancy at booking." Excluded women taking drugs other than iron and folic acid.	Larsson et al, 2008

Hemoglobin A1C	Decreases during pregnancy. Reaches its lowest point between 23 and 26 weeks, then begins to increase again.	Gronowski, 2004	White, non-fasting HbA1C, % --1st tri: 4.6-5.6 --2nd tri: 4.5-5.6 --3rd tri: 4.5-6.1 Asian, non-fasting HbA1C, %[13] --1st tri: 4.7-5.8 --2nd tri: 4.7-5.7 --3rd tri: 4.3-6.1	517	Uncomplicated singleton pregnancies. Excluded: "known or suspected renal, cardiac, liver, metabolic, or hematological diseases." Also, diabetes, any previous history of OB complications, spontaneous abortions, stillbirths, offspring with congenital anomalies, family history of diabetes.	Hartland et al., 1999
Homocysteine	Decreases by about 50% during pregnancy. Most of this decrease occurs in the first and second trimesters; homocysteine levels are stable during the third trimester.	Robinson, 2000	Plasma total homocysteine, μmol/L[4] --9 weeks: 5.50-16.12 --16 weeks: 4.28-12.40 --20 weeks: 4.25-12.64 --24 weeks: 4.03-12.55 --28 weeks: 3.93-12.06 --32 weeks: 4.38-11.73 --36 weeks: 4.46-11.73	108	Uneventful pregnancies and healthy term babies.	Velzing-Aarts et al., 2005

Blood pressure (systolic & diastolic)	Total decrease in mean arterial pressure of about 10 mm Hg. Lowest level by 24th week, rises slowly through the rest of pregnancy. Decrease in blood pressure is almost entirely due to fall in diastolic BP. There is relatively little change in systolic BP.	Chamberlain & Broughton-Pipkin, 1998	Systolic, 5th-95th centile (Caucasians), mm Hg: --Weeks 6-13: 90-128 --Weeks 14-25: 89-129 --Weeks 26-42: 90-132 Diastolic, 5th-95th centile (Caucasians), mm Hg: -- Weeks 6-13: 48-75 -- Weeks 14-25: 48-74 -- Weeks 26-42: 49-79	3234	No pregnancy-induced hypertension or preeclampsia. Other exclusion criteria: antihypertensive medication, emergency referral to the medical center.	Ochsenbein-Kolble et al., 2004
Heart rate	Considerably increased in early pregnancy, reaches maximum in 3rd trimester. Ultimately rises by 15-20 BPM to about 85 BPM at 35 weeks.	Gronowski, 2004; Chamberlain & Broughton-Pipkin, 1998; Hytten & Lind, 1973	24-hour heart rate, BPM --Early pregnancy: (< or = 6 weeks gestation): 80 +/- 6 (74-86) --Late pregnancy (9th month): 87 +/- 8 (79-95)	20	Singleton pregnancies, nonsmoking, carried pregnancy to term	Stein et al., 1999

Comparing levels of AL-related biomarkers in the NHANES samples with published reference ranges

Overall, the mean values for each biomarker in the NHANES sample of pregnant women were consistent with published reference ranges. Comparing the data from Tables 4, 7, and 8 with the expected changes and clinical reference ranges from Table 9, the following information can be reported for each Al-related biomarker:

C-reactive protein: In the NHANES sample, the mean CRP levels were significantly different in pregnant and non-pregnant women ($p<0.01$), and as expected from clinical reference materials, CRP levels were higher in pregnant women (Gronowski, 2004). The mean CRP level in the NHANES sample of pregnant women, 0.78 mg/dL, fell within the reference range observed by Larsson et al. (2008). The mean CRP levels in women in the first, second, and third trimesters of pregnancy were not significantly different ($p=0.69$); nor were the mean levels in early vs. late pregnancy ($p=0.17$). However, the mean level for each trimester and for early vs. late pregnancy fell into the week-specific reference ranges reported by Larsson et al (2008).

Albumin: In the NHANES sample, the mean albumin levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical references, albumin levels were lower in pregnant women than in non-pregnant women (Gronowski, 2004; Lockitch, 1993; Hytten & Lind, 1973). The mean albumin level in the NHANES sample of pregnant women, 3.60 g/dL, fell within the reference range observed by Larsson et el. (2008). The mean albumin levels in women in the first, second, and third

trimesters of pregnancy were significantly different ($p<0.01$), as were the levels in early vs. late pregnancy ($p<0.01$). The mean albumin level for each trimester and for early vs. late pregnancy fell into the week-specific reference ranges reported by Larsson et al. (2008).

Total cholesterol: In the NHANES sample, the mean cholesterol levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical references, the levels were higher in pregnant women than in non-pregnant women (Gronowski, 2004; Lockitch, 1993; Hytten & Lind, 1973). The mean total cholesterol level in the NHANES sample of pregnant women, 213.24 mg/dL, fell within the reference range observed by Belo et al. (2004). The mean total cholesterol levels in women in the first, second, and third trimesters of pregnancy were significantly different ($p<0.01$), as were the levels in early vs. late pregnancy ($p<0.01$). The mean total cholesterol level for each trimester fell into the trimester-specific reference ranges reported by Belo et al. (2004).

HDL cholesterol: In the NHANES sample, the mean HDL cholesterol levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical references, levels were higher in pregnant women than in non-pregnant women (Gronowski, 2004; Lockitch, 1993). The mean HDL cholesterol level in the NHANES sample of pregnant women, 64.38 mg/dL, fell within the reference range observed by Belo et al. (2004). The mean HDL cholesterol levels in women in the first, second, and third trimesters of pregnancy were significantly different ($p<0.01$), as were the levels in

early vs. late pregnancy ($p<0.01$). The mean HDL cholesterol level for the first and second trimesters fell into the trimester-specific reference ranges reported by Belo et al. (2004). The third-trimester mean in our sample, 67.61 mg/dL, was slightly higher than the upper value of the reference range reported by Belo et al. (2004) (66.4 mg/dL).

Creatinine: In the NHANES sample, mean creatinine levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical references, levels were lower in pregnant women than in non-pregnant women (Gronowski, 2004; Lockitch, 1993). The mean creatinine level in the NHANES sample of pregnant women, 48.22 μmol/L, fell within the reference range observed by Larsson et al. (2008). The mean creatinine levels in women in the first, second, and third trimesters of pregnancy were significantly different ($P<0.01$), as were the levels in early vs. late pregnancy ($p<0.01$). The mean albumin level for each trimester and for early vs. late pregnancy fell into the week-specific reference ranges reported by Larsson et al. (2008).

Hemoglobin A1C: In the NHANES sample, mean hemoglobin A1C levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical references, levels were lower in pregnant women than in non-pregnant women (Gronowski, 2004). The mean hemoglobin A1C level in the NHANES sample of pregnant women, 4.95%, fell within the reference range observed by Hartland et al. (1999). The mean hemoglobin A1C levels in women in the first, second, and third trimesters of pregnancy were significantly different ($p<0.01$), as were the levels in early

vs. late pregnancy ($p<0.01$). The mean hemoglobin A1C level for each trimester fell into the trimester-specific reference ranges reported by Hartland et al. (1999).

Homocysteine: In the NHANES sample, mean homocysteine levels were significantly different in pregnant and non-pregnant women ($p<0.01$). As expected from clinical reference materials, levels were lower in pregnant women than in non-pregnant women (Robinson, 2000). The mean homocysteine level in the NHANES sample of pregnant women, 4.44 μmol/dL, fell within the reference range observed by Velzing-Aarts et al. (2005). The mean homocysteine levels in women in the first, second, and third trimesters of pregnancy were significantly different ($p<0.01$), as were the levels in early vs. late pregnancy ($p<0.01$). The mean homocysteine level for each trimester fell into the trimester-specific reference ranges reported by Velzing-Aarts et al. (2005).

Systolic and diastolic blood pressure: In the NHANES sample, mean SBP and DBP were significantly different in pregnant and non-pregnant women ($p<0.01$ for both). As expected from clinical reference materials, SBP and DBP levels were lower in pregnant women than in non-pregnant women (Chamberlain & Broughton-Pipkin, 1998). The mean SBP and DBP levels in the NHANES sample of pregnant women, 108.34 mm Hg and 59.60 mm Hg, respectively, fell within the reference ranges observed by Ochsenbein-Kolble et al. (2004). The mean SBP and DBP levels in the first, second, and third trimesters of pregnancy were significantly different ($p<0.01$ for both). Mean DBP levels were also significantly different between early and late pregnancy ($p=0.01$), but mean

SBP levels were not (p=0.56). The mean SBP and DBP levels for each trimester fell into the trimester-specific reference ranges reported by Ochsenbein-Kolble et al. (2004).

Heart rate: In the NHANES sample, mean heart rate was significantly different in pregnant and non-pregnant women (p<0.01). As expected from clinical reference materials, heart rate was higher in pregnant women than in non-pregnant women (Gronowski, 2004; Chamberlain & Broughton-Pipkin, 1998; Hytten & Lind, 1973). The mean heart rate in the NHANES sample of pregnant women, 85.76 beats per minute, fell within the reference range observed by Stein et al. (1999). The mean heart rate in the NHANES sample of women in the first, second, and third trimesters of pregnancy were significantly different (p<0.01), as were mean heart rates in early vs. late pregnancy (p<0.01). The mean heart rate for women in early and late pregnancy fell into the specific early and late pregnancy reference ranges reported by Stein et al. (1999).

Research Aim 4: Comparing AL scores in pregnant and non-pregnant women

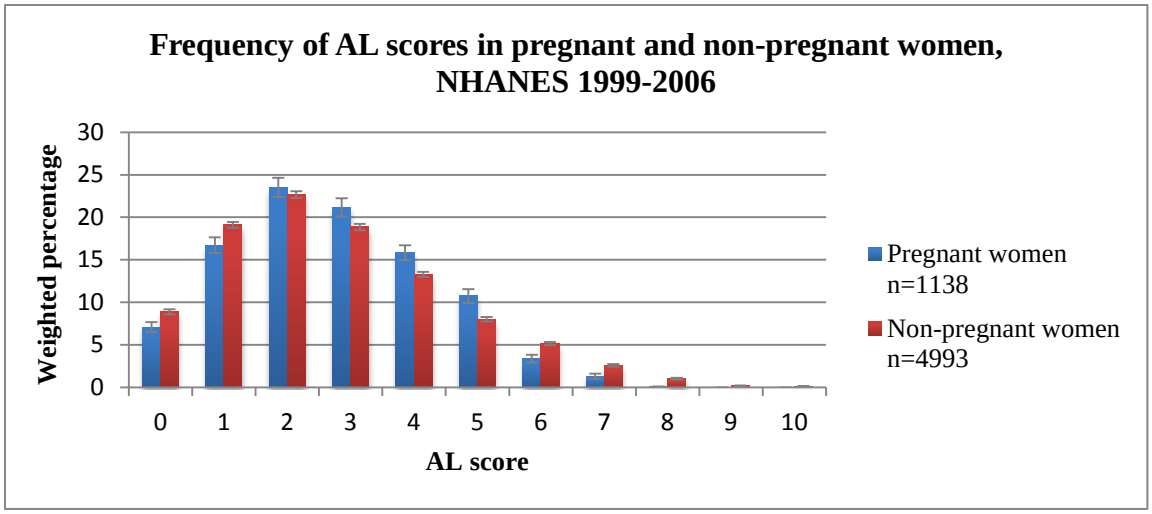
Based in the NHANES sample, pregnant and non-pregnant women had very similar AL scores (Table 10). The median AL score was higher in pregnant women than in non-pregnant women, but the mean scores differed very little.

Table 10: AL scores in pregnant and non-pregnant women

	Median AL score	**Mean AL score**	**Range**
Pregnant women	3	2.752	0-8.00
Non-pregnant women	2	2.749	0-10.00

The distributions of AL scores in pregnant and non-pregnant women appear very similar, although they are statistically different ($p<0.01$; Figure 1). This difference is probably due in large part to the sample sizes (>1000 in each group).

Figure 1: Frequency of AL scores in pregnant and non-pregnant women



Research Aim 5: Comparing AL scores among pregnant women in different stages of pregnancy

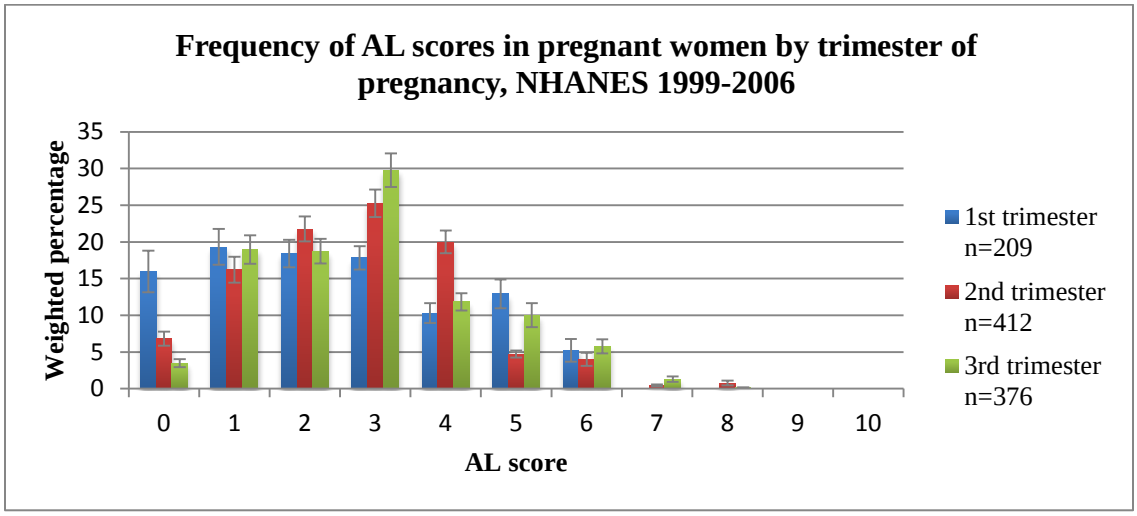
Women in different trimesters of pregnancy had similar mean and median AL scores (Table 11), although there appeared to be more variability in mean AL scores compared to the previous analysis of all pregnant and non-pregnant women. This variability may be due to the smaller sample sizes when pregnant women are considered by trimester.

Table 11: AL scores in pregnant women by trimester of pregnancy

	Median AL score	**Mean AL score**	**Range**
1st trimester	2	2.47	0-6.00
2nd trimester	3	2.73	0-8.00
3rd trimester	3	2.88	0-8.00

The distributions of AL scores in the samples pregnant women by trimester have the same general shape, although they are statistically different ($p<0.01$; Figure 2).

Figure 2: Frequency of AL scores in pregnant women by trimester of pregnancy



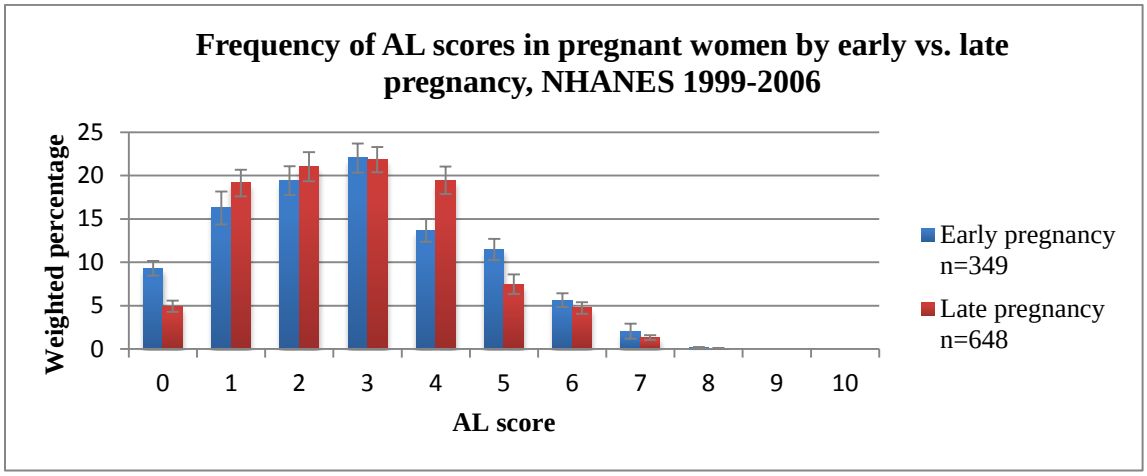
When pregnant women were grouped into early vs. late pregnancy, they had the same median AL score and very similar mean AL scores (Table 12).

Table 12: AL scores in pregnant women by early vs. late pregnancy

	Median AL score	**Mean AL score**	**Range**
Early pregnancy	3	2.83	0-8.00
Late pregnancy	3	2.80	0-8.00

The distributions of AL scores in pregnant women by early vs. late pregnancy have a similar shape, and they are not statistically different (p=0.37; Figure 3).

Figure 3: Frequency of AL scores in pregnant women by early vs. late pregnancy



Research Aim 6: Determining attributes of AL in pregnant women

This study categorized AL in several different ways to determine whether attributes of AL differ depending on how its levels are defined. In one analysis, high and low AL were defined using a cutoff AL score of 4. The full results of these analyses are presented below.

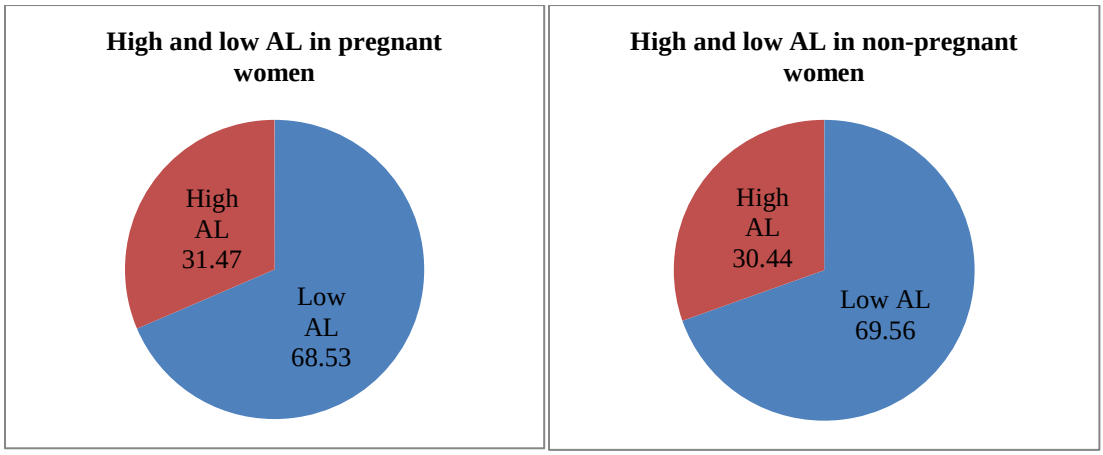
In another analysis, high and low AL were defined using a cutoff AL score of 3. The results of this analysis were very similar to results when a cutoff score of 4 was used. Data for that analysis are included in Appendix 3.

In third analysis, AL was categorized into three levels: high, medium, and low. The results of these analyses were similar to the results when AL was categorized only into high and low. These results are also included in Appendix 4.

The remainder of this section presents the attributes of AL using a cutoff of 4 to categorize AL scores as high or low. For ease of interpretation, only the percentage of high AL is included in the figures in this section. The corresponding proportions of low AL in each group are (1 - % high AL). Full frequency tables for high and low AL by age, race, ratio of family income to poverty level, and education level are included in Appendix 5.

Using an AL score of 4 as the cutoff to define low and high AL, approximately the same proportion of pregnant and non-pregnant women in the NHANES sample had low and high AL (p=0.68; Figure 4).

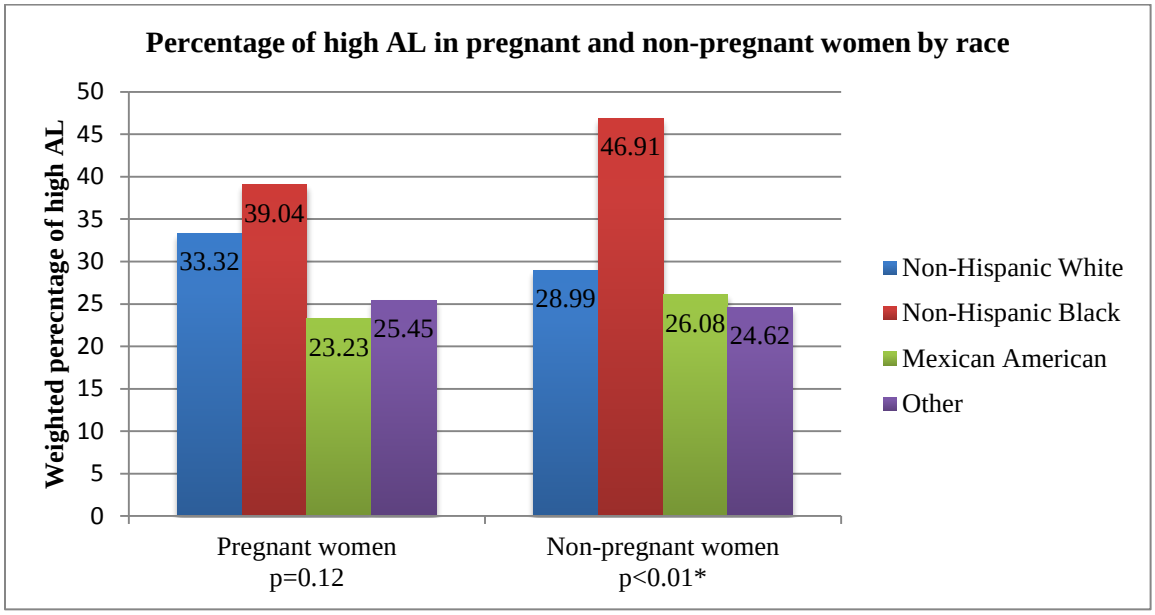
Figure 4: Frequency of high and low AL in pregnant and non-pregnant women



Race and AL

Considering the frequencies of high and low AL by race, non-pregnant women of different races had significantly different AL ($p<0.01$), but the proportions of high and low AL did not differ significantly among pregnant women ($p=0.12$; Figure 5).

Figure 5: Percentage of high AL in pregnant and non-pregnant women by race



In both pregnant and non-pregnant women, non-Hispanic Black women had a higher percentage of high AL scores compared with non-Hispanic White women. Mexican American women had a lower percentage of high AL scores than either non-Hispanic White or non-Hispanic Black women. However, these differences did not reach the level of statistical significance in the sample of pregnant women.

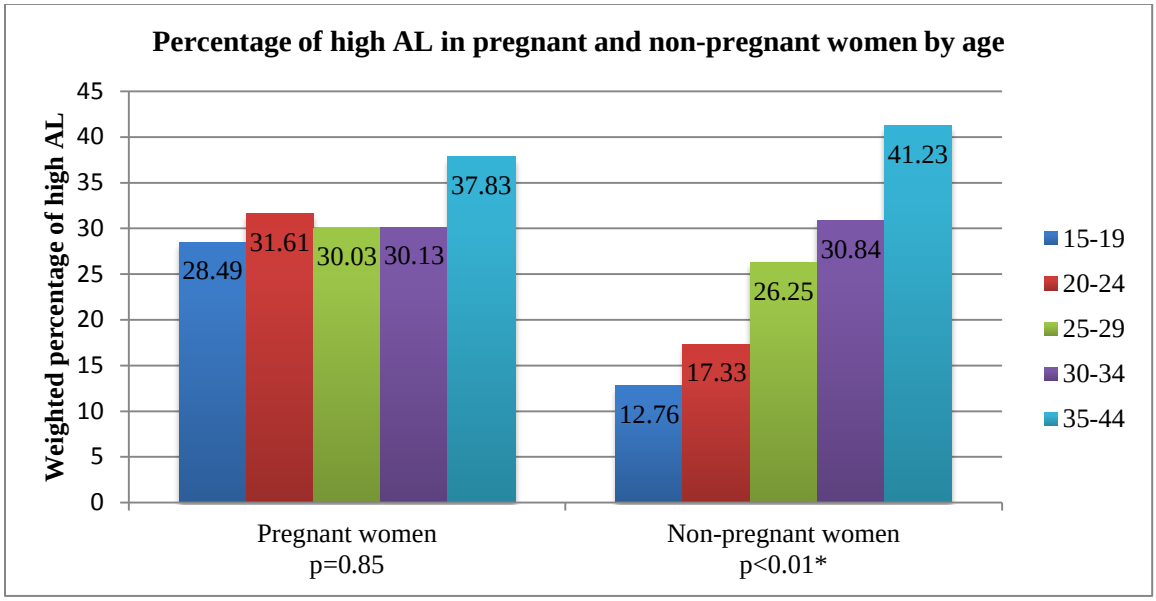
From previous studies of race and AL, it was expected that non-Hispanic Black women would have higher AL scores than non-Hispanic White or Mexican American women. The results of this analysis support that hypothesis in non-pregnant women. In

pregnant women, the results also suggest that non-Hispanic Black women have higher AL than women in other race groups, although the lack of statistical significance allows no firm conclusions to be drawn.

Age and AL

Considering the frequencies of high and low AL by age, non-pregnant women in different age groups had significantly different AL ($p<0.01$), but the proportions of high and low AL did not differ significantly by age in pregnant women ($p=0.85$; Figure 6).

Figure 6: Percentage of high AL in pregnant and non-pregnant women by age



In non-pregnant women, the percentage of high AL increased in each age group, as expected from previous studies that showed a direct correlation between AL score and age. However, the findings in pregnant women do not support a similar conclusion in that

group. Although pregnant women in the oldest age group (35-44) had a higher percentage of AL than women in the other age groups, the difference was not statistically significant.

When age was considered as a continuous variable instead of a categorical variable, results were similar. The mean age among pregnant women with high AL was not significantly different from the mean age among those with low AL ($p=0.10$). Among non-pregnant women, those with high AL were older than those with low AL ($p<0.01$). These data are available in Appendix 5.

Race, age, and AL

Geronimus et al. (2006) found that AL increases more quickly in Black women than in White women, suggesting that race may be an effect modifier of the relationship between age and AL—this is the basis of the weathering hypothesis. To determine whether the effect of age on AL was different in women of different races in this study, the data on high AL by age were stratified by race (Figure 7).

Figure 7: Percentage of high AL in pregnant and non-pregnant women by race and age

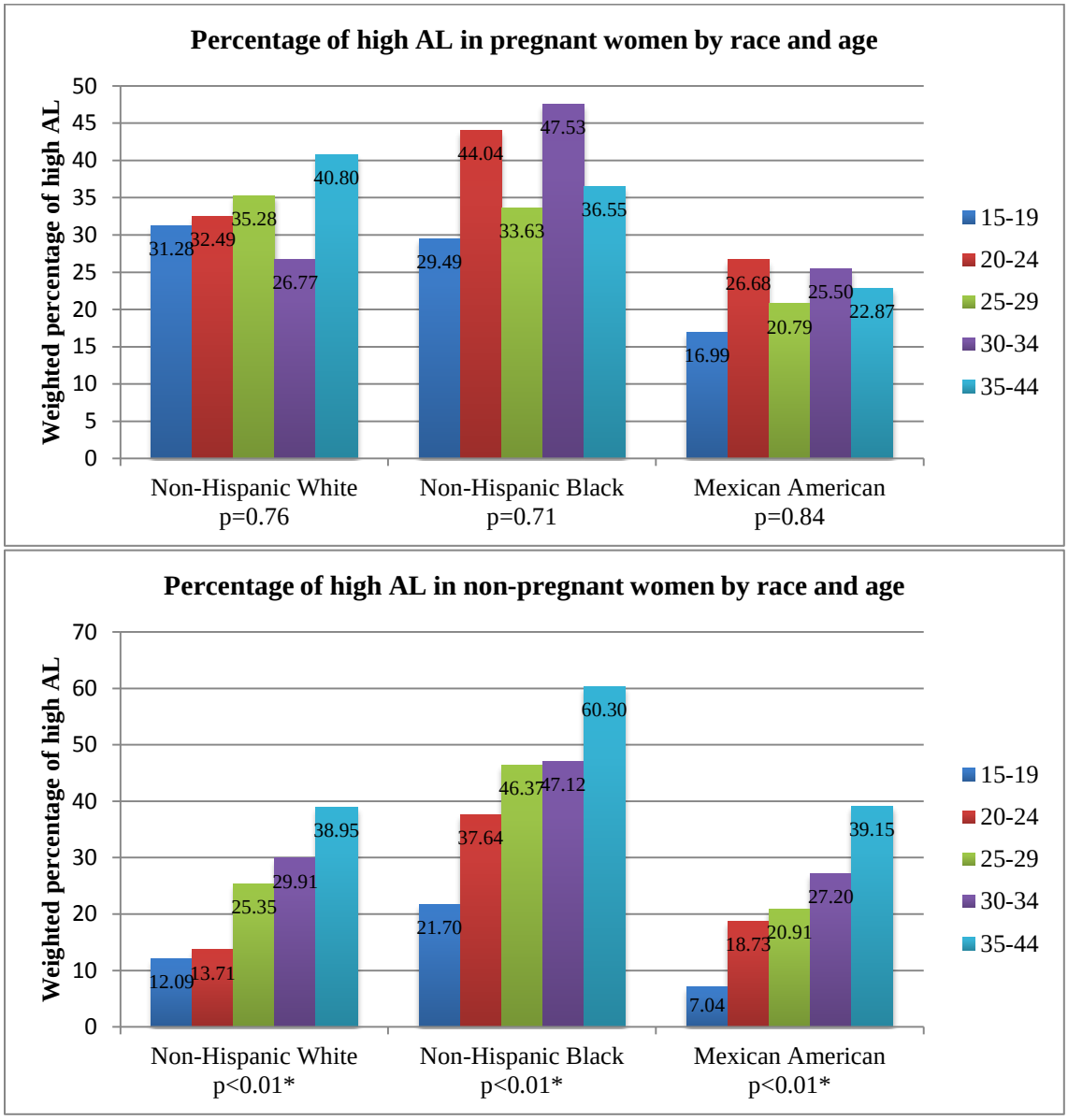


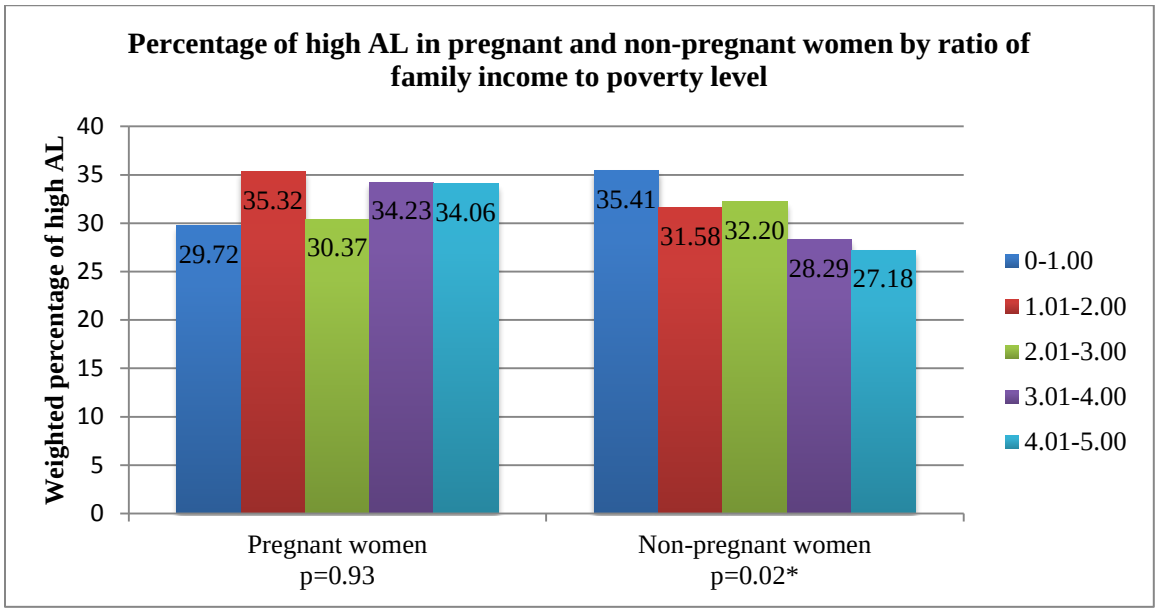
Figure 7 clearly shows that, in non-pregnant women, the percentage of high AL differed significantly by age in all race groups ($p<0.01$). AL increases in each age group for all races, and in every age group, non-Hispanic Black women have higher percentages of AL than women of other races. These findings are consistent with previous studies of AL. However, a similar pattern is not seen in the sample of pregnant women. In that

sample, the percentage of high AL did not differ significantly by age in any race group, and there was no clear trend of increasing AL with age in women of any race.

Income and AL

This study considered ratio of family income to poverty as an indicator of income level. This ratio ranged from 0 to 5, with higher scores indicating higher levels of income. Among the sample of non-pregnant women, percentage of high AL differed significantly by income level (p=0.02). However, there was no significant difference in the sample of pregnant women (p=0.93; Figure 8).

Figure 8: Percentage of high AL in pregnant and non-pregnant women by ratio of family income to poverty level



The proportion of non-pregnant women with high AL appeared to decrease with increasing income, which is consistent with previous research showing that AL is

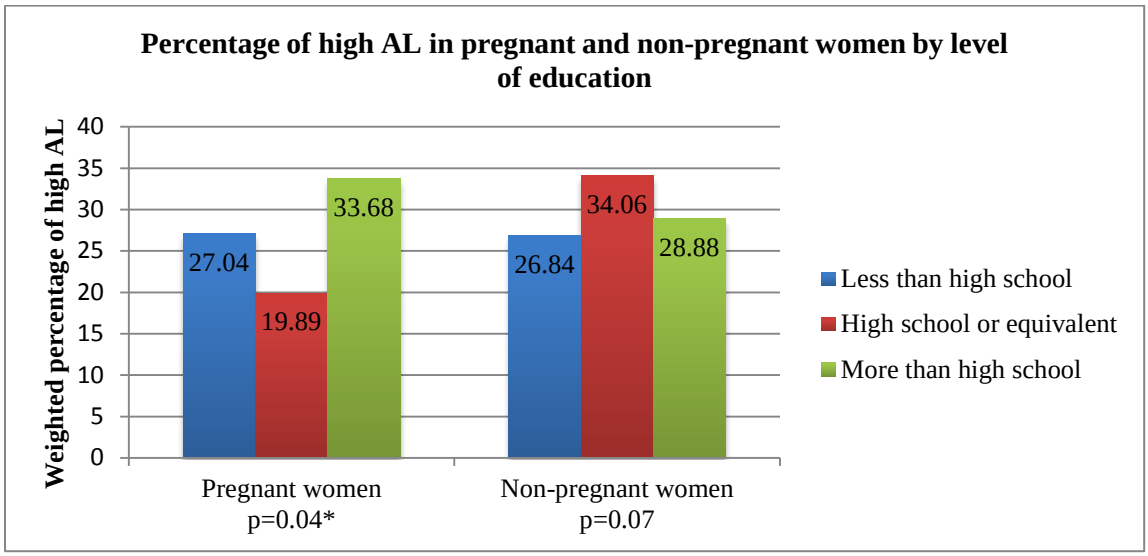
inversely correlated with income. Among pregnant women, however, income did not appear to have an effect on AL.

When ratio of family income to poverty level was considered as a continuous variable instead of a categorical variable, results were similar. The mean ratio among pregnant women with high AL was not significantly different than the mean ratio among those with low AL (p=0.57). Among non-pregnant women, women with high AL had a lower mean ratio than women with low AL (p<0.01). These data are available in Appendix 5.

Education level and AL

The percentage of high AL differed significantly among pregnant women (p=0.04) but not among non-pregnant women (p=0.07) in the NHANES sample (Figure 9).

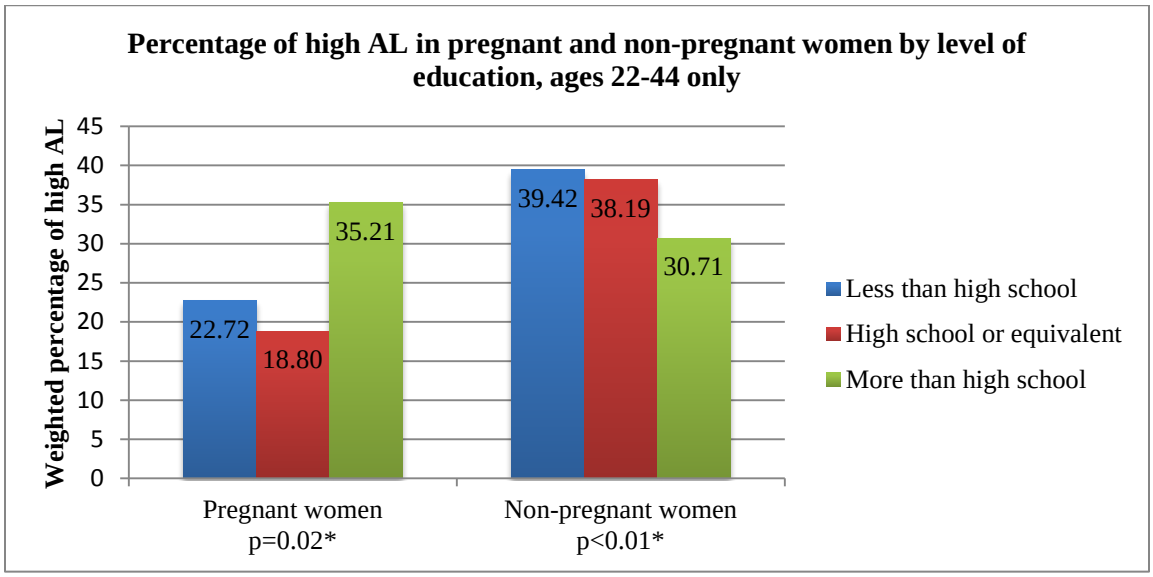
Figure 9: Percentage of high AL in pregnant and non-pregnant women by level of education



From previous studies, AL would have been expected to be higher among women of lower educational attainment. Although the percentage of high AL differed significantly by education level in pregnant women, these results did not show the expected pattern of decreasing AL with increasing education. Surprisingly, the expected pattern was not seen in the sample of non-pregnant women, either.

One possible explanation for this finding is that the women included in this study are females as young as age 15, so some participants have a high school or less than high school level of education simply because of their age. To assess this possibility, the analysis was repeated, restricting the age range to 22-44 years (Figure 10).

Figure 10: Percentage of high AL in pregnant and non-pregnant women by level of education, ages 22-44 only



When the youngest women are excluded from the sample, the difference in percentage of high AL becomes statistically significant in non-pregnant women ($p<0.01$), and it is more evident that the likelihood of having high AL decreases with increasing

levels of education. However, although the differences are also significant in the non-pregnant sample (p=0.02), the data still do not suggest that increasing levels of education are associated with lower AL. In fact, the pregnant women with the highest level of education (more than high school) also had the highest percentage of high AL.

All other analyses in this study were done with the full sample of pregnant and non-pregnant women (ages 15-44) because restricting the age range to 22-44 years decreased the sample size by 24% in the sample of pregnant women and 42% in the sample of non-pregnant women. This reduction would reduce power in the other analyses. Additionally, age is an important consideration in the study of AL, and comparisons between women at the youngest ages with those at older ages are of interest in the remainder of this study.

Logistic regression modeling

To consider all of the demographic variables together, a logistic regression model was created with AL category (high/low defined using a cutoff AL score of 4) as the response variable and age, race, ratio of family income to poverty level, and education level as predictor variables. The results of modeling the probability of having high AL in the samples of pregnant and non-pregnant women are presented in Tables 13 and 14.

Table 13: Logistic regression model: Race, age, income, and education level as predictors of high AL in pregnant women

Parameter	**Type 3 analysis of effects**		**Analysis of maximum likelihood estimates**			**Odds ratio estimates**	
	Wald χ^2	**Pr> χ^2**	**Estimate (SE)**	**Wald χ^2**	**Pr> χ^2**	**Point estimate**	**95% Wald confidence limits**
Intercept	--	--	-0.21 (0.36)	0.35	0.55	--	--
Race	4.39	0.22	--	--	--	--	--
NH White	--	--	Reference	--	--	--	--
NH Black	--	--	-0.22 (0.35)	0.41	0.52	0.80	0.41, 1.58
MA	--	--	-0.74 (0.36)	4.32	0.04*	0.48	0.24, 0.96
Other	--	--	-0.45 (0.46)	0.95	0.33	0.64	0.26, 1.58
Age	5.23	0.26	--	--	--	--	--
15-19	--	--	Reference	--	--	--	--
20-24	--	--	-0.51 (0.40)	1.66	0.20	0.60	0.28, 1.31
25-29	--	--	-0.70 (0.45)	2.45	0.12	0.50	0.21, 1.19
30-34	--	--	-0.51 (0.52)	0.99	0.32	0.60	0.22, 1.65
35-44	--	--	0.08 (0.46)	0.03	0.86	1.09	0.44, 2.67
Income (ratio I/P)	2.56	0.63	--	--	--	--	--
0-1.00	--	--	Reference	--	--	--	--
1.01-2.00	--	--	0.30 (0.50)	0.35	0.55	1.35	0.50, 3.61
2.01-3.00	--	--	-0.64 (0.47)	1.87	0.17	0.53	0.21, 1.32
3.01-4.00	--	--	-0.21 (0.48)	0.19	0.66	0.81	0.32, 2.06
4.01-5.00	--	--	-0.19 (0.32)	0.33	0.56	0.83	0.44, 1.56
Education	7.99	0.02*	--	--	--	--	--
< HS	--	--	Reference	--	--	--	--
HS or eq.	--	--	-0.49 (0.37)	1.76	0.18	0.62	0.30, 1.26
> HS	--	--	0.33 (0.33)	0.99	0.32	1.39	0.73, 2.67

Table 14: Logistic regression model: Race, age, income, and education level as predictors of high AL in non-pregnant women

Parameter	**Type 3 analysis of effects**		**Analysis of maximum likelihood estimates**			**Odds ratio estimates**	
	Wald χ^2	**Pr> χ^2**	**Estimate (SE)**	**Wald χ^2**	**Pr> χ^2**	**Point estimate**	**95% Wald confidence limits**
Intercept	--	--	-1.61 (0.18)	77.66	<0.01*	--	--
Race	63.70	<.0001*	--	--	--	--	--
NH White	--	--	Reference	--	--	--	--
NH Black	--	--	0.76 (0.12)	39.56	<0.01*	2.14	1.69, 2.72
MA	--	--	-0.25 (0.18)	2.01	0.16	0.78	0.55, 1.10
Other	--	--	-0.30 (0.22)	1.88	0.17	0.74	0.48, 1.14
Age	87.65	<.0001*	--	--	--	--	--
15-19	--	--	Reference	--	--	--	--
20-24	--	--	0.01 (0.21)	0.002	0.96	1.01	0.67, 1.53
25-29	--	--	0.95 (0.19)	25.34	<0.01*	2.59	1.79, 3.75
30-34	--	--	1.15 (0.21)	30.58	<0.01*	3.17	2.11, 4.77
35-44	--	--	1.66 (0.19)	75.77	<0.01*	5.26	3.62, 7.64
Income (ratio I/P)	22.97	0.0001*	--	--	--	--	--
0-1.00	--	--	Reference	--	--	--	--
1.01-2.00	--	--	-0.30 (0.15)	4.26	0.04*	0.74	0.56, 0.99
2.01-3.00	--	--	-0.36 (0.19)	3.37	0.07	0.70	0.48, 1.02
3.01-4.00	--	--	-0.59 (0.17)	11.57	<0.01*	0.55	0.39, 0.78
4.01-5.00	--	--	-0.64 (0.14)	21.11	<0.01*	0.52	0.40, 0.69
Education	1.67	0.43	--	--	--	--	--
< HS	--	--	Reference	--	--	--	--
HS or eq.	--	--	0.05 (0.15)	0.12	0.72	1.05	0.79, 1.40
> HS	--	--	-0.12 (0.16)	0.62	0.43	0.88	0.65, 1.20

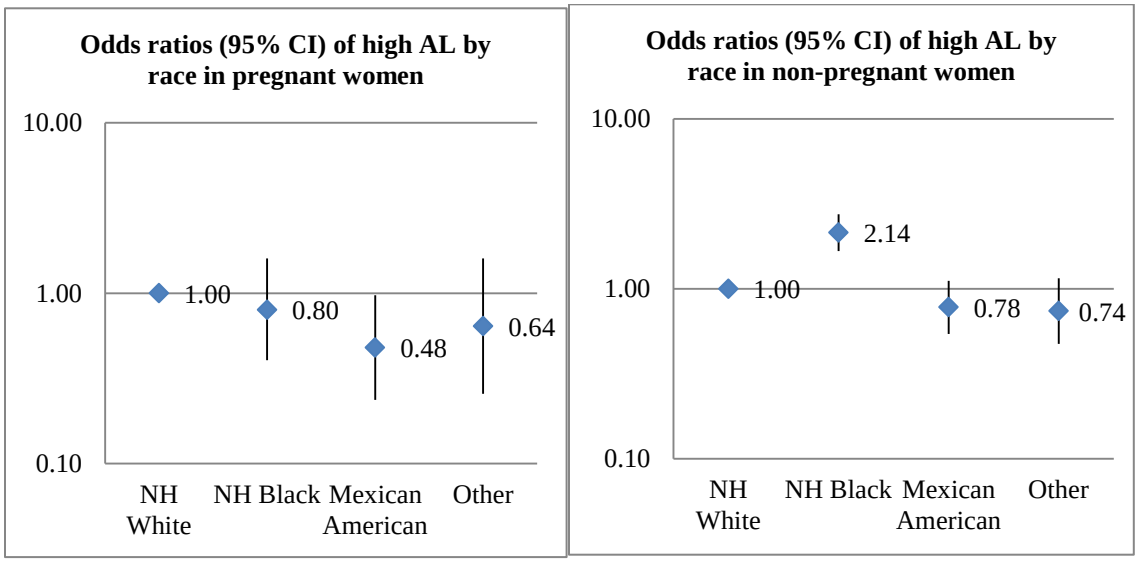
The type 3 analysis of effects in the NHANES sample of pregnant women suggests that of the four predictor variables in the model, only education level had a statistically significant relationship with the response variable, high/low AL ($p=0.02$). In the sample of non-pregnant women, the results were reversed: of the predictor variables in the model, all but education were statistically significantly associated with high/low AL (all $p<0.01$).

Results from the levels of each predictor variable in the model are presented below.

Race: In the sample of pregnant women, Mexican American women had smaller odds of having high AL compared with non-Hispanic White women (OR=0.48, 95% CI=0.03, 0.86) when the other variables were held constant. The odds of having high AL was not statistically different between non-Hispanic Black women or women of other races and non-Hispanic White women (Figure 11).

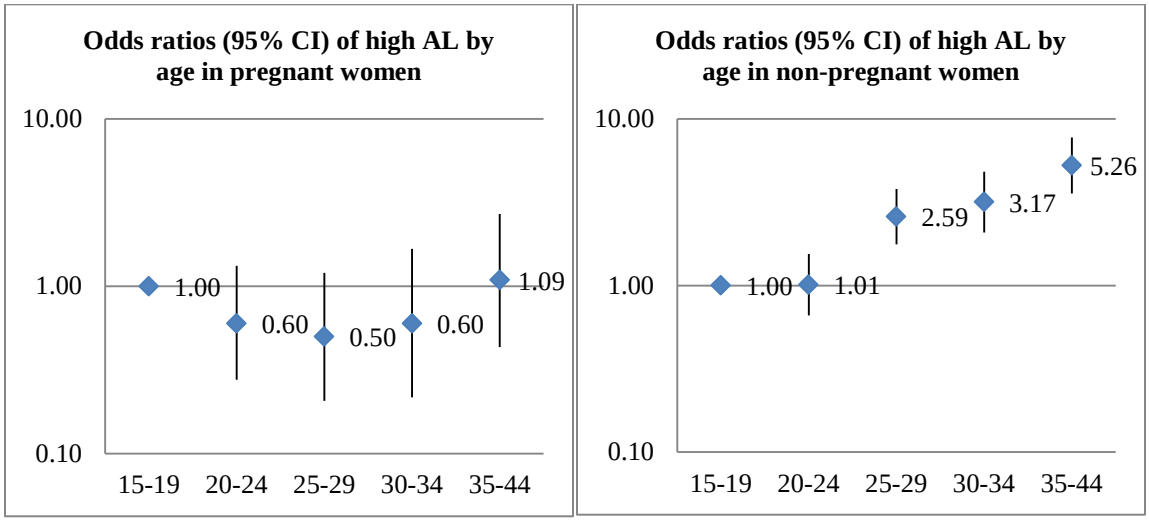
In the sample of non-pregnant women, non-Hispanic Black women had greater odds of having high AL compared with non-Hispanic White women (OR=2.14, 95% CI=1.69-2.72) when the other variables are held constant. The odds of having high AL was not statistically different between Mexican American women or women of other races and non-Hispanic White women (Figure 11).

Figure 11: Odds ratios and 95% confidence intervals of high AL by race in pregnant and non-pregnant women



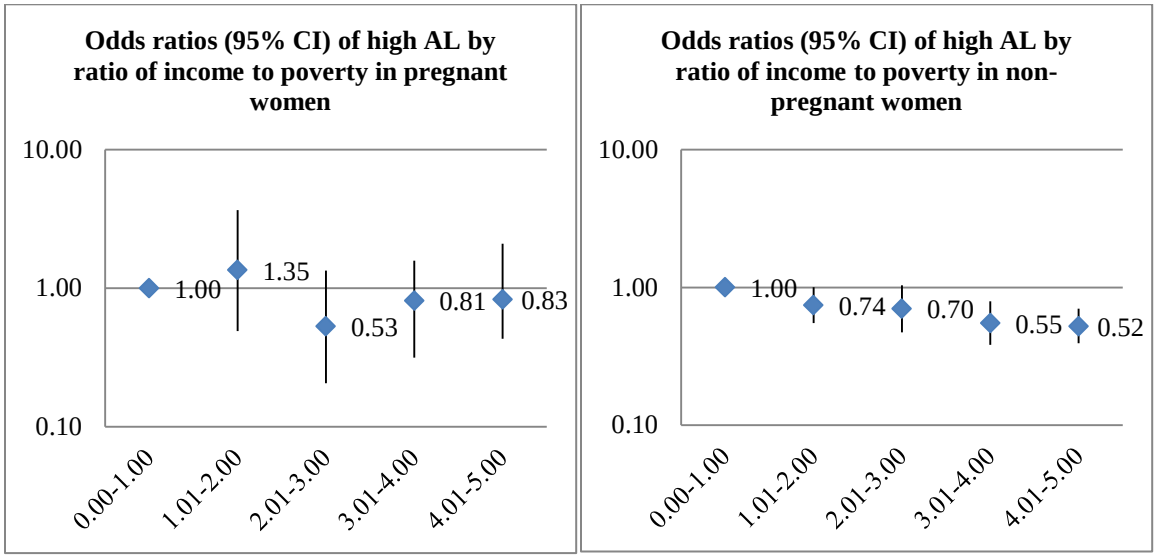
Age: In pregnant women, the odds of having high AL were not statistically associated with age in any age group (Figure 12). In non-pregnant women, the odds of having high AL increased with age starting with the 25-29 year-old age group (Age 25-29: OR=2.59, 95% CI=1.79, 3.75) when the other variables were held constant. By age 30-34, the odds of having high AL were more than three times the odds for 15-19 year-olds (OR=3.17, 95% CI=2.11, 4.77), and the odds in 35-44 year-olds were more than five times the odds for 15-19 year-olds (OR=5.26, 95% CI=3.62, 7.65).

Figure 12: Odds ratios and 95% confidence intervals of high AL by age in pregnant and non-pregnant women



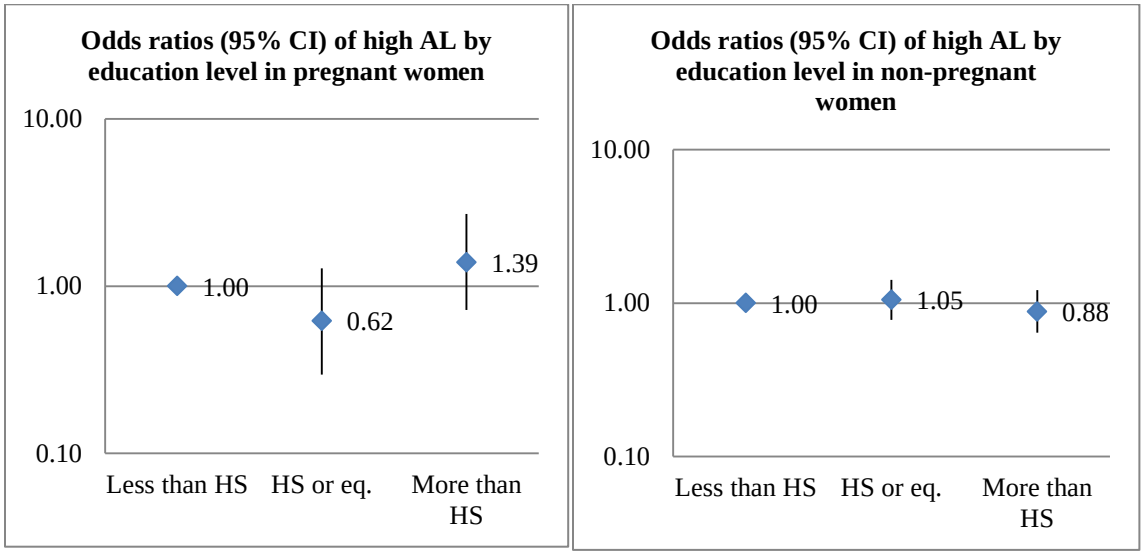
Income: In the sample of pregnant women, ratio of family income to poverty was not associated with the odds of having high AL in any income category (Figure 13). Among non-pregnant women, the odds of having high AL decreased with increasing income when the other variables were held constant. Women with the highest level of family income, 4.01 to 5.00 times the poverty level, are about half as likely to have high AL as those with the lowest level of family income, 0 to 1.00 times the poverty level (OR=0.52, 95% CI=0.40, 0.69).

Figure 13: Odds ratios and 95% confidence intervals of high AL by income in pregnant and non-pregnant women



Education: Education level was not associated with the odds of having high AL in for any level of education in either pregnant or non-pregnant women, when all other variables were held constant. This finding may be explained by the inclusion of high-school and college-age women in the samples, as discussed in the univariate analysis above. The odds ratios for both models are presented in Figure 14.

Figure 14: Odds ratios and 95% confidence intervals of high AL by education level in pregnant and non-pregnant women



Cluster analysis

A cluster analysis of the variables for AL score, race, age, ratio of family income to poverty, and education level in the sample of pregnant women yielded the dendrogram in Figure 15. This dendrogram suggests two major clusters of pregnant women based on these variables. Compared to the women in cluster 2, the women in cluster 1 are older, have higher incomes, are more likely to be white, and have higher levels of education ($p<0.01$ for all; Table 15). However, the median AL scores were not statistically different between the two groups ($p=0.12$).

Figure 15: Dendrogram illustrating clusters of AL score, age, race, income, and education level in pregnant women

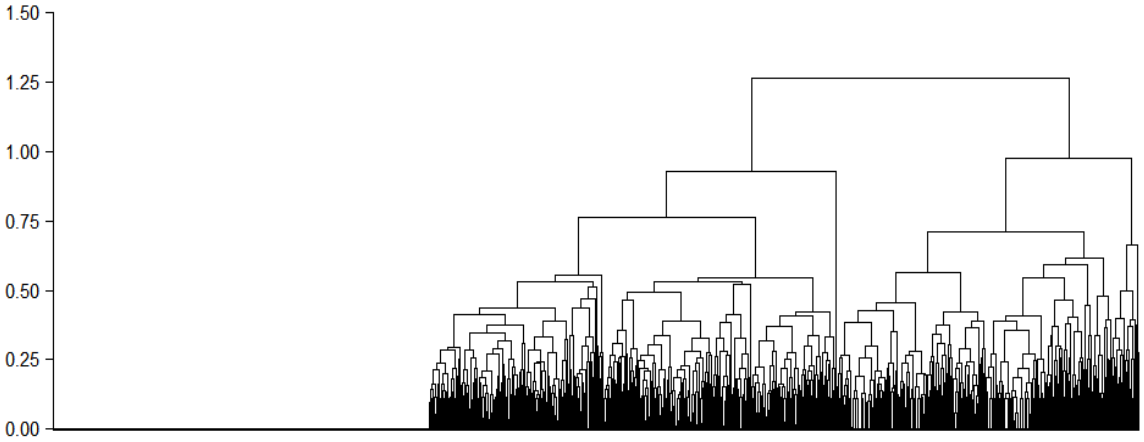


Table 15: Characteristics of clusters with regard to AL score, age, race, income, and education level in pregnant women

	Cluster 1	**Cluster 2**	**P value**
Number of observations	317	427	
Age	Mean=31.83 (SD=3.47, range 25-43)	Mean=22.25 (SD=3.24, range 15-28)	<0.01*
Race			<0.01*
NH White	58.68% (186)	35.60% (152)	
NH Black	8.20% (26)	20.10% (86)	
Mexican American	20.82% (66)	33.96% (145)	
Other	12.30% (39)	10.30% (44)	
Education level			<0.01*
Less than HS	13.56% (43)	44.73% (191)	
HS or equivalent	14.51% (46)	25.76% (110)	
More than HS	71.92% (228)	29.51% (126)	
Ratio of income to poverty	Mean=3.32 (SD=1.65, range 0-5)	Mean=1.68 (SD=1.35, range 0-5)	<0.01*
AL score	Mean=2.49 Median=2	Mean=2.61 Median=3	0.12

Chapter 6: Discussion

AL-related biomarkers in pregnant and non-pregnant women

The mean levels of each of the ten AL-related biomarkers differed significantly in the NHANES samples of pregnant and non-pregnant women. This result is not surprising, as most studies of AL exclude pregnant women because the levels of various biomarkers are assumed to change during pregnancy. However, there were other notable differences between the pregnant and non-pregnant women. The difference in mean age in the two groups is of particular interest. The mean age in the two groups differed by more than 3 years (27.39 years in pregnant women vs. 30.57 years in non-pregnant women, $p<0.01$). The age distributions in the two groups also look very different, with the highest percentage of pregnant women in the 25-29 age group and the highest percentage of non-pregnant women in the 35-44 age group (Appendix 6).

Because age is known to be associated with AL, the different age distributions in the two populations may have influenced the calculation of AL scores and interpretation of results in this study. Therefore, it could be informative to age-standardize the samples of pregnant and non-pregnant women. However, direct age standardization (the method recommended by NHANES) does not provide estimates of variance or weighted quartiles, which are necessary for calculating AL scores. Therefore, indirect age standardization would be necessary, and this approach would require recalculating the NHANES survey weights based on the birth rates for each age and race group. This approach is not recommended by NHANES; CDC researchers are currently working to calculate a new set of age-standardized weights for public use (J. Parker, personal

communication). Once these weights are available, the analyses in the current study could be performed again to see whether age-standardization provides a more accurate picture of the characteristics of AL in pregnant women.

Age-standardization is not an issue when comparing women in different stages of pregnancy, as mean ages did not differ by trimester ($p=0.54$) or in early vs. late pregnancy ($p=0.58$). Additionally, no significant differences were found in race, education, marital status, or income in women in different stages of pregnancy. However, mean the levels of most AL-related biomarkers were different in different stages of pregnancy, confirming that these biomarkers are not static during pregnancy. This finding suggests that it may be more informative to consider AL by stage of pregnancy than to lump all pregnant women together in a single group. Some AL-related biomarkers are known to increase or decrease throughout the course of pregnancy, whereas others fluctuate (e.g. blood pressure, which decreases during the second trimester but increases again during the third trimester [Chamberlain & Broughton-Pipkin, 1998]). Considering the levels of each biomarker by stage of pregnancy makes these patterns more apparent.

Normal ranges for AL-related biomarkers in pregnant women

The fact that normal reference ranges for the various biomarkers have been reported suggests that these levels change in predictable ways. Information from clinical reference materials and studies of healthy pregnant women indicate that levels of each biomarker change in similar ways in most pregnant women. The findings from the present study support that conclusion, as overall the mean values for each of the 10 AL-related biomarkers in the NHANES sample of pregnant women were consistent with

published reference ranges and clinical reference materials. Levels of each biomarker measured by trimester and in early vs. late pregnancy were also in line with reference ranges reported by week or by trimester.

Limitations of this approach included the questionable representativeness of the samples from which the reference ranges were calculated. The age, race, and other characteristics of the women included in each study were often not reported, and some reference ranges were calculated using only White women. Additionally, the reference range studies used different criteria for determining healthy pregnancies, and not all of them included a normal pregnancy outcome among their criteria.

The measurements of mean biomarker levels presented in Tables 4, 7, and 8 may have clinical utility independent of their usefulness as building blocks of an AL index in pregnant women. Although reference ranges were available from the literature for each biomarker, few of these studies have been conducted and some are more than a decade old. The availability of mean levels and distributions of these biomarkers in a nationally representative sample of pregnant women could be of use to laboratories and OB/GYN practitioners who seek to interpret lab results as part of patient care.

Comparing AL scores in pregnant and non-pregnant women and during different stages of pregnancy

Initial analysis of AL scores in pregnant and non-pregnant women suggested that the mean and distribution of AL in the two groups is very similar. Like non-pregnant women, most pregnant women have an AL score of 1, 2, or 3, and very few have scores of more than 5. These similarities would be expected if AL has the same attributes in

pregnant and non-pregnant women. However, the median AL scores in the two groups were different (3 in pregnant women vs. 2 in non-pregnant women), and the difference in the distribution of AL in the two groups was statistically significant ($p<0.01$). The significant difference may be a result of the large sample size of the two groups.

When AL was considered by stage of pregnancy, the means and distributions of AL were also quite similar. When the sample of pregnant women was split into early pregnancy (less than 5 months) and late pregnancy (5+ months), the two groups had the same median AL score and very close mean AL scores. The distribution of AL in the groups was not statistically different ($p=0.37$). However, when the sample of pregnant women was split into trimesters, the median AL scores were different in the 1st trimester compared to the 2nd and 3rd trimesters (2 in the 1st trimester vs. 3 in the later trimesters), and the distributions of AL in the three groups were significantly different ($p<0.01$). These findings suggest that it may be more precise to consider AL by trimester of pregnancy instead of analyzing all pregnant women as a single group. However, size of the NHANES sample of pregnant women in each trimester is smaller than considering all pregnant women together, which would reduce the power of a trimester-based analysis.

Attributes of AL in pregnant women

The main objective of this study was to determine whether AL has similar attributes in pregnant women as it does in non-pregnant women. Based on the results of the univariate and multivariate analyses, it does not appear that AL has the same attributes in pregnant women with regard to race, age, income, and education. In the sample of pregnant women, there were no significant differences in the percentage of

high AL in women of different races, age groups, or income levels ($p>0.05$ for all). Thus, when AL is measured this way, weathering as defined by Geronimus et al. (2006) is not seen in pregnant women. The only significant difference in the percentage of high AL in the sample of pregnant women was seen with education levels, and the results showed the opposite of what would have been expected: instead of being inversely correlated, pregnant women with the highest levels of education also had the highest AL ($p=0.02$).

Because this study is the first to attempt to measure AL in pregnant women, an exploratory cluster analysis was undertaken to identify whether AL scores clustered with variables for race, age, income, and education level in the NHANES sample of pregnant women. The cluster analysis suggested two major groupings within the sample of pregnant women. Compared with cluster 2, the women in cluster 1 were older, more likely to be non-Hispanic White, more likely to have greater than a high school level of education, and more likely to have higher income ($p<0.01$ for all). However, the median AL score did not differ significantly between the two clusters ($p=0.12$). These results were consistent with those of the univariate and multivariate logistic regression analyses, underscoring the finding that AL is not clearly associated with race, age, income, or education level in the NHANES sample of pregnant women.

The results of this study did not appear to be sensitive to the way in which AL was categorized. Results were similar whether AL was defined as high and low with a cutoff score of 3, as high and low with a cutoff score of 4, or with three levels (high, moderate, and low). These results underscore the finding of Kaestner et al. (2009) that it makes little difference to the overall analysis how high and low AL are defined.

The sample of non-pregnant women was analyzed as a way to internally validate the measurement of AL in this study. Levels of AL showed expected patterns with regard to age, race, income, and education (when the age was restricted to 22-44 year-old women) compared to previous studies (Chyu & Upchurch, 2011; Seeman et al., 2008; Geronimus et al., 2006; Crimmins et al., 2003; Seeman et al., 1997). Specifically, non-pregnant women with high AL were more likely to be non-Hispanic Black, to be older, to have lower income, and to have lower levels of education ($p<0.01$ for all). That the results in non-pregnant women were consistent with those of previous studies suggests that there were no major errors in the measurement and analysis of AL in this study.

Chapter 7: Conclusions

AL does not appear to have the same attributes in pregnant women as it does in non-pregnant women, although there are a few intriguing similarities. These similarities, as well as the potential explanations for the differences seen between the two groups, warrant future study.

There are several reasons why AL may not show similar patterns in pregnant women compared to non-pregnant women. One of the primary reasons may be related to the different age distributions of the pregnant and non-pregnant women, as discussed above. Another potential reason is that there may be chronic stress in younger pregnant women that is not experienced by their non-pregnant peers. According to the National Campaign to Prevent Teen and Unplanned Pregnancy (2009), teenagers who become pregnant tend to be more socioeconomically disadvantaged than non-pregnant women of the same age. Teenage mothers are more likely to be from single-parent families, have a family income below 200% of the federal poverty level, and to have a history of childhood sexual or physical abuse. Chronic stress beginning at younger ages may help explain why AL was not correlated with age in the pregnant women in this study. Although the differences were not statistically significant, the results of the logistic regression model suggested that pregnant women in the 20-24, 25-29, and 30-34 year-old age groups may have lower AL than pregnant women in the 15-19 year-old age group. It is possible that social factors related to teenage pregnancy contribute to chronic stress in the youngest pregnant women, which would help explain why AL has different attributes in that group.

Other reasons for the differences between AL in pregnant women and in non-pregnant women may involve the methodologies used to study AL in the two groups. First, it is possible that AL must be measured in a different way to be meaningful in pregnant women. For example, the ten biomarkers available in NHANES may not be appropriate to measure this construct in this population. Other factors, such as certain hormones, might be the proper biomarkers to assess AL in pregnant women. Alternately, it may be informative to split the existing biomarkers into categories (e.g. cardiovascular, immune, and metabolic, as suggested by Seeman et al. [2008]). One or more of these subsets of biomarkers may be more useful than all ten for measuring AL in pregnant women. It is also possible that AL is meaningful to measure in pregnant women, but only when pregnancy is considered by trimester or by early vs. late pregnancy. Because the various biomarkers do not remain static during pregnancy, important attributes of AL could be obscured if all pregnant women are lumped together into a single group. Lastly, it remains a possibility that AL is not meaningful to measure in pregnancy. However, further research is necessary before this conclusion can be drawn.

Strengths and limitations

The strengths of this study include the use of a nationally representative sample of pregnant and non-pregnant women from continuous NHANES. Pregnant women were oversampled from 1999 through 2006, so the study included a relatively large sample of pregnant women (Mirel et al., 2009). This study also conservatively identified pregnant and non-pregnant women using several reproductive health variables to minimize misidentification. A combination of questionnaire and laboratory data facilitated the

construction of a robust AL index and provided the necessary demographic and health history data for each participant.

Several limitations of this study are related to the nature of data available from NHANES. NHANES is a cross-sectional study, so the data could not be used to evaluate changes in AL over the life course or assess the relationship between AL during pregnancy and subsequent birth outcomes. Only a subset of biomarkers that might be relevant to AL were available in the NHANES data set; for example, no primary markers of AL such as catecholamines or cortisol are included. This study relied on questionnaire responses regarding medication use and demographic characteristics, so it was subject to the inherent limitations of self-reported data.

Another limitation of the study is the possibility of unmeasured confounders. Because this study was a preliminary look at AL in pregnant women, the logistic regression model included only race, age, education, and income. Future studies may also wish to include possible confounding variables such as cigarette smoking, alcohol and drug use, and dietary factors. Other reproductive factors, such as parity and history of adverse birth outcomes might also be relevant. Data on these variables are available in the NHANES data set.

Chapter 8: Future Directions

The results of the current study raise several interesting questions about AL and pregnancy that could be addressed in future analyses. These include:

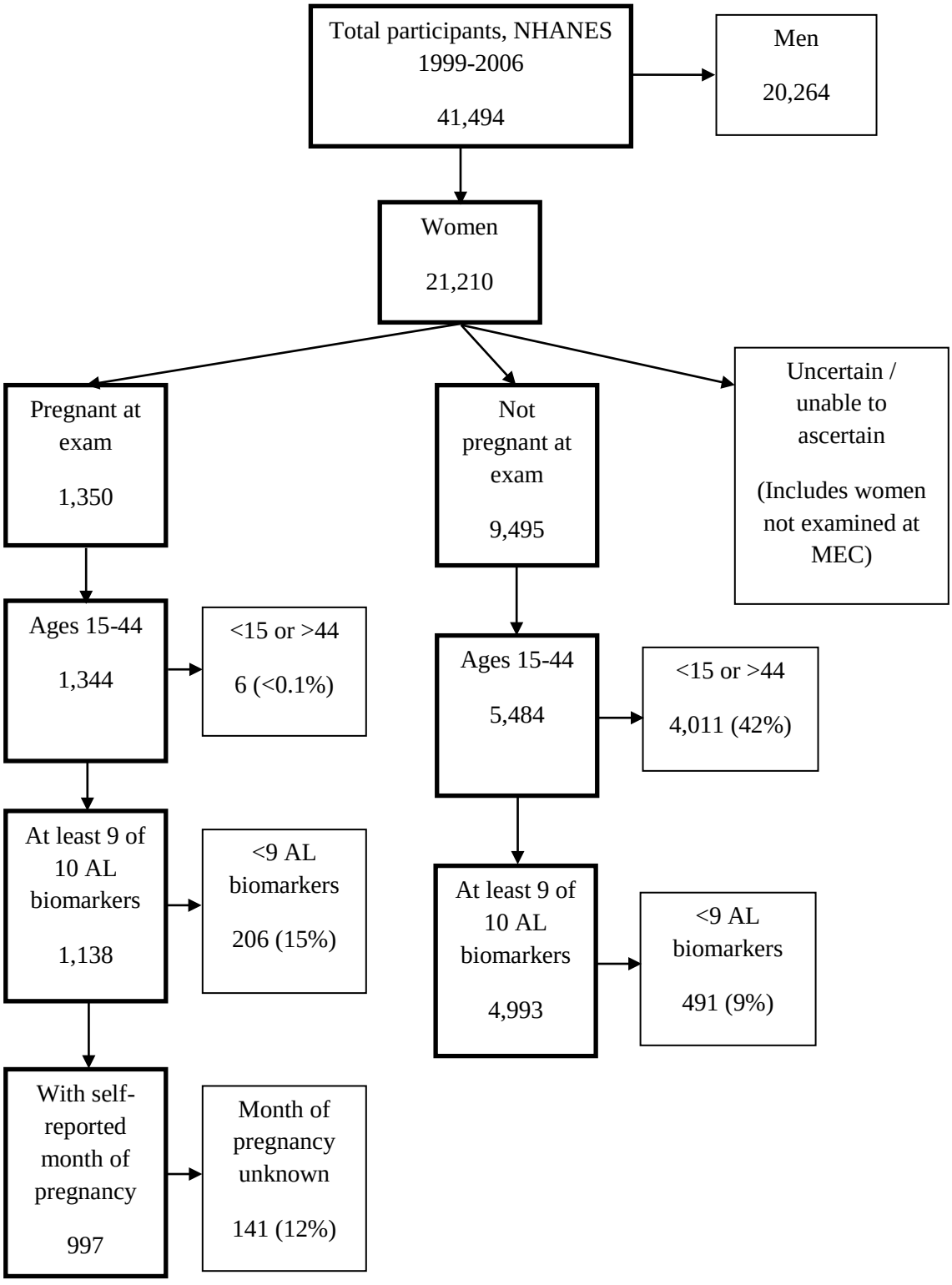
- Does age-standardization of the samples of pregnant and non-pregnant women change the distribution of AL scores in these groups, and does it affect the attributes of AL with regard to race, age, income, and education?
- Does analyzing AL in pregnant women based on stage of pregnancy, particularly by trimester, give a more precise picture of AL than considering all pregnant women as a single group?
- If younger pregnant women experience more chronic stress than their non-pregnant peers, and thus have different patterns of AL compared to non-pregnant women, does restricting the analysis to older women (i.e. 22 to 44 years) yield different results? Additionally, it might be interesting to compare the attributes of AL in the youngest group of pregnant women to those of the oldest group of pregnant women to determine whether there is support for the hypothesis that younger pregnant women have higher levels of AL due to more chronic stress compared with their non-pregnant counterparts.
- Does using a different set of AL-related biomarkers, or splitting the existing set of biomarkers into immune, cardiovascular, and metabolic categories, provide a more useful way to measure AL in pregnant women?

If further research is able determine an accurate and useful way to measure AL during pregnancy, future studies could be undertaken to see whether levels of AL can identify high-risk pregnancies and/or predict adverse birth outcomes. As a first step, it would be interesting to link the data for pregnant women in the NHANES data set with state birth certificate records to determine birth outcomes for each pregnancy. This data linkage would open up a wealth of research opportunities into risk factors for birth outcomes, including AL.

This project represents the first foray into studying AL during pregnancy. Although the results yielded few conclusions about the meaningfulness of AL in pregnant women, they suggest that there are both interesting similarities and major differences in the attributes of AL in pregnant and non-pregnant women. Exploring these similarities and differences provides an interesting jumping-off point for future study.

Appendices

Appendix 1: Samples of pregnant and non-pregnant women chosen from all NHANES participants, 1999-2006



Appendix 2: Distributions of AL scores in pregnant and non-pregnant women, NHANES 1999-2006

The following tables present the frequency, weighted percentage, and standard error of the percentage for each AL score (0-10) in different groups. * indicates the result is significant at $p<0.05$.

AL scores in pregnant and non-pregnant women

	Pregnant women		**Non-pregnant women**	
AL score	**Frequency**	**Weighted percentage (SE of percentage)**	**Frequency**	**Weighted percentage (SE of percentage)**
0	85	7.09 (1.17)	520	8.89 (0.62)
1	209	16.74 (1.85)	1044	19.13 (0.68)
2	272	23.52 (2.28)	1146	22.67 (0.81)
3	240	21.18 (2.15)	925	18.87 (0.75)
4	176	15.85 (1.72)	608	13.27 (0.6)
5	97	10.76 (1.62)	374	8.03 (0.53)
6	41	3.40 (0.89)	222	5.17 (0.38)
7	15	1.33 (0.59)	109	2.62 (0.29)
8	3	0.13 (0.07)	37	1.04 (0.2)
9	0	0	5	0.18 (0.09)
10	0	0	3	0.13 (0.07)
Totals:	1138	100%	4993	100%

Wilcoxon rank sum test p=.0057*

AL scores in pregnant women by trimester

	1st trimester		**2nd trimester**		**3rd trimester**	
AL score	**Frequency**	**Weighted percentage (SE of percentage)**	**Frequency**	**Weighted percentage (SE of percentage)**	**Frequency**	**Weighted percentage (SE of percentage)**
0	29	15.97 (5.67)	32	6.82 (1.93)	20	3.49 (1.06)
1	52	19.32 (4.87)	65	16.20 (3.53)	69	18.95 (3.88)
2	53	18.42 (3.76)	98	21.76 (3.39)	88	18.74 (3.33)
3	33	17.83 (3.19)	98	25.25 (3.75)	84	29.78 (4.58)
4	23	10.31 (2.71)	69	20.00 (3.12)	58	11.83 (2.39)
5	14	12.92 (3.93)	32	4.73 (0.95)	34	10.01 (3.26)
6	5	5.23 (3.09)	12	4.01 (1.80)	17	5.76 (1.90)
7	0	0	4	0.44 (0.25)	5	1.30 (0.77)
8	0	0	2	0.79 (0.66)	1	0.14 (0.14)
9	0	0	0	0	0	0
10	0	0	0	0	0	0
Totals:	209	100%	412	100%	376	100%

Kruskal-Wallis test $p<.0001$*

AL scores in pregnant women by early vs. late pregnancy

	Early pregnancy		**Late pregnancy**	
AL score	**Frequency**	**Weighted percentage (SE of percentage)**	**Frequency**	**Weighted percentage (SE of percentage)**
0	25	9.30 (1.68)	45	4.94 (1.26)
1	60	16.26 (3.80)	115	19.14 (3.04)
2	92	19.40 (3.31)	139	21.02 (3.38)
3	69	22.02 (3.37)	145	21.83 (2.88)
4	58	13.67 (2.61)	112	19.46 (3.17)
5	30	11.48 (2.42)	47	7.48 (2.23)
6	11	5.64 (1.58)	34	4.74 (1.31)
7	3	2.07 (1.75)	10	1.31 (0.56)
8	1	0.16 (0.16)	1	0.08 (0.08)
9	0	0	0	0
10	0	0	0	0
Totals:	349	100%	648	100%

Wilcoxon rank sum test p=0.37

Appendix 3: Attributes of AL categorized as high/low using a cutoff AL score of 3

The following tables present the frequency of high and low AL by race, age, income, and education level. In these analyses, high and low AL were defined using a cutoff AL score of 3. Low AL was defined as a score less than 3; high AL was defined as a score greater than or equal to 3.

All counts are number of women in sample; all percentages are weighted using NHANES weights to reflect the U.S. population. * indicates the result is significant at $p<0.05$.

Frequency of high and low AL in pregnant and non-pregnant women

	Pregnant women	**Non-pregnant women**
High AL	47.36% (566)	50.68% (2710)
Low AL	52.64% (572)	49.32% (2283)
Totals	100% (1138)	100% (4993)

Rao-Scott chi-square = 1.39 p=0.24

AL category by race group in pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican American**	**Other**	**Totals**
Low AL	48.49% (246)	44.61% (65)	55.62% (197)	35.39% (58)	47.36% (566)
High AL	51.51% (259)	55.39% (106)	44.39% (143)	64.61% (64)	52.64% (572)
Totals	100% (505)	100% (171)	100% (340)	100% (122)	100% (1138)

Rao-Scott chi-square: 5.34 p=0.15

AL category by race group in non-pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican American**	**Other**	**Totals**
Low AL	51.52% (1026)	32.83% (506)	57.65% (913)	59.32% (265)	50.68% (2710)
High AL	48.48% (867)	67.17% (744)	42.35% (496)	40.68% (176)	49.32% (2283)
Totals	100% (1893)	100% (1250)	100% (1409)	100% (441)	100% (4993)

Rao-Scott chi-square: 91.11 p<.0001*

AL category by age category in pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	42.43% (75)	46.83% (112)	49.93% (180)	46.73% (139)	46.74% (60)	47.36% (566)
High AL	57.57% (84)	53.17% (125)	50.07% (163)	53.27% (121)	53.26% (79)	52.64% (572)
Totals	100% (159)	100% (237)	100% (343)	100% (260)	100% (139)	100% (1138)

Rao-Scott chi-square: 0.68 p=0.95

AL category by age category in non-pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	68.97% (1209)	62.81% (296)	56.95% (297)	47.23% (270)	40.34% (557)	50.68% (2710)
High AL	31.03% (590)	37.19% (211)	43.05% (237)	52.77% (314)	59.66% (931)	49.32% (2283)
Totals	100% (1880)	100% (507)	100% (534)	100% (584)	100% (1488)	100% (4993)

Rao-Scott chi-square: 126.63 p<.0001*

AL category by mean age (continuous) in pregnant women

	Mean age	**SE**	**N**
Low AL	27.35	0.43	566
High AL	27.42	0.48	572
Totals	27.39	0.32	1138

ANOVA: F=0.04 p=0.85

AL category by mean age (continuous) in non-pregnant women

	Mean age	**SE**	**N**
Low AL	28.56	0.23	2710
High AL	32.64	0.22	2883
Totals	30.57	0.17	4993

ANOVA: F=287.66 p<.0001*

Age and AL, stratified by race in pregnant women

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic White	**Low AL**	44.72% (19)	47.39% (39)	48.62% (78)	51.71% (77)	45.97% (33)	48.49% (246)
	High AL	55.28% (21)	52.61% (55)	51.38% (84)	48.29% (56)	54.03% (43)	51.51% (259)
	Totals	100% (40)	100% (94)	100% (162)	100% (133)	100% (76)	100% (505)

Rao-Scott chi-square = 0.34 p=0.99

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic Black	**Low AL**	35.94% (16)	39.28% (10)	57.48% (23)	33.22% (8)	63.45% (8)	44.61% (65)
	High AL	64.06% (31)	60.72% (27)	42.52% (23)	66.78% (19)	36.55% (6)	55.39% (106)
	Totals	100% (47)	100% (37)	100% (46)	100% (27)	100% (14)	100% (171)

Rao-Scott chi-square = 5.51 p=0.24

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	54.25% (36)	62.28% (54)	60.63% (55)	51.03% (41)	38.71% (11)	57.14% (206)
	High AL	45.75% (27)	37.72% (34)	39.37% (36)	48.97% (25)	61.29% (21)	42.86% (134)
	Totals	100% (63)	100% (88)	100 (91)	100% (66)	100% (32)	100% (340)

Rao-Scott chi-square = 3.86 p=0.43

Age and AL, stratified by race in non-pregnant women

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic White	**Low AL**	69.82% (367)	66.10% (128)	56.64% (133)	47.38% (120)	42.01% (278)	51.52% (1026)
	High AL	30.18% (162)	33.90% (78)	43.36% (107)	52.62% (134)	57.99% (386)	48.47% (867)
	Totals	100% (529)	100% (206)	100% (240)	100% (254)	100% (664)	100% (1893)

Rao-Scott chi-square = 69.48 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic Black	**Low AL**	55.68% (315)	36.79% (42)	36.80% (39)	31.60% (43)	21.52% (67)	32.83% (506)
	High AL	44.32% (251)	63.21% (72)	63.20% (67)	68.40% (99)	78.48% (255)	67.17% (744)
	Totals	100% (566)	100% (114)	100% (142)	100% (106)	100% (322)	100% (1250)

Rao-Scott chi-square = 66.74 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	78.94% (502)	64.84% (89)	65.27% (95)	54.65% (75)	43.40% (152)	57.65% (913)
	High AL	21.05% (130)	35.16% (47)	34.73% (51)	45.35% (57)	56.60% (211)	42.35% (496)
	Totals	100% (632)	100% (136)	100 (146)	100% (132)	100% (363)	100% (1409)

Rao-Scott chi-square = 58.62 p<.0001*

Ratio of family income to poverty (categorical) by AL category in pregnant women

	<1	**1-2**	**2-3**	**3-4**	**4-5**	**Totals**
Low AL	40.79% (137)	48.72% (136)	51.50% (63)	41.74% (53)	46.67% (133)	46.03% (522)
High AL	59.21% (159)	51.28% (118)	48.50% (83)	58.26% (64)	53.33% (120)	53.97% (544)
Totals	100% (296)	100% (254)	100% (146)	100% (117)	100% (253)	100% (1066)

Rao-Scott chi-square = 2.07 p=0.72

Ratio of family income to poverty (categorical) by AL category in non-pregnant women

	<1	**1-2**	**2-3**	**3-4**	**4-5**	**Totals**
Low AL	47.55% (690)	50.47% (621)	46.81% (347)	52.53% (307)	53.45% (556)	50.51% (2521)
High AL	52.45% (603)	49.53% (537)	53.19% (329)	47.47% (253)	46.55% (434)	49.49% (2156)
Totals	100% (1293)	100% (1158)	100% (676)	100% (560)	100% (990)	100% (4677)

Rao-Scott chi-square = 7.08 p=0.13

Ratio of family income to poverty by AL category in pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.69	0.13	522
High AL	2.55	0.14	544
Totals	2.62	0.10	1066

ANOVA: F=1.84 p=0.18

Ratio of family income to poverty by AL category in non-pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.81	0.06	2521
High AL	2.66	0.05	2156
Totals	2.74	0.05	4677

ANOVA: F=10.48 p=0.0012*

AL category by education level in pregnant women

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	39.52% (128)	62.68% (101)	49.66% (191)	48.87% (420)
High AL	60.48% (127)	37.24% (72)	50.34% (180)	50.13% (379)
Totals	100% (255)	100% (173)	100% (371)	100% (799)

Rao-Scott chi-square: 6.38 p=0.04*

AL category by education level in non-pregnant women

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	55.12% (940)	47.19% (387)	53.21% (708)	52.28% (2035)
High AL	44.88% (587)	52.81% (368)	46.79% (665)	47.72% (1620)
Totals	100% (1527)	100% (755)	100% (1373)	100% (3655)

Rao-Scott chi-square: 6.06 p=0.048*

Appendix 4: Attributes of AL categorized into three levels (high/moderate/low)

The following tables present the frequency of high, moderate, and low AL by race, age, income, and education level. In these analyses, three categories of AL were defined as follows: Low AL was defined as a score of 0 or 1; moderate AL was defined as a score of 2 or 3; and high AL was defined as a score of 4 or greater.

All counts are number of women in sample; all percentages are weighted using NHANES weights to reflect the U.S. population. * indicates the result is significant at $p<0.05$.

Frequency of high, moderate, and low AL in pregnant and non-pregnant women

	Pregnant women	**Non-pregnant women**
Low AL	23.84% (294)	28.02% (1564)
Moderate AL	44.70% (512)	41.54% (2071)
High AL	31.47% (332)	30.44% (1358)
Totals	100% (1138)	100% (4993)

Rao-Scott chi-square = 3.09 p=0.21

AL category by race in pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican-American**	**Other**	**Totals**
Low AL	26.72% (134)	18.14% (29)	29.61% (101)	10.61% (30)	23.84% (294)
Moderate AL	39.96% (208)	42.82% (74)	47.16% (167)	63.94% (63)	44.70% (512)
High AL	33.32% (163)	40.04% (68)	22.23% (72)	25.45% (29)	31.47% (332)
Totals	100% (505)	100% (171)	100% (340)	100% (122)	100% (1138)

Rao-Scott chi-square: 21.56 p=0.0015*

AL category by race in non-pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican-American**	**Other**	**Totals**
Low AL	27.62% (559)	18.44% (269)	34.44% (572)	35.27% (164)	28.02% (1564)
Moderate AL	43.40% (831)	34.66% (504)	39.48% (557)	40.11% (179)	41.54% (2071)
High AL	28.99% (503)	46.91% (477)	26.08% (280)	24.62% (98)	30.44% (1358)
Totals	100% (1893)	100% (1250)	100% (1409)	100% (441)	100% (4993)

Rao-Scott chi-square: 96.41 p<.0001*

AL category by age category in pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	21.64% (40)	23.69% (63)	28.24% (93)	21.88% (70)	19.96% (28)	23.84% (294)
Moderate AL	49.87% (77)	44.69% (102)	41.73% (149)	47.99% (126)	42.20% (58)	44.70% (512)
High AL	28.49% (42)	31.61% (72)	30.03% (101)	30.13% (64)	37.83% (53)	31.47% (332)
Totals	100% (159)	100% (237)	100% (343)	100% (260)	100% (139)	100% (1138)

Rao-Scott chi-square: 3.51 p=0.90

AL category by age category in non-pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	40.75% (772)	36.74% (172)	32.26% (177)	27.46% (160)	20.08% (283)	28.02% (1564)
Moderate AL	46.49% (860)	45.93% (229)	40.99% (201)	41.70% (238)	38.69% (543)	41.54% (2071)
High AL	12.76% (248)	17.33% (106)	26.45% (156)	30.84% (186)	41.23% (662)	30.44% (1358)
Totals	100% (1880)	100% (507)	100% (534)	100% (584)	100% (1488)	100% (4993)

Rao-Scott chi-square: 184.41 p<.0001*

AL category by mean age (continuous) in pregnant women

	Mean age	**SE**	**N**
Low AL	27.03	0.40	294
Moderate AL	27.27	0.48	512
High AL	27.81	0.66	332

ANOVA: F=2.88 p=0.09

AL category by mean age (continuous) in non-pregnant women

	Mean age	**SE**	**N**
Low AL	27.93	0.31	1564
Moderate AL	29.86	0.32	2071
High AL	33.96	0.27	1358

ANOVA: F = 337.34 p<.0001*

Age and AL, stratified by race in pregnant women

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic White	**Low AL**	20.29% (9)	31.06% (24)	28.29% (38)	26.73% (44)	23.05% (19)	26.72% (134)
	Moderate AL	48.43% (17)	36.44% (35)	36.44% (71)	46.50% (61)	36.15% (24)	39.96% (208)
	High AL	31.28% (14)	32.49% (35)	34.49% (53)	26.77% (28)	40.80% (33)	33.32% (163)
	Totals	100% (40)	100% (94)	100 (62)	100% (133)	100% (76)	100% (505)

Rao-Scott chi-square = 3.09 p=0.92

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic Black	**Low AL**	21.17% (10)	9.01% (3)	27.20% (11)	16.04% (3)	15.05% (2)	18.14% (29)
	Moderate AL	49.33% (22)	46.95% (18)	39.17% (17)	36.43% (11)	48.40% (6)	42.82% (74)
	High AL	29.49% (15)	44.04% (16)	33.63% (18)	47.53% (13)	36.55% (6)	39.04% (68)
	Totals	100% (47)	100% (37)	100% (46)	100% (27)	100% (14)	100% (171)

Rao-Scott chi-square = 4.37 p=0.82

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	26.81% (18)	28.78% (30)	44.49% (33)	20.99% (17)	14.31% (3)	29.61% (101)
	Moderate AL	56.20% (34)	44.54% (40)	34.72% (37)	53.50% (37)	62.82% (19)	47.16% (167)
	High AL	16.99% (11)	26.68% (18)	20.79% (21)	25.50% (12)	22.87% (10)	23.23% (72)
	Totals	100% (63)	100% (88)	100% (91)	100% (66)	100% (32)	100% (340)

Rao-Scott chi-square = 14.58 p=0.07

Age and AL, stratified by race in non-pregnant women

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic White	**Low AL**	40.18% (213)	37.28% (68)	30.67% (71)	26.05% (66)	20.86% (141)	27.62% (559)
	Moderate AL	47.73% (250)	49.01% (104)	43.99% (102)	44.05% (114)	40.19% (261)	43.40% (831)
	High AL	12.09% (66)	13.71% (34)	25.35% (67)	29.91% (74)	38.95% (262)	28.99% (503)
	Totals	100% (529)	100% (206)	100% (240)	100% (254)	100% (664)	100% (1893)

Rao-Scott chi-square: 92.71 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic Black	**Low AL**	29.23% (163)	19.75% (21)	19.02% (20)	21.24% (29)	12.11% (36)	18.44% (269)
	Moderate AL	49.07% (281)	42.61% (51)	34.60% (37)	31.64% (45)	27.60% (90)	34.66% (504)
	High AL	21.70% (122)	37.64% (42)	46.37% (49)	47.12% (68)	60.30% (196)	46.91% (477)
	Totals	100% (566)	100% (114)	100% (106)	100% (142)	100% (322)	100% (1250)

Rao-Scott chi-square: 64.85 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	50.68% (328)	42.86% (59)	43.50% (64)	30.84% (45)	21.48% (76)	34.44% (572)
	Moderate AL	42.28% (260)	38.41% (52)	35.59% (51)	41.97% (55)	39.37% (139)	39.48% (557)
	High AL	7.04% (44)	18.73% (25)	20.91% (31)	27.20% (32)	39.15% (148)	26.08% (280)
	Totals	100% (632)	100% (136)	100% (146)	100% (132)	100% (363)	100% (1409)

Rao-Scott chi-square: 78.36 p<.0001*

Ratio of family income to poverty (categorical) by AL category in pregnant women

	<1	1-2	2-3	3-4	4-5	Totals
Low AL	14.14% (62)	25.80% (70)	29.43% (38)	26.53% (31)	22.78% (67)	22.88% (268)
Mod AL	56.14% (141)	38.88% (113)	40.20% (60)	39.24% (47)	43.17% (115)	44.39% (476)
High AL	29.72% (93)	35.32% (48)	30.37% (48)	34.23% (39)	34.06% (71)	32.73% (322)
Totals	100% (296)	100% (254)	100% (146)	100% (117)	100% (253)	100% (1066)

Rao-Scott chi-square = 8.16 p=0.42

Ratio of family income to poverty (categorical) by AL category in non-pregnant women

	<1	1-2	2-3	3-4	4-5	Totals
Low AL	26.93% (296)	30.28% (381)	22.89% (186)	26.29% (163)	29.25% (311)	27.60% (1437)
Mod AL	37.67% (522)	38.13% (442)	44.91% (297)	45.42% (249)	43.56% (443)	41.76% (1953)
High AL	35.41% (375)	31.58% (335)	32.20% (193)	28.29% (148)	27.18% (236)	30.64% (1287)
Totals	100% (1293)	100% (1158)	100% (676)	100% (560)	100% (990)	100% (4677)

Rao-Scott chi-square = 19.51 p=0.01*

Ratio of family income to poverty by AL category in pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.81	0.17	268
Moderate AL	2.49	0.14	476
High AL	2.66	0.16	322

ANOVA F=0.72 p=0.40

Ratio of family income to poverty by AL category in non-pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.77	0.09	1347
Moderate AL	2.82	0.06	1953
High AL	2.59	0.05	1287

ANOVA F=9.63 p=.002*

AL category by education level in pregnant women

	Less than high school		**High school or equivalent**		**More than high school**		**Totals**	
Low AL	22.81%	(70)	39.08%	(61)	23.88%	(103)	26.23%	(234)
Moderate AL	50.15%	(112)	41.73%	(69)	42.94%	(170)	44.33%	(351)
High AL	27.05%	(73)	19.19%	(43)	33.68%	(98)	29.44%	(214)
Totals	100%	(255)	100%	(173)	100%	(371)	100%	(799)

Rao-Scott chi-square: 9.34 p=0.0532

AL category by education level in non-pregnant women

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	30.79% (556)	25.56% (211)	28.84% (408)	28.54% (1175)
Moderate AL	42.38% (644)	40.38% (303)	42.29% (556)	41.87% (1503)
High AL	26.84% (327)	34.06% (241)	28.88% (409)	29.59% (977)
Totals	100% (1527)	100% (755)	100% (1373)	100% (3655)

Rao-Scott chi-square: 6.46 p=0.17

Appendix 5: Attributes of AL categorized as high/low using a cutoff AL score of 4

The following tables present the frequency of high and low AL by race, age, income, and education level. In these analyses, high and low AL were defined using a cutoff AL score of 4. Low AL was defined as a score less than 4; high AL was defined as a score greater than or equal to 4.

All counts are number of women in sample; all percentages are weighted using NHANES weights to reflect the U.S. population. * indicates the result is significant at $p<0.05$.

Frequency of high and low AL in pregnant and non-pregnant women

	Pregnant women	**Non-pregnant women**
Low AL	68.53% (806)	69.56% (3635)
High AL	31.47% (332)	30.44% (1358)
Totals	100% (1138)	100% (4993)

Rao-Scott chi-square = 0.17 p=0.68

AL category by race in pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican American**	**Other**	**Totals**
Low AL	66.68% (342)	60.96% (103)	76.77% (268)	74.55% (93)	68.53% (806)
High AL	33.32% (163)	39.04% (68)	23.23% (72)	25.45% (29)	31.47% (332)
Totals	100% (505)	100% (171)	100% (340)	100% (122)	100% (1138)

Rao-Scott chi-square: 5.79 p=0.12

AL category by race in non-pregnant women

	Non-Hispanic White	**Non-Hispanic Black**	**Mexican American**	**Other**	**Totals**
Low AL	71.01% (1390)	53.09% (773)	73.92% (1129)	75.38% (343)	69.56% (3635)
High AL	28.99% (503)	46.91% (477)	26.08% (280)	24.62% (98)	30.44% (1358)
Totals	100% (1893)	100% (1250)	100% (1409)	100% (441)	100% (4993)

Rao-Scott chi-square: 71.77 p<.0001*

AL category by age category in pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	71.51% (117)	68.39% (165)	69.97% (242)	69.87% (196)	62.17% (86)	68.53% (806)
High AL	28.49% (42)	31.61% (172)	30.03% (101)	30.13% (64)	37.83% (53)	31.47% (332)
Totals	100% (159)	100% (237)	100% (343)	100% (260)	100% (139)	100% (1138)

Rao-Scott chi-square: 1.34 p=0.85

AL category by age category in non-pregnant women

	15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Low AL	87.24% (1632)	82.67% (401)	73.25% (378)	69.16% (398)	58.77% (826)	69.56% (3635)
High AL	12.76% (248)	17.33% (106)	26.25% (156)	30.84% (186)	41.23% (662)	30.44% (1358)
Totals	100% (1880)	100% (507)	100% (534)	100% (584)	100% (1488)	100% (4993)

Rao-Scott chi-square: 165.66 p<.0001*

AL category by mean age (continuous) in pregnant women

	Mean age	**SE**	**N**
Low AL	27.19	0.35	806
High AL	27.82	0.66	332
Totals	27.39	0.32	1138

ANOVA: F=2.78 p=0.10

AL category by mean age (continuous) in non-pregnant women

	Mean age	**SE**	**N**
Low AL	29.09	0.24	3635
High AL	33.96	0.27	1358
Totals	30.57	0.17	4993

ANOVA: F=352.08 p<.0001*

Age and AL, stratified by race in pregnant women

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic White	**Low AL**	68.73% (26)	67.51% (59)	64.72 (109)	73.23% (105)	59.20% (43)	66.68% (342)
	High AL	31.28% (14)	32.49% (35)	35.28% (53)	26.77% (28)	40.80% (33)	33.32% (163)
	Totals	100% (40)	100% (94)	100% (162)	100% (133)	100% (76)	100% (505)

Rao-Scott chi-square = 1.87 p=0.76

		15-19	**20-24**	**25-29**	**30-34**	**35-44**	**Totals**
Non-Hispanic Black	**Low AL**	70.52% (32)	55.96% (21)	66.37% (28)	52.47% (14)	63.45% (8)	44.61% (65)
	High AL	29.49% (15)	44.04% (16)	33.63% (18)	47.53% (13)	36.55% (6)	55.39% (106)
	Totals	100% (47)	100% (37)	100% (46)	100% (27)	100% (14)	100% (171)

Rao-Scott chi-square = 2.13 p=0.71

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	83.01% (52)	73.32% (70)	79.21% (70)	74.50% (54)	77.13% (22)	76.77% (268)
	High AL	16.99% (11)	26.68% (18)	20.79% (21)	25.50% (12)	22.87% (10)	23.23% (72)
	Totals	100% (63)	100% (88)	100 (91)	100% (66)	100% (32)	100% (340)

Rao-Scott chi-square = 1.43 p=0.84

Age and AL, stratified by race in non-pregnant women

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic White	**Low AL**	87.91% (463)	86.29% (172)	74.65% (173)	70.09% (180)	61.05% (402)	71.01% (1390)
	High AL	12.09% (66)	13.71% (34)	25.35% (67)	29.91% (74)	38.95% (262)	28.99% (503)
	Totals	100% (529)	100% (206)	100% (240)	100% (254)	100% (664)	100% (1893)

Rao-Scott chi-square = 81.26 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Non-Hispanic Black	**Low AL**	78.30% (444)	62.36% (72)	53.63% (57)	52.88% (74)	39.70% (126)	53.09% (773)
	High AL	21.70% (122)	37.64% (42)	46.37% (49)	47.12% (68)	60.30% (196)	46.91% (477)
	Totals	100% (566)	100% (114)	100% (142)	100% (106)	100% (322)	100% (1250)

Rao-Scott chi-square = 62.86 p<.0001*

		15-19	20-24	25-29	30-34	35-44	Totals
Mexican American	**Low AL**	92.96% (588)	81.27% (111)	79.09% (115)	72.80% (100)	60.85% (215)	73.92 % (1129)
	High AL	7.04% (44)	18.73% (25)	20.91% (31)	27.20% (32)	39.15% (148)	26.08% (280)
	Totals	100% (632)	100% (136)	100 (146)	100% (132)	100% (363)	100% (1409)

Rao-Scott chi-square = 63.10 p<.0001*

Ratio of family income to poverty (categorical) by AL category in pregnant women

	<1	**1-2**	**2-3**	**3-4**	**4-5**	**Totals**
Low AL	70.28% (203)	64.68% (183)	69.63% (98)	65.77% (78)	65.94% (182)	67.27% (744)
High AL	29.72% (93)	35.32% (71)	30.37% (48)	34.23% (39)	34.06% (71)	32.73% (322)
Totals	100% (296)	100% (254)	100% (146)	100% (117)	100% (253)	100% (1066)

Rao-Scott chi-square = 0.83 p=0.93

Ratio of family income to poverty (categorical) by AL category in non-pregnant women

	<1	**1-2**	**2-3**	**3-4**	**4-5**	**Totals**
Low AL	64.59% (918)	68.42% (823)	67.80% (483)	71.71% (412)	72.82% (754)	69.36% (3390)
High AL	35.41% (375)	31.58% (335)	32.20% (193)	28.29% (148)	27.18% (236)	30.64% (1287)
Totals	100% (1293)	100% (1158)	100% (676)	100% (560)	100% (990)	100% (4677)

Rao-Scott chi-square = 11.53 p=0.02*

Ratio of family income to poverty by AL category in pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.60	0.11	744
High AL	2.66	0.16	322
Totals	2.62	0.10	1066

ANOVA: F=0.32 p=0.57

Ratio of family income to poverty by AL category in non-pregnant women

	Mean ratio of income/poverty	**SE**	**N**
Low AL	2.80	0.05	3390
High AL	2.59	0.05	1287
Totals	2.74	0.05	4677

ANOVA: F=18.42 p=<.0001*

AL category by education level in pregnant women

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	72.96% (182)	80.81% (130)	66.32% (273)	70.56% (585)
High AL	27.04% (73)	19.89% (43)	33.68% (98)	29.44% (214)
Totals	100% (255)	100% (173)	100% (371)	100% (799)

Rao-Scott chi-square: 6.45 p=0.04*

AL category by education level in non-pregnant women

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	73.16% (1200)	65.94% (514)	71.12% (964)	70.41% (2678)
High AL	26.84% (327)	34.06% (241)	28.88% (409)	29.59% (977)
Totals	100% (1527)	100% (755)	100% (1373)	100% (3655)

Rao-Scott chi-square: 5.45 p=0.07

AL category by education level in pregnant women, age restricted to 22-44 years

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	77.28% (107)	81.20% (89)	64.79% (254)	69.61% (450)
High AL	22.72% (35)	18.80% (30)	35.21% (92)	30.39% (157)
Totals	100% (142)	100% (119)	100% (346)	100% (607)

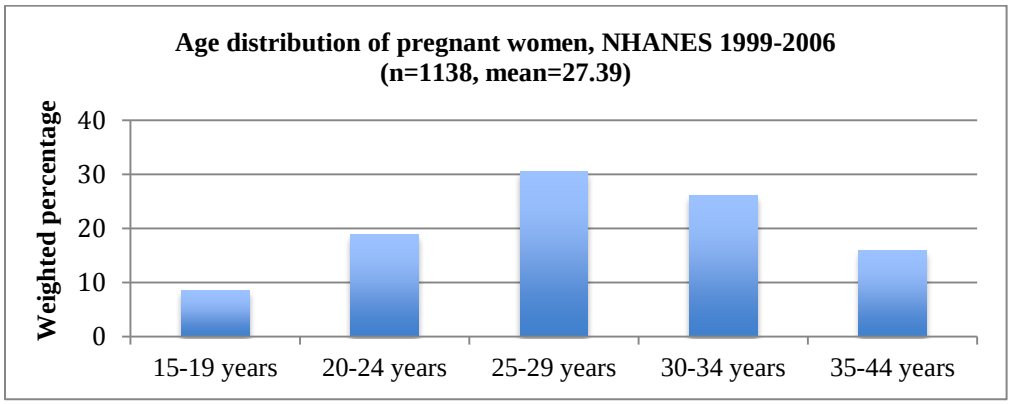
Rao-Scott chi-square: 8.22 p=0.0164*

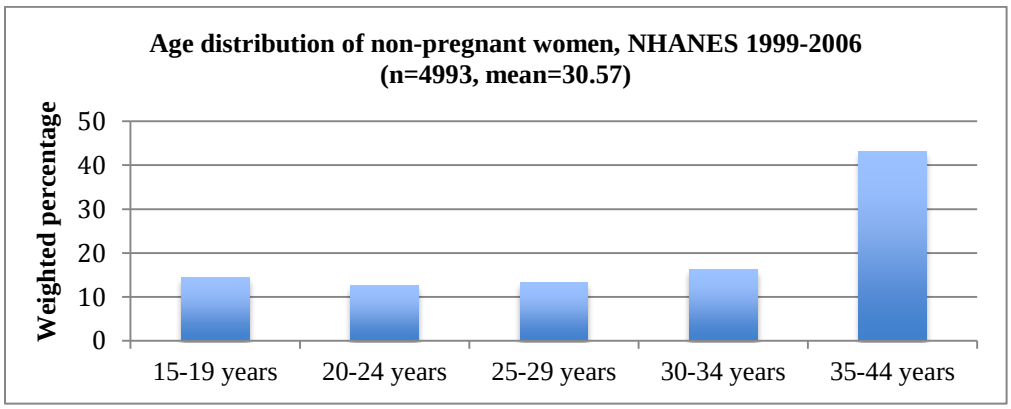
AL category by education level in non-pregnant women, age restricted to 22-44 years

	Less than high school	**High school or equivalent**	**More than high school**	**Totals**
Low AL	60.58% (327)	61.81% (281)	69.29% (750)	66.12% (1358)
High AL	39.42% (200)	38.19% (202)	30.71% (374)	33.88% (776)
Totals	100% (527)	100% (483)	100% (1124)	100% (2134)

Rao-Scott chi-square: 9.59 p=0.0083*

Appendix 6: Age distributions of pregnant and non-pregnant women, NHANES 1999-2006





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