BLOOD PRESSURE AND TOBACCO EXPOSURE AMONG RURAL ADOLESCENTS AGES 15-18

by

LUZ HUNTINGTON-MOSKOS

ANNE TURNER-HENSON, COMMITTEE CHAIR
SUSAN DAVIES
CONNIE KOHLER
MARTI RICE
XIAOGANG SU

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LUZ HUNTINGTON-MOSKOS

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ABSTRACT

High blood pressure is a prevalent precursor to cardiovascular disease. An estimated 3% of US adolescents have hypertension. In addition, prehypertension is predictive of hypertension in adolescents, with progression between these stages at approximately 7% per year. Tobacco use/exposure is strongly linked to cardiovascular risk and disease in adults. Further, rural communities have higher tobacco use prevalence and fewer community policies restricting tobacco use. Little is known about the effects of tobacco exposure on blood pressure and the mediating effects of inflammation (salivary C-reactive protein (CRP)) in rural adolescents. The purpose of this study is to determine the relationships between tobacco exposures, inflammation, and blood pressure among rural adolescents ages 15-18 after controlling for age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal stage, and weight status.

A convenience sample of 148 adolescents ages 15-18 was recruited from two rural high schools (88 female and 60 male, all Caucasian). Adolescents were measured for blood pressure, weight status (BMI, waist circumference), tobacco exposure (self-report, salivary cotinine), and inflammation (salivary CRP). Self-report measures of tobacco exposure included the Uptake Continuum and Peer and Family Smoking Index.
The study found 25% of adolescent males and 11.4% of adolescent females had elevated systolic blood pressures. A fifth of the sample (22%) had elevated salivary cotinine levels indicative of secondhand smoke exposure. Nearly half of the rural adolescent participants stated that their family members smoke cigarettes. Salivary cotinine levels had a significant association with smoking exposure by family members ($X^2 = 10.81, p = .001$), though not with smoking exposure by peers ($X^2 = 1.21, p = .271$). Age, gender, waist circumference and salivary cotinine were found to contribute to 36.4% of the variance in systolic blood pressure and 19.1% of the variance in diastolic blood pressure. No evidence was found to support inflammation as a mediator between tobacco exposure and blood pressure.

Tobacco exposure and elevated blood pressure are contributors to cardiovascular risk. Prehypertensive blood pressure measurements during adolescence are predictive of hypertension. In adolescents, elevated blood pressure measurements accompanied by other cardiovascular risk factors, such as tobacco use/exposure, are of concern. As part of the effort to improve rural public health, the health of rural adolescents must be made visible.

Key Words: blood pressure, tobacco, inflammation, adolescents, rural
DEDICATION

I would like to dedicate this dissertation to my children, Violet and Catalina Moskos. Once we decided that mom’s return to graduate school would benefit our family, the both of you encouraged me the entire way. I really appreciate the quiet moments watching a movie when we cuddled on the couch together; those were the moments when I found the strength to recharge and keep moving forward. All three of us had homework to finish and we got it done. My own dad helped me understand that education is a great path toward building a full life. I hope this journey has shown you that education can help you mold your own lives into anything you can dream up. I couldn’t have done this without both of you by my side.
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CHAPTER ONE
INTRODUCTION

Hypertension, a major public health challenge (Institute of Medicine, 2010a), is the most prevalent precursor to cardiovascular disease in the United States (US). An individual’s cardiovascular health is defined by seven metrics including blood pressure levels and smoking status as well as body mass index, nutritional intake, physical activity level, blood glucose and total cholesterol (Lloyd-Jones et al., 2010). Hypertension is costly, with estimated direct and indirect costs at $93.5 billion annually (Gillespie, Kuklina, Briss, Blair, & Hong, 2011; Heidenreich et al., 2011). Approximately 78 million US adults have high blood pressure (Go et al., 2013). Although related, a distinction can be made between the concepts of high blood pressure and hypertension. A single elevated blood pressure reading can be referred to as a high blood pressure or categorized as hypertensive; however, this single, categorized blood pressure reading does not equate to a diagnosis of hypertension. A diagnosis of hypertension results from an average of two or more properly measured, seated blood pressure readings which occur on two or more occasions (Chobanian et al., 2003).

There is growing concern regarding hypertension and high blood pressure among youth. Rates of hypertension among US children and adolescents have been estimated to be 2% to 5% (Hansen, Gunn, & Kaelber, 2007; Loeffler, Navas-Acien, Brady, Miller, & Fadrowski, 2012; McNiece et al., 2007; National High Blood Pressure Education
Researchers focused solely on adolescents have reported hypertension rates at 3% (Loeffler, et al., 2012; Sugiyama et al., 2007). Two decades of research have established that a blood pressure elevation in childhood can track through adolescence and into adulthood (Bao, Threefoot, Srinivasan, & Berenson, 1995; Chen & Wang, 2008; Kollias, Pantsiotou, Karpertas, Roussias, & Stergiou, 2011; National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004; Toschke, Kohl, Mansmann, & von Kries, 2009). The persistence of blood pressure elevations from childhood through adolescence and into adulthood is cause for alarm and, yet, also presents a significant opportunity for the prevention of cardiovascular disease.

Blood pressure (BP) measurements in children and adolescents must take into account age, gender and height in order to determine the percentile and, thus, interpret the measurements as normotensive, pre-hypertensive and hypertensive (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). With both adults and children, a diagnosis of hypertension requires an average of two or more properly measured, seated blood pressure measurements on two or more occasions. To prevent hypertension, the guidelines set forth by the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004) recommend maintaining blood pressure below the 95% percentile. In practice, these necessary, additional steps to determine the percentile for blood pressure classification have contributed to an under
diagnosis of hypertension among children and adolescents in the US (Hansen, et al., 2007).

Blood pressure, tobacco exposure and cardiovascular health have been repeatedly linked (Ford, Nonnemaker, & Wirth, 2008; Institute of Medicine, 2010b; Jefferis et al., 2010; Venn & Britton, 2007; Wilkinson, Lee, & Arheart, 2007). The concept of tobacco exposure includes self-reported individual tobacco use behaviors, such as cigarette smoking and/or smokeless tobacco use, and sources of secondhand smoke exposure (United States Department of Health and Human Services, 2010c). The term “secondhand smoke” refers to a mixture of side stream smoke from a lit cigarette and mainstream smoke exhaled by an active smoker (United States Department of Health and Human Services, 2006). The prevalence of tobacco exposure among adolescents is substantial. The 2011 findings for the Youth Risk Behavior Surveillance Survey (YRBS) reported that ever smoking levels among 9th to 12th graders are as high as 45% while current smoking levels (within the last 30 days) are at 18% (Eaton et al., 2012). Although the prevalence of current smoking levels has decreased significantly since 1991 (27.5%), the nation’s adolescents have not reached the 16% target goal set by Healthy People 2020 (United States Department of Health and Human Services, 2010a).

Smokeless tobacco use includes the use of products such as chewing tobacco, which requires regular spitting, and moist snuff, which requires no spitting (White, Oliffe, & Bottorff, 2012). The prevalence of smokeless tobacco use for those 12 years and older has remained stable at approximately 3.0%; however, an increase in use was noted among male adolescents from 3.4% in 2002 to 4.4% in 2007 (Substance Abuse and Mental Health Services Administration, 2009). Among individuals over 12 years of age,
past month smokeless tobacco use was substantially higher in rural counties (8.4%) compared to large metropolitan counties (1.9%) and small metropolitan counties (4.7%) (Substance Abuse and Mental Health Services Administration, 2009). The 2011 Youth Risk Behavior Surveillance Survey found an 8% prevalence of smokeless tobacco use among adolescents nationwide (Eaton, et al., 2012). Again, this prevalence rate exceeds the 6.9% target goal set by Healthy People 2020 (United States Department of Health and Human Services, 2010a). Smokeless tobacco use is higher among adolescents who smoke (Newman & Shell, 2005). Despite these high rates of tobacco use among adolescents, there is a limited amount of research on smokeless tobacco use among adolescents in general (Newman & Shell, 2005); but even less is known about the prevalence among rural adolescents.

Secondhand smoke exposure must be included in a discussion of tobacco exposure. To date, empirical evidence indicates that there is no safe, minimum dose of secondhand smoke: All secondhand smoke is considered a risk to health (United States Department of Health and Human Services, 2006). Approximately 88 million individuals are exposed to secondhand smoke in the US; this includes 32 million youth between ages of 3 and 19 years (Kaufmann et al., 2010). Secondhand smoke exposure declined 70% since the 1980’s; however, adolescents continue to be exposed to secondhand smoke in their homes (Marano, Schober, Brody, & Zhang, 2009). The baseline level of secondhand smoke exposure for adolescents ages 12 to 17 is 45.5% according to Healthy People 2020 (United States Department of Health and Human Services, 2010a). The 2007-2008 National Health and Nutrition Examination Surveys reported that 46.5% of nonsmoking
adolescents ages 12-19 had elevated serum cotinine levels (Kaufmann, et al., 2010), indicating significant secondhand smoke exposure.

An adolescent’s tobacco use and exposure can be quantified using both subjective and objective measures. Subjective, self-report measures are frequently used in youth tobacco research (Arrazola, Dube, & Engstrom, 2012; Eaton, et al., 2012; Johnston, O'Malley, Bachman, & Schulenberg, 2013) as they are less expensive, particularly for large studies, and a measure of validity has been established (Wills & Cleary, 1997). However, underage tobacco use is illegal and adolescents may struggle with providing valid responses on self-report measure. Adolescent have a strong need anonymity and confidentiality with regard to tobacco use and exposure questionnaires (Dolcini, Adler, & Ginsberg, 1996). Thus, objective measures of tobacco use and exposure, such as the biomarker of salivary cotinine, can provide biochemical verification of responses on self-report measures (Caraballo, Giovino, & Pechacek, 2004). Since objective measures of tobacco (e.g. salivary cotinine) lack information regarding the specific source of exposure and subjective, self-report measures often lack the measurement specificity found with biomarkers, the inclusion of both objective and subjective tobacco use and exposure measures adds strength to a research design.

Cotinine is the principal metabolite of nicotine and the biomarker of choice when studying smoking status and secondhand smoke exposure (Benowitz, Bernert, Caraballo, Holiday, & Wang, 2009). Established cut-points related to cotinine levels in the human body allow researchers to objectively determine tobacco usage and extent of secondhand smoke exposure (see Figure 1).
Figure 1. Discriminating Smokers from Non-smokers. Copyright 2010 by Salimetrics, LLC

Cotinine can be measured in serum, urine, and saliva (Society for Research on Nicotine and Tobacco, 2002) and can assist in quantifying the extent of tobacco exposure but not the exact source of the exposure (Benowitz, et al., 2009). The use of salivary cotinine is well suited to school-based studies as it is less invasive (Granger et al., 2007). Cotinine cutpoints have been established to assist in quantifying the extent of tobacco exposure and these cutpoints differ between adults and adolescents. With salivary specimens from adolescents, the cut-point of 1.0 ng/ml or less is indicative of light
secondhand smoke exposure (Benowitz, et al., 2009). Salivary cotinine levels between 1.0 ng/ml and 3.0 ng/ml are indicative of either light smoking behavior or heavy secondhand smoke exposure. Finally, the salivary cotinine cut-point of 3.0 ng/ml or greater is indicative of active smoking or substantial secondhand smoke exposure specific in an adolescent (Benowitz, et al., 2009).

Compared to urban areas, rural communities experience tobacco-related disparities including higher prevalence of cigarette smoking, higher levels of smokeless tobacco use and higher secondhand smoke exposure (American Legacy Foundation, 2009; Vander Weg, Cunningham, Howren, & Cai, 2011). Rural communities have a number of challenges related to accomplishing sustained tobacco control including limited transportation, low income, fewer individuals with health insurance, limited health care access and proximity to tobacco growers (American Legacy Foundation, 2009; U.S. Department of Health and Human Services, 2011). In 2002-2007, the Monitoring the Future survey found that current cigarette smoking reported by adolescents in non-metropolitan (rural) statistical areas was significantly greater than those adolescents living in large metropolitan statistical areas, 27.8% and 20.9%, respectively (King, Dube, & Tynan, 2012). In 2007, a third of children from rural areas reported living with a smoker compared to only 24.4% of children from urban areas (U.S. Department of Health and Human Services, 2011). The increased use of tobacco in rural communities may have implications for rural adolescents with regard to their blood pressure and cardiovascular health.

Tobacco exposure, quantified using self-reported cigarette smoking and/or smokeless tobacco use and through sources of secondhand smoke exposure, has been
linked to negative cardiovascular effects through a number of mechanisms including inflammation as well as oxidative stress, hemodynamic effects, endothelial dysfunction, thrombosis, and hyperlipidemia (Institute of Medicine, 2010b). The smoke resulting from tobacco use is thought to have a multiplicative, negative effect on cardiovascular health when coupled with untreated hypertension (U.S. Department of Health and Human Services, 1983). Inflammation may be the common denominator for the “multiplicative, negative effect” involving tobacco exposure and hypertension. Acute and systemic inflammation functions as part of the body’s immune reaction in response to infection, trauma, cancer and various inflammatory diseases. In addition to linking tobacco exposure and negative cardiovascular effects, inflammation serves as a link between the hypertension and atherosclerosis (Libby, Ridker, & Maseri, 2002). “Atherosclerosis is a generalized, chronic inflammatory vessel disorder” thought to be responsible for the development of negative cardiovascular outcomes such as stroke and myocardial infarction (Nordestgaard & Zacho, 2009, p. 521).

C-reactive protein (CRP) is a biomarker used in the measurement of low-grade chronic inflammation and has been used to define atherosclerotic risk (Libby, et al., 2002). Elevated levels of C-reactive protein (CRP) indicative of systemic inflammation have been positively associated with smoking status in adults (Tracy et al., 1997; United States Department of Health and Human Services, 2010c) and adolescents (Azar & Richard, 2011; Juonala et al., 2006; O'Loughlin et al., 2008). A significant positive correlation between levels of cotinine (indicating metabolized nicotine) and levels of CRP in youth ages 6-18 has been reported (Wilkinson, et al., 2007). CRP appears to mediate the relationship between tobacco exposure and blood pressure (Lieb et al., 2008)
as elevated levels of CRP have been linked to the future development of hypertension in non-hypertensive adults (Lieb, et al., 2008; Sesso et al., 2003; Wang et al., 2007). Elevated CRP levels are considered “a marker for the extent of atherosclerosis or for the inflammatory activity and vulnerability of atherosclerotic plaques” (Nordestgaard & Zacho, 2009).

In addition to the process of inflammation, multiple factors influence an individual’s blood pressure and their level of tobacco exposure. In this study, the confounding variables to be controlled for include age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status, and weight status. Age and gender are both integral to classifying the blood pressure measurements of individuals under age 18 (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Diastolic blood pressure, in particular, is often lower in women compared to men (Oparil & Miller, 2005). Blood pressure also appears to differ by ethnicity with African Americans having a higher frequency and earlier onset of hypertension (Oparil & Wright Jr, 2005). While adjusting for weight status, Rosner and colleagues (2009) found evidence of ethnic differences in blood pressure among male youth but not among female youth. Hispanic and African American male youth have a higher prevalence of blood pressure elevation compared to Caucasian male youth (Rosner, et al., 2009).

Along with the biological factors of age, gender and ethnicity, the contextual factor of socioeconomic status is an important covariate when studying the outcome of blood pressure among adolescents. In a prospective cohort design including children from Holland ages 5 to 6 (n=3024), the effects of socioeconomic status on the development of
hypertension were found to begin in childhood; higher blood pressures and more prehypertension were found in children from lower socioeconomic status families (van den Berg, van Eijsden, Galindo-Garre, Vrijkotte, & Gemke, 2013). The main findings from the prospective Cardiovascular Risk in Young Finns Study state that socioeconomic status, childhood body mass index and parental risk factor status are independent predictors of hypertension along with adult obesity and dyslipidemia (Juonala, Viikari, & Raitakari, 2013). A systematic review of literature regarding childhood socioeconomic circumstances and cardiovascular risk in adulthood found that low socioeconomic status during childhood is an independent risk factor for developing cardiovascular disease in adulthood (Galobardes, Smith, & Lynch, 2006). The variable of socioeconomic status captures information regarding an individual’s context for living, often including income and education level. In addition, socioeconomic status may also assist in quantifying the stress related to living and, thus, impact an individual’s health (McEwen & Tucker, 2011).

Parental history of hypertension is an important risk factor related to cardiovascular disease; in fact, it is an integral part of the Framingham Hypertension Risk Score used to assess hypertensive risk in non-hypertensive adult populations. Developed from the Framingham Heart Study findings, the Framingham Hypertensive Risk Score is calculated from a patient’s age, gender, systolic and diastolic blood pressure, body mass index, history of smoking and parental history of hypertension (Parikh et al., 2008). These seven items are considered key indicators in the future development of hypertension among adults (Kivimaki et al., 2009; Parikh, et al., 2008). Both paternal and
maternal hypertension are strong, independent factors in the development of hypertension in adulthood (Wang et al., 2008) and elevations in childhood (Oikonen et al., 2011).

The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents specifically identifies parental history of hypertension as a primary risk factor for hypertension among youth (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Among a sample of Canadian youth, the variable of family history of hypertension was strongly associated with blood pressure, both systolic and diastolic, in adolescent males and younger females (Shi, de Groh, & Morrison, 2012).

Another biological factor to consider when studying blood pressure among youth is puberty. During puberty, body tissues are endocrinologically active and, as such, influence cardiovascular and metabolic processes in adolescents (Siervogel et al., 2003). Optimum endothelial microvascular function is important to the prevention of atherosclerosis and, thus, the maintenance of ideal blood pressure. Pubertal status has been found to be a predictor of endothelial microvascular function in a sample of healthy, normotensive children and adolescents (Radtke et al., 2012). Body mass index along with systolic and diastolic BP were found to increase significantly with increasing pubertal status (Radtke, et al., 2012). Research by Shankar and colleagues (2005) found greater rates of increased systolic blood pressure in youth during puberty; the rates of increase were three to six times greater in male adolescents and two to four times greater in female adolescents compared to the time period prior to pubertal growth. Early maturing girls have been found to have higher blood pressure and body mass index compared to their peers ages 9-15 (Chen & Wang, 2009). In a sample of obese children and adolescents,
cardiovascular risk factors (including hypertension) increased significantly with the onset of puberty (Reinehr & Toschke, 2009). With research focused on blood pressure outcomes among adolescents, pubertal status is a confounding variable and efforts should be made to control for it within a research design.

Finally along with age, gender, ethnicity, parental history of hypertension and pubertal status, the influence of weight status on blood pressure in adolescents must be acknowledged. In this study, weight status was measured using both body mass index and waist circumference. An estimated 16.9% of US children and adolescents ages 2-19 are obese (Ogden, Carroll, Kit, & Flegal, 2012). Obese adolescents are more likely to experience high blood pressure (Din-Dzietham, Liu, Bielo, & Shamsa, 2007; Ford, et al., 2008; Paradis et al., 2004; Shi, et al., 2012). The relationship between blood pressure and weight status is more complex than it appears. With a nationally representative sample of US adolescents, a study by Sugiyama and colleagues (2007) found that systolic blood pressure positively correlates with body mass index while diastolic blood pressure negatively correlates with body mass index. In the Bogalusa Heart Study with 24,092 examination data points analyzed, obesity was found to be an independent predictor of blood pressure data; however, the increases in blood pressure did not keep pace with increases in obesity over the same time period (Freedman et al., 2012). From 1974 to 1993, the prevalence of obesity increased from approximately 6% to 17% while mean levels of systolic blood pressure did not change and mean levels of diastolic blood pressure decreased by 2 mmHg (Freedman, et al., 2012).
Statement of the Problem

Hypertension and blood pressure elevations are contributors to cardiovascular disease (Chobanian, et al., 2003). Primary hypertension exists among adolescents at a prevalence rate of approximately 3% (Loeffler, et al., 2012; Sugiyama, et al., 2007). Aside from diagnosed hypertension, single measurement blood pressure elevations appear to occur at a more frequent rate. Elevated blood pressure measurements have been reported at 37% for youth 6 to 17 years of age (Sorof et al., 2001). In addition, tobacco exposure experienced in the daily lives of adolescents contribute to an increased risk for cardiovascular disease as adults (Institute of Medicine, 2010b; Kavey et al., 2003; Lloyd-Jones, et al., 2010; Mbulo, 2008). Daily tobacco exposure results from a number of sources including cigarette use, smokeless tobacco use, and secondhand smoke exposure. With a higher smoking prevalence and fewer smoking restrictions noted in rural communities (American Legacy Foundation, 2009; Berg et al., 2006; Institute of Medicine, 2005), rural adolescents experience more tobacco exposure compared to their urban peers. Healthy People 2020 objectives highlight tobacco use as one of the ten leading health priorities and underscore the need to increase overall cardiovascular health for all Americans (United States Department of Health and Human Services, 2010b). The Rural Healthy People 2010 document ranks tobacco use as its sixth leading rural health priority (Stevens, Colwell, & Hutchison, 2003). In general, research within rural areas and national research data with adolescents continues to be rare (Bushy, 2008; Mulye et al., 2009) and, as a result, research findings on tobacco exposure and blood pressure among rural adolescents is an even greater rarity. In order to reduce cardiovascular risk related to tobacco exposure for the next generation of adults in rural
communities, knowledge about the extent of tobacco exposure among rural adolescents and their effect on blood pressure is needed.

Purpose of the Study

The purpose of this study was to examine the influence of tobacco exposure on blood pressure in rural adolescents ages 15-18 while controlling for age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status and weight status. This study was also designed to investigate the mediating role of C-reactive protein in the relationship between tobacco exposure and blood pressure in this population.

Research Questions and Hypotheses

Research Question 1

Is there a relationship between individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure in rural adolescents ages 15-18?

Hypothesis 1

There is a positive relationship between individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure among rural adolescents ages 15-18.
Research Question 2
Do individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure influence cotinine levels in rural adolescents ages 15-18?

Hypothesis 2
Individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure will positively influence cotinine levels among rural adolescents ages 15-18.

Research Question 3
How much of the variance in blood pressure is explained by cotinine levels (salivary cotinine) in rural adolescents ages 15-18 after controlling for age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status and weight status?

Hypothesis 3
Tobacco exposure, quantified using the salivary biomarker cotinine, will account for a significant amount of variability in blood pressure among rural adolescents ages 15-18, when controlling for age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status and weight status.

Research Question 4
Does C-reactive protein mediate the relationship between salivary cotinine and blood pressure in rural adolescents ages 15-18?
Hypothesis 4

C-reactive protein will mediate the relationship between salivary cotinine and blood pressure in rural adolescents, ages 15-18.

Significance of the Problem

Cardiovascular diseases, including heart disease and stroke, are the leading cause of death in the US, resulting in greater than 700,000 deaths annually (Murphy, Xu, & Kochanek, 2013). Hypertension and blood pressure elevations are contributors to cardiovascular disease (Chobanian, et al., 2003). Among adolescents, the prevalence rate of hypertension is approximately 3% (Loeffler, et al., 2012; Sugiyama, et al., 2007) and rates of blood pressure elevations may be as high as 37% (Sorof, et al., 2001). Longitudinal data and systematic reviews of research have provided evidence that blood pressure elevations in childhood track through adolescence and into adulthood (Bao, et al., 1995; Chen & Wang, 2008; Toschke, et al., 2009). The origins of cardiovascular disease appear to begin prior to adulthood; therefore, additional research is needed to further understand blood pressure during adolescence.

Cardiovascular morbidity and mortality is relevant to the health and well being of individuals living in both urban and rural communities nationwide. Referred to as “the forgotten fifth”, individuals with a rural residence comprise 20% of the US population and are a sizeable minority group (Pruitt, 2009). In fact, 75% of the nation’s counties and 75% of the nation’s land mass are rural (Hart, Larson, & Lishner, 2005). Even with limited data, researchers acknowledge that a spectrum of significant risk behaviors exist
among rural adolescent populations (Curtis, Waters, & Brindis, 2011). In the state of Montana, researchers reported an increased risk of cigarette use (p < .0001, OR = 1.09, 95% CI = 1.06, 1.11) and smokeless tobacco use (p < .0001, OR = 1.08, 95% CI = 1.05, 1.12) for adolescents in rural counties (Hanson et al., 2009). Multiple years of data from the Youth Risk Behavior Surveillance Survey (YRBS) has revealed that rural adolescents are more likely to be daily cigarette smokers compared to their urban and suburban counterparts (Lutfiyya et al., 2008). Nationwide, rural adolescents experience tobacco-related disparities (American Legacy Foundation, 2009).

Greater attention to health outcomes in rural communities is necessary. Without improved healthcare infrastructure and a population health focus, the chronic disease burden in rural communities will be substantial (Institute of Medicine, 2005). Tobacco exposure and elevated blood pressure are important contributors to cardiovascular disease. A research focus on blood pressure and tobacco exposure among rural adolescents will provide valuable evidence to plan intervention efforts and develop health policy in rural communities.

Conceptual Definitions of Variables

For the purpose of clarity within this study, the conceptual definitions for the independent variable of tobacco exposure, the possible mediating variable of systemic inflammation and the dependent variable of blood pressure were established. The conceptual definitions are presented in Table 1.
Table 1
*Concepts and Definitions*

<table>
<thead>
<tr>
<th>Concept</th>
<th>Conceptual Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure</td>
<td>The force exerted on the walls of arteries as blood is pumped from the heart though the circulatory system (National Heart Lung and Blood Institute, 2012).</td>
</tr>
<tr>
<td></td>
<td>A systolic or diastolic blood pressure measurement greater than or equal to the 90th percentile according to the age, gender and height of an adolescent. The blood pressure measurement from a single occasion does not constitute a diagnosis of prehypertension or hypertension (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).</td>
</tr>
<tr>
<td>Tobacco Exposure</td>
<td>The exposure that results from individual smoking behaviors, such as cigarette smoking and smokeless tobacco use, as well as secondhand smoke exposure.</td>
</tr>
<tr>
<td></td>
<td>Secondhand smoke is a mixture of side stream smoke from a lit cigarette and mainstream smoke exhaled by an active smoker (United States Department of Health and Human Services, 2006).</td>
</tr>
<tr>
<td>Systemic Inflammation</td>
<td>A chronic inflammatory state that contributes to the atherosclerotic process, contributing to blood pressure elevations and hypertension (Libby, et al., 2002).</td>
</tr>
</tbody>
</table>

*Blood Pressure*

adolescent participant in this study, a systolic or diastolic blood pressure greater than or equal to 90\textsuperscript{th} percentile according to age, gender and height was categorized as a prehypertensive blood pressure reading. In addition, a systolic or diastolic blood pressure greater than or equal to 95\textsuperscript{th} percentile was categorized as a hypertensive. This categorization served to identify blood pressure elevations on a single occasion and did not constitute a diagnosis of prehypertension or hypertension (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

*Tobacco Exposure*

The variable of tobacco exposure encompasses both individual smoking behaviors, such as cigarette smoking and smokeless tobacco use, as well as secondhand smoke exposure. Secondhand smoke is defined as a mixture of side stream smoke from a lit cigarette and mainstream smoke exhaled by an active smoker (United States Department of Health and Human Services, 2006). In relation to the expanded biobehavioral interaction model (Kang, Rice, Park, Turner-Henson, & Downs, 2010), the variable of tobacco exposure is present in the behavioral domain as well as the environment domain with both impacting the biologic domain and health outcomes.

*Systemic Inflammation*

The process of systemic inflammation is an integral step in the development of atherosclerosis (Ambrose & Barua, 2004). Atherosclerosis is the biological mechanism underlying the development of hypertension. Notable levels of the inflammatory biomarker C-reactive protein are indicative of a low grade, chronic level of inflammation and risk of atherosclerotic complications (Libby, et al., 2002). Tobacco exposure places
the human body in a chronic inflammatory state (United States Department of Health and Human Services, 2010c). Cigarette smoking is thought to impact all phases of the atherosclerotic process including endothelial dysfunction and acute cardiovascular events (Ambrose & Barua, 2004). A significant positive correlation has been noted between the serum cotinine (indicative of secondhand smoke) and serum CRP among child and adolescents nonsmokers (Wilkinson, et al., 2007). Additional research is needed to further define the role of inflammation in the relationship between tobacco exposure and blood pressure, particularly as it relates to the developmental period of adolescence.

Theoretical Framework

This research study used a biobehavioral approach to explore the relationship between tobacco exposure, such as cigarette smoking, smokeless tobacco use and secondhand smoke exposure, and blood pressure in rural adolescents. In addition, the possible mediating role of C-reactive protein between tobacco exposure and blood pressure was addressed with rural adolescents ages 15-18. The expanded biobehavioral interaction model (Kang, et al., 2010) provides the theoretical foundation for this research study. This model succeeds in unifying three theories focused on the phenomenon of stress and serves to underscore the importance of biological interaction. The integrated theories in this model include Selye’s physiological model of stress, Lazarus and Folkman’s cognitive appraisal model of stress and coping theory, and McEwen’s stress, allostasis and allostatic load model (Kang, et al., 2010). This model provides a comprehensive approach to biology and behavior; the theoretical underpinnings provided by Selye and McEwen are particularly relevant to this study.
Through the lens of Selye’s physiological model of stress and McEwen’s stress, allostasis and allostatic load model, tobacco exposure is viewed as the stressor on the human body. Selye developed the concept of stress along with the theory entitled the general adaptation syndrome (GAS). In this theory, a stressor is defined as an agent that produces stress at any time (Selye, 1976). Selye describes the concept of stress as a chronologically-developed response to stressors, consisting of three phases: the alarm reaction, the stage of resistance and the stage of exhaustion. Selye’s theory addresses the body’s nonspecific response to stressors, which may include “the secretion of adrenocorticotropic hormone (ACTH), corticoids and catecholamines, thymic-lymphatic involution, eosinopenia and peptic ulceration” (Selye, 1976, p. 53). These nonspecific effects can be elicited by any number of different agents that place an increased demand on the body. This broad category of agents, which can act as stressors, includes tobacco exposure. In this study, the stressor of tobacco exposure is thought to promote systemic inflammation and atherosclerotic risk, which in turn instigates elevations in blood pressure (Libby, et al., 2002).

The allostatic load model, as discussed by McEwen and Wingfield (2003), focuses on the concept of allostasis, the process by which complex physiological systems adapt to physical, psychosocial, and environmental challenges. The concept of allostasis is central to survival and is integral to the potentially detrimental concept of allostatic load, defined as “the wear and tear on the brain and body resulting from alldynamic overactivity as well as dysregulation of the mediators of allostasis” (McEwen & Tucker, 2011, p. S131). The human body can develop a detrimental allostatic load due to stressful experiences and unfavorable lifestyle choices such as excessive eating, poor sleep,
alcohol use and smoking behaviors (McEwen & Tucker, 2011). The cardiovascular system is noted to be one of the body systems most susceptible to stress and the effects of allostatic load (McEwen & Tucker, 2011).

The expanded biobehavioral interaction model presents five domains that impact health outcomes. The domains include the individual, environmental, psychosocial, behavioral and physiological domains. This model emphasizes the dynamic interplay present between domains and acknowledges both the unidirectional and bidirectional relationships that occur between domains. This research study explored the relationship between variables in the individual, environmental, behavioral and physiological domains. The individual self-reported tobacco use behaviors of cigarette smoking and smokeless tobacco use (found in the behavioral domain) have a bidirectional relationship with the sources of secondhand smoke exposure found in the environmental domain of the model. The increased levels of cotinine resulting from individual tobacco use and sources of secondhand smoke exposure instigate increased levels of inflammation (specifically C-reactive protein) in the physiological domain. The biomarker of C-reactive protein functions as a mediator in the physiological domain and serves to increase blood pressure in the health outcomes portion of the model. Kang and colleagues (2010) note that variables within the biological domain often function as a mediator of health outcomes. In this research study, systemic inflammation (quantified using the salivary C-reactive protein biomarker) is the potential mediator between tobacco exposure and blood pressure for the study population.

Although the expanded biobehavioral interaction model provides a comprehensive foundation for the research study, it is important to acknowledge that the
model is complex. The model works to integrate five domains of influence to provide a better understanding of health outcomes. As a result of this integration, the model loses a measure of parsimony; however, this is often the case with models and theories that strive to be comprehensive.

Conceptual Framework

With the expanded biobehavioral model as the theoretical foundation, a conceptual framework was developed for this study that integrates the study variables and illustrates the relationships between variables (see Figure 2).

Figure 2. Diagram of Conceptual Framework. Rectangle on the left denotes tobacco exposure including self-reported individual tobacco use and sources of secondhand smoke exposure. The abbreviation CRP refers to C-reactive protein. Brackets above the outcome variable of blood pressure contain the covariates, with SES referring to socioeconomic status and PHH referring to parental history of hypertension.
Assumptions

In this study, the following assumptions were made:


2. **Tobacco exposure, including cigarette smoking, smokeless tobacco and secondhand smoke, are harmful to one’s health** (US Department of Health and Human Services, 2006; US Department of Health and Human Services, 2010).

3. **Adolescents 15-18 years of age can be active participants in research and, therefore, answer questions in a thoughtful manner.**

Summary

The improvement of cardiovascular health is seen as a pivotal public health issue and a primary goal for the year 2020 (Lloyd-Jones, et al., 2010). Healthcare professionals note the importance of early assessment of cardiovascular health, particularly among children and adolescents (Kavey, et al., 2003). This study adds to research knowledge on tobacco exposure and blood pressure among rural adolescents. Due to the greater smoking prevalence and limited smoking restrictions in rural communities (American Legacy Foundation, 2009; Berg, et al., 2006; Institute of Medicine, 2005), this study addressed the effect of tobacco exposure on blood pressure specific to rural adolescents. Also addressed in this study is the possible mediating role of systemic inflammation in the relationship between tobacco exposure and blood pressure for this population. Addressing these gaps in the literature provides insight into the extent of tobacco
exposure among adolescents and advances knowledge to improve the cardiovascular health of adolescents and, thus, the next generation of adults in rural communities.
CHAPTER TWO
REVIEW OF THE LITERATURE

In this chapter, the review of the literature addresses blood pressure and its relationship to tobacco exposure, as well as examining systemic inflammation as a possible mediating variable. To begin with, epidemiological evidence specific to the US is presented specific to blood pressure and includes the concepts of hypertension and high blood pressure. The epidemiological evidence on tobacco exposure in the US, in the form of cigarette smoking, smokeless tobacco use and secondhand smoke exposure is also reviewed. The literature reviewed serves to clarify what is known about the relationships between blood pressure, tobacco exposure and systemic inflammation. Research literature on the pertinent confounding variables of age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status and weight status adds to the discussion. Finally, to provide additional context to the research questions, literature specific to rural health and adolescents is discussed.

The search strategy for this review of the literature incorporates the use of the following databases: PubMed, CINAHL, and PsycINFO. Journal articles published since 2008 were reviewed for their relevance to this study. In addition to these specific databases, the archives of the Journal of Adolescent Health and the Journal of Rural Health were searched to enhance the thoroughness of finding research literature specific
to rural adolescents in the US. The bibliographies of relevant articles were reviewed to find additional sources and seminal works.

Blood Pressure in the US

The burden of cardiovascular disease in the US has been described as “large and expensive”, associated with approximately 2 million heart attacks and strokes resulting in 800,000 fatalities annually (Wright, Wall, Briss, & Schooley, 2012, p. 591). The relationship between blood pressure and cardiovascular disease is continuous; as blood pressure increases so does the risk of heart attack, heart failure, stroke, and kidney disease (Chobanian, et al., 2003; Turnbull, Kengne, & MacMahon, 2010). As a source of preventable death in the US, the lowering of blood pressure is a pivotal public health issue to address. A clear understanding of the prevalence of high blood pressure and hypertension within different US population groups is essential to the improvement of cardiovascular health.

Blood Pressure among Adults

To fully describe the state of blood pressure in the US, the concepts of high blood pressure and hypertension must be clearly defined. It is important to clarify that a single blood pressure reading categorized as hypertensive does not equate to a diagnosis of hypertension. A diagnosis of adult hypertension results from an average of two or more properly measured, seated blood pressure readings over 140/90 mmHg, which occur on two or more occasions (Chobanian, et al., 2003).

The National Health and Nutrition Examination Survey (NHANES) offers multiple years of nationally representative data on blood pressure to assist in proper
surveillance. Crim and colleagues (2012) reviewed 19 studies that used NHANES data to determine the prevalence rate of blood pressure and hypertension. In a review of 2007-2008 NHANES data, the adult hypertension prevalence rate was found to be 29.8% (Crim, et al., 2012). Roger and colleagues (2012) reported that approximately 76 million individuals over the age of 20 have high blood pressure in the US. With longitudinal data from adult participants in the Framingham Heart Study, researchers determined that blood pressure levels greater than 115/75 mmHg present greater risk for negative cardiovascular events; in fact, the cardiovascular risk doubles with each 20 mmHg increase in systolic pressure or each 10 mmHg increase in diastolic pressure (Falkner, 2010; Vasan et al., 2001). “[T]obacco smoking and high blood pressure are the leading risk factors of mortality, responsible for nearly one in five and one in six deaths in US adults, respectively” (Danaei et al., 2009, p. 18).

Along with morbidity and mortality, a substantial economic burden results from the large number of affected adults; an estimated $50.6 billion was spent in 2008 for direct and indirect costs related to high blood pressure (Roger, et al., 2012). Hypertension is viewed as the most expensive contributor to cardiovascular disease with the future costs attributed to hypertension projected to increase to $200.3 billion by 2030 (Heidenreich, et al., 2011).

**Blood Pressure among Adolescents**

Although blood pressure-related research among children and adolescents is limited (Din-Dzietham, et al., 2007), rates of hypertension among US children and adolescents have been estimated to be approximately 2% to 5% (Hansen, et al., 2007; Loeffler, et al., 2012; McNiece, et al., 2007; National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).
The age ranges used for studies on blood pressure in US children and adolescents vary widely including 1-17 years (Rosner, et al., 2009), 8-17 years (Din-Dzietham, et al., 2007; Lande, Pearson, Vermilion, Auinger, & Fernandez, 2008), 12-17 years (Loeffler, et al., 2012) and 12-19 years (Sugiyama, et al., 2007). For blood pressure prevalence rates specific to the adolescent population, Sugiyama and colleagues (2007) used cross sectional data from the 1999-2002 National Health and Nutrition Examination Survey (NHANES) (n=4,508) and reported elevated systolic, diastolic and hypertension rates of 2.0%, 1.4% and 3.3%, respectively. With 1999-2006 NHANES data, Loeffler and colleagues (2012) reported that 3.3% of adolescents ages 12-17 (n=6,036) had an elevated blood pressure.

A single blood pressure measurement classified in the hypertensive range for a child or adolescent is cause for concern. Longitudinal studies in the US and Greece provide evidence that a blood pressure elevation in childhood can track through adolescence and into adulthood (Bao, et al., 1995; Chen & Wang, 2008; Kollias, et al., 2011; National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004; Toschke, et al., 2009). An intervention study by Sorof and colleagues (2001) reported that single measurement blood pressure elevations occur more frequently, as 37% of their study population (n=140) was found to have an elevated blood pressure measurement. The pilot study completed in preparation for this dissertation research reported a 35% prevalence rate of elevated blood pressures in a sample of 40 rural adolescents ages 15-19 (Huntington-Moskos, 2012). Surveillance of blood pressure and hypertension among adolescents presents a valuable opportunity to
impact future cardiovascular morbidity and mortality through prevention efforts by reducing blood pressure earlier in the lifespan.

Although nationally representative data regarding blood pressure among children and adolescents is available from the National Health Statistics Center, validity issues arise when reviewing multiple years of survey data. Din-Dzietham and colleagues (2007) compared national survey data from 1963-2002, and found a number of areas of concern specific to blood pressure measurement. The validity concerns included (1) a lack of repeated blood pressure measurements, (2) zero-end digit preference, (3) inappropriate cuff size, and (4) inconsistent measurement of confounding variables such as physical activity and pubertal status (Din-Dzietham, et al., 2007). The findings by Hansen and colleagues (2007) stress the reality of under diagnosis by pediatric clinicians related to blood pressure among adolescents, pointing out that the additional steps necessary to classify a blood pressure reading for an individual under 18 years of age has led to widespread under diagnosis in years passed. The National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004) issued guidelines to stress the importance of quality blood pressure measurement in epidemiological studies as well as the clinical setting. These guidelines serve to promote standardization of research protocols, assisting in quality improvement of blood pressure measurement moving forward.

**Blood Pressure among Rural Adolescents**

Blood pressure research involving rural adolescents is scarce. Since 2006, a total of five research studies were found to involve blood pressure among rural adolescents (Adams et al., 2008; King, Meadows, Engelke, & Swanson, 2006; Moore et al., 2009;
Moore, Stephens, Wilson, Wilson, & Eichner, 2006; Rodriguez et al., 2010). These studies that are both regional and multiethnic, involve both genders in grades 9-12 and have fairly large sample sizes ranging from 700 to 1800 participants. Unfortunately, none of the studies report data on tobacco exposure and the relationship to blood pressure among the participants or elaborate on how the concept of rurality was defined.

The available studies on blood pressure among rural adolescents are almost exclusively focused on the predictor of weight status. Each of the five studies found weight status to be a significant predictor of an elevated blood pressure measurement. Moore and colleagues (2009) reported a prevalence rate of 13.8% for hypertensive blood pressure measurements in a multiracial, low income, rural population of children and adolescents ages 5-17 years old; while Rodriguez and colleagues found that 17.8% of their multiethnic, rural sample of adolescents ages 13-17 had an elevated systolic blood pressure. King and colleagues (2006) found the relationship between body mass index and blood pressure to be stronger among Caucasian adolescents compared to African American adolescents (16% vs. 8%, $\chi^2 = 1.8$, $p = .18$). Finally, a study by Adams and colleagues (2008) noted that within their sample of rural adolescents grades 9 through 12, those that were identified as overweight had nearly a 7 times greater risk for elevated blood pressure compared to their normal weight peers.

The predominance of blood pressure studies focused on weight status is understandable as the trend toward obesity among rural adolescents is greater when compared to their urban peers (Adams, et al., 2008; King, et al., 2006). However, even though research has established weight status as an independent predictor of blood pressure elevation, findings from cross-sectional and longitudinal research studies have
reported that increases in obesity rates do not reflect parallel increases in blood pressure among children and adolescents (Freedman, et al., 2012; King, et al., 2006); therefore, additional contributing factors must be addressed.

Tobacco Exposure in the US

The concept of tobacco exposure includes the use of cigarettes and smokeless tobacco products as well as exposure to secondhand smoke. Tobacco use is considered the most preventable cause of morbidity and mortality in the US (Fenelon & Preston, 2012; United States Department of Health and Human Services, 2004). Annually, an estimated 443,000 deaths are attributed to tobacco use including nearly 50,000 deaths related to secondhand smoke exposure (Centers for Disease Control and Prevention, 2008). Furthermore, this loss of life results in $96 billion in direct medical costs and $97 billion in lost productivity annually in the US (Centers for Disease Control and Prevention, 2008). An alternative statistical analysis of the Centers for Disease Control and Prevention’s 2000-2004 data produced findings with an estimated tobacco-related mortality closer to 380,000 deaths annually and on the decline (Rostron, 2013). It is possible that tobacco-related mortality is declining, however, researchers reviewing two decades of nationally-representative data found that the relative risk of death for smokers has increased rapidly; the risk of death for smokers compared to nonsmokers increased by 25.4% between 1987 and 2006 (Mehta & Preston, 2012). Clearly, these findings represent a substantial loss of life and a large economic impact for the nation.

Tobacco Exposure among Adults

One in four adults in the US uses tobacco products (King, et al., 2012). This prevalence rate is derived from 2009-2010 data from the National Adult Tobacco Survey
(NATS) with tobacco products defined to include any of the following: cigarettes, cigars, cigarillos, pipe smoking, water pipe smoking and/or smokeless tobacco products—including chewing tobacco or snus. One in five adults in the US is a current cigarette smoker (King, Dube, Kaufmann, Shaw, & Pechacek, 2011; King, et al., 2012).

In an analysis of the 2009-2010 NATS dataset and considering the demographics of the nationally-representative sample, King and colleagues (2012) reported that tobacco use was higher among men compared to women (32.2% vs. 18.5%). Individuals with less education had higher levels of tobacco use and those with a general education degree (GED) had the highest prevalence level of tobacco use at 51.5%. Differences in rates were reported by race/ethnicity categories. Non-Hispanic Asians had the lowest prevalence rate at 10.9% while the group classified as non-Hispanic other had the highest prevalence rate at 37.2%. In this 2009-2010 NATS dataset, King and colleagues (2012) reported that geographic location has a strong influence on prevalence rates across the nation. Tobacco use in the state of Kentucky was the highest at 37.4% and the lowest prevalence was in Utah at 14.1%. The state of Indiana was reported to have a tobacco use prevalence rate of 29.2%, with cigarette use specifically at 24.5% (King, et al., 2012).

Tobacco use in the US also includes smokeless tobacco products. An analysis of the 2009-2010 National Adult Tobacco Survey (NATS) data reveals an overall prevalence rate of 3.4% for smokeless tobacco use (King, et al., 2012). A further distinction can be made between different types of smokeless tobacco products. The use of chewing tobacco requires the regular spitting (expectorating) of the liquid that accumulates in the mouth while moist snuff requires no expectoration and any resulting liquid can be swallowed (White, et al., 2012). Findings specific to each type of smokeless
tobacco product are available using National Health Survey data from 2000, 2005, and 2010. The regular use of chewing tobacco was found to be 1.3%, 1.1%, and 1.2% for each respective year while the regular use of moist snuff was found to be 1.4%, 1.6%, and 2.0% (Bhattacharyya, 2012). Prevalence rates indicative of experimentation with smokeless tobacco among adults are higher with ever use of chewing tobacco at 7.1%, 8.5%, and 9.2% and ever use of moist snuff at 4.4%, 7.5%, and 8.4% respectively (Bhattacharyya, 2012). While the use of chewing tobacco has stabilized at approximately 1% of the population, the use of moist snuff appears to be increasing in popularity. The prevalence of moist snuff use was reported at 2% and experimentation rates were on an upward trend at 8%. Demographic characteristics that are strongly associated with the use of smokeless tobacco include male gender, non-Hispanic Caucasian ethnicity, and having less than a high school education (Bhattacharyya, 2012; King, et al., 2012).

Adults have been reported to use of a variety of tobacco products, described as combustible tobacco products and smokeless tobacco products. This phenomenon of using multiple tobacco products is known as dual use, concurrent use or polytobacco use (Mushtaq, Williams, & Beebe, 2012; Rath, Villanti, Abrams, & Vallone, 2012). Mushtaq, Williams and Beebe (2012) completed a gender-stratified analysis of the 2010 Behavioral Risk Surveillance Survey (BRFSS) and report that dual use was found in 1.6% of male participants, while 17.4% of men reported exclusive smoking and 4.2% reported exclusive smokeless tobacco use. Dual use is rarely reported in women (0.3%), with 14.8% female participants reporting exclusive smoking and 0.5% reporting exclusive smokeless tobacco use (Mushtaq, et al., 2012). While dual use in women is rare, Mushtaq and colleagues (2012) noted that the 0.3% prevalence rate represents half a million
The prevalence of dual use appears to have an even stronger hold among young adults. Using data from the Legacy Young Adult Cohort Study (n=4,201), nearly a quarter of young adults (23%) reported current tobacco use and, of these, 30% reported dual use (Rath, et al., 2012). Although dual use of tobacco is a growing area of research, the pathway by which dual use of tobacco develops is not fully understood (Mushtaq, et al., 2012).

The harmful effects linked to secondhand smoke exposure are numerous. Nonsmokers exposed to secondhand smoke increase their risk of lung cancers by 20-30% and their risk of heart disease by 25-35% (United States Department of Health and Human Services, 2007a). Using data from the 2007-2008 National Health and Nutrition Examination Survey (NHANES), it is estimated that 88 million individuals over 3 years of age are exposed to secondhand smoke (Kaufmann, et al., 2010). With an analysis of multiple years of NHANES data (1999 to 2006), researchers found that approximately 40.5% of US adults had serum cotinine levels indicative of secondhand smoke exposure (Max, Sung, & Shi, 2009). In particular, the researchers noted the “astonishingly high” prevalence rate for 62.1% non-Hispanic Blacks adults (Max, et al., 2009, p. 1640). In an analysis of the 2006 NHANES data, Max, Sung and Shi (2012) reported secondhand smoke exposure resulted in over 42,000 deaths. Specific racial/ethnic disparities were noted in this study, as African Americans accounted for 13% of all secondhand smoke-related deaths and African American infants accounted for 24% of the 36% of all infant deaths related to secondhand smoke exposure (Max, et al., 2012). In addition to the number of deaths, secondhand smoke exposure resulted in a loss of nearly 600,000 years
of potential life lost and $6.6 billion of lost productivity (Max, et al., 2012). Clearly, there is a substantial social and economic impact related specifically to secondhand smoke exposure in the US.

**Tobacco Exposure among Adolescents**

A number of surveillance studies with nationally representative samples provide ongoing prevalence data related to adolescent tobacco use in the US; these studies include the National Youth Tobacco Survey (NYTS), the Youth Risk Behavior Surveillance Survey (YRBS), the Monitoring the Future Survey (MTF), and the National Survey on Drug Use and Health (NSDUH). Conducted since 2000, the NYTS is a school-based, nationally representative survey focused on youth and their self-reported tobacco use. Using the NYTS dataset, 23.2% of adolescents attending high school reported current tobacco use (Arrazola, et al., 2012). Between 2000 and 2011, researchers noted a significant downward trend in tobacco use among this age group, including a decrease in current tobacco use from 34.4% to the current level of 23.2%; a decrease in current combustible tobacco use from 14% to 6.3% and a decrease in current cigarette use from 27.9% to 15.8% (Arrazola, et al., 2012).

The Centers for Disease Control and Prevention developed the YRBS to collect prevalence data on health risk behaviors among adolescents including tobacco use. Since 1991, the YRBS has been administered biennially and the data sampling plan for this study is representative of in-school adolescents grades 9 through 12; the YRBS does not capture surveillance data related to out of school youth (Brener et al., 2013). The 2011 YRBS findings revealed ever smoking levels among 9th to 12th graders were as high as
45% while current smoking levels (within the last 30 days) were reported at 18% (Eaton, et al., 2012).

Prevalence data from the YRBS is valuable in a number of ways including:
1) identification of trending over time, 2) determination of the co-occurrence of risk behaviors, 3) comparison between local, state and national findings, 4) determination of prevalence rates among groups by age and race/ethnicity and 5) assistance in gaging progress towards a number of Healthy People objectives (Brener, et al., 2013). Although the prevalence of current smoking levels has decreased significantly from the 1991 high of 27.5% to the current level of 18% (Centers for Disease Control and Prevention, 2009; Eaton, et al., 2012), US adolescents have not reached the desired prevalence level of 16% set by Healthy People 2020 (United States Department of Health and Human Services, 2010a).

According to the 2012 Monitoring the Future Survey, approximately 40% of US adolescents have tried cigarettes by 12th grade, and 17% of 12th graders were classified as current smokers (Johnston, et al., 2013). This prevalence rate for current cigarette use among 12th graders appears to be well aligned with the 18% prevalence rate noted in the 2011 YRBS. For trends in tobacco use among adolescents, an analysis of the Monitoring the Future data found that peak tobacco use for 12th grade adolescents occurred in 1997 at 37% and has decreased to the current level at 17% in 2012 (Johnston, et al., 2013).

An analysis of the 2011 YRBS found an 8% prevalence of smokeless tobacco use among adolescents nationwide (Eaton, et al., 2012). Researchers analyzing the 2011 NYTS found that smokeless tobacco use is particularly high among high school males compared to their female counterparts, 12.9% versus 1.6% respectively (Arrazola, et al.,
2012). Again, the 2011 findings exceed the 6.9% target goal set by Healthy People 2020 (United States Department of Health and Human Services, 2010a). In addition, research has noted that smokeless tobacco use is higher among adolescents who smoke (Newman & Shell, 2005).

With a younger and larger study population, researchers using the NSDUH reported that smokeless tobacco use has remained stable at approximately 3.0% for those individuals 12 years and older; however, an increase in use was noted among male adolescents from 3.4% in 2002 to 4.4% in 2007 (Substance Abuse and Mental Health Services Administration, 2009). Among individuals over 12 years of age, past month smokeless tobacco use was substantially higher in rural counties (8.4%) compared to large metropolitan counties (1.9%) and small metropolitan counties (4.7%) (Substance Abuse and Mental Health Services Administration, 2009). Despite the high rates of tobacco use among adolescents, there is a limited amount of research on smokeless tobacco use among adolescents (Newman & Shell, 2005).

Of the approximate 88 million individuals exposed to secondhand smoke in the US, 32 million are youth ages 3-19 years (Kaufmann, et al., 2010). Secondhand smoke exposure has had an overall decline of 70% since the 1980’s; however, the main source of secondhand smoke exposure for adolescents continues to be the home environment (Marano, et al., 2009). The 2007-2008 National Health and Nutrition Examination Surveys reported that 46.5% of nonsmoking adolescents ages 12-19 had elevated serum cotinine levels, indicating significant secondhand smoke exposure (Kaufmann, et al., 2010). The 2010 baseline level of secondhand smoke exposure for adolescents ages 12 to 17 is 45.5% and a target goal of 41% has been set in the Healthy People 2020 objectives.
(United States Department of Health and Human Services, 2010a). To date, the research suggests that there is no safe, minimum dose of secondhand smoke; all secondhand smoke exposure is considered a risk to health (United States Department of Health and Human Services, 2006). Adolescents continue to experience a high-level of secondhand smoke exposure and, whether this exposure occurs in the home or community, it has detrimental implications for the future cardiovascular health of youth.

Adolescence is a critical time period for the development of tobacco use behavior. With approximately 88% of regular cigarette smokers reporting their first cigarette use at or before 18 years of age, addressing tobacco use behavior during adolescence is of great importance (United States Department of Health and Human Services, 2012). After decades of steady decline in tobacco use, the pace of declining cigarette use has slowed and the decline in smokeless tobacco use has stalled (United States Department of Health and Human Services, 2012). To positively impact continued health and wellness, a renewed focus on the adolescent tobacco use is vital.

Tobacco Exposure among Rural Adolescents

Significant tobacco-related disparities have been noted to rural America including a higher prevalence of smoking and smokeless tobacco use as well as higher secondhand smoke exposure (American Legacy Foundation, 2009; Vander Weg, et al., 2011). A number of unique challenges to tobacco control exist in rural communities including a lack of services, limited transportation, low income, low insurance rates, limited health care access and proximity to tobacco growers (American Legacy Foundation, 2009). Due to the established culture and tradition of tobacco use, rural communities require well-tailored approaches to enforcing tobacco-free policies and cessation services (American Legacy Foundation, 2009).
The Behavioral Risk Factor Surveillance System (BRFSS) dataset includes nationally representative sample of adults over 18 years old has been used to complete a secondary analysis to determine the prevalence of smoking among rural adults (Doescher, Jackson, Jerant, & Hart, 2006; Vander Weg, et al., 2011). Researchers analyzing the BRFSS from 2006 and 2008 found significantly higher tobacco use prevalence among rural adults compared to their urban and suburban counterparts. Current smoking prevalence was 22.2% in rural areas compared to 17.3% in suburban areas and 18.1% in urban areas. Similarly higher prevalence rates for current smokeless tobacco use were found with 5.9% in rural areas compared to 3.6% in suburban areas and 2.2% in urban areas (Vander Weg, et al., 2011). State-specific prevalence rates for the Midwest region, including Kentucky, Indiana and Ohio, have been reported to be at least 28% for rural residents (Doescher, et al., 2006). All these prevalence rates for rural adults far exceed the Healthy People 2020 goal of 12% adult smoking prevalence.

Home smoking restrictions are an effective strategy to reduce secondhand smoke exposure (Berg, et al., 2006); however, rural adults have reported significantly lower rates of home smoking restrictions compared to their urban peers. Approximately 70.5% of rural adults reported that smoking was “not allowed anywhere” in their home compared to 78.7% of suburban adults and 79.0% of urban adults (Vander Weg, et al., 2011). In a regional sample of white, rural smokers, researchers explored which factors are associated with home smoking restrictions (Berg, et al., 2006). The study involved telephone surveys to determine demographics, smoking history, chronic conditions and home smoking restrictions. The sample, itself, was found to be a heavy smoking population, with 78.6% of participants reportedly smoking more than a pack a day.
Slightly less than half of the participants (46.4%) reported no established home smoking restrictions. Participants with nonsmoking friends, a nonsmoking partner, and/or children under age 6 reported a greater presence of home smoking restrictions. Participants with chronic health illnesses reported less home smoking restrictions (Berg, et al., 2006).

In an earlier study, McMillen, Breen and Crosby (2004) compared the secondhand smoke exposure prevalence found in urban and rural counties. The Social Climate Survey for Tobacco Control used telephone surveys with random digit dialing procedures to collect data in a representative sample of 3,009 US adults. Using both bivariate and multivariate statistical techniques, researchers found that adults in rural communities experience greater secondhand smoke exposure than their urban counterparts. Adults in rural communities are less likely to have smoke-free homes or cars and reside in communities with no smoke-free workplaces, stores or restaurants (McMillen, et al., 2004). The absence of smoke-free environments certainly has implications for adolescents in rural communities as well. The study limitations related to self-report, underrepresentation of unknown populations and the subjective nature of the secondhand smoke exposure estimates, as no biomarkers were used for the purpose of objective measurement (McMillen, et al., 2004).

Research that explores the influence of rural residence on tobacco use in adolescents is rare (Coomber et al., 2011; Lutfiyya, et al., 2008; Newman & Shell, 2005). Lutfiyya and colleagues (2008) completed a secondary analysis using the YRBS dataset from the years 1997-2003. The use of multiple years of cross sectional data allowed the researchers to sufficiently power the study for the variable of rural residence. The researchers’ data analyses revealed that rural adolescents are more likely to be daily
cigarette smokers compared to both their urban (OR=.33, CI=.31, .35) and suburban counterparts (OR=.34, CI=.32, .36). Through the use of logistic regression, rural adolescent smokers were more likely to be 1) Caucasian, 2) female, 3) have used smokeless tobacco, 4) smoked their first cigarette by age 12 or younger and 5) have smoked at school within the last month. In a cross-national study with samples from Washington State, USA and Victoria, Australia, Coomber and colleagues (2011) also found that the variable of rurality impacts prevalence rates of tobacco use among adolescents. Lifetime and current substance use, including tobacco use, was significantly higher among rural adolescents compared to urban adolescents with an odds ratio equal to 1.31 (Coomber, et al., 2011). The limitations of the study by Lutfiyya and colleagues includes the use of self-reported data, no sampling of out-of-school youth, and no data collection regarding parental education level to understand the influence of socioeconomic status along with rural residence (Lutfiyya, et al., 2008) while the study by Coomber and colleagues (2011) is limited in its generalizability due to its the use of regional samples.

The social and cognitive processes that influence rural adolescent smoking have been studied with sample of 1673 rural adolescents from northern Iowa. This regional study explored eight variables: 1) peer smoking norms, 2) adult smoking norms, 3) drug refusal assertiveness, 4) drug refusal techniques, 5) life skills, 6) pro-smoking attitudes, 7) risk taking tendency, and 8) family management practices (Epstein, Botvin, & Spoth, 2003). Using gender-stratified statistical analyses, researchers found pro-smoking attitudes and the smoking behaviors of adults and peers influenced the current smoking status in both the male and female adolescents. Although the cross-sectional design,
regional sample, and predominantly Caucasian participants significantly limits generalizability (Epstein, et al., 2003), these findings speak to the generational and culture influences that may be present in rural communities.

More research is needed to further describe tobacco exposure and the related health outcomes among rural adolescents. A heavy reliance on self-report measures of tobacco use and exposure is found in the literature. Researchers must incorporate the use of objective measures of tobacco use and exposure particularly among adolescents, who are using tobacco products illegally if under the age of 18 years old. In addition, research studies specific to rural adolescents are limited in size and scope. The available studies clearly support the assertion that greater levels of tobacco use and exposure exist among adolescents living rural communities. More research is needed to build knowledge regarding rural adolescents and factors related to their cardiovascular risk, including tobacco exposure and blood pressure prevalence.

Blood Pressure and Cigarette Smoking

Cigarette smoking is associated with hemodynamic changes in the human body including an increase in blood pressure related to nicotine’s activation of the sympathetic nervous system (Benowitz, 2003; United States Department of Health and Human Services, 2010c). Using decades of longitudinal data, the Framingham Hypertensive Risk Score was developed to assess the four-year risk of hypertension among non-hypertensive adults. Smoking status contributes to an adult’s Hypertensive Risk Score along with a patient’s age, gender, systolic and diastolic blood pressure, body mass index, and parental history of hypertension (Parikh, et al., 2008). The Framingham Hypertensive
Risk Score has been further validated using large, longitudinal datasets of adult participants including the Whitehall II study (Kivimaki, et al., 2009) and the Multi-Ethnic Study of Atherosclerosis (MESA) study (Muntner et al., 2010).

Specific to the adolescent population, Levent, Ozyurek and Ulger (2004) found evidence that tobacco smoking adolescents have increased aortic stiffness compared to their non-tobacco using counterparts. Aortic stiffness is considered an early sign of atherosclerosis and the smoking of tobacco products is an important contributor to the atherosclerotic process for adolescents (Levent, et al., 2004).

Research focused on adolescents and the influence of cigarette smoking on their blood pressure is rare. Studies with cross sectional research designs have largely found no significant association between cigarette smoking and blood pressure in adolescents (Fasting, Nilsen, Holmen, & Vik, 2008; Hujova et al., 2011; Katona et al., 2011; Nur et al., 2008). Fasting and colleagues (2008) completed a study with over 5,000 Norwegian adolescents ages 13-19 years. Smoking prevalence among adolescents was 20%, however, only 4% of these smokers reported having a smoking habit of one pack year or more (Fasting, et al., 2008). The researchers found that adolescent smokers with the lowest smoking exposure in their sample had lower systolic blood pressure compared to their nonsmoking peers. Fasting and colleagues (2008) hypothesized that this finding may be a transient phenomenon among adolescents and would require further research. Adolescent smokers in this study were also more likely to be overweight or obese (males \( p = 0.01 \) and female smokers \( p = 0.003 \)) thus, pointing to a clustering of adolescent risk behaviors.

Flouris, Faught and Klentrou (2008) discussed the clustering of cardiovascular
risk factors in their research involving adolescent smokers. With an age and gender-matched sample of 119 adolescents, Flouris and colleagues (2008) completed a research study to assess the prevalence of cardiovascular disease risk among regular adolescent smokers. In this study, an adolescent participant was considered a regular smoker if they confirmed by self-report that they had smoked more than 100 cigarettes in their lifetime. The researchers found that the adolescent smokers had a significantly higher prevalence of cardiovascular disease and smoking in their family history \( (p < 0.001) \). Weight status was higher among adolescent smokers but there was no statistically significant difference between the two groups regarding the traditional cardiovascular risk factors of high cholesterol and elevated blood pressure (Flouris, et al., 2008). With these findings, the researchers postulated that the individuals categorized as adolescent smokers had adopted a ‘smoker lifestyle’ early on, prior to smoking initiation. The researchers describe the smoker lifestyle as encompassing unhealthy dietary habits, a sedentary approach to physical activity, a history of cardiovascular disease and a pro-smoking influence from family members (Flouris, et al., 2008).

Nur and colleagues (2008) completed a cross sectional study with random sampling to gain insight regarding the prevalence of hypertension and its pertinent risk factors among high school students in Sivas, Turkey. They completed the necessary follow-up for adolescents with elevated blood pressures and determined a 4.4% prevalence rate. However, no significant correlation was found between the presence of hypertension and smoking status. The multivariate regression analyses found only weight status to be independent predictor of blood pressure in this sample of adolescents (Nur, et al., 2008).

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Seeking to determine cardiovascular disease risk factors in relation to cigarette smoking among Roma children ages 7-18, Hujova and colleagues (2011) completed a cross-sectional, bi-ethnic study with 174 Roma youth and 131 non-Roma youth. This sample had a smoking prevalence rate of 19% and, additionally, 67.8% of Roma parents were found to be smokers compared to 38.9% of non-Roma parents (Hujova, et al., 2011). This percentage of smoking parents would have significant implications for secondhand smoke exposure among the participants; however, no data was collected specific to secondhand smoke exposure. The researchers found that, although the blood pressure measurements for smoking Roma youth trended higher than nonsmoking Roma youth, the findings were not statistically significant (Hujova, et al., 2011). It must be noted that only one blood pressure measurement was taken for each adolescent participant and the researchers provided no discussion regarding attention to cuff size while using a mercury sphygmomanometer.

The Debrecen Hypertension Study was a cross-sectional, school-based study of Hungarian adolescents examining factors which influence blood pressure including gender, age, height, weight, family history, low birth weight, smoking, alcohol consumption, sports, salt intake, and stress (Katona, et al., 2011). This study included a large, representative sample of adolescents ages 15-18 (n=10,194). The researchers found neither the systolic nor the diastolic blood pressure was influenced by smoking status; there was no significant difference between regular smokers and nonsmoking adolescents in the sample (Katona, et al., 2011). In fact, the researchers found that smoking had an opposite effect by slightly decreasing systolic blood pressure by 1.02 mm Hg (Katona, et al., 2011). The researchers offered little as far as possible explanation for this seemingly
paradoxical finding.

The research study by Kollias and colleagues (2009) has a cross sectional design as well, however, it offers some trending data as two cohorts \(n = 446\) in 2004 and \(n = 558\) in 2007) were analyzed. The purpose of this study was to assess the prevalence of high blood pressure and investigate modifiable dietary and lifestyle factors related to blood pressure in a sample of Greek adolescents ages 12-17 years (Kollias, et al., 2009). The researchers found that smoking was positively associated with increased blood pressure levels in the study population; in particular, diastolic blood pressure among male adolescents was positively associated with smoking status in 2007 cohort. It is important to note that the sample contained a small percentage of smokers; only 5% reported smoking less than 10 cigarettes/day and another 5% reported smoking greater than 10 cigarettes/day (Kollias, et al., 2009).

Researchers have explored the effect of cigarette smoking on blood pressure in adolescents in two different longitudinal studies (Dasgupta et al., 2006; Ford, et al., 2008). Dasgupta and colleagues (2006) sought to further investigate the sex differences found in early determinants of high systolic blood pressure for a sample of 1,267 Canadian adolescents in 7th, 9th and 11th grade from the McGill University Study on the Natural History of Nicotine Dependence in Teens (NDIT). The original goal of this longitudinal investigation was to examine the natural course of nicotine dependence in adolescents over a 5-year period using a convenience sample (Dasgupta, et al., 2006). The use of tobacco, in the form of cigarettes, was measured by self-report for 3 months prior to each data collection time point (Dasgupta, et al., 2006). In the study, adolescents were dichotomously categorized as “tobacco use–ever” or “tobacco use–never”.
Researchers found the systolic blood pressure was slightly lower among female adolescents who were tobacco users while male adolescent tobacco users had higher systolic blood pressure values throughout adolescence (Dasgupta, et al., 2006). The researchers reported an odds risk for tobacco use (OR, 0.82; 95% CI, 0.53 to 1.26) that was not significantly associated with high systolic blood pressure (Dasgupta, et al., 2006). Additionally, Dasgupta and colleagues (2006) reported a significant sex-tobacco use interaction term in their statistical analyses, which they attributed either to chance or possibly a 3-way interaction between sex, tobacco use, and overweight (Dasgupta, et al., 2006). The limitations in this longitudinal study include no data collection regarding ethnicity or pubertal status; thus, these covariates could not be controlled (Dasgupta, et al., 2006).

Ford, Nonnemaker, and Wirth (2008) used data from Waves I and III of the National Longitudinal Study of Adolescent Health (ADD Health) (n=14,322) to determine whether or not tobacco use, lower levels of physical activity, higher levels of physical inactivity, and higher BMI during adolescence would predict an increased risk in high blood pressure and cholesterol by young adults. Self-report measures were used to collect data on tobacco use as well as diagnosis of high blood pressure and high cholesterol. Adolescent tobacco use did not independently predict high blood pressure in young adulthood; however, the significance level was very close to significance with a $p$ value of 0.057 (Ford, et al., 2008). When trying to interpret the lack of significance for adolescent smoking as it relates to young adult blood pressure, the authors speculated that differences in patterns of cigarette use for adolescents versus young adults might be responsible (Ford, et al., 2008). It is interesting to note that a study of young adults
(n = 14,229) using the same longitudinal dataset (ADD Health) found smoking to be an independent correlate of systolic blood pressure and the authors stressed the importance of smoking cessation as an important preventive approach for young adults (Brummett et al., 2011).

Few research studies explore the relationship between blood pressure and cigarette smoking among adolescents. Of the available studies, a greater number have no significant relationship and only two studies (one cross sectional and one longitudinal) found significant positive associations between blood pressure and cigarette smoking in adolescents. However, it must be emphasized that all of the available studies, both cross sectional research designs and longitudinal research designs alike, used self-report measures for smoking status with no standard wording for the smoking-related questionnaire items. A biobehavioral approach that incorporates cotinine as a biomarker and uses validated question items for cigarette smoking has the potential to reveal different findings.

Blood Pressure and Smokeless Tobacco Use

To understand the effect of smokeless tobacco use on the health of adults, the American Heart Association developed a policy statement with the stated purpose of reviewing the scientific evidence on smokeless tobacco use and the potential cardiovascular risks that may result (Piano et al., 2010). Much like cigarette smoke, smokeless tobacco use is associated with hemodynamic changes including a significant increase in heart rate; however, the research appears to support only a transient change in blood pressure among adults; no lasting effect was noted (Piano, et al., 2010). The
evidence suggests that cardiovascular risks may be lower when using smokeless tobacco products compared to cigarette smoking; the policy statement stresses that smokeless tobacco products are “not without harm” (Piano, et al., 2010, p. 1539). In fact, a meta-analysis of research studies involving mostly adult male participants was completed by Boffetta and Straif (2009) and sought to determine if smokeless tobacco users have an increased risk of death from myocardial infarction or stroke. The researchers reported that there is “consistent evidence of a moderate increase in risk of fatal myocardial infarction and fatal stroke” (Boffetta & Straif, 2009, p. 5).

Research focused on the influence of smokeless tobacco on blood pressure among adolescents is exceedingly rare. Kumar, Deshmukh and Garg (2012) completed a research study to determine the prevalence of hypertension and prehypertension for adolescents ages 10-19 years old in the rural district of Wardha in Central India. Using a random sample of adolescents (n = 990), the researchers found a prevalence rate of 3.4% for hypertension and 10.6% for prehypertension after three blood pressure screenings. The sample itself included a large percentage of adolescents who use chew tobacco (42.3%) as well as a very small percentage of overweight or obese adolescents (0.07%). Among the adolescents who self-reported the use of chew tobacco, 4.1% were prehypertensive and 16.0% hypertensive. The odds of being pre-hypertensive was 2.72 times (95% CI: 1.75-4.23) more for those adolescents that use chew tobacco compared to the nontobacco using adolescents (Kumar, et al., 2012). In the multivariate regression analysis, the researchers found that the variable of chewing tobacco use did not enter into the final model (Kumar, et al., 2012). These findings are of particular interest because
confounding related to weight status would be minimal as the majority of the sample was within the normal weight range.

**Blood Pressure and Secondhand Smoke Exposure**

Secondhand smoke has the potential to exert “significant cardiovascular toxicity” due to both acute and chronic exposure (Institute of Medicine, 2010b, p. 83). Only minutes of exposure to secondhand smoke can impact the cardiovascular system. Just five minutes of secondhand smoke exposure negatively affected the elasticity of the aorta in non-smokers (Stefanadis et al., 1998) and 30 minutes of exposure was found to significantly reduce coronary flow velocity in nonsmokers (Otsuka et al., 2001). Endothelial dysfunction, inflammation and plaque activation increase with exposure to secondhand smoke (Barnoya & Glantz, 2005; Benowitz, 2003; Institute of Medicine, 2010b) leading to atherosclerosis and greater risk for increased blood pressure (Oparil, Zaman, & Calhoun, 2003).

Similar to smokeless tobacco, few research studies have focused on secondhand smoke exposure and the influence on adolescent blood pressure. Agrabasli and colleagues (2008) explored the concepts of weight status, parental smoking and blood pressure among middle adolescents ages 15–17. The researchers used current smoking status of family members as a proxy measure for secondhand smoke exposure in the home. Using two cross-sectional samples of Turkish adolescents from 1989–1990 ($n = 673$) and 2004–2005 ($n = 640$), researchers reported that self-report data on current smoking history of family members was not significantly associated with either systolic or diastolic blood pressure (Agirbasli, et al., 2008). Alongside this finding, researchers
reported a decrease in the prevalence of high blood pressure between the two time periods; 16% were classified as hypertensive in 1989–1990 while fifteen years later in 2004–2005 only 8% were classified as hypertensive ($p < 0.001$). It must be noted that the data collected on smoking status in this research study did not use an objective measure but rather relied solely on self-report data.

Moss, Lucht, Kip and Reis (2010) focused their research on the acute physiologic effects of secondhand smoke on children ages 7-18. In this nonrandomized, controlled research study, the researchers sought to investigate whether exhaled carbon monoxide, heart rate, and blood pressure would increase acutely in children exposed to secondhand smoke compared to children who were not exposed (Moss, et al., 2010). The researchers studied the child and adolescent participants in parent-child dyads with twenty dyads in the exposed group and 20 dyads in the unexposed group. Inclusion in the exposed group was determined by biochemical verification of the parent’s smoking status. In the data collection phase of this study, a parent/child dyad from the exposed group was brought to a hotel room to simulate a controlled, home environment. The parent from the exposed group smoked 1 cigarette at a distance of seven feet from the child while the child watched television or entered into conversation for entertainment. Researchers reported that the absolute level of blood pressure was similar before and after the stated intervention, irrespective of exposure status (systolic blood pressure $r = .11$, $p = .50$ and diastolic blood pressure $r = −0.09$, $p = .59$). The findings indicated a weak, insignificant relationship between a brief exposure of secondhand smoke and acute blood pressure change for children and adolescents in this sample (Moss, et al., 2010). Although the sample size in this study is very small, this study offers beginning insight into the effects
of acute secondhand smoke exposure in children and adolescents.

With a sample of preschool children ages 5-6 years old, the research by Simonetti and colleagues (2011) has contributed to the understanding of chronic secondhand smoke exposure. With a cross sectional research design and a convenience sample of 4,236 preschool children, Simonetti and colleagues sought “to elucidate whether casual blood pressure measurements provide meaningful information about risk factor exposure at a very young age” (Simonetti, et al., 2011, p. 292). Data related to the potential for secondhand smoke exposure was collected as self-report data from parents on current smoking status and the amount of cigarettes per day. Parental smoking was found to independently affect systolic blood pressure ($p = 0.001$) even after the researchers statistically controlled for body mass index, parental hypertension, and birth weight. Parental smoking increased the likelihood of a systolic blood pressure in the top 15% of the population by 21% (2% to 44%; $p = 0.02$). The researchers reported an increase in both systolic and diastolic blood pressure with an increase in the number of parent-related risk factors including parental obesity, hypertension, and smoking (Simonetti, et al., 2011). Simonetti and colleagues (2011) noted that this study provides evidence that secondhand smoke raises blood pressure among preschool aged children and that parental smoking, hypertension, and obesity appear to work together to influence blood pressure in preschool children.

Blood Pressure, Tobacco Exposure and Inflammation

An ongoing inflammatory response is the foundation for the development of atherosclerosis and provides a pathophysiological link between atherosclerosis and
hypertension (Libby, et al., 2002). The measurement of low-grade chronic inflammation is accomplished using the inflammatory biomarker C-reactive protein (CRP), which assists in defining atherosclerotic risk (Libby, et al., 2002). In a large, prospective cohort study with 2,459 middle-aged adult participants diagnosed with coronary heart disease and 3,969 matched participants as controls, CRP was a moderate predictor of coronary heart disease with an odds ratio of 1.36 (1.16-1.58) (Danesh et al., 2004). While controlling for various confounders such as age, sex and socioeconomic status, Danesh and colleagues (2004) reported the odds ratio for coronary heart disease among current smokers compared to never smokers as 1.75 (1.51-2.03) and among those with elevated systolic blood pressure (131 to 147 mmHg) to be 1.50 (1.30-1.74). Among adults ages 18-34 years, evidence suggests that high serum C-reactive protein levels are associated with the accelerated progression of atherosclerosis independent of the traditional cardiovascular disease risk factors (Zieske et al., 2005).

Tobacco exposure from cigarette smoking and/or secondhand smoke exposure predispose an individual towards negative cardiovascular outcomes (Ambrose & Barua, 2004). Cigarette smoking increases an individual’s risk for negative cardiovascular outcomes related to atherosclerosis including acute coronary syndromes, sudden death, and stroke (Ambrose & Barua, 2004). The chronic use of cigarettes is thought to be responsible for the initiation (or even the instigation) of an inflammatory process, thus promoting atherosclerosis (Ambrose & Barua, 2004; Virdis, Giannarelli, Neves, Taddei, & Ghiadoni, 2010).

To investigate inflammation specific to cardiovascular outcomes, C-reactive protein (CRP) is a biomarker that is frequently cited in the nicotine and tobacco research
literature (Institute of Medicine, 2010b; Jefferis, et al., 2010; Venn & Britton, 2007; Wilkinson, et al., 2007). Venn and Britton (2007) examined the relationship between serum cotinine (a biomarker indicative of tobacco use and exposure) and serum CRP levels in never-smoking adults participating in a large, nationally representative cross-sectional study (n = 7,599 adults 17 years of age or older). No statistically significant relationship was found between cotinine and CRP levels (Venn & Britton, 2007). Venn and Britton (Venn & Britton, 2007) postulate that this lack of statistical significance may result from the fact that the NHANES III (from which the sample was developed) research design did not incorporate high-sensitivity laboratory methods for CRP analysis. In contrast, Panagiotakos and colleagues (2004) conducted a large scale study with a stratified, random sample of Greek adults (n = 3,355) and found elevated C-reactive protein levels in adults exposed to secondhand smoke at least three days per week (p = 0.03) (Panagiotakos, et al., 2004). This study, however, relied on self-report to determine the extent of secondhand smoke exposure experienced, not an objective biobehavioral measure such as the biomarker cotinine.

There is a paucity of adolescent research that examines the influence of tobacco exposure and inflammation on cardiovascular outcomes such as blood pressure. With participants ages 6–18 years (n = 5,044), a significant positive correlation was found between cotinine and C-reactive protein levels (Wilkinson, et al., 2007). The sample for this study was derived from the 1999-2002 National Health and Nutrition Examination Survey (NHANES), which is a well-established, nationally representative data set for the US. Although this positive correlation appears to affirm a relationship between tobacco exposure and inflammation, further research with more complex statistical analyses is
needed to fully understand the impact of tobacco exposure on inflammation among children and adolescents.

Using the NHANES data from 1999 to 2000, Ford (2003) examined the relationship between C-reactive protein concentration and risk factors of cardiovascular disease. In this child-focused approach to NHANES data, systolic and diastolic blood pressures were collected in children 8 to 17 years of age and smoking status data was incorporated for children 12 to 17 years of age (Ford, 2003). Ford (2003) found statistically significant relationships between C-reactive protein concentration and age, body mass index ($r = 0.43, p \leq 0.05$), and systolic blood pressure ($r = 0.23, p \leq 0.05$) in both sexes; body mass index was the best predictor of C-reactive protein concentration overall. In this study, no association was found between C-reactive protein and smoking status in adolescents 12-17 years old (Ford, 2003).

Knoflach and colleagues (2003) completed research with a sample of 141 males 17-18 yrs. old to examine the relationships between traditional vascular risk factors (i.e. smoking and blood pressure), markers of inflammation, and immune reactivity with intima-media thickness as the outcome variable. Intima-media thickness is an ultrasound measure used in this study to quantify the degree of atherosclerotic progress (Knoflach, et al., 2003). With regards to data collection, the mean of three blood pressure measurements was calculated to quantify blood pressure and smoking status was self-reported by the adolescent participant (Knoflach, et al., 2003). Researchers found that high intima-media thickness, indicative of atherosclerosis, was associated with high diastolic blood pressure, and positive smoking status. Although not a statistically significant findings, the levels of the inflammatory biomarker CRP were greater in
participants with high intima-media thickness compared to participants with low intima-
media thickness (Knoflach, et al., 2003).

Researchers from the Cardiovascular Risk in Young Finns Study sought to
examine whether or not C-reactive protein (CRP) levels track into adulthood and whether
these levels in childhood can predict intima-media thickness in adulthood (Juonala, et al.,
2006). The serum samples used to establish baseline CRP levels in this study were
collected in 1980 and kept frozen at -20°C (Juonala, et al., 2006). The researchers found
that smoking status correlated significantly with childhood CRP levels. With a
statistically weak but significant relationship, childhood CRP values were found to be
predictive of adult CRP levels independent of traditional cardiovascular risk factors such
as blood pressure or smoking (Juonala, et al., 2006). Finally, Juonala and colleagues
found that childhood CRP levels were not significantly related to adult carotid intima-
media thickness; however, childhood systolic blood pressure, body mass index and
smoking were predictive of adult intima-media thickness (Juonala, et al., 2006). Potential
validity concerns were acknowledged as a limitation when discussing the use of 25-year
old frozen serum samples to assess baseline CRP levels (Juonala, et al., 2006).

The research of McMahan and colleagues (2007) sought to provide further
validity to the risk score developed from the autopsy findings in the Pathobiological
Determinants of Atherosclerosis in Youth (PDAY) Study. The risk score incorporates the
coronary risk factors of gender, age, serum lipoprotein concentrations, smoking,
hypertension, obesity, and hyperglycemia (McMahan, et al., 2007). Like many studies
already discussed in this review of the literature, this study used self-report measures to
determine smoking status. An average of at least three separate blood pressure
measurements were used in the statistical analyses. In 1986, risk factors were measured in participants from the Cardiovascular Risk in Young Finns (n=1,279) who were 12 years old or older and follow up measurements were completed in 2001, including intima-media thickness measurements. PDAY risk scores were calculated early in life (circa 1986) as well as the calculated change in risk score 15 years later in 2001. McMahan and colleagues (2007) reported that the PDAY risk score (which incorporates smoking and hypertension) and the change in the risk score over a 15-year period were both significant predictors of carotid artery intima–media thickness.

Wijnstok and colleagues (2010) completed research examining cardiovascular risk among adolescents in Northern Ireland in the Young Hearts Projects (n = 2,017). The aim of the study was to further understand the relationship between inflammation, premature atherosclerosis, and childhood adiposity while incorporating novel biomarkers including high sensitivity C-reactive protein (hsCRP), intercellular adhesion molecules (sICAM), and soluble vascular adhesion molecules (sVCAM) (Wijnstok, et al., 2010). With a focus on childhood adiposity, 95 obese subjects were identified within the larger study sample and matched with 95 overweight and 95 normal-weight adolescents according to age, sex, and cigarette smoking for a total of three matched groups (Wijnstok, et al., 2010). As an outcome variable indicative of cardiovascular risk, the researchers used a clustered cardiovascular risk score that incorporates mean arterial blood pressure, low density to high-density lipid cholesterol ratio, cardiorespiratory fitness, skinfolds, and triglyceride levels. The researchers reported that all three biomarkers, including high sensitivity C-reactive protein, had significant associations with the calculated clustered cardiovascular risk outcome variable (Wijnstok, et al.,
The best prediction model included all three biomarkers of hsCRP, sICAM, and sVCAM with a variance of 26%. The main finding of this study noted that the inflammation biomarkers were strong predictors for CVD risk in adolescents compared to the lifestyle risk factors (Wijnstok, et al., 2010). Although this study does not address tobacco exposure, it is important to emphasize that this study is rare in that it is focused on cardiovascular risk, relevant biomarkers, and the adolescent population.

When determining the relationship between smoking behavior and arterial stiffness, the literature presents inconsistent findings (van de Laar et al., 2011). A study by van de Laar and colleagues (2011) examined smoking behavior in adolescence and young adulthood and its relationship with arterial stiffness in young adulthood. As a secondary aim, the study examined the influence of inflammation and endothelial dysfunction. With a sample of 424 individuals from the Northern Ireland Young Hearts Project, smoking status was assessed at adolescence (age 15) and again in young adulthood (at approximately age 22). The researchers reported findings confirming that adolescents who smoked had higher aortic stiffness, inflammation and endothelial dysfunction levels in young adulthood independently of other adolescent and adult lifestyle behaviors. The researchers state that this significant association between smoking status and inflammation may be a reflection of the duration of smoking and not necessarily adolescence as a critical time period (van de Laar, et al., 2011).

Only one study was found that incorporated active and passive smoke exposure with the outcome variable of salivary C-reactive protein (CRP). This study involved 45 first year university students with a mean age of 18.89 years and was completed as pilot work for a larger study centered on the use of CRP as a salivary biomarker (Azar &
Richard, 2011). The researchers controlled for recent infection, certain medications (statins, aspirin and oral contraception), age and body mass index. Smoking status was determined by self-report as well as salivary cotinine verification. The statistical analysis was completed by category of smoker including nonsmokers, passive smokers and active smokers. In this pilot work, Azar and Richard found some evidence of a dose-response relationship between total smoke exposure and CRP where active smokers had higher CRP levels than nonsmokers (t (14) = 3.57, p = 0.00). However, the difference between active smokers and passive smokers did not reach statistical significance (t (32) = 1.24, p = 0.22.). The researchers emphasize the need for continued research with salivary CRP, as it is a less invasive biomarker that causes less distress for patients compared to the use of serum biomarkers (Azar & Richard, 2011).

Research studies that examine the relationships between blood pressure, tobacco exposure and inflammation are rare, particularly with regards to the adolescent population. Differences in methodology, including self-report of tobacco use, failure to incorporate the variable of secondhand smoke exposure and use of high sensitivity C-reactive protein as a biomarker make comparisons between studies challenging. Only one study that incorporated the use of salivary C-reactive protein as a biomarker of systemic inflammation was found. Findings from the available studies do not provide conclusive evidence regarding the role of inflammation as a mediator between blood pressure and tobacco exposure.
Potential Confounding Variables

To focus primarily on the relationship between blood pressure and tobacco exposure, this study was designed to control for the following confounding variables: age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status, and weight status. The literature on these identified confounding variables will be reviewed so as to understand how these variables independently influence blood pressure in the adolescent.

Age

The concept of age captures the chronological and maturational development of an individual. Age is an integral component to the categorization of the blood pressure measurements of individuals under 18 years old along with their gender and height. The normative distribution of blood pressure standards that are based on age, gender and height allow for a more a precise classification of blood pressure (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). A number of research studies with adolescent blood pressure as the outcome measure have determined age to be an important confounding variable to acknowledge (Dasgupta, et al., 2006; Din-Dzietham, et al., 2007; Freedman, et al., 2012; Lande, et al., 2008; Menezes et al., 2011; Sugiyama, et al., 2007).

Gender

In the US, the prevalence of blood pressure varies by gender (Oparil & Miller, 2005). During early adulthood, women have lower systolic blood pressure compared to their male counterparts. After age 60, men have the lower systolic pressure measurements compared to females. With regards to diastolic blood pressure, adult women tend towards
slightly lower diastolic blood pressures compared to adult men, regardless of age. With regards to gender, diastolic blood pressure is often lower in women compared to men (Oparil & Miller, 2005).

Similar to age, the concept of gender figures prominently in the classification of blood pressure measurements in those individuals under the age of 18 years old (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). With evidence suggesting that systolic blood pressure measurements are higher among men and boys compared to women and girls, Dasgupta and colleagues (2006) investigated whether gender differences affect systolic blood pressure in adolescents. The sample for this study was derived from a longitudinal cohort of Canadian adolescents followed over a period of five years. Absolute systolic blood pressure values changed significantly for the male adolescents in the sample, while changing very little for the female adolescents over the same time period. The findings suggest that the risk for high systolic blood pressure increased by 19% annually for male adolescents with no increased risk for female adolescents. Male adolescents were more likely to have high systolic blood pressure in youth with this risk increasing in magnitude during the adolescent period, and this increase contributes to the higher prevalence of hypertension among men in adulthood (Dasgupta, et al., 2006).

*Ethnicity*

Blood pressure also varies according to an individual’s ethnic background. A diagnosis of hypertension is more common, more severe, and often begins at a younger age among African American individuals compared to their non-Hispanic, Caucasian individuals. (Oparil & Wright Jr, 2005). African American adults experience a greater
prevalence of hypertension compared to both non-Hispanic Caucasian and Hispanic individuals (Egan, Zhao, & Axon, 2010; Roger, et al., 2012). The physiological and social mechanisms behind these noted ethnic differences remain unclear (Oparil & Wright Jr, 2005).

Rosner and colleagues (2009) completed research to examine the prevalence of blood pressure elevations among ethnic groups of children and adolescents including African American, Caucasian and Hispanic. This study used the Pediatric Task Force database, which contains information from over 75,000 office visits involving nearly 60,000 children and adolescents. The data related to ethnicity was collected by self-report. The prevalence of systolic blood pressure elevation was not found to be significantly different between African American and Caucasian males (OR: 0.96; \( p < 0.39 \)). In contrast, Hispanic males had a higher prevalence rate of systolic blood pressure elevations compared to Caucasian males (OR: 1.49; \( p < 0.001 \)). Hispanic and African American females had higher systolic blood pressures compared to Caucasian females (\( p < 0.003 \)). When statistically adjusting for weight status, Hispanic males continued to have a greater rate of systolic blood pressure elevations (OR: 1.29; 95% CI: 1.14 to 1.47; \( p < 0.001 \)), followed by African American males (OR: 0.91; 95% CI: 0.83 to 1.00; \( p < 0.06 \)) compared to Caucasian males (Rosner, et al., 2009). In contrast, females had no significant ethnic differences in systolic blood pressure when adjusted for weight status (Rosner, et al., 2009).

**Parental History of Hypertension**

Longitudinal research with adult populations has underscored the importance of parental history of hypertension when considering the risk for hypertension in the next
generation. The Framingham Heart Study was developed in 1948 as a community-based, prospective cohort study with an original enrollment of 5209 adults and a second cohort, known as the Framingham Offspring Study, with 5124 adults that includes the children of the original cohort (Parikh, et al., 2008). Decades of data from this longitudinal study were used to develop the Framingham Hypertensive Risk Score; a tool that takes into account a patient’s parental history of hypertension along with their age, gender, systolic and diastolic blood pressure, body mass index, and history of smoking (Parikh, et al., 2008). These seven factors are key indicators in the future development of hypertension among adults (Kivimaki, et al., 2009; Parikh, et al., 2008). In addition to the Framingham Heart Study, the Johns Hopkins Precursors Study provides longitudinal data to support both paternal and maternal hypertension as strong, independent factors in the development of hypertension in adulthood (Wang, et al., 2008).

The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents specifically identifies parental history of hypertension as a primary risk factor for hypertension among youth (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). In a sample of 1,850 Canadian children ages 6-17 years, researchers designed the Canadian Health Measures Survey to explore the influence of family history of hypertension. A strong association was found between the family history of hypertension and both systolic and diastolic blood pressure in adolescent males and younger females (Shi, et al., 2012). The variable of parental history of hypertension is valuable as it serves as a proxy to acknowledge the genetic influences at play in an adolescent’s blood pressure.
Socioeconomic Status

Social inequities, experienced even as early as 5 years old, influence the development of hypertension (van den Berg, et al., 2013). In a sample of Dutch children ages 5 to 6 years (n = 3024), approximately 13.2% of children with high-educated mothers had blood pressures in the pre-hypertensive range, compared to a full fifth (20.8%) of children with mid-educated mothers and over a quarter (26.6%) of the children with low-educated mothers (van den Berg, et al., 2013). In addition, a systematic literature review of 40 research studies found “a robust, inverse association” between childhood socioeconomic circumstances and cardiovascular risk in adulthood (Galobardes, et al., 2006, p. 91).

The Cardiovascular Risk in Young Finns Study began in 1980 and includes 30 years of data to assist in the understanding of childhood risk factors that influence cardiovascular outcomes in adulthood (Juonala, et al., 2013). This prospective study has a sample representative of Finland’s youth including 3,596 children and adolescents ages 3-18 years. Using the variable of family income as a proxy for socioeconomic status, one of the main findings from the prospective Cardiovascular Risk in Young Finns Study states that childhood socioeconomic status is an independent predictor of hypertension in adulthood (Juhola et al., 2012; Juonala, et al., 2013).

Pubertal Status

The process of puberty involves a series of endocrine changes that result in a physiological transition for childhood into adulthood (Patton & Viner, 2007). Puberty appears to influence cardiovascular and metabolic processes in adolescents through the endocrine active in body tissues (Siervogel, et al., 2003). Blood pressure and body mass
index increase significantly during the process of puberty (Chen & Wang, 2009; Radtke, et al., 2012). The changes in blood pressure during puberty may influence the future levels of blood pressure during adulthood (Shankar, et al., 2005).

In a longitudinal study involving a sample of 715 adolescents, Shankar and colleagues (2005) examined the changes in blood pressure related specifically to pubertal growth. The researchers identified two visits where the maximum change in height occurred and used this change to estimate each adolescent participant’s age of maximum height velocity. The age of maximum height velocity was then used to estimate the adolescent participant’s onset and end of puberty growth. Through serial blood pressure measurements, the researchers found that the rates of increase in blood pressure during puberty were three to six times greater in male adolescents and two to four times greater in female adolescents compared to the time period prior to pubertal growth. In addition, the increase found in male adolescents was significantly greater compared to their female counterparts \( p < 0.001 \) (Shankar, et al., 2005). These findings suggest that pubertal status is a confounding variable that must be controlled for when exploring the relationship between blood pressure and tobacco exposure in rural adolescents.

**Weight Status**

Weight status is a confounding variable that has a substantial influence on the outcome of blood pressure in adolescents. Obese adolescents are more likely to experience high blood pressure (Din-Dzietham, et al., 2007; Ford, et al., 2008; Paradis, et al., 2004; Shi, et al., 2012). An estimated 18.4% of US adolescents ages 12-19 are obese (Ogden, et al., 2012).

Although research has confirmed a strong, positive relationship between blood
pressure and weight status, the relationship does not appear to be a straightforward, linear one. For instance, Sugiyama and colleagues (2007) completed research using a nationally representative sample of US adolescents and found that systolic blood pressure positively correlates with body mass index while diastolic blood pressure negatively correlates with body mass index. Using longitudinal data from the Bogalusa Heart Study that includes 24,092 examination data points, obesity was found to be an independent predictor of blood pressure data; however, the increases in blood pressure did not parallel the increases in obesity over the same time period (Freedman, et al., 2012). Over a time period of two decades (1974 to 1993), the prevalence of obesity increased from approximately 6% to 17% compared to no change in systolic blood pressure and a decrease in diastolic blood pressure decreased by 2 mmHg (Freedman, et al., 2012). Although weight status appears to be a significant contributing variable to the outcome of blood pressure, it is clear that a combination of factors influence blood pressure in adolescents.

In this study, the confounding variables of age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status, and weight status were statistically controlled. A research design that controls these particular covariates allows for a clearer understanding of the specific relationship between blood pressure and tobacco exposure among rural adolescents.

Adolescence

This research study examines the influence of tobacco exposure on blood pressure and takes place within the context of adolescence. The onset of puberty often denotes the
beginning of adolescence and this unique period of growth and development is typically captured within the age range of 10 and 19 years (Sawyer et al., 2012). “Health in adolescence is the result of interactions between prenatal and early childhood development and the specific biological and social-role changes that accompany puberty, shaped by social determinants and risk and protective factors that affect the uptake of health-related behaviours.” (Sawyer, et al., 2012, p. 1630). The need for a renewed focus on adolescence has risen in the literature, stressing that adolescence serves as a basis for future health (Sawyer, et al., 2012).

With this renewed focus on adolescence comes the realization that “the vulnerability of adolescents is relatively unexamined” compared to the vulnerabilities to health present in the prenatal and early childhood periods of growth and development (Santelli, Sivaramakrishnan, Edelstein, & Fried, 2013, p. S42). More research is needed to explore the health-related vulnerabilities found in adolescence, including those related to tobacco use and exposure. Greater than 80% of established adult smokers state that they tried their first cigarette before 18 years of age (United States Department of Health and Human Services, 2012). Once an adolescent becomes an established smoker, their risk of chronic disease acquisition increases with the duration of smoking behavior (United States Department of Health and Human Services, 2012). With regards to secondhand smoke exposure, adolescents continue to be at the mercy of adults when considering smoking restrictions in the home and workplace. Research completed by Dove, Dockery and Connolly (2010) examined the effect of smoke-free air laws on children and adolescents (ages 3-19) who lived with and without a smoker in the home. With a nationally representative sample from the National Health and Nutrition Survey
the researchers found that smoke-free air laws are an effective intervention to reduce secondhand smoke exposure among youth with no secondhand smoke exposure in the home; however, youth who lived with a smoker were found to have the highest detectable cotinine levels. No significant reduction in their cotinine levels was found related to the presence of smoke-free air laws (Dove, et al., 2010). The negative impact of tobacco use and exposure on long term cardiovascular outcomes among youth is well established in scientific literature (United States Department of Health and Human Services, 2012).

There are opportunities to address vulnerability in adolescence as it related to tobacco use and exposure. The challenge comes in “[m]aking adolescents and their health visible” to parents, school administrators, policymakers and adolescents themselves (Sawyer, et al., 2012, p. 1637). The urgency to make the health needs of adolescents visible is even greater for adolescents living in rural areas of the United States.

Rural Health

Rurality is considered a social determinant of health and a root cause affecting health outcomes (Lutfiyya et al., 2012). Individuals in rural communities are more likely to smoke, be less physically active, eat a less nutritious diet, and be more overweight than individuals who live in a suburban communities (Eberhardt, Ingram, & Makuc, 2001). Rural, tobacco-growing communities, in particular, experience disparities related to tobacco use, secondhand smoke, and weak smoke-free air policies (Hahn, Rayens, & York, 2013). The smaller the rural community and the more tobacco grown in the area, the less ready the community is for smoke-free air policy change (Hahn, et al., 2013).
These patterns of health behavior impact rural youth. Exposure to secondhand smoke affects a third of youth from small rural communities and 35% of youth from large rural communities compared to only 24.4% of urban children (United States Department of Health and Human Services, 2011).

Additionally, rural adolescents face extensive barriers to healthcare including “isolation, insufficient financial resources, lack of available services, impaired geographic accessibility, and concerns for confidentiality” (Curtis, et al., 2011, p. 61). The pattern of health behaviors in rural communities suggests that rural culture is a determinant of health (Hartley, 2004). To effectively tackle rural health disparities, a population health approach is required, and researchers interested in improving health in rural areas must acknowledge the impact of rural residence (Hartley, 2004).

Summary

An individual’s blood pressure and tobacco use and exposure influence his/her cardiovascular health risk (Danaei, et al., 2009). US adult hypertension prevalence rates are estimated at nearly 30% (Crim, et al., 2012) while the prevalence rate among US adolescents is estimated at 3% (Loeffler, et al., 2012; Sugiyama, et al., 2007). With regards to tobacco usage, approximate one-quarter of US adults use tobacco products (King, et al., 2012) and just under a quarter of US adolescents (23.2%) report current tobacco use (Arrazola, et al., 2012). These statistics represent a sizeable US population and result in a significant impact on national healthcare costs.

Research examining blood pressure, tobacco exposure and the role of inflammation in adolescents is rare. The available research does not provide conclusive
evidence regarding the relationship between these variables as the sample age ranges, blood pressure procedural differences and varying methodologies make comparisons challenging. Reliance on self-report of smoking behaviors and secondhand smoke exposure is rampant. There is also a significant reliance on school-based studies which does not allow for the discussion of risk among out-of-school youth. Although rural communities have been noted for having few smoke-free air policies (Hahn, Rayens, Okoli, Love, & Kim, 2004) and higher rates of obesity (United States Department of Health and Human Services, 2011) that can impact blood pressure, no studies were found that examined blood pressure, tobacco exposure, and inflammation among rural adolescents.
CHAPTER THREE

METHODOLOGY

In this study, the relationship between tobacco exposure and blood pressure in adolescents ages 15-18 living in a rural area was examined. The dependent variable of blood pressure was examined in relationship to the independent variable of tobacco exposure with systemic inflammation as a potential mediator. The concept of tobacco exposure is comprised of self-reported individual tobacco use, such as cigarette smoking and smokeless tobacco use, as well as sources of secondhand smoke exposure (United States Department of Health and Human Services, 2010c). “Secondhand smoke” is defined as the mixture of side stream smoke from a lit cigarette and mainstream smoke exhaled by an active smoker is (United States Department of Health and Human Services, 2006). The confounding variables that were controlled for include age, gender, ethnicity, parental history of hypertension, weight status, pubertal stage, and socioeconomic status. A pilot study (n = 40) was completed to address feasibility issues and informed the present study. In this chapter, the research design, sample and setting are discussed. Each instrument and its corresponding protocols are reviewed in the context of the study along with the data collection procedures, data management and data analysis.
Design

This study used a predictive, cross-sectional design. A cross-sectional design was used, as the data for this study was collected at one time-point only (Burns & Grove, 2009). This design was appropriate for the study, as it provided information on the relationship between tobacco exposure and blood pressure.

The predictive aspect of this research design provides a framework from which to quantify the level of the relationship between the independent variable and dependent variable (Burns & Grove, 2009). The predictive research design assisted the principal investigator in understanding the size of the contribution made by tobacco exposure with regard to blood pressure in this population. With this research design, the potential for third variable effects between tobacco exposure and blood pressure in this population can be examined. Data were collected on C-reactive protein as a potential mediator of blood pressure. The data collected from a sample of rural adolescents in this study occurred in a naturalistic setting with no manipulation of the environment nor intervention provided and is, thus, considered a nonexperimental design (Polit & Beck, 2008).

Setting and Sample

Characteristics of the Sample

A nonprobability, convenience sample of adolescents was recruited from two high schools located in a rural county in a Midwestern state. Convenience sampling, a common sampling technique, allows for easier accessibility to participants (Burns & Grove, 2009).
Study participants were selected from two high schools with similar demographic characteristics. School A had 339 students enrolled in grades 9-12 with 33.6% of the population participating in the National School Lunch Program, receiving either free or reduced lunches (Indiana Department of Education, 2011-2012). The ethnic composition of School A during 2011 to 2012 was 98.3% Caucasian, 1.2% Latino and 0.6% Multiracial. By comparison, School B had 458 students enrolled in grades 9-12 with 39.3% participating in the National School Lunch Program. The majority of students in School B (94.8%) were Caucasian with approximately 5.1% of the student population reporting some ethnic diversity including Hispanic, Asian, Black and Multiracial (Indiana Department of Education, 2011-2012).

The principal investigator met with school administrators for each of the two high schools (Appendix A) to determine the appropriate avenues for participant recruitment. Study purpose, sample inclusion and exclusion criteria, informed consent process, data collection procedures and participant incentives were discussed with each school’s administration. The principal investigator also met with the nursing staff at each school to determine the school policies and procedures for reporting any blood pressure abnormalities. The recruitment methods from the completed pilot study yielded the necessary number of adolescent participants; therefore, the methods using small classroom discussion in science and agriculture courses were implemented for the larger study.

The inclusion criteria for the pilot and larger study included adolescent participants who were: (1) in the 9th-12th grade, (2) ages 15-18 years, (3) able to understand, speak, and respond in English, (4) willing to participate, and (5) obtained
written parental consent. Exclusion criteria for the study were as follows: (1) non-English speaking adolescents or parents, (2) diagnosis of acute infection and current antibiotic use in last 48 hours, (3) current use of nicotine replacement therapy, and (4) self-report pregnancy. Data collection regarding C-reactive protein levels was needed; therefore, adolescents with a current, acute infection (by self-report) were excluded, as their C-reactive protein levels would be episodically elevated. To maintain the validity of salivary cotinine measure, adolescents currently using nicotine replacement therapy for smoking cessation (by self-report) were excluded from the study. Adolescents who self-reported being pregnant were excluded due to their potential altered ability to metabolize nicotine which may result in less valid cotinine levels (Benowitz, et al., 2009).

Setting Characteristics

In a rural county of a Midwestern state, School A is located in the geographic center of the rural county on a school campus, which includes grades kindergarten through 12th grade. School B is located in a small town at the southern end of the county and contains grades 9 through 12.

The concept of rural is multifaceted and difficult to define (Hart, et al., 2005). The definition of rurality provided by the Rural Urban Commuting Area (RUCA) codes (University of Washington Rural Health Research Center, 2005) as well as the Rural Health Grants Eligibility Analyzer (Wagner et al.) was used to define the rural county setting. RUCA codes are a taxonomy that uses the standard Census Bureau Urbanized Area and Urban Cluster definitions as well as work commuting data to characterize different Census tracts as rural or urban (Hart, et al., 2005; University of Washington Rural Health Research Center, 2005). RUCA codes are a favorable methodology for defining rurality,
as the codes are sensitive enough to differentiate rural areas according to economic integration (Hart, et al., 2005). This method of defining rurality is consistent with the method used in the national report entitled “The Health and Well-Being of Children in Rural Areas: A Portrait of the Nation 2007” (U.S. Department of Health and Human Services, 2011).

Power Analysis

In determining an appropriate sample size for this study, a significance level of 0.05 and a medium effect size was selected. A previous pilot study using a biobehavioral approach to total smoke exposure and C-reactive protein in adolescents reported significant findings using an alpha set at 0.05, power at 0.8 and a moderate-to-large effect size of 0.50 to 0.80 (Azar & Richard, 2011). An apriori power analysis was completed for this study using the G*Power statistical software (Faul, Erdfelder, Buchner, & Lang, 2009). A medium effect size using 134 was deemed the most appropriate and feasible sample size for this research study. The principal investigator increased the final sample size by ten percent to compensate for any missing data or possible inadequate saliva collection for participants. Thus, the final sample size for the study was 148 participants.

Protection of Vulnerable Subjects

Informed consent serves as a cornerstone to safeguard participants in research. Previous studies have reported that children as young as seven years old are capable of providing their own assent in the research process (Broome, 1999). For the sound implementation of assent procedures, several factors must be considered. First, a researcher working with adolescents of different ages must have an understanding of their
range of developmental and cognitive abilities (Santelli et al., 2003). A researcher must acknowledge the potential of a power differential between an adolescent and their parents as well as between an adolescent and the health care provider/principal investigator (Broome, 1999; Trivedi, 2005). Finally, a researcher must understand an adolescent’s motivations to participate or to decline involvement in research (Leikin, 1993). Adolescents have a unique perspective and the researcher who appreciates this perspective is more likely to build a successful research relationship with an adolescent participant.

The pilot research plan related to this study was submitted to the IRB at the University of Alabama at Birmingham (UAB) in the spring of 2012. An addendum to the IRB-approved pilot study was submitted in the fall of 2012 to allow for the completion of the larger dissertation study. The UAB IRB approved all protocols, informed consent documents, interview scripts, and study measures prior to initiating any data collection (Appendix A). With a sample population of adolescents at least 15 years old, the UAB IRB advised the principal investigator to use one informed consent document cosigned by the adolescent participant and their parent/guardian (Appendix B). Researchers have noted that parental consent process can result in a study sample with fewer males, fewer minorities, fewer youth from low income families and fewer smokers (Dent, Galaif, Sussman, & Stacy, 1993). As stipulated by the UAB IRB, both signatures were required for the adolescent to participate. If the parent/guardian did not provide written informed consent then, the adolescent was not enrolled in the study. If the parent/guardian provided written consent and the adolescent does not actively consent as well, then, enrollment did not occur.
The informed consent document stated that study participation was not mandatory and that the adolescent participant could withdraw from the study at any time without penalty. The principal investigator defined confidentiality for all adolescent participants. The principal investigator informed participants that all data collection documents and salivary specimens were labeled with a unique study number; no names were used in order to maintain confidentiality. The principal investigator used the recommended UAB IRB template to develop the informed consent document. The informed consent document for this study had a Flesch-Kincaid Grade Level of 11.2, which raises concern regarding the ability to recruit adolescent participants with lower reading proficiency. A fifth grade reading level is recommended to help allay literacy concerns.

Each adolescent with an informed consent document signed by both adolescent and parent/guardian was enrolled as a study participant. Once an adolescent participant completed the data collection process, which included four self-report questionnaires, height, weight, waist circumference, blood pressure and passive drool saliva collection, he/she was given a $10 gift card to compensate for his/her time and effort. Compared to incentives given within the adolescent health research community, 10 US dollars is a reasonable monetary incentive and this amount served to acknowledge the time and effort invested by the adolescent (Borzekowski, Rickert, Ipp, & Fortenberry, 2003; Santelli, et al., 2003).

To ensure confidentiality, all study measures including saliva specimen containers and other data forms were labeled with a unique subject identification code. Collected data did not include the name of the adolescent or any personal identifying information on any of the data collection forms. Personal identifying information linking the
adolescent to the unique subject identification code was recorded on a log sheet. The log sheet containing the personal identifying information was stored in a locked cabinet separate from the data forms. Data were coded to remove the direct identifiers of the adolescent. All data were reported as a group aggregate with no participant individually identified. Data storage adhered to the UAB IRB policy. Data were entered into a password-protected database on an IRB-approved encrypted flash drive (Iron Key), with hard copies kept separate from the database. Hard copies of data were secured in a locked drawer in a locked office at the principal investigator’s place of employment (Indiana University Southeast School of Nursing).

Salivary specimens were shipped for processing to Salimetrics, State College, PA. The salivary specimens had a unique subject identification number on the specimen container. As per Salimetrics company policy, the collected salivary specimens were destroyed by Salimetrics 30 days after the corresponding salivary assays were completed and the data were reported to the principal investigator.

Instrumentation

The study’s research design incorporated a biobehavioral approach, using objective and subjective measures in the data collection. The dependent variable of blood pressure was measured using an automatic oscillometric blood pressure machine. Subjective and objective instruments were used to measure the independent variables; tobacco exposure was measured by self-report questionnaire and by the salivary biomarker cotinine. One salivary specimen was collected from each adolescent with a passive drool technique; this salivary specimen was used to quantify cotinine, a
biomarker of tobacco exposure, and C-reactive protein levels, a biomarker of systemic inflammation. Data on the identified covariates in this study were collected using two methods: (1) self-report questionnaires for age, gender, ethnicity, parental history of hypertension, and pubertal stage; (2) physiologic measures for weight, height and waist circumference to determine weight status. Each variable, and its corresponding measure, is summarized in Table 2.
### Table 2

*Instrumentation for Study Variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Type</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Pressure (Systolic, Diastolic)</td>
<td>Dependent</td>
<td>Oscillometric Blood Pressure Device Dinamap Pro 100 Series</td>
</tr>
<tr>
<td>Tobacco Exposure</td>
<td>Independent</td>
<td>Demographic Questionnaire Uptake Continuum Peer and Family Smoking Index High Sensitivity Salivary Cotinine Quantitative Enzyme Immunoassay Kit</td>
</tr>
<tr>
<td>Systemic Inflammation</td>
<td>Mediator</td>
<td>Salivary C-Reactive Protein ELISA Kit</td>
</tr>
<tr>
<td>Age</td>
<td>Covariate</td>
<td>Demographic Questionnaire</td>
</tr>
<tr>
<td>Gender</td>
<td>Covariate</td>
<td>Demographic Questionnaire</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Covariate</td>
<td>Demographic Questionnaire</td>
</tr>
<tr>
<td>Socioeconomic Status (National School Lunch Program participation)</td>
<td>Covariate</td>
<td>Demographic Questionnaire</td>
</tr>
<tr>
<td>Pubertal Status</td>
<td>Covariate</td>
<td>Self-Rating Scale for Pubertal Development</td>
</tr>
<tr>
<td>Weight Status</td>
<td>Covariate</td>
<td>Waist Circumference (inches) Body Mass Index (height in inches, weight in pounds)</td>
</tr>
<tr>
<td>Parental History of Hypertension</td>
<td>Covariate</td>
<td>Demographic Questionnaire</td>
</tr>
</tbody>
</table>

**Demographic information**

Adolescent participants completed a self-report, demographic questionnaire (Appendix C) created by the principal investigator to collect data on the study covariates.
and to screen participants for the aforementioned inclusion/exclusion criteria for the study. This demographic questionnaire contained ten items to identify age, grade, gender, ethnicity, housing type, and parental history of hypertension. With regard to exclusion criteria, adolescents answered items related to current antibiotic use, nicotine replacement therapy and pregnancy status. With a binary response of “yes” or “no”, one question served to determine the adolescent’s smokeless tobacco use.

**Blood pressure**

Blood pressure (systolic and diastolic) was measured using the Dinamap Pro Series 100 (GE Medical Systems Information Technologies, Inc., Milwaukee, Wisconsin), an automated oscillometric blood pressure machine. The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents served as a resource for the protocol (Appendix D) and to accurately identify adolescent participants with any blood pressure abnormalities (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The age-specific charts provided in this report were used to categorize blood pressure readings as normotensive, pre-hypertensive, or hypertensive.

A comparison study completed by Holt and colleagues (2011) analyzed the differences between direct arterial blood pressure, oscillometric measurements and Doppler ultrasound/sphygmomanometric blood pressure measurements in children/adolescents from birth to 17 years old ($n = 40$) in a pediatric intensive care unit. Researchers found differences in oscillometric blood pressure measurements outside the normotensive range; as the oscillometric readings were higher during hypotension and lower during hypertension when compared with the arterial and Doppler ultrasound
methods (Holt, et al., 2011). Auscultation and sphygmomanometry remain the preferred method for obtaining blood pressure measurement in children/adolescents; however, there are stated difficulties regarding hearing the appropriate Korotoff’s sounds (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The need for precise identification of Korotoff’s sounds limits a researcher’s ability to use sphygmomanometry when collecting data in a field setting (e.g. school) and with a large sample of energetic, and possibly talkative adolescents.

Oscillometric blood pressure devices and sphygmomanometry determine blood pressure in different manners and, therefore, do not produce interchangeable blood pressure readings. An oscillometric device measures arterial blood pressure oscillation during a cardiac cycle and then, with the use of a algorithm, the systolic and diastolic blood pressure is estimated (Chang, Rabinowitz, & Shea, 2003). The use of oscillometric blood pressure devices has a number of advantages including ease of use, convenience, elimination of both observer bias and digit preference (Chang, et al., 2003). These devices require regular calibration and maintenance and are affected by environmental and patient factors, such as temperature and appropriate cuff size selection respectively (Butani & Morgenstern, 2003). No established normative data currently exists to assist in recommending the use of oscillometric devices in children (Butani & Morgenstern, 2003). Few oscillometric blood pressure devices have been established for accuracy in children. When evaluating individual oscillometric models, researchers confirmed that there is not sufficient validation data to warrant pass-fail labels on oscillometric devices for use in children (Barker, Shiell, & Law, 2000).
Due to the stated advantages of oscillometric blood pressure devices, this study used the Dinamap Pro 100 Series to assess the blood pressure of the adolescent participants. The Dinamap Pro 100 oscillometric blood pressure device has been used in large scale epidemiologic studies as with the adult population in the Multi-Ethnic Study of Atherosclerosis (MESA) (Chang, et al., 2003; Ni et al., 2006). When comparing mercury sphygmomanometry and use of the Dinamap Pro 100, the blood pressure values were found to correlate well with \( r = 0.81 \) for diastolic blood pressure and \( r = 0.89 \) for systolic blood pressure (Ni, et al., 2006). In addition, approximately 20% of participants from a large-scale study were classified with high systolic blood pressure using both blood pressure measurement methods (Ni, et al., 2006). In another study to evaluate the Dinamap Pro 100 (Chang, et al., 2003), researchers noted that lower blood pressure readings were found in younger participants which added some measure of validity to the measurements and the researchers found no significant statistical difference in the blood pressure readings from three individual Dinamap Pro 100 devices. The researchers did concede that the Dinamap Pro 100 device tended towards underestimating blood pressure (Chang, et al., 2003).

**Weight Status**

Although data regarding height was needed to interpret the blood pressure, each adolescent’s height, weight and waist circumference were also collected to address the covariate of weight status (Appendix D). The weight and height measurements were used to calculate the participant’s body mass index (BMI). Each adolescent participant had their height measured using the SECA 213 portable stadiometer and their weight measured to the nearest tenth of a pound using the SECA 803 digital body weight scale.
(SECA, Inc., Hamburg, Germany). The standardized procedures for body weight allow for the use of an electronic digital scale that measures to the nearest 100 grams (Heyward & Wagner, 2004). With these measurements, BMI was be calculated using the standard equation of weight in pounds divided by height in inches squared and multiplied by 703 (Centers for Disease Control and Prevention, 2011). BMI is a common measure of weight status and is frequently found in the research that explores weight status trends among children and adolescents in the US (Ogden et al., 2006; Ogden, Carroll, & Flegal, 2008; Ogden, et al., 2012).

The Centers for Disease Control and Prevention’s BMI for age growth charts were used as a reference due to their age and gender specificity (Centers for Disease Control and Prevention, 2011). Within this system of classification, percentile ranges determine the weight status of children and adolescents; a BMI greater than the 95th percentile is considered obese and a body mass index greater than the 85th percentile is considered overweight. The use of percentile ranges in classifying BMI in children and adolescents is justified for two reasons: (1) the presence of body fat amounts change with growth and (2) the distribution of body fat differs between male and female children. It is important to note that BMI was originally developed for use with adults and the metric’s use with children only began in the late 1990’s (Flegal & Ogden, 2011). Body mass index is considered a screening tool and has not been developed for the purposes of diagnosis (Flegal & Ogden, 2011).

In addition to BMI as a measure of total body fat, excess abdominal fat and waist circumference are important in the discussion of weight status. In a review of existing evidence, abdominal fat was more indicative of greater health risk than fat distributed in
peripheral areas of the body (Li, Ford, Mokdad, & Cook, 2006; NHLBI Obesity Education Initiative Expert Panel, 2000). Previous research has found BMI and waist circumference to be strong predictors of cardiovascular risk in adults (Janssen, Katzmarzyk, & Ross, 2004) and children (Choy et al., 2011; Savva et al., 2000). Each adolescent was measured for waist circumference using the QM2000 circumference measuring tape (Quick Medical, Inc., Issaquah, WI). As part of a standard research protocol (Appendix D), the iliac crest and the umbilicus were used as landmarks to obtain an accurate waist circumference measurement (Choy, et al., 2011; Savva, et al., 2000). The mean waist circumference for this sample was compared to available national data to assist in interpretation. Body mass index and waist circumference were considered covariates in the statistical analyses for this study.

Self-Rating Scale for Pubertal Development

The Self-Rating Scale for Pubertal Development is an eight-item measure developed by Carskadon and Acebo (1993). This self-administered, written scale served to estimate pubertal stage without the use of pictorial representations or interviews (Appendix E). The scale has general puberty items on height, body hair, skin changes as well as gender-specific items on facial hair, voice changes, breast development and start of menstruation. Of the eight items present in the scale, two items are specific to male participants and three items are specific to female participants. This measure was developed using the previous work of Petersen and colleagues (1987), which incorporated verbal interviews based on Tanner’s Stages. Each scale item offers the adolescent participant five ordinal response options including: (1) “has not yet begun”, (2) “has barely started”, (3) “is definitely underway”, (4) “seems completed”, and
(5) “I don’t know”. The two items on the scale focus on menstruation, asking if menstruation has begun for the female adolescent and at what age.

The scoring of this instrument requires that each response be assigned a point value; then, the points are added for all responses to equal a scoring range between 0-20. The higher the point values on the scale, the more advanced the stage of puberty. The scores for male adolescents are categorized as follows: (1) prepubertal = 3, (2) early pubertal = 4 or 5 with no 3-point responses, (3) midpubertal = 6-8 with no 4-point responses, (4) late pubertal = 9-11 and (5) postpubertal= 12. The female puberty categories combine point totals along with the presence or absence of menstruation as follows: (1) prepubertal = 2 and no menarche, (2) early pubertal = 3 and no menarche, (3) midpubertal = >3 and no menarche, (4) late pubertal = ≤ 7 and menarche, and (5) postpubertal= 8 and menarche.

Carskadon and Acebo (1993) established the questionnaire’s psychometric properties with the use of a pretest compared to observations by a pediatrician. The researchers found the Spearman correlation coefficient to indicate a strong relationship between the adolescent self-report and the observations by the pediatrician (r = 0.841 - 0.868). Using comparisons between student, teacher and parent versions, internal consistency of the measure was found to have a fairly high Cronbach alpha level, with a range of 0.67-0.70 for the student version and 0.68-0.78 for the parent version. Correlations between parents and sixth grade boys achieved statistical significance at p<0.001. This same level of statistical significance was achieved between parents and both fifth and sixth grade girls (Carskadon & Acebo, 1993). In a pilot study completed in the spring of 2012, the reliability statistics for this instrument included a Cronbach alpha
level of 0.704 for the male participants in the sample. As female participants only
answered four questions Likert-style questions and with a smaller number of participants,
no reliability statistics could be calculated for the female participants.

A notable strength of this pubertal developmental measure is its appropriateness
in the school setting. Researchers note past objections from parents and school
administrators on the use of direct observation for Tanner staging as well as the use of
pictorial representations (Brooks-Gunn, Warren, Rosso, & Gargiulo, 1987; Carskadon &
Acebo, 1993; Duke, Litt, & Gross, 1980; Gulledge, 2010; Schlossberger, Turner, &
Irwin, 1992). This measure allows adolescent participants to self-report their pubertal
stage in a manner more acceptable to parents and school leadership. The self-report
method has also been deemed to have sufficient accuracy when a rough estimate of
pubertal stage is needed (Morris & Udry, 1980; Schlossberger, et al., 1992).

*Tobacco Exposure*

The National Cancer Institute’s Measures Guide for Youth Tobacco Research
provides guidance and support for tobacco research among adolescents (National Cancer
Institute, n.d.). This guide represents a professional consensus of psychometrically-sound
measures for use in this area of research. The measures selected for the guide meet the
following inclusion criteria: English language, quantitative score, developmentally
appropriate, available psychometric findings and disseminated in a peer-reviewed
publication. This study incorporated two measures from the National Cancer Institute’s
Measures Guide for Youth Tobacco Research; the Uptake Continuum (Appendix F) to
categorize smoking behavior and the Peer and Family Smoking Index (Appendix G) to
describe smoking influences and possible environmental exposure.
The Uptake Continuum self-report questionnaire is a 10-item instrument used in previous large-scale studies to categorize smoking behaviors in adolescents ages 12 to 18 years (Choi, Gilpin, Farkas, & Pierce, 2001). Tobacco researchers Choi, Gilpin, Farkas and Pierce (2001) developed this measure to specifically account for the concepts of self-efficacy and intention to smoke. The smoking behavior categorization includes seven stages: (1) Committed Never Smoker, (2) Susceptible Never Smoker, (3) Puffer, (4) Non-Current Experimenter, (5) Current Experimenter, (6) Non-Current Established Smoker and (7) Current Established Smoker. Each adolescent participant’s smoking behavior was categorized through the scoring of this measure. This instrument categorizes adolescents in a standardized manner and acknowledges that becoming a current established smoker is a dynamic process that may take many years (Choi, et al., 2001).

Using two large scale \( n = 7960 \) and \( n = 3376 \), longitudinal studies, Choi and colleagues (2001) published findings regarding the predictive validity of the Uptake Continuum categorization. These findings state that less than 6% of the adolescents in the Committed Never Smoker category progress to established smoking over a 3- to 4-year period. Among those adolescents categorized as Susceptible Never Smokers or Puffers, 12% progress to established smoking over a 3- to 4-year period. Adolescents in the Susceptible Never Smoker category were 2-3 times more likely to initiate smoking over a 3- to 4-year period compared those in the Committed Never Smoker category. Finally, the predictive validity findings of the instrument establish that 80% of adolescents in the Current Established Smoker category will still be smoking in 3 to 4 years (Choi, et al., 2001).
The Uptake Continuum scale was piloted by the principal investigator in a sample of rural adolescents \((n = 40)\) ages 15-19 in the spring of 2012. The instrument’s internal consistency for this sample population was adequate with a Cronbach \(\alpha\) of 0.775. As with any self-report instrument, the reliability of an adolescent’s responses must be considered; however, this study included the objective measure of salivary cotinine to provide biochemical verification of the adolescent participant self-report data.

**Peer and Family Smoking.** The Peer and Family Smoking tool is a five-item self-report index to quantify the number of smokers present among an adolescent’s parents and/or peers (Pierce, Choi, Gilpin, Farkas, & Berry, 1998). This index has multiple choice responses that result in categorical variables related to family and peer exposure to smoking behavior. The development of this instrument involved a sample of 1,752 adolescents ages 12-17 (Pierce, et al., 1998). An analysis of a number of detailed questions was completed and later simplified into a concise, five-item index. This index is used to inquire about the number of smokers in an adolescent’s immediate family or closest friends. The first three items in the index require yes or no responses regarding the smoking behavior of parents, step-parents, guardians and siblings. The final two items require the adolescent to identify whether they have male and/or female friends who smoke. If the adolescent confirms they have friends who smoke, they must quantify the number of male and female friends who smoke. With only 5 items that require yes/no response choices or numerical answers, this instrument does not allow for the calculation of reliability statistics such as the Cronbach’s \(\alpha\).

The Peer and Family Smoking Index is scored as either no tobacco exposure or exposure for family and peers separately (Pierce, et al., 1998). Previous studies have not
found peer and family smoking to be a strong predictor of an adolescent’s progression through the smoking uptake continuum; however, this instrument provides a measure of the social influence for smoking present among family and peers (Pierce, et al., 1998). In addition, this study used this instrument as a measure for potential secondhand smoke exposure. Secondhand smoke exposure as defined in this study is a mixture of sidestream smoke from a lit cigarette and mainstream smoke exhaled by an active smoker (United States Department of Health and Human Services, 2006). To support the validity of this measure, saliva was collected to determine cotinine levels and provide an additional measure for secondhand smoke exposure in participants using the established cutpoints of salivary cotinine. Due to the simple yes/no responses and the small number of items on the index, no internal consistency statistics were calculated to determine reliability.

**Salivary Biomarkers**

The use of salivary specimens in a research design provides an objective, less invasive method of data collection (Granger, et al., 2007) and, therefore, is deemed more appropriate for a school-based data collection as compared to serum or urine collection. An adolescent can effectively accomplish the collection of whole saliva by the passive drool technique into a collection tube (Granger, et al., 2007; Huntington-Moskos, 2012). Salivary specimens were collected to determine both cotinine and C-reactive protein levels among adolescent participants. Adolescent participants met the aforementioned exclusion criteria to ensure the effective and valid use of these two specific biomarkers.

**Cotinine.** When working to quantify tobacco exposure among rural adolescents, the biomarker cotinine provides biochemical verification of adolescent self-report.
Cotinine is the principal metabolite of nicotine and the biomarker of choice when studying smoking status and secondhand smoke exposure (Avila-Tang et al., 2012; Benowitz, et al., 2009). Cotinine can be detected in an individual for 3 to 4 days after tobacco exposure, having a half-life of approximately 16 hours (Avila-Tang, et al., 2012; Benowitz, et al., 2009). The longer half-life of cotinine compared to nicotine and cotinine’s moderate cost for analysis are noted advantages to its use in tobacco research (Avila-Tang, et al., 2012; Society for Research on Nicotine and Tobacco, 2002). Cotinine is a highly specific and sensitive biomarker of tobacco use when in the absence of nicotine replacement therapy (Society for Research on Nicotine and Tobacco, 2002). Serum cotinine and salivary cotinine levels are highly correlated and have high specificity and sensitivity (Avila-Tang, et al., 2012). In fact, serum and salivary cotinine are stated to have a 96–97% sensitivity and 99–100% specificity, respectively (Society for Research on Nicotine and Tobacco, 2002).

Although cotinine is an effective biomarker in determining tobacco use, it does not provide information on the exact exposure source (Benowitz, et al., 2009). Tobacco exposure can derive from active cigarette smoking, smokeless tobacco use, secondhand smoke exposure and nicotine replacement therapy (Jarvis, Fidler, Mindell, Feyerabend, & West, 2008). No known biomarker for tobacco exposure can perfectly differentiate between nonsmokers, active smokers and individuals exposed to heavy secondhand smoke (Avila-Tang, et al., 2012).

Research describing tobacco exposure as determined by cotinine levels is subject to individual variations in nicotine metabolism due to genetic variation, age, gender, ethnicity, timing of specimen collection (e.g. day of the week) and individual smoking
topography (Avila-Tang, et al., 2012). This research design acknowledged the sources of inter-subject variability for cotinine by considering the timing of the salivary specimen collection. Salivary specimens were collected early in the calendar year when the home environment is closed and less ventilated. Although research has identified the home as a significant source of secondhand smoke exposure for adolescents (Marano, et al., 2009), the salivary specimens were collected in the school setting. The collection of salivary specimens early in the week would introduce more variability due to differing activity during the weekend; therefore, salivary specimen collection took place on a Thursday or Friday between 8am and noon to more accurately reflect secondhand smoke exposure during a typical school week.

Immediately after collecting a salivary specimen, the specimen was placed on ice in a cooler. Once a day of data collection was completed, salivary specimens were transported to a regional university lab and stored in a -20C freezer (Salimetrics LLC, 2012). All salivary specimens were overnight shipped to Salimetrics (State College, PA, USA) for testing of cotinine in duplicate using a highly sensitive enzyme immunoassay. The Salimetrics Cotinine Enzyme Immunoassay (EIA) is sensitive enough to detect salivary cotinine at a level of 0.05ng/ml. Salimetrics has guidelines for interpretation of cotinine levels based on the work of Neal Benowitz and colleagues (Benowitz, et al., 2009). A cotinine cut-point of 3.0 ng/ml will be used to distinguish between tobacco exposure due to active smoking and smoke exposure due to secondhand smoke specific to the adolescent (Benowitz, et al., 2009). Cotinine levels between 1.0 ng/ml and 3.0 ng/ml were considered a range of overlap that may be indicative of light smoking behavior or heavy secondhand smoke exposure (Benowitz, et al., 2009; Salimetrics LLC,
Cotinine levels under 1.0 ng/ml were categorized as light secondhand smoke exposure. The Salimetrics Cotinine EIA uses 20 uL of saliva sample per determination and has a lower limit of sensitivity of 0.15 ng/mL. The performance characteristics reported for Salimetrics Cotinine EIA include an intra-assay coefficient of variation ranging from 4.5%-8.6%. This range is acceptable as the intra-assay coefficient of variation should be below 10%. The Salimetrics Cotinine EIA inter-assay precision ranges from 4.21% to 9.04% with an acceptable inter-assay coefficient of variation being <15% (Salimetrics LLC, 2010b).

*C-reactive protein.* A number of biomarkers have been studied across disciplines for their use in quantifying systemic inflammation. Kang and colleagues (2010) noted that C-reactive protein (CRP) is among the most common biomarkers of systemic inflammation along with interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor. As such, many nicotine and tobacco research studies have incorporated CRP as a biomarker of systemic inflammation (Institute of Medicine, 2010b; Jefferis, et al., 2010; Venn & Britton, 2007; Wilkinson, et al., 2007). CRP, as a salivary biomarker, serves as an objective measure and offers a less invasive method to collect data on inflammation; it is, therefore, an appropriate biomarker for this school-based study (Azar & Richard, 2011). Although Dillon and colleagues (2010) found no correlation between salivary and serum CRP in a sample of healthy adults, the research conducted by Ouellet-Morin and colleagues (2010) found a moderately strong correlation and noted that salivary CRP may have predictive value. In a longitudinal study completed by Out and colleagues (2012), correlations between salivary and plasma CRP levels were significant at wave 1 \( (r (120) = .38, p < .01) \) and wave 4 \( (r (107) = .53, p < .01) \). The study findings also
supported the salivary biomarker’s ability to reliably discriminate between the high and low levels of plasma CRP when using the clinically relevant cutoff point of 3 mg/L (Out, et al., 2012).

As with the biomarker cotinine, all saliva samples were placed on ice in a cooler when collected. The specimens were then stored in a -20C freezer and later shipped to Salimetrics, LLC (State College, PA) for processing. Each salivary specimen was analyzed for CRP in duplicate using the Salimetrics C-Reactive Protein ELISA kit. The level of CRP in saliva is dependent on each individual’s unique saliva flow rate; therefore, the salivary flow rate was reported to Salimetrics to assist in the accurate processing of specimens. The assay uses a saliva volume of 50 ul of X10 dilution per determination and has a lower limit of sensitivity of 10 pg/mL with a calibrator range of 93.75 to 3000 pg/mL. (Salimetrics LLC, 2010c). The picogram (pg) is a measure of mass indicating one trillionth (10^{-12}) of a gram (picogram, 2013). The performance characteristics reported for Salimetrics CRP Elisa kit include an intra-assay coefficient of variation ranging from 1.9%-5.9%; an acceptable range considering it is well below the 10% standard. The Salimetrics CRP Elisa kit has an inter-assay precision range from 3.7% to 11.2%, which is acceptable as it falls below the <15% standard (Salimetrics LLC, 2010c).
Table 3

Psychometrics for Study Measures

<table>
<thead>
<tr>
<th>Instrument Name and Reading Level</th>
<th>Number of Items</th>
<th>Reliability</th>
<th>Validity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake Continuum 4.1</td>
<td>10</td>
<td>Pilot tested in spring 2012 with a sample of rural adolescents (n=40); Cronbach $\alpha=0.775$.</td>
<td>Predictive validity established with less than 6% of Committed Never Smokers progressing to Established Smoking over a 3-4 year period (Choi, et al., 2001).</td>
</tr>
<tr>
<td>Peer and Family Smoking Index 4.7</td>
<td>5</td>
<td>This is an index. Unable to calculate Cronbach $\alpha$ due to small number of questions and binary response.</td>
<td>Construct validity established through the simplification of a more detailed instrument (Pierce, et al., 1998).</td>
</tr>
<tr>
<td>Self-Rating Scale for Pubertal Development 2.9</td>
<td>8</td>
<td>Cronbach $\alpha=0.67-0.70$ for student version and Cronbach $\alpha=0.68-0.78$ for the parent version (Carskadon &amp; Acebo, 1993).</td>
<td>Validated against Tanner staging completed by trained physicians (Carskadon &amp; Acebo, 1993). Pilot tested in spring 2012 with a sample of rural adolescents (n=40); Cronbach $\alpha=0.704$ for male adolescents.</td>
</tr>
</tbody>
</table>
Pilot Study

A pilot study to address feasibility issues was conducted in the spring of 2012. An apriori power analysis was completed to establish an appropriate sample size for this pilot. Since the purpose for the pilot study was to pinpoint any feasibility issues in the research design, the principal investigator used a large effect size with a significance level of .10. This apriori power analysis with a large effect size yielded a suggested sample size of 36 participants, containing ideally 9 smoking participants and 27 non-smoking participants. The adolescent participants were recruited from a rural high school in a Midwestern state. The completion of the pilot study afforded the principal investigator the opportunity to address any issues regarding the recruitment and data collection processes.

In order to recruit participants, a brief recruitment presentation was developed and took place in two separate settings: (1) one session in a large study hall room containing approximately 50 sophomore students (ages 15-16) from the health education and personal finance elective courses; and (2) four separate sessions in a small classroom setting for junior and senior students (ages 15-19) in advanced science courses. During this brief recruitment presentation, the principal investigator discussed the pilot study goals, data collection procedures, risks, benefits and incentives. The principal investigator discussed the concept of confidentiality and outlined how confidentiality would be maintained throughout the study. Students were given the opportunity to ask questions once the presentation was completed. Study packets, containing a parent letter with the pilot study goals and the informed consent document, were distributed at the end of the recruitment presentation. Students were asked to review the enclosed materials with their
parent or guardian and determine their interest in study participation. Both the adolescent participant and their parent/guardian gave informed, written consent. Potential adolescent participants were given one week to return the informed consent document signed by their parent or guardian.

This pilot study included a school-based, convenience sample in which the principal investigator collected data from 40 adolescents ages 15-19. A ten percent increase over the calculated sample size requirement was implemented to compensate for possible missing data and/or inadequate saliva specimen collection. During the 2011-2012 academic school year, the ethnic composition of this rural high school was 98.3% Caucasian, 1.2% Latino and 0.6% Multiracial. The 2011-2012 total enrollment for grades 9-12 was 339 students with approximately one-third of the student population (33.6%) participating in the National School Lunch Program, receiving either free or reduced-priced lunches (Indiana Department of Education, 2011-2012). Of the 40 recruited adolescent participants, 26 (60%) were female and 14 (35%) were male; all (100%) were Caucasian.

The data collection was completed in the high school conference room by the principal investigator and a trained research assistant. The trained research assistant was recruited from a regional baccalaureate nursing program. The research assistant’s training included the completion of initial Institutional Review Board (IRB) training and a thirty-minute orientation to the established study protocols. A privacy screen was set up inside the conference room to separate an adolescent completing the physiologic data collection from an adolescent completing the self-report questionnaires. The use of a privacy screen during waist circumference measurement addressed any body image concerns and also
provided privacy for the adolescent participant during salivary specimen collection. In addition, weight measurements were written directly on data collection sheets with no verbal discussion to maintain privacy. The principal investigator completed data collection related to blood pressure, waist circumference and salivary specimen collection while the trained research assistant collected data on height and weight.

The instrumentation for this pilot study mirrors that of the larger study and includes the physiological measurements of blood pressure, height, weight, and waist circumference. The SECA 803 digital body weight scale (SECA, Inc., Hamburg, Germany) was properly zeroed out and the SECA 213 portable stadiometer was assembled by the principal investigator per manufacturer guidelines (SECA, 2011). Data collection of waist circumference involved a specific protocol (Appendix D) using the umbilicus and the iliac crest as landmarks for accurate measurement (Janssen, et al., 2004; Savva, et al., 2000). Written, self-report measures were also part of the pilot study instrumentation including a 7-item Demographic Questionnaire, the Uptake Continuum scale, the Peer and Family Smoking Index, and the Self-Rated Scale for Pubertal Development. The readability statistics for the 7-item demographic questionnaire included a Flesch Reading Ease Score of 78.5% indicating easy readability and a Flesch Kincaid Grade Level of 4.2, which is well below the fifth grade reading level. A salivary specimen was collected from each participant via passive drool technique. The collected salivary specimens were shipped to Salimetrics (State College, PA) and analyzed for the biomarkers of cotinine and CRP.

Forty adolescent participants enrolled in the pilot study and all were able to complete the data collection procedures without difficulty. Each adolescent was able to
provide a salivary specimen in a cryovial using the passive drool technique. The data collection procedures were completed for each adolescent within twenty minutes. All salivary specimens were successfully analyzed, having adequate saliva in each cyrovial.

The data collected with this study instrumentation yielded the following descriptive statistics: The mean systolic blood pressure was 115 mmHg (range of 92-141 mmHg) and diastolic blood pressure mean was 64mmHg (range of 54.5-80.5 mmHg). The mean cotinine level was 0.96 ng/ml with a range of 0 to 3.27ug/ml. With regard to weight status, the mean body mass index was 23.67 (range of 17.58-34.81) and the mean waist circumference was 82.8 cm (range of 67.9-109.54 cm).

The principal investigator took the average of the each participant’s two blood pressure measurements and then, using age, gender and height, categorized the systolic and diastolic blood pressure to determine blood pressure percentile (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Approximately, 35% of the adolescent participants had systolic blood pressure readings at or above the 90th percentile which was a higher than expected prevalence rate for this sample. Pearson correlation coefficients were calculated and revealed a significant positive correlation between systolic blood pressure and body mass index ($r = 0.366$) and waist circumference ($r = 0.387$). No significant correlations were found between cotinine and C-reactive protein ($r = 0.12,$) or systolic blood pressure ($r = -0.182$). The alpha was set at 0.05 for all correlational analyses. With tobacco exposure, 35% percent of adolescent participants had a cotinine level indicative of secondhand smoke exposure or light smoking behavior (1-3 ng/ml). Only one adolescent had a level of cotinine indicative of regular smoking (3.27 ng/ml) and only one
adolescent in the sample had no detectable level of cotinine present. The Uptake Continuum was found to have adequate internal consistency (Cronbach $\alpha = 0.775$).

With two scheduled data collection days, the pilot study was complete. In preparation for the larger study, two questions were added to the demographic questionnaire regarding race/ethnicity and smokeless tobacco use. To assist with exclusion criteria, another two questions were added to determine the current use of antibiotics and nicotine replacement therapy. The larger study was adjusted to include only half days of data collection between the times of 8 am and 12 noon. This shortened data collection time period allowed for more comparability between samples. The principal investigator adjusted the saliva collection procedure to allow for a more exact time of collection; this adjustment allowed for a more exact flow rate calculation. All adolescents were able to complete physiological measurements and self-report measures.

Procedures for Data Collection

Recruitment

The principal investigator met with the principals from two rural high schools to determine the most appropriate venues for recruitment. The principal investigator advocated for recruitment in a small classroom setting, as it encouraged more questions from potential adolescent participants. Recruitment talking points were revised based on the pilot presentation used to discuss study goals, data collection procedures, risks, benefits and incentives. The concept of confidentiality was defined during the recruitment presentation and the principal investigator stated the commitment to maintaining confidentiality throughout the research study process. Participants were informed that the
collected data were delinked from any direct participant identifiers to maintain confidentiality during the recruitment presentation; while parents were informed of this via the parent letter (Appendix H). The principal investigator stressed to any interested adolescent that their participation was not mandatory and they may withdraw from the study at any time without penalty. Study packets, containing a parent letter (Appendix H) and an informed consent document (Appendix B), were distributed at the end of the recruitment presentation.

**Informed Consent**

The informed consent documents (Appendix B) along with a letter directed to parents (Appendix H) were distributed to students. Potential adolescent participants were instructed to have their parent/guardian review the form at home and provide active informed consent if they support the participation of their adolescent. The adolescent was instructed to return the signed informed consent to the designated box in the main office. With the guidance of the principal investigator, the adolescent participant reviewed the informed consent document and signed it on the day of data collection. The informed consent document was distributed to adolescents to take home a minimum of two weeks prior to the scheduled data collection dates to allow for ample review time. The informed consent document was reviewed with each adolescent participant on the day of data collection to complete the informed consent process.

**Data Collection**

For this research study, the data collection of physiologic measures and self-report questionnaires took place in the school setting, in a large conference room. Privacy screens were set up inside the room to provide privacy for the adolescent completing the
physiologic measures. A research assistant was recruited from a regional, baccalaureate nursing program and completed initial IRB training. The role of the trained research assistant was (1) to bring enrolled participants to the data collection area, and (2) to issue the monetary incentive once data were collected. Each participant was provided with a study questionnaire packet, a pencil with eraser and a blank piece of paper to use as a cover sheet.

The set up of equipment included a zeroed out SECA 803 digital body weight scale (SECA, Inc., Hamburg, Germany) and assembly of the SECA 213 portable stadiometer based on manufacturer guidelines (SECA, 2011). The data collection related to waist circumference adhered to the established protocol (Appendix D), using the umbilicus and the iliac crest as appropriate landmarks (Janssen, et al., 2004; Li, et al., 2006; Savva, et al., 2000). Body image is of great concern during the adolescent stage of development; therefore, the principal investigator and the trained research assistant strictly adhered to use of privacy screens, were alert to cues regarding an adolescent’s comfort level and strove to communicate clearly with adolescent participants during data collection.

Blood pressure measurements were completed using an oscillometric blood pressure device, namely the Dinamap Pro Series 100 (GE Medical Systems Information Technologies, Inc., Milwaukee, Wisconsin). The blood pressure protocol (Appendix D) established by the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents was used to standardize the procedure between adolescent participants. A selection of appropriate size blood pressure cuffs was available to account for variability in the body type/size of participants; the three
different cuff sizes included a small adult cuff, a standard adult cuff and a large adult cuff. To ensure appropriate cuff size selection, the bladder length of the cuff covered a minimum of 80% of the adolescent’s arm circumference and the inflatable bladder width itself covered at least 40% of the arm circumference midway between the olecranon and the acromion (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The principal investigator visually assessed the bladder length and width with each individual adolescent participant. The blood pressure was measured on the right arm with each adolescent participant.

The protocol for the blood pressure measurement (Appendix D) stated that each adolescent participant: (1) be seated in a chair with a supported back for five minutes prior to the first blood pressure reading, (2) have both feet placed on the floor, and (3) place the cubital fossa of the right arm at heart level (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Two separate blood pressure measurements were taken with the participant in a resting position for two minutes between measurements (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The blood pressure cuff remained on the right arm of the adolescent participant in between the two measurements. The adolescent participant was monitored for any sign of anxiety or discomfort during the blood pressure readings. The principal investigator ensured a moderate ambient temperature in the room and provided age-appropriate reading materials for participants during blood pressure measurement. All physiologic measurements were completed before the self-report instruments including those related to tobacco use and exposure.
A salivary specimen was collected from each adolescent participant individually. The adolescent was instructed to rinse his/her mouth with water prior to sample collection. Each adolescent was given a pre-labeled tube (unique identifier study code number) with a cut straw inside and rubber band wrapped around the tube. The adolescent participant was instructed that the cut straw must remain in the tube. A timer was set to measure the amount of time needed for the passive drool collection; each participant was timed individually. With the straw pulled out slightly, the adolescent participant drooled into the straw in the specimen tube. Once the cyrovial was full to the instructed level, the adolescent was asked to remove the straw from the tube, discard it in the trash receptacle and hand the tube to the principal investigator. The principal investigator collected each tube, verified adequate specimen volume, capped off the tube securely and placed the tube in a designated cooler on ice. The principal investigator checked that used straws were properly discarded. At the end of the day, salivary specimens were placed in a freezer (−20 degrees Celsius) appropriate for biological specimens at Indiana University Southeast. Once the data collection was complete, specimens identified by unique study code number only were shipped overnight on dry ice to Salimetrics LLC (State College, PA) for processing.

The blood pressure data for each participant was reviewed for any abnormalities. If the principal investigator found a blood pressure abnormality, the school nurse was notified and the blood pressure was documented in the health file maintained by the school. The parent or guardian of a participant with a blood pressure abnormality was sent a letter (Appendix I) detailing the blood pressure readings and a recommendation for follow-up with a primary care provider. To facilitate a greater understanding of
adolescent blood pressure, a brochure detailing the National Heart, Lung and Blood Institute (NHLBI) guidelines for child and adolescent blood pressure (United States Department of Health and Human Services, 2007b) accompanied the parent/guardian notification letter.

Data Management and Analysis

Data Management

The data collection took place during multiple days over a three-month period at two separate high schools. As each adolescent participant completed the data collection, each questionnaire was quickly reviewed for unintentional omissions and any potential errors in documentation. The principal investigator reviewed each participant’s survey packet to ensure the integrity of the data collection. All research data were stored in a locked cabinet at a regional university, which was the principal investigator’s place of employment. All computerized data were stored on an encrypted, password-protected flash drive (Iron Key), which is approved for use by the UAB IRB. Only the adolescent participant’s unique study identification number identified the data and, as such, all data was de-linked from the adolescent participant’s name.

Data entry was completed solely by the principal investigator. Prior to data collection, a codebook was developed to incorporate all physiologic measurements and questionnaire items. The cells containing any missing data values were left empty in the data file. The principal investigator completed two separate data entry sessions resulting in a working data file and a merge data file. These data files were merged and compared as a means of checking for any data entry errors. Any necessary corrections were to the
working file and it was saved, unmanipulated, as a master data file. Once this process was completed, the corrected working file was used to transform variables and begin statistical analyses.

Data Analysis

The Statistical Package for the Social Sciences (SPSS 20.0) software package used for data analyses. All statistical analyses were set with an alpha of 0.05 for two-tailed tests to minimize the probability of a Type 1 error (Polit & Beck, 2008).

Descriptive statistics were used to examine the sample demographic characteristics. Frequency statistics and histograms assisted in characterizing the data regarding age, gender, ethnicity, parental history of hypertension, free/reduced lunch participation, exposure to smoking by family and exposure to smoking by peers. The principal investigator analyzed the descriptive statistics to determine the mean and range for BMI, waist circumference, cotinine, CRP, systolic and diastolic blood pressure. Reviewing the frequencies, percentages, means, standard deviations and range also assisted in identifying any missing or invalid values in the dataset. The data associated with each variable was reviewed for normal distribution and the presence of outliers. This review of the data was necessary to promote adherence to the assumptions for regression analysis including existence, independence, linearity, normality, homoscedasticity, and multicollinearity (Tabachnick & Fidell, 2007).

As a means of understanding the relationships between variables, the principal investigator developed a Pearson’s correlation matrix with the variables of cotinine, CRP, systolic blood pressure and diastolic blood pressure. Multiple linear regression was used to develop a predictive model for systolic and diastolic blood pressure. Finally, a
mediation analysis was completed to determine if CRP, a biomarker of inflammation, acts as a mediator of systolic and/or diastolic blood pressure. This data analysis is discussed further in the statistical analysis plan to follow.

Statistical Analysis Plan

Research Question 1. Is there a relationship between individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure in rural adolescents ages 15-18?

Hypothesis 1. There is a positive relationship between individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure among rural adolescents ages 15-18. For hypothesis 1, the prevalence of tobacco exposure in this sample of rural adolescents ages 15-18 was determined using descriptive statistics. A Chi-square Test for Independence was used to analyze the relationship between individual tobacco use and the self-reported sources of secondhand smoke exposure (family, peers) in this sample of rural adolescents.

Research Question 2. Do individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure influence cotinine levels?

Hypothesis 2. Individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure will positively influence cotinine levels among rural adolescents ages 15-18. For hypothesis 2, a Chi-square Test for Independence was used to determine the association between tobacco exposure (salivary cotinine) and the self-reported sources of secondhand smoke exposure in this sample of rural adolescents. A Mann Whitney U test was also used to compare the
salivary cotinine levels between participant’s smoking status, categorized as “ever smoker” and “never smoker”. The 7 categories resulting from the Uptake Continuum measure were collapsed into the dichotomous categories of “ever smoker” and “never smoker” due to the small number of established smokers in the sample.

Research Question 3. How much of the variance in blood pressure is explained by tobacco exposure (salivary cotinine) in rural adolescents ages 15-18?

Hypothesis 3. Tobacco exposure, quantified by salivary cotinine levels, will account for a significant amount of variability in blood pressure among rural adolescents ages 15-18, when controlling for age, gender, ethnicity, parental history of hypertension, socioeconomic status, pubertal status and weight status. For hypothesis 2, the statistical technique of hierarchical multiple regression was used to determine the amount of variability contributed by tobacco exposure on the dependent variable of blood pressure, both systolic and diastolic. When using the technique of multiple linear regression, the principal investigator controlled for the variables of age, gender, and weight status. To control for the confounding variables, the principal investigator entered age, gender, and weight status on block 1 and tobacco exposure (salivary cotinine) on block 2.

Research Question 4. Does the process of inflammation mediate the relationship between tobacco exposure and blood pressure in rural adolescents ages 15-18?

Hypothesis 4. Systemic inflammation, quantified as the biomarker C-reactive protein, will mediate the relationship between tobacco exposure and blood pressure in rural adolescents ages 15-18. To complete the necessary steps in a mediation analysis related to hypothesis 4, univariate regression models was used as described by Baron and Kenny (1986). A mediating variable is one example of a third variable that influences the
relationship between the independent and dependent variables in a research study; other examples of third variables include moderating variables and confounding variables (MacKinnon, 2011). More specifically, a mediating variable is seen as an intermediate step on a causal pathway between the stated independent and dependent variables of a research study (Baron & Kenny, 1986; MacKinnon, 2011). When describing a simple mediation model, Baron and Kenny (1986) state that the outcomes of behaviors are often mediated by different processes that are internal to an organism; therefore, a biomarker may be appropriately identified as a potential mediator for the outcome of blood pressure. C-reactive protein, as a biomarker of systemic inflammation, is hypothesized to be a mediating variable between tobacco exposure, quantified using salivary cotinine, and the dependent variable of blood pressure, specifically systolic and diastolic blood pressure.

According to Baron and Kenny (1986), three separate conditions must be met to complete a simple mediation analysis. First of all, a significant statistical relationship must exist between the independent variable and the mediating variable. This first condition is diagrammed as pathway “a” in Figure 3. Second, a significant relationship must exist between the mediating variable and the dependent variable, represented as pathway “b” in Figure 3. Finally, the third condition that must be met is a significant relationship between the independent variable and the dependent variable, noted as pathway “c”.
The steps outlined by Kenny and Baron (1986) assist in confirming the presence of a mediation pathway but does not speak to the strength of the mediating relationship (Dudley, Benuzillo, & Carrico, 2004). The indirect effect of the mediating variable is represented by the pathway ab presented in Figure 3. If a mediating relationship is found, Sobel’s test is a statistical technique used to determine the amount of variance accounted for by the mediating variable (Dudley, et al., 2004; MacKinnon & Dwyer, 1993). Sobel’s test provides a means to determine if the indirect effect of the mediator is significance.

Summary

The methodology for this research study is addressed within this chapter. Details regarding the research design, sample, setting, data collection techniques and instrumentation have been provided. The instrumentation includes both self-report questionnaires and physiologic measures. The combination of these two types of measurements were used to add strength to the research design and allow for triangulation of data findings. To ensure the protection of human subjects, the steps taken to inform adolescent participants and their parents/guardians are outlined in the chapter.
This research study seeks to add to the body of knowledge regarding tobacco exposure, inflammation and blood pressure among rural adolescents.
CHAPTER FOUR

FINDINGS

The study findings are addressed in this chapter. The purpose of this study was to determine the influence of tobacco exposure on blood pressure in a sample of rural adolescents ages 15-18 after controlling for the variables of age, gender, ethnicity, parental history of hypertension, weight status, pubertal stage, and socioeconomic status. C-reactive protein as a possible mediating variable between tobacco exposure and blood pressure was also addressed. In this chapter, the sample and setting characteristics, descriptive statistics for study variables, and reliability of the study instruments are described to provide context for the study findings. A summary of findings is discussed specific to each research question and its accompanying hypotheses.

Sample and Setting Characteristics

Adolescent participant recruitment took place at two high schools in a rural county in a Midwestern state. As predetermined by the school principals, potential study participants were accessed through their biology, chemistry and agriculture classes. The sample for this research study was recruited during the months of January, February and into the middle of March 2013. The a priori power analysis, which was based on a medium effect size, determined the necessary sample size for this study to be 136 participants. The recruited sample for the study was 150, which was 10% above the
sample size recommended by the apriori power analysis. This slight oversampling assisted with the possibility of missing data, including inadequate saliva amounts for assay analysis.

A total of 400 recruitment packets were distributed within these classes with an approximate even distribution between the two high schools. Over a six-week period, 150 adolescents returned informed consent documents with parent signatures: 84 from School A (56.1%) and 66 from School B (43.9%). One student was absent during all scheduled data collection days and, therefore, was not part of the final sample. An additional student participated in the data collection but was later excluded due to self-reported pregnancy status. The final sample was composed of 148 adolescent participants (88 female and 60 male). As 400 recruitment packets were distributed within the accessed classrooms, a 37.5% response rate was calculated for this study. The descriptive statistics related to the demographic composition of the sample are presented in Table 4.
Table 4  
*Demographic Characteristics of Sample (N = 148)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>$M (SD)$</th>
<th>$N (%)$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 years</td>
<td></td>
<td>25 (16.9)</td>
</tr>
<tr>
<td>16 years</td>
<td></td>
<td>52 (35.1)</td>
</tr>
<tr>
<td>17 years</td>
<td></td>
<td>42 (28.4)</td>
</tr>
<tr>
<td>18 years</td>
<td></td>
<td>29 (19.6)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>88 (59.5)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>60 (40.5)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td>142 (95.9)</td>
</tr>
<tr>
<td>Multiracial</td>
<td></td>
<td>6 (4.1)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latino</td>
<td></td>
<td>6 (4.8)</td>
</tr>
<tr>
<td>Non Latino</td>
<td></td>
<td>139 (95.2)</td>
</tr>
<tr>
<td><strong>Socioeconomic Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free Lunch</td>
<td></td>
<td>28 (19.0)</td>
</tr>
<tr>
<td>Reduced Lunch</td>
<td></td>
<td>13 (8.8)</td>
</tr>
<tr>
<td>No NSLP participation</td>
<td></td>
<td>106 (72.1)</td>
</tr>
</tbody>
</table>

Note. $SD =$ standard deviation, $N =$ number of participants. NSLP = National School Lunch Program. Due to missing values, percentages may not total 100%.

* This variable had one missing value ($N = 147$).
** This variable had two missing values ($N = 146$).

The sample was predominantly female (59.5%), Caucasian (95.9%) and non-Mexican American (93.9%). Age of the study participants was well distributed with 16.9% 15 year-olds, 35.1% 16 year-olds, 28.4% 17 year-olds and 19.6% identified as 18 year-old participants. The racial/ethnic composition of this study sample was similar to that of both participating high schools. The ethnic composition of School A was 98.3% Caucasian, 1.2% Latino and 0.6% Multiracial and School B was 94.8% Caucasian students with approximately 5.1% reporting ethnic diversity including Hispanic, Asian, Black and Multiracial (Indiana Department of Education, 2011-2012).

The National School Lunch Program (NSLP) was used as a proxy variable to determine socioeconomic status of the participants in the sample. Approximately 28% of
the study sample received either free or reduced lunch, which is lower than the NSLP participation rates for both School A (33.6%) and School B (39.3%).

Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>M (SD)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental History of Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>12 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>39 (26.4)</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>4 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Neither</td>
<td>43 (29.1)</td>
<td></td>
</tr>
<tr>
<td>I don’t know</td>
<td>50 (33.8)</td>
<td></td>
</tr>
<tr>
<td>Pubertal Status*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid Pubertal</td>
<td>14 (10.4)</td>
<td></td>
</tr>
<tr>
<td>Late Pubertal</td>
<td>84 (62.2)</td>
<td></td>
</tr>
<tr>
<td>Post Pubertal</td>
<td>37 (27.4)</td>
<td></td>
</tr>
<tr>
<td>Weight Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>34 (5.1)</td>
<td></td>
</tr>
</tbody>
</table>

Note. SD = standard deviation, N = number of participants. Due to missing values, percentages may not total 100%.

*Variable had 13 missing value (N = 135).

Self-report data regarding parental history of hypertension was collected from adolescent participants using one multiple-choice item on the demographic questionnaire. Findings regarding parental history of hypertension, pubertal status and weight status are presented in Table 5. Nearly a third of study participants (29.1%) reported that neither parent had hypertension while another third of participants reported knowledge that one of their parents, either mother or father, had hypertension. Approximately 33.8% of adolescent participants reported they did not have any knowledge regarding whether their parents had hypertension or not.

Data on pubertal status was collected from adolescent participants using the Self-Rating Scale for Pubertal Development (Carskadon & Acebo, 1993). This self-report measure contains 8 items including 2 items specific to male participants and 3 items specific to female participants. The majority of the adolescent participants reported that
they had completed puberty or were experiencing late puberty. Approximately ten percent of the sample reported experiencing the middle of puberty. The adolescent participants \((n = 148)\) answered all questions related to the *Self-Rating Scale for Pubertal Development*; however, some participants chose the “I don’t know” option (8.8%) and, statistically, this was treated as a missing value. A notable amount of missing values (39.9%) was found specific to the breast development question for female participants.

Weight status was treated as a confounding variable of blood pressure. To quantify weight status, the principal investigator collected data on height, weight and waist circumference. For this sample, the mean waist circumference was 34.2 inches with a standard deviation of 5.1 inches. The mathematical equation used to calculate body mass index (BMI) included the weight in pounds divided by the height in inches squared and multiplying that result by 703 (Centers for Disease Control and Prevention, 2011). The mean BMI for this study was 25 with 5.6 as the calculated standard deviation. The BMI data collected from this study sample was further categorized according to guidelines provided by the Centers for Disease Control and Prevention (2011). Over half the study sample had BMI values that fell into the normal weight range and a quarter had BMI values categorized as obese. The prevalence for each BMI category in this sample is presented in Table 6.

Table 6
*Categorization of Body Mass Index \((N = 148)\)*

<table>
<thead>
<tr>
<th>Weight Status</th>
<th>BMI Percentile*</th>
<th>(N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;5\textsuperscript{th}</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Normal weight</td>
<td>(\geq 5\textsuperscript{th} - &lt; 85\textsuperscript{th})</td>
<td>85 (57.4)</td>
</tr>
<tr>
<td>Overweight</td>
<td>(\geq 85\textsuperscript{th} - &lt; 95\textsuperscript{th})</td>
<td>26 (17.6)</td>
</tr>
<tr>
<td>Obese</td>
<td>(\geq 95\textsuperscript{th})</td>
<td>37 (25.0)</td>
</tr>
</tbody>
</table>

Note. \(N\) = number of participants.

*Centers for Disease Control and Prevention, 2011*
Instrument Reliability

Reliability statistics for the internal consistency of measures were completed for the *Uptake Continuum* and for the *Self-Administered Rating Scale for Pubertal Development* (see Table 7). A Cronbach’s α ranging from 0.8 – 0.9 indicates strong internal consistency between the items in a measure (Burns & Grove, 2009). The *Uptake Continuum* measure was found to have a strong internal consistency with a Cronbach’s α = 0.80. The reliability statistics for the pubertal development measure were reported by gender, as the Cronbach’s α was found to be adequate for males (0.72) and low for females (0.42) in this sample. The inadequate Cronbach’s α of 0.42 may be partially explained by (1) the small number of items on the scale (4 items) and (2) the larger amount of unanswered items including the item specific to breast development.

Table 7  
*Instrument Reliability*

<table>
<thead>
<tr>
<th>Instrument</th>
<th>No. of items</th>
<th>Cronbach’s α coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking Uptake Continuum</td>
<td>12</td>
<td>0.80</td>
</tr>
<tr>
<td>Self-Administered Rating Scale for Pubertal Development: Males</td>
<td>5</td>
<td>0.72</td>
</tr>
<tr>
<td>Self-Administered Rating Scale for Pubertal Development: Females</td>
<td>4</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Descriptive Statistics of Study Variables

The relationship between the independent variable of tobacco exposure and the dependent variable of blood pressure was examined. Salivary C-reactive protein (CRP) was also examined as a potential mediator between blood pressure and tobacco exposure. Therefore, the variables most central to this study include blood pressure, salivary
cotinine and salivary CRP. Table 8 contains the descriptive statistics for these variables.

<table>
<thead>
<tr>
<th>Study Variable</th>
<th>Type of Variable</th>
<th>Actual Range</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>Dependent</td>
<td>88-145</td>
<td>116</td>
<td>117</td>
<td>11</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>Dependent</td>
<td>48-95</td>
<td>66</td>
<td>67</td>
<td>7</td>
</tr>
<tr>
<td>Cotinine (ng/ml)*</td>
<td>Independent</td>
<td>0-274.52</td>
<td>0.24</td>
<td>5.1</td>
<td>28.3</td>
</tr>
<tr>
<td>C-reactive Protein (pg/ml)</td>
<td>Potential Mediator</td>
<td>1130-300000</td>
<td>2943.0</td>
<td>13309.6</td>
<td>37470.9</td>
</tr>
</tbody>
</table>

Note. SD = standard deviation, N = number of participants.
*Variable had 1 missing value (N = 147).

The two blood pressure measurements taken with each adolescent participant were averaged and categorized according to *The Fourth Report on Diagnosis, Treatment and Evaluation of High Blood Pressure in Children and Adolescents* (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The variable of blood pressure was analyzed separately for systolic and diastolic values. The systolic blood pressure ranged from 88 to 145 mmHg with a mean of 117 mmHg. The diastolic blood pressure range was 48 to 95 mmHg with a mean of 67 mmHg. According to the classification system, 16.9% of adolescent participants had elevated systolic blood pressures and 3.4% had elevated diastolic blood pressures. Although these percentages are derived from single blood pressure measurements, they do exceed the 3% prevalence of hypertension among adolescents (Loeffler, et al., 2012; Sugiyama, et al., 2007). The majority of adolescent participants had blood pressures in the normotensive range. The prevalence for each blood pressure category is found in Table 9.
Table 9
*Categorization of Blood Pressure Measurements (N = 148)*

<table>
<thead>
<tr>
<th>Category*</th>
<th>Blood Pressure Percentile</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic Blood Pressure</strong></td>
<td>Normotensive</td>
<td>&lt;90&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Prehypertensive</td>
<td>≥ 90&lt;sup&gt;th&lt;/sup&gt; - &lt;95&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>≥ 95&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure</strong></td>
<td>Normotensive</td>
<td>&lt;90&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Prehypertensive</td>
<td>≥ 90&lt;sup&gt;th&lt;/sup&gt; - &lt;95&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hypertensive</td>
<td>≥ 95&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note. N = number of participants.
*National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004

In this study, tobacco exposure was conceptualized as (1) self-reported individual tobacco use behaviors, such as cigarette smoking and/or smokeless tobacco use; (2) self-reported sources of secondhand smoke exposure and (3) salivary cotinine levels as a biomarker. The variable of smoking status was derived for the response to items on the *Uptake Continuum* measure (Choi, et al., 2001). Adolescents who identified themselves (via 13 multiple-choice items) as committed nonsmokers or susceptible nonsmokers were categorized as never smokers. On the other hand, adolescents who reported one puff of experimentation with cigarette smoking, more advanced smoking experimentation, or established cigarette smoking behavior were categorized as ever smokers. The smokeless tobacco use prevalence for this sample was derived from one item on the demographic question, which asked the adolescent participant, “*Do you use smokeless tobacco products also known as chewing tobacco, spit tobacco, ‘chew’, ‘chaw’, ‘dip’?*” Finally, data regarding the sources of secondhand smoke exposure from an adolescent’s family and/or peers were collected using the dichotomous responses from the *Peer and Family Smoking* index (Pierce, et al., 1998). Data regarding individual, self-reported tobacco use
behaviors and sources of secondhand smoke exposure from family and/or peers are presented in Table 10.

Table 10

**Tobacco Use and Exposure Characteristics of Sample (N = 148)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking Status</td>
<td></td>
</tr>
<tr>
<td>Never Smoker</td>
<td>88 (59.5)</td>
</tr>
<tr>
<td>Ever Smoker</td>
<td>60 (40.5)</td>
</tr>
<tr>
<td>Smokeless Tobacco Use</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>No</td>
<td>145 (98.0)</td>
</tr>
<tr>
<td>Smoking Exposure by Family*</td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td>68 (47.6)</td>
</tr>
<tr>
<td>No Exposure</td>
<td>75 (52.4)</td>
</tr>
<tr>
<td>Smoking Exposure by Peers**</td>
<td></td>
</tr>
<tr>
<td>Exposure</td>
<td>57 (50.0)</td>
</tr>
<tr>
<td>No Exposure</td>
<td>57 (50.0)</td>
</tr>
</tbody>
</table>

Note. N = number of participants. Due to missing values, percentages may not total 100%.
* Variable had 5 missing value (N = 143).
** Variable had 34 missing values (N = 114).

Approximately 60% of adolescent participants reported never smoking, not even one puff of cigarette smoking related to experimentation. Only 3 individual participants (2%) reported using smokeless tobacco products; of these 3 participants, one identified themselves as a dual user of tobacco. Dual tobacco use was “defined as concurrent use of more than one tobacco product” (Rath, et al., 2012, p. 2). When asked if parents and/or siblings smoked, just under half of study participants (47.6%, n =143) reported smoking by family members. With regard to smoking among peers, half of adolescent participants (50%, n = 114) reported that they had either male or female friends who smoke. Although half of participants reported having friends/peers who smoke, this was not reflected in the prevalence rate of smokers in this study.

With the **Peer and Family Smoking** index, adolescent participants were given the option to select “I don’t know” as their response, which was statistically treated as a missing value. Due to the selection of the “I don’t know” response, there were 5 missing values (3.4%) related to the variable of secondhand smoke exposure by family and 34
missing values (23.0%) related to secondhand smoke exposure by peers.

Tobacco exposure was also quantified using the objective measure of salivary cotinine. The maximum level of cotinine found in this study was 275 ng/ml, which is well above the 3 ng/ml cutpoint denoting regular smoking behavior or heavy secondhand smoke exposure in an adolescent (Benowitz, et al., 2009; Salimetrics LLC, 2010a). The adolescent participant with a salivary cotinine level of 275 ng/ml identified themselves as a current experimenter with smoking behavior, reporting smoking fewer than 100 cigarettes in a lifetime but did smoke within the last 30 days. The mean for salivary cotinine was 5.1 ng/ml with a standard deviation of 28.3 ng/ml. The distribution for the salivary cotinine variable was positively skewed; therefore, the median of 0.24 ng/ml is also reported.

The cotinine findings were categorized according to the cutpoints established by Benowitz and colleagues (Benowitz, et al., 2009). Although the biomarker of cotinine can serve to quantify the extent of tobacco use and/or exposure, it does not specifically identify the source of tobacco use and/or exposure. As reported in Table 11, approximately 12% of adolescent participants had levels of cotinine (1.0 - 2.99 ng/ml) that were most likely indicative of secondhand smoke exposure. Another ten percent had levels (≥ 3.0 ng/ml) most likely indicative of tobacco use, such as cigarette smoking, but may also indicate heavy secondhand smoke exposure. Over 75% of participants had cotinine levels (< 1.0 ng/ml) indicative of minimal to no exposure.
Salivary C-reactive protein has been used in tobacco research as a measure of low-grade inflammation (Azar & Richard, 2011). In this sample of rural adolescents ages 15 to 18 years old, the findings related to salivary CRP had a large range and standard deviation. The mean was 13309.6 pg/ml with a standard deviation of 37470.9 pg/ml. With a positively skewed distribution, the median of 2943.0 pg/ml is also reported. There is a paucity of research regarding the use of salivary C-reactive protein particularly among adolescents (Azar & Richard, 2011; Ouellet-Morin, Danese, Williams, & Arseneault, 2010; Out, et al., 2012). Unlike salivary cotinine, there are no established cutpoints available to categorize the salivary CRP findings; however, >3mg/L is the considered a clinically relevant cutpoint for serum CRP (Out, et al., 2012).

Correlations among Study Variables

As a starting point to describe the relationships between study variables, bivariate correlations were completed. Pearson’s product-moment correlation coefficient was completed between the dependent variable of blood pressure (systolic blood pressure and diastolic blood pressure) and age, salivary cotinine, salivary C-reactive protein, body mass index and waist circumference while Spearman’s Rank Order (rho) correlation was completed for the variable of pubertal status. The correlational analyses involving

Table 1
Categorization of Salivary Cotinine Levels (N = 147)

<table>
<thead>
<tr>
<th>Category*</th>
<th>Salivary Cotinine Level (ng/ml)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No exposure</td>
<td>0 ng/ml</td>
<td>59 (40.1)</td>
</tr>
<tr>
<td>Minimal exposure</td>
<td>0.1 - 0.99 ng/ml</td>
<td>55 (37.4)</td>
</tr>
<tr>
<td>Secondhand smoke exposure</td>
<td>1.0 – 2.99 ng/ml</td>
<td>18 (12.2)</td>
</tr>
<tr>
<td>Regular smoking behavior</td>
<td>≥ 3.0 ng/ml</td>
<td>15 (10.2)</td>
</tr>
</tbody>
</table>

*Benowitz et al., 2009
salivary cotinine and C-reactive protein were completed with the log-transformed variable so as to meet the assumption of normality. The association between blood pressure and the dichotomous form of the variables gender, socioeconomic status and parental history of hypertension was calculated using the Chi-square test for independence.

Significant, positive correlations were found between the variables of systolic and diastolic blood pressure \((r = .633, p < .01)\); age and pubertal status \((r = .314, p < .01)\); and body mass index and waist circumference \((r = .935, p < .01)\). These significant findings add a measure of validity as the physiological relationships between these variables are well established. With regard to the salivary biomarkers, cotinine had a positive, low-to-moderate correlation with waist circumference \((r = .232, p < .01)\) and a positive but low correlation with body mass index \((r = .195, p < .05)\). The salivary biomarker CRP did not correlate with any study variable.

The two measures of weight status both had significant correlations with blood pressure. Body mass index had a moderate, positive correlation with systolic blood pressure \((r = .471, p < .01)\) and diastolic blood pressure \((r = .355, p < .01)\). Waist circumference also had a moderate, positive correlation with systolic blood pressure \((r = .455, p < .01)\) and diastolic blood pressure \((r = .388, p < .01)\). The correlation matrix for the study variables is presented in Table 12.
Table 12
*Correlation Matrix: Blood Pressure and Study Covariates*

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>DBP</th>
<th>Cotinine(^a)</th>
<th>CRP(^b)</th>
<th>AGE</th>
<th>PUB(^c)</th>
<th>BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>.633**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine(^a)</td>
<td>-0.17</td>
<td>-0.061</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP(^b)</td>
<td>-0.064</td>
<td>-0.130</td>
<td>0.023</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.022</td>
<td>0.124</td>
<td>-0.028</td>
<td>-0.015</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUB(^c)</td>
<td>-0.065</td>
<td>0.122</td>
<td>-0.065</td>
<td>0.081</td>
<td>0.314**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.471**</td>
<td>0.355**</td>
<td>0.195*</td>
<td>0.092</td>
<td>-0.026</td>
<td>0.136</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.455**</td>
<td>0.388**</td>
<td>0.232**</td>
<td>0.086</td>
<td>-0.025</td>
<td>0.132</td>
<td>0.935**</td>
<td>1</td>
</tr>
</tbody>
</table>

*Note. SBP = systolic blood pressure, DBP = diastolic blood pressure, COT = salivary cotinine, CRP = salivary C-reactive protein, BMI = body mass index and WC = waist circumference. *p < .05, **p < .01
\(^a\)Data was log transformed twice
\(^b\)Natural log transformed data
\(^c\)Spearman rho*

Chi-square test for independence was completed using the dichotomous forms of the variables for gender, parental history of hypertension and socioeconomic status. The dependent variable of blood pressure (both systolic and diastolic) was transformed into a dichotomous variable using the mean as a cutpoint to distinguish between low and high blood pressure. The Chi-square test for independence indicated no significant association between blood pressure, either systolic or diastolic, and the variables of parental history of hypertension or socioeconomic status. A significant association was found between
gender and systolic blood pressure \( (X^2 = 25.74, p = .000) \); while no association was found between gender and diastolic blood pressure.

Analyses for Research Question 1

Research Question 1: Is there a relationship between individual, self-reported tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure in rural adolescents ages 15-18? A Chi-square test for independence was completed to determine if a relationship existed between self-reported individual cigarette smoking behavior and the secondhand smoke exposure resulting from family smokers and peer smokers. Statistical analyses were completed using the dichotomous variable for self-reported smoking status. Collapsing the seven cigarette smoking behavior categories from the Uptake Continuum measure into never vs. ever smoking categories created the variable of self-reported smoking status.

For sources of secondhand smoke exposure, two variables were created from the self-reported responses to the Peer and Family Smoking measure; one variable for smoking by family members including parents, stepparents and/or siblings and another variable for smoking by peers. The Peer and Family Smoking measure is scored as either no exposure or exposure for family and peers separately (Pierce, et al., 1998). Two variables derived from the Peer and Family Smoking measure were coded as (1) no exposure and (2) exposure; thus, resulting in a dichotomous variable.

The Chi-square test for independence indicated a significant association between self-reported smoking status and smoking exposure by family \( (X^2 = 5.57, p = .018) \). A
significant association was also found between self-reported smoking status and peers ($X^2 = 26.56, p = .000$).

With regard to smokeless tobacco use, only 3 adolescent participants (2%) in this sample reported using smokeless tobacco products. No meaningful statistical analysis could be completed regarding smokeless tobacco use, as there were too few cases in the dataset.

The analyses completed for research question 1 offers support for hypothesis 1. This study found a significant association between self-reported individual tobacco use (cigarette smoking only) and the sources of secondhand smoke exposure among rural adolescents ages 15-18.

Analyses for Research Question 2

Research Question 2: Do individual tobacco use (self-reported cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure influence cotinine levels? Using the variables of tobacco exposure (via salivary cotinine) and smoking exposure by family as peers, a Chi-square test for independence was completed. The Chi-square test for independence indicated a significant association between tobacco exposure (salivary cotinine) and self-reported smoking exposure by family only ($X^2 = 10.81, p = .001$). No significant association was found between tobacco exposure (salivary cotinine) and self-reported smoking exposure by peers ($X^2 = 1.21, p = .271$). As with research question 1, there were too few cases of smokeless tobacco use to complete a meaningful statistical analysis involving the variable of smokeless tobacco use.
To explore the dataset specific to the variable of salivary cotinine, Mann Whitney U tests were also completed between salivary cotinine and the variables of gender, never vs. ever smoking status, smoke exposure by peer and smoke exposure by family. None of the dichotomous variables of gender, never vs. ever smoking status, or smoke exposure by peers were significantly related to an adolescent’s salivary cotinine level (see Table 13).

Table 13

*Mann Whitney U Test for Salivary Cotinine Level and Study Variables*

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Never vs. Ever Smoking Status</th>
<th>Smoke Exposure by Peers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann Whitney U</td>
<td>2487.5</td>
<td>2189.5</td>
<td>1341.5</td>
</tr>
<tr>
<td>Z</td>
<td>-.495</td>
<td>-1.70</td>
<td>-1.51</td>
</tr>
<tr>
<td>p-value</td>
<td>.620</td>
<td>.089</td>
<td>.131</td>
</tr>
</tbody>
</table>

However, the variable of smoking exposure by family, a variable used as a proxy for sources of secondhand smoke exposure, did have a significant relationship with the salivary cotinine level (see Table 14). The variable of smoking exposure by family describes whether or not an adolescent reported smoking behavior by parents, stepparents, guardians and/or siblings.

Table 14

*Mann Whitney U Test for Salivary Cotinine Level and Smoking Exposure by Family*

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann Whitney U</td>
<td>1516.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Rank</td>
<td></td>
<td>Exposure</td>
<td>86.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Exposure</td>
<td>57.99</td>
</tr>
<tr>
<td>Sum of Ranks</td>
<td></td>
<td>Exposure</td>
<td>5861.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Exposure</td>
<td>4291.5</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td>-4.186</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
<td>.000**</td>
</tr>
</tbody>
</table>

*Note.** *p* < .05
The analyses completed for research question 2 offer partial support for hypothesis 2. Hypothesis 2 states that individual tobacco use (cigarette smoking and smokeless tobacco use) and the sources of secondhand smoke exposure will positively influence cotinine levels among rural adolescents ages 15-18. This study found a significant association between tobacco exposure (via salivary cotinine) and smoking exposure by family ($X^2 = 10.81, p = .001$). A significant difference in the mean salivary cotinine levels was also found between adolescents who reported smoking exposure by family ($p = .000$). However, there was no significant difference in mean salivary cotinine levels by adolescent who reported smoking exposure by peers ($p = .271$).

Analyses for Research Question 3

Research Question 3: How much of the variance in blood pressure is explained by tobacco exposure (salivary cotinine) in rural adolescents ages 15-18? The variable of blood pressure was statistically analyzed as both a categorical variable and a continuous variable. Ordinal regression analysis (with blood pressure as a categorical variable) was completed to address this research question. With guidance from *The Fourth Report on Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents*, the systolic blood pressures were categorized using age, gender and height to determine the percentile (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). The first category, identified as normotensive, was used for systolic blood pressures below the 90\(^{th}\) percentile. The second category, identified as prehypertensive, was used for systolic blood pressure equal to or above the 90\(^{th}\) but less than the 95\(^{th}\) percentile. The third and
The ordinal regression analysis findings indicate that the categories of normotensive, prehypertensive and hypertensive were significantly different from one another. The model has a significant p-value = .000; 2) a Pseudo $R^2$ using Nagelkerke
was .294; and 3) a nonsignificant Goodness of Fit Test. These findings are indicative of a good model fit for this model, which includes the variables of age, gender and tobacco exposure while controlling for weight status. Because no significant association was found between parental history of hypertension, socioeconomic status or pubertal status, these variables were not included in the model. Ethnicity was also excluded from the model due to the lack of variability found within the sample.

An ordinal regression analysis specific to the categorized diastolic blood pressure was not completed. Only five adolescent participants had elevated diastolic blood pressures, thus, there were too few cases in the prehypertensive and hypertensive categories to complete a meaningful ordinal regression analysis.

To further explore the predictors that contribute to blood pressure levels in this sample of rural adolescents, hierarchical multiple regression analyses were completed using blood pressure as a continuous variable. The multiple regression analyses were completed twice; each analysis included a different variable for weight status. The full model including the variable of waist circumference accounted for a similar amount of variability in both systolic and diastolic as compared to the model with body mass index as the variable accounting for weight status. However, the significant F change values were improved with the full model including waist circumference and tobacco exposure: (1) $p = .0.98$ versus $p = .053$ for the full model with systolic blood pressure as the outcome and (2) $p = .094$ versus $p = .046$ for diastolic blood pressure as the outcome variable. The hierarchical multiple regression findings for the full model with waist circumference as the variable accounting for weight status are presented.
Prior to the hierarchical multiple regression analysis with systolic blood pressure as the outcome variable, the normal distribution of the systolic blood pressure was confirmed with a review of the histogram for this variable. The resulting $R$-squared value and the F statistic from the multiple linear regression analysis are presented in Tables 16 and 17, respectively. The confounding variables of age, gender and waist circumference were entered in Block 1 resulting in a model with the $R$-squared value of .347, $F (3, 143) = 25.31$ ($p = .000$). Model 1 provides evidence that the confounding variables of body mass index, age and gender contribute 34.7% of the variance in systolic blood pressure levels.

Table 16

Hierarchical Multiple Linear Regression with Systolic Blood Pressure

<table>
<thead>
<tr>
<th>Model</th>
<th>$R$</th>
<th>$R^2$</th>
<th>Adjusted $R^2$ Change</th>
<th>$F$ Change</th>
<th>$df1$</th>
<th>$df2$</th>
<th>Sig F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.589$^a$</td>
<td>.347</td>
<td>.333</td>
<td>.347</td>
<td>25.31</td>
<td>3</td>
<td>143</td>
</tr>
<tr>
<td>2</td>
<td>.603$^b$</td>
<td>.364</td>
<td>.346</td>
<td>.017</td>
<td>3.80</td>
<td>1</td>
<td>142</td>
</tr>
</tbody>
</table>

$^a$ Predictors: (Constant), waist circumference, age, gender.

$^b$ Predictors: (Constant), waist circumference, age, gender, log transformed cotinine.
The full model (Model 2) included the confounding variables of age, gender, and waist circumference on Block 1 and the transformed cotinine variable on Block 2. The full model was significant, $F(4, 142) = 20.305, p = .000$. The $R^2$-squared value for the full model is .364 with a $R^2$-squared Change value of 1.7%, which explains the contribution of the transformed cotinine variable. The full model provides evidence that age, gender, and waist circumference along with cotinine level contribute 35.7% of the variance in systolic blood pressure, a notable but marginal increase compared to Model 1 ($p = .053$).

The regression coefficients for the full model are presented in Table 18. Gender has significant $t$-test ($5.613, p < .000$) as well as waist circumference ($t = 6.856, p < .000$) indicating that these variables contributed significantly to the model. The transformed cotinine level did not reach significant with a $p$-value of .053.
Table 18
*Regression Coefficients for Multiple Linear Regression with Systolic Blood Pressure*

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Collinearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Constant</td>
<td>70.851</td>
<td>13.297</td>
<td>5.328</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>.097</td>
<td>.726</td>
<td>.009</td>
<td>.133</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>8.219</td>
<td>1.464</td>
<td>.377</td>
<td>5.613</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>.997</td>
<td>.145</td>
<td>.472</td>
<td>6.856</td>
</tr>
<tr>
<td></td>
<td>COT</td>
<td>-3.559</td>
<td>1.826</td>
<td>-.134</td>
<td>-1.95</td>
</tr>
</tbody>
</table>

*Note.* WC = waist circumference, COT = log transformed cotinine.

A hierarchical multiple regression analysis was also completed with diastolic blood pressure as the outcome variable. Before the analysis was completed, the normal distribution of the diastolic blood pressure was confirmed with a review of a histogram.

The resulting $R^2$-squared value and the $F$ statistic from the multiple linear regression analysis are presented (see Tables 19 and 20 respectively).

Table 19
*Hierarchical Multiple Linear Regression with Diastolic Blood Pressure*

<table>
<thead>
<tr>
<th>Model</th>
<th>$R$</th>
<th>$R^2$</th>
<th>Adjusted $R^2$</th>
<th>$F$ Change</th>
<th>$df$1</th>
<th>$df$2</th>
<th>Sig $F$ Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.410&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.168</td>
<td>.151</td>
<td>.168</td>
<td>9.647</td>
<td>3</td>
<td>143</td>
</tr>
<tr>
<td>2</td>
<td>.438&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.191</td>
<td>.169</td>
<td>.023</td>
<td>4.070</td>
<td>1</td>
<td>142</td>
</tr>
</tbody>
</table>

<sup>a</sup>Predictors: (Constant), waist circumference, age, gender.

<sup>b</sup>Predictors: (Constant), waist circumference, age, gender, log transformed cotinine.
Table 20  
**ANOVA Table: Hierarchical Multiple Linear Regression with Diastolic Blood Pressure**

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression</td>
<td>1085.837</td>
<td>3</td>
<td>361.946</td>
<td>9.647</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>5365.118</td>
<td>143</td>
<td>37.518</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6450.956</td>
<td>146</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Regression</td>
<td>1235.336</td>
<td>4</td>
<td>308.834</td>
<td>8.408</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>5215.619</td>
<td>142</td>
<td>36.730</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6450.956</td>
<td>146</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Predictors: (Constant), gender, age, waist circumference  
<sup>b</sup>Predictors: (Constant), gender, age, waist circumference, log transformed cotinine

The confounding variables of age, gender and waist circumference were entered in Block 1 and resulted in a $R^2$ value of .168, $F(3, 143) = 9.647, p = .000$. Model 1 provides evidence that age, gender and waist circumference contribute 16.8% of the variance in diastolic blood pressure for this sample of adolescents.

The full model (Model 2) included the log transformed cotinine variable along with gender, age and body mass index. The statistics for this model resulted in a significant $F$ statistic, $F(4, 142) = 8.408, p = .000$. The $R^2$ value for the full model was .191 with a $R^2$ Change value of 2.3%, which explains the contribution of the transformed cotinine variable and had a Sig $F$ Change of .046. The full model provides evidence that age, gender and waist circumference along with cotinine level contribute 19.1% of the variance in diastolic blood pressure; the difference between Model 1 and Model 2 did reach significance with $p = .046$.  

135
The regression coefficients for the full model are presented in Table 21. The variables of waist circumference and cotinine in the full model both reach significance with a t-test statistic of 5.495 ($p = .000$) and -2.017 ($p = .000$) respectively. Unlike the full model for systolic blood pressure, the transformed cotinine variable did reach significance ($p = .046$) in the full model for diastolic blood pressure with this sample of adolescents.

Table 21
Regression Coefficients for Multiple Linear Regression with Diastolic Blood Pressure

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>Collinearity Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>B</td>
<td></td>
<td>Tol.</td>
</tr>
<tr>
<td>2</td>
<td>Constant</td>
<td>33.472</td>
<td>9.271</td>
<td>3.611</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>.880</td>
<td>.506</td>
<td>.131</td>
<td>1.738</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>.240</td>
<td>1.021</td>
<td>.018</td>
<td>.235</td>
</tr>
<tr>
<td></td>
<td>WC</td>
<td>.557</td>
<td>.101</td>
<td>.427</td>
<td>5.495</td>
</tr>
<tr>
<td></td>
<td>COT</td>
<td>-2.568</td>
<td>1.273</td>
<td>-.157</td>
<td>-2.02</td>
</tr>
</tbody>
</table>

Note. WC= waist circumference, COT= log transformed cotinine.

With regards to hypothesis 3, tobacco exposure, analyzed with the log transformed variable of cotinine, contributed a notable but small amount of variability in both systolic and diastolic blood pressure among rural adolescents ages 15-18, when controlling for age, gender and waist circumference. The hierarchical multiple linear regression model including age, gender, waist circumference and cotinine supported hypothesis 3.
Analyses for Research Question 4

Research Question 4: Does inflammation mediate the relationship between tobacco exposure and blood pressure in rural adolescents, ages 15-18? A series of univariate regression models, for the statistical process described by Baron and Kenny (1986), was completed to determine if C-reactive protein functions as a mediator between tobacco exposure and blood pressure when controlling for gender, age and body mass index. In the first step, the univariate regression model was completed to determine if the independent variable of tobacco exposure was a significant predictor of systemic inflammation quantified by C-reactive protein (the proposed mediator).

Table 22

<table>
<thead>
<tr>
<th>Model</th>
<th>$R$</th>
<th>$R^2$</th>
<th>Adjusted $R^2$</th>
<th>$F$ Change</th>
<th>$df1$</th>
<th>$df2$</th>
<th>Sig $F$ Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.023$^a$</td>
<td>.001</td>
<td>-.006</td>
<td>.001</td>
<td>1</td>
<td>145</td>
<td>.786</td>
</tr>
</tbody>
</table>

$^a$Predictors: (Constant), log transformed cotinine.

As presented in Table 22, the model summary showed that cotinine did not significantly predict C-reactive protein. The mediation analysis was stopped here as no significant relationship was found between cotinine and C-reactive protein. With regard to hypothesis 4, salivary C-reactive protein does not appear to mediate the relationship between tobacco exposure and blood pressure in this sample of rural adolescents, ages 15-18.
Summary

A number of statistical analyses were completed to test the four research questions and the accompanying hypotheses presented in this chapter. This study found a significant association between individual tobacco use (cigarette smoking) and the sources of secondhand smoke exposure (family, peer) among rural adolescents ages 15-18; thus, supporting hypothesis 1. Partial support for hypothesis 2 was found with the significant association between smoking exposure by family members and salivary cotinine level. With regard to blood pressure, both systolic and diastolic blood pressure were used as outcome variables in hierarchical multiple regression models. In support of hypothesis 3, the model including age, gender, waist circumference and cotinine contributed 36.4% of the variance in systolic blood pressure; cotinine contributed 1.7% to this total variance. For diastolic blood pressure the model including age, gender and waist circumference contributed 16.8% variance to the outcome. When cotinine was added, the model contributed 19.1% of the variance in diastolic blood pressure. Finally, with regard to hypothesis 4, no significant relationship was found between cotinine and C-reactive protein. Due to this lack of significance, the proposed mediation analysis for research question 3 was stopped. C-reactive protein, as a biomarker of systemic inflammation, does not appear to mediate the relationship between tobacco exposure and blood pressure in this sample of rural adolescents ages 15-18.
CHAPTER FIVE

DISCUSSION

A discussion of the study findings is presented in this chapter. The purpose of this study was to determine the influence of tobacco exposure on blood pressure in a sample of rural adolescents ages 15-18 after controlling for the variables of age, gender, parental history of hypertension, weight status (BMI, waist circumference), pubertal stage, and socioeconomic status. The proposed mediating role of C-reactive protein in the relationship between tobacco exposure and blood pressure is also addressed in this discussion. The findings are discussed specific to the descriptive statistics of the sample and the four research questions that guided this study. A discussion of the conceptual framework, study limitations and the implications for professional practice, policy and future research direction are included.

Major Findings

In this study involving rural adolescents ages 15-18, significant associations were found between individual tobacco use (cigarette smoking) and secondhand smoke exposure (family, peers). A significant association between tobacco exposure (salivary cotinine) and smoking exposure by family was also found. This study found support for three of the four stated hypotheses was provided.
In support of hypothesis 1, a significant association between individual tobacco use (cigarette smoking) and both smoking exposure by family ($X^2 = 5.57, p = .018$) and peers ($X^2 = 26.56, p = .000$) was found. A significant association between tobacco exposure (via salivary cotinine) and adolescent participants who reported smoking exposure by family members ($X^2 = 10.81, p = .001$) provided partial support for hypothesis 2, which states that individual tobacco use and the sources of secondhand smoke exposure will positively influence cotinine levels among rural adolescents ages 15-18.

In the predictive model of systolic blood pressure, the variables of age, gender, waist circumference and cotinine were found to contribute 36.4% of the variance ($p = .000$) with cotinine contributing 1.7% ($p = .053$) of this total variance. Furthermore, the predictive model involving diastolic blood pressure included age, gender, waist circumference and cotinine resulted in a total variance of 19.1% ($p = .000$); the addition of cotinine contributed significantly to the full model ($p = .046$). Particularly the predictive model for diastolic blood pressure provided support for hypothesis 3, which states that tobacco exposure, quantified by salivary cotinine level, will account for a significant amount of variability in blood pressure among rural adolescents ages 15-18.

No significant relationship was found between salivary cotinine (tobacco exposure biomarker) and salivary C-reactive protein resulting in a lack of support for the mediation model hypothesized in research question 4. Thus, the evidence did not support hypothesis 4, which postulated C-reactive protein as a mediator in the relationship between tobacco exposure and blood pressure in this sample of rural adolescents ages 15-18.
Demographics

The final study sample was comprised 148 adolescent participants (88 female and 60 male). According to the U.S. Census Bureau, there were approximately 1,163 adolescents between the ages of 15 and 19 in this rural county in 2010 (U.S. Census Bureau, 2010). While this was a nonprobability sample, the final sample of adolescents (n = 148) accounted for roughly 10% of the county’s adolescents in this age group. The calculated response rate for the study was low at 37.5%. As a result, only 28% of participants were low-income individuals and only 10% were smokers. Low socioeconomic status (Howe et al., 2010; Kivimaki et al., 2006; Marin, Chen, & Miller, 2008) (van den Berg, et al., 2013) and smoking behavior (Dasgupta, et al., 2006; Flouris, et al., 2008) are important risk factors related to blood pressure in adolescence. Therefore, a greater inclusion of low-income individuals and adolescent smokers would have strenghten the study findings.

Confounding Variables

The descriptive statistics were reviewed for each of the confounding variables including age, gender, socioeconomic status, pubertal status, weight status (body mass index and waist circumference) and parental history of hypertension. The age distribution of adolescent participants was fairly even, with each age (15, 16, 17 and 18 year-olds) well represented in the sample. The rural county represented in this study is 99% Caucasian and 1% Hispanic or Latino (U.S. Census Bureau, 2010). The study participants were representative of the county in terms of race/ethnicity. As with the entire rural county, the vast majority of the study sample was Caucasian at 96%. In terms of race, the remaining 4% of adolescent participants identified themselves as Multiracial. Compared
to the ethnicity of the county, Latino adolescents were overrepresented at 4.8% of the sample. There was very little variability in ethnicity in this sample; therefore, this variable for excluded for statistical analyses. Tobacco researchers have noted that females and Caucasian youth are more likely to participate in tobacco-focused research studies (Diviak, Wahl, O'Keefe, Mermelstein, & Flay, 2006). This demographic composition held true for this study as well as the sample was highly Caucasian (96%), as well as predominately female (60%).

With regard to socioeconomic status, 27.9% self-reported participation in the National School Lunch Program (NSLP), receiving free or reduced lunch. NSLP participation is based on family income and a student receives a “free lunch if income is at most 130% of the federal poverty level; reduced price if between 130% and 185%; [and] full price if above 185%” (Mirtcheva & Powell, 2009, p. 486). NSLP participation was used as a proxy for socioeconomic status. The adolescent participants were recruited from schools with NSLP participation rates at 33.6% (School A) and 39.3% (School B) (Indiana Department of Education, 2011-2012). This study sample was found to have a higher overall socioeconomic status compared to the high schools from which the sample was recruited. As a group, the adolescent participants had higher socioeconomic status and lower smoking prevalence. One-fifth of the sample had salivary cotinine levels indicative of secondhand smoke or regular smoking behavior. The socioeconomic status and the tobacco use/exposure has implications for the study findings as social inequities experienced early in life influence the development of hypertension (van den Berg, et al., 2013).

With the confounding variable of pubertal status, more than half of the adolescent
participants (56.8%) reported themselves to be in late puberty while a quarter reported they were post pubertal. Just under ten percent of the sample (9.5%) considered themselves to be midpubertal. The frequencies reported for pubertal status only reflect the data from 135 adolescent participants, as 13 participants (8.8%) reported “I don’t know” for certain key questions on the *Self Administered Rating Scale for Pubertal Development* resulting in missing values and the inability to categorize the participant’s pubertal development. The small number of items in the measure and the large number of missing values among females contributed to the measure’s low reliability among female adolescent participants (Cronbach’s α = 0.42).

A previous study with younger adolescents also reported suboptimal Cronbach’s alpha statistics for this measure, .656 and .521 for male and female early adolescents respectively (Lam, 2012). While this study involved older adolescents 15-18 years old, the psychometric analysis for the *Self Administered Rating Scale for Pubertal Development* measure was originally completed with a large sample (*n* = 698) of younger adolescents 10-12 years old (Carskadon & Acebo, 1993). Carskadon and Acebo, the developers of this measure, acknowledge that missing values can be a frequent occurrence particularly when the measure is used with younger adolescents (Carskadon & Acebo, 1993). In this study, the majority of missing values for female participants resulted from the breast development question. Without data for the breast development question, the principal investigator was unable to categorized a female participant as late pubertal or postpubertal thus resulting in a missing value. Carskadon and Acebo (1993) state that missing values are a reality when using this measure, as adolescents often have difficulty determining exactly when puberty has started or finished. Use of a different
measure for pubertal status may be advisable, however, very few measures suitable for a school-based study exist (Lam, 2012).

Weight status is a strong predictor of elevated blood pressure in adolescents (Khoury et al., 2012; Muntner, He, Cutler, Wildman, & Whelton, 2004; Sugiyama, et al., 2007). Within this sample of 148 rural adolescents, 17.6% had a body mass index (BMI) categorized as overweight (BMI between 85th and 95th percentile) and an additional 25% had a BMI over the 95th percentile, which was categorized as obese. Thus, a total of 42.6% of participating adolescents exceeded the normal weight range. This sample prevalence far exceeds the 2011 estimate for obesity among Indiana high school students, which was estimated to be 13.4% (Centers for Disease Control and Prevention, 1991-2011). Additionally, the 2009-2010 estimate of obesity among US adolescents ages 12 to 19 year old, which was estimated to be 18.4%, was also exceeded (Ogden, et al., 2012).

Body mass index is a prevalent measure used to discuss weight status among children and adolescents; however, it is important to note that body mass index is more a measure of excess weight (which made include muscle mass) than excess fat (Flegal & Ogden, 2011).

Two measures of weight status were included in the design of this study, body mass index and waist circumference. Abdominal obesity is measured by waist circumference is a measure of and considered more reflective of visceral fat than body mass index in children (Brambilla et al., 2006). Waist circumference is also considered a better predictor of cardiovascular risk in children and adolescents than body mass index (Janssen, et al., 2004; Savva, et al., 2000). The waist circumference mean for the entire sample was 34.2 inches, with little difference in means by gender (34.0 inches for
females and 34.4 inches for males). Using 2003-2004 data from the National Health and Nutrition Examination Survey, the national mean waist circumference for boys (ages 12-17) was reported to be 31.7 inches while the mean waist circumference for girls was 31.2 inches (Li, et al., 2006). Although the national means provided by Li and colleagues (2006) are a decade old, the waist circumference means from this study sample exceeded the national means reported for adolescents ages 12-17.

In this study, adolescent self report of parental history of hypertension served as a proxy for the genetic influences of hypertension. Over a quarter of adolescent participants (29.1%) reported that neither of their parents had hypertension. Thirty-four percent of adolescent participants reported they had one parent with hypertension while another 2.7% reported that both their parents had hypertension. Previous research has reported a strong association between family history of hypertension and blood pressure in adolescent males and younger females (Shi, et al., 2012). No significant association was found between parental history of hypertension and blood pressure of adolescent participants in this study. However, over a third of adolescent participants reported that they did not know if their parents had hypertension. Although this lack of knowledge equated to missing values for this analysis, it also highlights a potential opportunity for improved health communication between parents and their adolescents.

Descriptive Statistics Related to Blood Pressure

Although the majority of blood pressure readings were found to be within normotensive range, 16.9% of adolescents had prehypertensive or hypertensive systolic blood pressure readings. More specifically, 8.8% of the systolic blood pressures were prehypertensive and 8.1% were hypertensive. A small number of participants had
elevated diastolic pressures (1.4% prehypertensive and 2% hypertensive). Increases in systolic blood pressure during youth have been linked to pubertal growth (Shankar, et al., 2005). The increased prevalence of systolic blood pressure is in line with literature stating that systolic blood pressure elevations are more common in children (Sorof, 2002). In fact, in a review of the literature, Sorof (2002) reported a three to eight-fold higher prevalence of systolic hypertension compared to diastolic hypertension in youth.

Falkner and colleagues (2008) examined blood pressure variability using a national database of blood pressure measurements from adolescents, who were 13 years old at baseline and 17 years old at follow-up (n = 8535). Researchers reported prehypertensive measurements in approximately 20% in adolescent males and 13% in adolescent females (Falkner, et al., 2008). In an analysis by gender, this study sample of rural adolescents had 13.3% of males and 5.7% of females with prehypertensive systolic blood pressures. In addition, 11.7% of males and 5.7% of females had hypertensive systolic blood pressure readings. Researchers have established that prehypertension is predictive of hypertension with progression between these stages at approximately 7% per year in adolescents (Falkner, et al., 2008). Longitudinal research evidence provides support that a blood pressure elevation in childhood can track through adolescence and into adulthood (Bao, et al., 1995; Chen, Wang, Appel, & Mi, 2008; Toschke, et al., 2009). Thus, the prevalence of prehypertensive and hypertensive blood pressure readings found in this sample of rural adolescents has implications for the development of hypertension in adulthood. The prevalence of elevated blood pressure readings in this sample of rural adolescents coupled with the predictive value of prehypertension in the
development of hypertension provides a compelling case for renewing efforts regarding the prevention of hypertension among youth.

*Descriptive Statistics Related to Tobacco Exposure*

Approximately 60% of adolescent participants in this study identified themselves as never smokers and the remaining adolescents (40.5%) reported experiencing at least one puff of smoke from a cigarette. This prevalence is lower than ever smoking for adolescents nationwide; 44.7% of US adolescents in grades 9-12 reported experiencing at least one or two puffs of smoke (Eaton, et al., 2012). For smokeless tobacco use, three individuals reported use which equated to 2% of the sample. The use of smokeless tobacco in the sample is also lower than reported use nationwide at 7.7% of US adolescents reported use of smokeless tobacco products (Eaton, et al., 2012). The lower prevalence rates within this study sample may be related to the higher socioeconomic status found in this sample, as higher levels of smoking among adolescents is associated with lower socioeconomic status (Soteriades & DiFranza, 2003). However, 40% of the adolescent participants confirmed experimenting with cigarettes and, therefore, smoking behavior continues to represent a significant opportunity for tobacco use prevention among rural youth.

The majority of the adolescent participants in this study (77.6%) had minimal exposure to tobacco smoke as shown by a salivary cotinine level ≤ 1.0 μg/ml; while, one fifth of the sample had salivary cotinine levels over 1.0 μg/ml. Secondhand smoke exposure was found in 12.2% of adolescent participants by salivary cotinine levels and 10% with levels indicative of regular smoking or heavy secondhand smoke exposure. The prevalence of secondhand smoke exposure in this sample appears to be well below the
baseline level of 45.5% reported in *Healthy People 2020* (United States Department of Health and Human Services, 2010a). This low prevalence may be a result of the low response rate for the study and the low participation of low-income individuals and adolescent smokers. Even so, the Office of the U.S. Surgeon General has asserted that there is no safe, minimum dose of secondhand smoke and all secondhand smoke exposure is considered a risk to health (United States Department of Health and Human Services, 2006).

*Descriptive Statistics Related to Salivary C-reactive Protein*

Salivary specimens were collected without difficulty from participating adolescents to determine levels of salivary C-reactive protein (CRP). There is a paucity of research regarding salivary CRP (Ouellet-Morin, et al., 2010; Out, et al., 2012) and its use in adolescent research (Azar & Richard, 2011). Salivary CRP highly correlates with serum CRP and is associated with atherosclerosis, arterial stiffness and metabolic syndrome in adults (Labat et al., 2013). A clinically relevant cutpoint of 3mg/L for serum CRP has been established (Out, et al., 2012; Pearson et al., 2003); however, the absence of established cutpoints to categorize salivary CRP findings limits the principal investigator’s ability to interpret the available data in the same manner in which salivary cotinine levels were interpreted. Continued development of this biomarker is necessary as the feasibility of its use in school-based studies is favorable.

*Findings Related to Research Questions*

Nearly 50% of adolescent participants reported smoking exposure by their family members including parents, step-parents, guardians and/or siblings. This finding supports
the work of Vander Weg and colleagues (2011), which states that adults living in rural areas are more likely to be smokers compared to their urban and suburban counterparts. Vander Weg and colleagues (2011) found significant differences in both current and lifetime cigarette use, with rural adults being more likely to be ever smokers (47.8% versus 42.5% suburban and 41.2% urban) and current smokers (22.2% versus 17.3% suburban and 18.1% urban; \( p = .001 \)). As found in this study, the higher prevalence of smoking among rural adults impacts the secondhand smoke exposure; the potential, emerging smoking behaviors and, ultimately, the cardiovascular health of the rural adolescents.

Adolescent cigarette smoking was significantly associated with both family smoking exposure and peer smoking exposure. In addition, a significant association was found between an adolescent’s salivary cotinine level and their self-reported smoking exposure from family members. This finding is in line with the work of Marano and colleagues (2009), which found that the home environment where family members interact continue to be the prominent source of secondhand smoke exposure for youth nationwide.

No significant difference was found between the salivary cotinine levels of adolescents who identified themselves as never versus ever smokers. This finding may be related to an adolescent’s infrequent smoking pattern or it may suggest that, regardless of smoking experimentation or established cigarette smoking behavior, rural adolescents are at risk for tobacco exposure in their communities and, more likely, in their own homes via secondhand smoke exposure. No significant difference in salivary cotinine levels was found by gender suggesting that, among rural adolescents, males and females are equally
at risk for secondhand smoke exposure. As this sample had very little ethnic diversity, no implications can be suggested regarding ethnicity.

Not only do adolescents experience secondhand smoke exposure in their homes, but smoking initiation among adolescents has been strongly linked with parental smoking (Gilman et al., 2009). In fact, a meta-analysis of 58 national and international studies found that the risk of smoking in adolescence was increased by 62% (OR 1.62, 95% CI 1.49 to 1.75) with the presence of one parent smoker in the household (Leonardi-Bee, Jere, & Britton, 2011). The risk increased even further if the smoking parent was the mother of the family (OR 2.19, CI 95% 1.73 to 2.79). The effect of sibling smoking was also associated with significant risk for smoking among adolescents (OR 2.30, CI 95% 1.85 to 2.86; 23 studies). Strong evidence was found regarding a dose-response relationship between an adolescent’s risk of smoking and the number of smoking parents (Leonardi-Bee, et al., 2011). The findings from this study support a strong familial influence regarding individual tobacco use and secondhand smoke exposure for this sample of rural adolescents as well.

The third research question and hypothesis were focused on how much variance in blood pressure was contributed by tobacco exposure among rural adolescents. Ordinal regression analyses were completed using systolic blood pressure as the dependent variables. The full model, including age, gender, weight status (BMI, waist circumference), and cotinine, was significant in the prediction of increasing systolic blood pressure levels, contributing 29% of the variance in the categorized blood pressure levels.

Hierarchical multiple linear regression analyses were also completed to determine the amount of variance in blood pressure, both systolic and diastolic, explained by
cotinine while controlling for age, gender and waist circumference. The confounding variables of age, gender and waist circumference explained a significant amount of variance in both systolic and diastolic pressures; these findings are consistent with previous research (Dasgupta, et al., 2006; Din-Dzietham, et al., 2007; Heys, Lin, Lam, Leung, & Schooling, 2013; Shi, et al., 2012; Sugiyama, et al., 2007). The cotinine variable added a small but significant change to the model including age, gender and waist circumference alone.

Even more notable is the strength of the hierarchical multiple linear regression model that included waist circumference as the variable for weight status. The model with age, gender, cotinine and waist circumference accounted for 36.4% of the variance in systolic blood pressure while the model using body mass index accounted for 35.7%. With diastolic blood pressure, a similar finding resulted as the model with age, gender, cotinine and waist circumference accounted for 19.1% of the variance while the model using body mass index accounted for 16.1%. Cotinine only made a significant contribution to the diastolic blood pressure variance in the model which included waist circumference ($p = .046$). The strength of the contribution of waist circumference with regard to blood pressure in adolescents adds support to the work of Savva and colleagues (Savva, et al., 2000), which states that waist circumference is a superior predictor of cardiovascular risk in youth compared to body mass index.

In step 2 of the hierarchical multiple linear regression model, the cotinine variable contributed to the variance in the systolic ($p = .053$) and diastolic blood pressures ($p = .046$) for this sample of rural adolescents. This contribution to the variance in blood pressure has clinical significance specific to the clustering of cardiovascular risk factors in
adolescents. Flouris, Faught and Klentrou (2008) reported evidence supporting the concept of a smoker’s lifestyle among adolescents. The concept of a smoker’s lifestyle simply acknowledges the enhanced potential for the clustering of cardiovascular risk factors among adolescent smokers. From a large pool of Canadian adolescents ages 12-15, Flouis, Faught and Klentrou worked with a sample of adolescent smokers ($n = 119$) who confirmed smoking over 100 cigarettes in their lifetime. This sample of adolescent smokers was then matched by age and gender to a group of adolescents nonsmokers. The researchers found that the adolescent smokers had increased body mass index values, decreased aerobic fitness and the male adolescents had increased diastolic blood pressures. In addition, adolescent smokers had a higher incidence of parents and siblings who smoke compared to their age and gender-matched counterparts (Flouris, et al., 2008). Promoting a comprehensive approach to cardiovascular health that includes better nutritional habits, increased physical activity along with the avoidance of tobacco use and exposure, may be of particular importance to adolescents living with smokers (Flouris, et al., 2008). Rural adults are more likely to be smokers (Vander Weg, et al., 2011) and smoking behavior appears to have an intergenerational influence (Chassin et al., 2008); therefore, this comprehensive approach is highly relevant to adolescents living in rural communities where the intergenerational influence of smoking behavior may be more prevalent.

The fourth and final research question focused on exploring the possible mediating role of C-reactive protein with regards to the relationship between blood pressure and tobacco exposure in rural adolescents. No significant relationship was found between tobacco exposure (in the form of salivary cotinine) and salivary C-reactive
protein. This finding was surprising and inconsistent with previous findings (Wilkinson, et al., 2007). The lack of relationship between cotinine and C-reactive protein precluded the need for further mediation analysis testing.

Conceptual Framework

The expanded biobehavioral interaction model (Kang, et al., 2010) served as a strong foundation for the research design of this study. Of the model’s five domains, the individual, environmental, and behavioral domains were central to the relationship between blood pressure and tobacco exposure. The model’s emphasis on the dynamic interplay between domains and the acknowledgement of both unidirectional and bidirectional relationships appear to hold as this study found support for the relationships between self-reported, individual tobacco use and sources of secondhand smoke exposure. Hypothesis 1 and 2 provided support for the bidirectional relationship between individual, self-reported tobacco use found in the behavioral domain and the sources of secondhand smoke exposure found in the environmental domain of the model.

In the predictive model developed for systolic and diastolic blood pressure related to hypothesis 3, the variables of age, gender, waist circumference and salivary cotinine explained a significant amount of variance. This finding also adds support to the use of the expanded biobehavioral model as it highlights the use of variables from the individual, behavioral and environmental domains in understanding the health outcome of blood pressure.

Hypothesis 4 stated that salivary C-reactive protein mediates the relationship between tobacco exposure and blood pressure in this sample of rural adolescents, ages
In the context of the expanded biobehavioral model, increased levels of salivary cotinine resulting from individual tobacco use and sources of secondhand smoke exposure were expected to instigate increased levels of C-reactive protein in the physiological domain, acting as a mediator and affect the health outcome of blood pressure.

No support was found for the role of C-reactive protein as a mediator in the physiological domain. However, future research may need to focus on (1) recruiting a greater number of adolescent smokers, (2) the duration of the adolescent’s smoking behavior and (3) a small effect size to capture the significance of the relationship between salivary cotinine and salivary C-reactive protein. An alternative biomarker for systemic inflammation such as particulate matter related to cigarette smoking (Butz et al., 2011) may also be explored as a mediator between tobacco exposure and blood pressure.

Study Limitations

As with any research endeavor, there were several research design and method limitations in this study to be acknowledged and discussed, including the use of one-time data collection, self-report measures, low response rate, recruitment of tobacco users and a regional, convenience sample. To begin with, the one-time data collection provides only a snapshot description of the variables of interest. This is particularly relevant for blood pressure as the outcome variable. Ideally, blood pressure measurements would be collected in a prospective manner. The Fourth Report on Diagnosis Treatment and Evaluation of High Blood Pressure in Children and Adolescents recommends that blood pressure measurements be taken on three or more occasions and be categorized using
gender, age, and height to accurately determine the presence of hypertension (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Single blood pressure measurements tend to overestimate the prevalence of hypertension (Falkner, et al., 2008). However in the study of blood pressure among youth, single blood pressure measurements have value. Falkner and colleagues (2008) found that single prehypertensive blood pressure measurements were predictive of hypertension in adolescents. With these single blood pressure measurements, the rate of progression from prehypertension to hypertension in adolescents was determined to be approximately 7% per year (Falkner, et al., 2008).

Due to the cross-sectional nature of this data, no causal inferences can be made between the study variables in this study. A number of criteria must be met before causal inferences can be made. These criteria include the establishment of a temporal, empirical relationship between variables (Polit & Beck, 2008). A prospective, longitudinal study would provide the temporal aspect necessary to help establish cause; however longitudinal studies require enhanced access to participants, more time and funding to be successfully implemented and completed.

With regard to study limitations, the use of self-report measures has some inherent difficulties. Self-report data can raise reliability concerns due to poor memory recall. With this adolescent sample, poor memory recall could be a contributor to the lack of knowledge regarding parental history of hypertension. The accuracy of offspring reports regarding the presence of hypertension in parents was studied with adult participants \( n = 791 \) male and 837 female in the Framingham Offspring Study, a multigenerational, population-based study (Murabito et al., 2004). Researchers found that
offspring self report of parental high blood pressure had a low negative predictive value of 53-55\% (Murabito, et al., 2004). When reporting the presence of parental hypertension, adolescents ages 11-15 had low accuracy compared to their accuracy in reporting parental history of heart attacks, stroke and diabetes (Hastrup, Phillips, Vullo, Kang, & Slomka, 1992). The researchers postulated that the less obvious self-care measures and the fewer hospitalizations directly attributed to hypertension (as compared to heart attack, cancer or stroke) could explain adolescents’ low accuracy in reporting parental hypertension (Hastrup, et al., 1992). Future research may require the involvement of parents for more accurate data collection on the variable of parental history of hypertension. This lack of knowledge may also be viewed as an opportunity to raise awareness among youth regarding the existence of hypertension in their family’s health history.

There are unique challenges with the use of self-report measures specific to youth tobacco research. The recruitment of tobacco users can be influenced by the legality issues related to underage tobacco use; an adolescent can have concerns related to anonymity and confidentiality with regard to tobacco use and exposure questionnaires (Dolcini, et al., 1996). The principal investigator strove to allay these concerns during the recruitment and informed consent process by verbally stating a strong commitment to upholding confidentiality throughout the research process.

In addition to confidentiality concerns, the clarity of questions and the specified timeframe of tobacco use can contribute to inefficient use of self-report measures in youth tobacco research (Dolcini, et al., 1996). Adolescents may be sporadic users of tobacco and, therefore, the timeframe specified in research questions may not capture the tobacco
use (Dolcini, et al., 1996). To offset these concerns, the *Uptake Continuum* measure was used to categorize smoking behavior among adolescents into seven possible categories of experimentation and established use. It is important to note that this measure has been validated with a large sample of adolescents (Choi, et al., 2001). Both self-report measures and biochemical verification (via the use of cotinine as a salivary biomarker) to determine tobacco exposure were used to ensure a measure of validity in this study.

Future research should strongly consider the use of both self-report smoking status and salivary cotinine when studying adolescent tobacco use; these subjective and objective measures complement each other and provide a clearer understanding of tobacco use and exposure among adolescents.

The generalizability of these study findings is limited by 1) the regional, convenience sample and 2) the low prevalence of adolescent smokers. The study sample was recruited from two high schools in one rural, Midwestern county; generalizing the findings beyond this setting must be done with caution. Future research efforts should involve a national multisite, rural school study to improve our understanding of tobacco exposure and blood pressure for rural adolescents across the nation.

Only 15 adolescent participants in this study had salivary cotinine levels indicative of regular smoking behavior; this equated to approximately ten percent of the sample. This prevalence is low and does not represent the adolescent smoking rates in the Midwest. The 2011 adolescent smoking rate related to current use in the state of Indiana was 18.1% and 24.1% in the near-neighboring state of Kentucky (Centers for Disease Control and Prevention, 1991-2011). The low prevalence of regular smoking behavior in this study sample reduces the generalizability of the study findings.
Future research efforts will need to explore recruitment techniques used to engage more adolescents with established smoking behaviors. One strategy mentioned in the literature to achieve enhanced enrollment of adolescent smokers involves a more active role for school counselors and administrators in identifying adolescent smokers in a school setting (Kealey et al., 2007). Implementing this recruitment technique would require a principal investigator to develop and maintain strong rapport with their recruitment site. An implied consent protocol, which asks parents to opt-out if they wish to exclude their adolescent’s participation, has been advocated for by some researchers in the field (Diviak, et al., 2006). This opt-out for parental consent eliminates any concern an adolescent may have with disclosing their smoking status to their parents and this technique may be more appropriate for tobacco research involving older adolescents.

Because this study required a biological specimen from each adolescent participant in the form of saliva, written parental consent was more appropriate. Both the parents and the adolescent participants in this study were informed that biological specimens were collected, stored for 30 days and then destroyed.

Finally, limitations regarding the use of salivary C-reactive protein as an emerging biomarker for systemic inflammation must be discussed. Literature supporting the use of salivary C-reactive protein is just beginning to emerge (Dillon, et al., 2010; Labat, et al., 2013; Ouellet-Morin, et al., 2010) particularly with regard to the adolescent population (Azar & Richard, 2011). A larger sample size or the use of additional exclusion criteria may be necessary when using salivary C-reactive protein as a biomarker of systemic inflammation. The principal investigator screened participants for recent infection with one question on the demographic questionnaire. Additional
exclusion criteria should be considered in future research and could include screening for oral contraceptive use (Juonala, et al., 2006), presence of peridontal disease (Azar & Richard, 2011), and disease processes such as diabetes, cystic fibrosis and irritable bowel syndrome (O'Loughlin, et al., 2008). Stricter exclusion criteria are needed for its effective use of salivary C-reactive protein in research involving youth. Further research on the validity of salivary C-reactive protein is needed as its use has great potential for school-based research.

Implications

Tobacco exposure and elevated blood pressure are important contributors to cardiovascular disease risk among adolescents (Kavey, et al., 2003; United States Department of Health and Human Services, 2010c). Previous research has reported single measurement blood pressure elevations in approximately 20% of adolescent males and 13% of adolescent females (Falkner, et al., 2008). This study focused on rural adolescents found prehypertensive systolic blood pressures among 13.3% of males and 5.7% of females and hypertensive blood pressures among 11.7% of males and 5.7% of females with the use of single blood pressure measurements. Although the rural adolescents from this study appear to have a lower prevalence of blood pressure elevations, research using longitudinal data and systematic review has provided evidence that blood pressure elevations in childhood track through adolescence and into adulthood (Bao, et al., 1995; Chen & Wang, 2008; Toschke, et al., 2009). In addition, prehypertensive blood pressure measurements are predictive of hypertension and the progression from prehypertensive to hypertensive blood pressure measurements in adolescents is approximately 7% per year.
An elevated blood pressure measurement, accompanied by other cardiovascular risk factors, is notable and cause for concern among adolescents. Rural adolescents experience a spectrum of significant risk behaviors (Curtis, et al., 2011) and rural adolescents, in particular, experience tobacco-related disparities nationwide (American Legacy Foundation, 2009; Lutfiyya, et al., 2008; Vander Weg, et al., 2011). Even with the higher socioeconomic status found in this study sample, 40% of participating adolescents identified themselves as ever smokers, having experimented with at least one puff of tobacco smoke. Approximately 22% of the sample had salivary cotinine levels indicative of secondhand smoke exposure; of this 22%, ten percent had salivary cotinine levels indicative of regular smoking behavior. Nearly 50% of adolescents reported having a family member who smokes, including parents, stepparents, guardians, and/or siblings. Although the United States (US) has made great strides in reducing secondhand smoke exposure through comprehensive smoke free legislation (American Lung Association, 2013; King, Dube, & Homa, 2013), these statistics attest to the continued presence and influence of tobacco in the lives of these rural adolescents.

In this study, smoking behavior among parents and/or siblings, as reported by the adolescent, had a significant relationship to increased salivary cotinine levels for rural adolescents. Interventions tailored towards (1) improving smoking restrictions in the home environment and (2) smoking cessation programs directed toward family members would positively impact the tobacco exposure experienced by rural adolescents. For example, role conflict can be experienced by parents who are smokers; researchers have found this increased role conflict can serve as a motivation to quit smoking (Friebely et
In addition to this motivation, it is conceivable that adolescents could serve as advocates for smoking cessation among their parents if the relationship allows for open and supportive communication.

In this study, the strongest predictor of elevated blood pressure was weight status (BMI, waist circumference). Evidence supported that tobacco exposure influence blood pressure in this adolescent population, but to a smaller degree compared to weight status. The promotion of optimal cardiovascular health behaviors early in life is of utmost importance and this includes the prevention of both tobacco exposure and elevated blood pressures among adolescents (Shay et al., 2013). Improvements in the cardiovascular health of adolescents in both rural and urban communities will require “[a] broad social and cultural shift in the definition of normative childhood behavior toward frequent physically active play, healthy food choices, and abstinence from tobacco use…” (Shay, et al., 2013, p. 1373). This shift will require the support of all adults present in the lives of adolescents including parents, teachers, healthcare providers as well as the influence of media, industry and government (Shay, et al., 2013).

No evidence was found regarding the role of inflammation as a mediator between tobacco exposure and blood pressure. However, the use of salivary C-reactive protein may require more stringent exclusion criteria or a smaller effect size. Another consideration for future research may be the use of an alternative measure related to inflammation. An environmentally-focused study on asthma symptoms and secondhand smoke exposure among youth ages 6-12 years old found evidence “suggesting that the nonnicotine particle-bound components of tobacco smoke are the major contributors to increased risk of systemic inflammatory diseases.” (Butz, et al., 2011). A burning
cigarette releases fine particulate matter (PM$_{2.5}$) that is easily inhaled into an individual’s lungs (Van Deusen et al., 2009, p. 635) and appears to be responsible for a number of negative health outcomes including lung cancer. Inflammation is thought to be the mechanism by which fine particulate matter causes negative health outcomes (Li, Rittenhouse-Olson, Scheider, & Mu, 2012). Future research regarding tobacco exposure and blood pressure should explore inflammation by way of fine particulate matter.

These findings also provide support for health policy related to “tobacco free living” (National Prevention Council, 2011, p. 28). The National Prevention Strategy developed by the Office of the Surgeon General (2011) has put forth four recommendations to promote tobacco free living including (1) the support of comprehensive tobacco free legislation, (2) the full implementation of the 2009 Family Smoking Prevention and Tobacco Control Act, (3) the expansion of tobacco cessation services and (4) the use of media to educate and encourage tobacco free living. These recommendations are evidence-based and serve to prevent youth from using tobacco and to reduce exposure to secondhand smoke for all. The implementation of comprehensive tobacco free legislation has been linked to increased voluntary smoke-free home rules in homes with smokers (OR 7.76, 95% CI 5.27, 11.43) as well as homes without smokers (OR 4.12, 95% CI 3.28, 5.16) (Cheng, Glantz, & Lightwood, 2011).

This study provides evidence that rural adolescents continue to experiment with tobacco products (40% self-reported ever smokers); continue to be exposed to secondhand smoke (22% elevated salivary cotinine levels) as confirmed by the objective measure of salivary cotinine; and that this tobacco exposure can impact adolescent blood pressure. Although great strides have been made to reduce the impact of tobacco across
the United States, tobacco-related health policy, including comprehensive tobacco free legislation, is needed to protect the health of rural adolescents.

Summary

This study examined the influence of tobacco exposure on blood pressure in a sample of rural adolescents ages 15-18 after controlling for the variables of age, gender, parental history of hypertension, weight status, pubertal stage, and socioeconomic status. In this relationship between tobacco exposure and blood pressure, a possible mediating role of C-reactive protein was also explored. The study found that 13.3% of males and 5.7% of females had prehypertensive systolic blood pressures and, additionally, 11.7% of males and 5.7% of females had hypertensive systolic blood pressure measurements. A fifth of the sample had elevated salivary cotinine levels indicative of secondhand smoke exposure and/or regular smoking behavior. Nearly half of rural adolescents stated that their parents, stepparents, guardians, and/or siblings were smokers. Both waist circumference and tobacco exposure were found to contribute to elevated blood pressure but tobacco appears to contribute to a smaller degree. No evidence was found regarding salivary C-reactive protein as a mediator between tobacco exposure and blood pressure.

Due to higher prevalence rates of tobacco use and obesity, the potential for an increased chronic disease burden in rural communities is of great concern (Institute of Medicine, 2005). The health of rural adolescents, in particular, requires greater attention from the research community. There is a great need to “[make] adolescents and their health visible” (Sawyer, et al., 2012, p. 1637). This is particularly true for adolescents
living in rural communities all across the United States; their future cardiovascular health depends on it.
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Appendix A

Institutional Review Board Approval

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator: HUNTINGTON-MOSKOS, LUZ G
Co-Investigator(s):
Protocol Number: X120305020
Protocol Title: Blood Pressure and Tobacco Exposures Among Rural Adolescents: A Pilot Study

The IRB reviewed and approved the above named project on 4-15-13. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.
IRB Approval Date: 4-15-13
Date IRB Approval Issued: 4-15-13

Marilyn Doss, M.A.
Vice Chair of the Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.
Appendix B

Informed Consent Document


IRB PROTOCOL: X120305020

INVESTIGATOR: Luz Huntington-Moskos, MS, RN, CPN; Anne Turner-Henson, DSN, RN, FAAN (faculty mentor)

SPONSOR: The University of Alabama at Birmingham School of Nursing

For Children/Minors (persons under 19 years of age) participating in this study, the term You addresses both the participant ("you") and the parent or legally authorized representative ("your child").

Explanation of Procedures

You are being asked to participate in a study. This research study is designed to examine the effects of tobacco exposures on blood pressure in adolescents, ages 15 to 18 years. This study will enroll 150 participants from regional high schools, who are in the 9th through 12th grades; 15, 16, 17, 18 years of age; and capable of completing the surveys. The first 150 participants who are eligible will be enrolled in this study.

If you are willing to participate in this study, please read and sign this consent form.

All data collection will occur at the school, during school hours. All information will be collected during a particular class on one school day. It will take approximately 20 minutes to get all of this information. By participating, you will not miss class information or schoolwork. You will be asked to complete 4 short questionnaires; provide samples of saliva (spit); and have your height, weight, waist circumference and blood pressure measured.

The saliva (spit) will be used to measure your cotinine level (cotinine is a broken down version of nicotine) and your C-reactive protein level (a measure of inflammation related to blood pressure). This saliva collection and the measurement of your height, weight, and waist circumference will be conducted in a private room or behind a screen.

You will be asked to complete 4 questionnaires: 1) a demographic information form 2) a questionnaire regarding any smoking behavior that may be present 3) a questionnaire asking the number (no names) of friends or family members that you know who smoke and 4) a questionnaire that asks general questions about
your physical growth and pubertal development. You will be asked to complete this set of study questionnaires that should take about 10 minutes.

**Risks and Discomforts**

You may possibly experience some psychological discomfort or potential embarrassment related to being weighed, measured for height and waist circumference, or during the completion of the pubertal development, and tobacco exposure-related questionnaires; however, the risk involved is minimal. Significant efforts will be made to limit the potential for other students’ knowledge of your weight, height, or waist circumference through the use of privacy screens. If you become upset or anxious during any parts of the data collection procedures, the data collection procedures will be stopped. You will be informed that you can stop at any time and you do not have to answer all the questions. With the consent of the participant, the school counselor may be notified as per school policy. If you are found to have an elevated blood pressure reading, the school nurse will be notified and the elevated blood pressure will be documented and follow-up arranged per school policy. A letter will be sent home to notify your parents of the elevated blood pressure reading. It is important to note that one elevated blood pressure reading alone does not indicate a condition of high blood pressure.

**Benefits**

You may not benefit directly from participation in this research. However, findings from this study will assist health professionals in developing better programs for promoting the health of adolescents, particularly in rural areas. Through the data collection completed in this study, you will be aware of your own blood pressure measurement.

**Alternatives**

The alternative is for you not to participate in the study.

**Confidentiality**

Information obtained for this study will be kept confidential to the extent allowed by law. The research results may be published for scientific purposes; however, your identity will not be revealed in any way by name. Research information that identifies you may be shared for ensuring compliance with the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB) and others who are responsible for ensuring compliance with laws and regulations related to research, including the Office for Human Research Protections (OHRP). In order to minimize the risk of a breach in confidentiality, all questionnaires will be coded with unique study code numbers. The study data will be stored on a password-protected, encrypted jump drive and locked in a cabinet that will be accessible only to the principal investigator of this study.

**Refusal or Withdrawal without Penalty**

You are taking part in this study by choice. There will be no penalty if you decide not to take part in the study. You may choose not to be in the study, or you may withdraw (stop) the study at any time before it is over. This will not affect your class standing or class grades. If you would like to withdraw, please notify Ms. Huntington-Moskos.
Cost of Participation
There will be no cost to you for your participation in this study.

Payment for Participation in Research
The adolescent participant will receive a small incentive for participating in the study. This incentive will be a gift card to Wal-Mart. The incentive is valued at $10.00. This incentive will be given to the adolescent participant once they have completed all physiological measures (saliva collection, blood pressure, height, weight and waist circumference) and the four short questionnaires.

Questions
If you have any questions, concerns, or complaints about the research, please contact Ms. Luz Huntington-Moskos, MS, RN, CPN at (812) 719-2042. She will be glad to answer any of your questions. You may also contact the supervising faculty member associated with this study, Anne Turner-Henson DSN, RN, FAAN at (205) 934-7533. If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the Office of the IRB (OIRB) at (205) 934-3789 or 1-800-822-8816. If calling the toll-free number, press the option for “all other calls” or for an operator/attendant and ask for extension 4-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday. You may also call this number in the event the research staff cannot be reached or you wish to talk to someone else.

Legal Rights
You are not waiving any of your legal rights by signing this informed consent document.

Signatures
You are making a decision whether or not to have your adolescent participate in this study. Your signature indicates that you have read (or been read) the information provided above and decided to allow your adolescent to participate. You will receive a copy of this signed informed consent document.

Signature of Parent or Guardian
Date

Signature of Participant (age 15-18)
Date

Signature of Principal Investigator
Date
Appendix C

Demographic Questionnaire

Blood Pressure and Tobacco Exposures among Rural Adolescents

Thank you for taking the time to complete this questionnaire. The information that you provide is important to this study. This particular questionnaire should only take about 5 minutes of your time. Please remember your answers will be confidential (private).

1. Age
   a. 15    b. 16    c. 17    d. 18

2. Grade
   a. 9th    b. 10th    c. 11th    d. 12th

3. Gender
   a. Female
   b. Male

4. Race
   a. Caucasian/White
   b. African American/Black
   c. Asian/Pacific Islander
   d. Multiracial/Other

5. Ethnicity
   a. Latino/Hispanic
   b. Non-Latino/Non-Hispanic

6. What type of home do you live in?
   a. House
   b. Apartment
   c. Mobile Home/Trailer
7. Do either of your parents have high blood pressure?
   a. Mother
   b. Father
   c. Both
   d. Neither. My parents do not have high blood pressure.
   e. I don’t know

8. Do you receive free or reduced lunch at school?
   a. Free
   b. Reduced
   c. No, I do not receive free or reduced lunch at school.

9. Are you currently pregnant?
   a. Yes
   b. No

10. Do you use smokeless tobacco products also known as chewing tobacco, spit tobacco, “chew”, “chaw”, “dip”?
    a. Yes
    b. No

11. Are you currently using Nicotine Replacement Therapy, such as nicotine patches, gum or lozenges?
    a. Yes
    b. No

12. Are you currently on antibiotics for a recent infection, such as strep throat or a sinus infection, etc.?
    a. Yes
    b. No

Thank You!
Appendix D

Protocols for Physiological Measurements

Protocol for Measurement of Height

**Equipment:**

Stadiometer

Data collection sheet

1. Inform the adolescent that you will be measuring his/her height.

2. Request that the adolescent take off his/her shoes.

3. The child should stand at a right angle to the vertical rod of the stadiometer.

4. The adolescent should stand with his/her weight evenly distributed between both feet, and the arms hanging by the sides with the palms facing the thighs. The heels are together, touching the vertical board of the stadiometer. The feet are spread at a 60-degree angle to each other.

5. Inform the adolescent that his/her head, scapula, and buttocks touching the vertical board.

6. Request that the head be erect with eyes focused straight ahead.

7. Tell the adolescent to take a deep breath and then lower the horizontal board of the stadiometer to the most superior point on the head, compressing the hair.

8. Measure the height to the nearest 1/4 inch.

9. Record the height in the space on the data collection form.


Adapted from the LITTLE PASS program, Dr. Marti Rice, PI, The University of Alabama at Birmingham.
Protocol for Measurement of Height

**Equipment:**

Electronic digital scale

Data collection sheet

1. Be sure that the scales are calibrated to zero before the adolescent stands on the scale.

2. Ask one adolescent at a time to come to the private area.

3. Ask the adolescent to remove his/her shoes.

4. Ask the adolescent to step up on the scale once the digital display shows all zeros.

5. Ask the adolescent to stand so that the body weight is evenly distributed between the feet.

6. Record weight to the nearest 1/4 of a lb. on the data collection sheet.


Adapted from the LITTLE PASS program, Dr. Marti Rice, PI, The University of Alabama at Birmingham.
Protocol for Measurement of Waist Circumference

Equipment:

Tape measure
Marking pen

Data collection sheet Privacy Screen

1. Tell the adolescent that you are going to measure his/her waist.

2. Be sure that the waist is measured in a private area so that only the participant and data collector(s) are present.

3. Have the adolescent raise his/her shirt or blouse.

4. In order to determine the level at which the waist circumference is measured, the data collector must stand to the right of the participant and palpates the upper hip bone to locate the right iliac crest.

5. Just above the uppermost lateral border of the right iliac crest, a horizontal mark is drawn, then crossed with a vertical mark on the midaxillary line.

6. The measuring tape is placed in the horizontal plane around the abdomen at the level of this marked point on the right side of the trunk.

7. The plane of the tape is parallel to the floor and the tape is snug, but does not compress the skin.

8. Tell the adolescent to breathe normally and measure the circumference to the nearest 1/16 inch.

9. Record the measurement in the appropriate boxes on the data collection sheet.


Adapted from the LITTLE PASS program, Dr. Marti Rice, PI, The University of Alabama at Birmingham.
Protocol for Collection of Saliva

Equipment:

Cryovial
Straw
Cooler with ice for specimens

1. When the participant comes into the data collection room, have a small cup of water available for each participant to rinse mouth. The water may be swallowed or spit into a garbage-bag-lined trash can.

2. For each participant, pass out the pre-labeled tubes with the straw inside and the rubber band wrapped around the outside of the tube. Give each adolescent a tissue to use since saliva can be ropey and it can be hard to finish with a string of saliva coming from the mouth.

3. Instruct the participant to take the cap off the tube and put it down in front of them. Explain that the straw provided needs to remain inside the tube but may be pulled out slightly. Explain that saliva is what is produced in the mouth and does not come from clearing the throat or coughing up sputum.

4. Have the participants drool into the straw in the saliva specimen tube. The data collector will start the time. Instruct the adolescent to continue to drool until cryovial is ¾ full. Inform the adolescent that they may stop the timer when this volume is acquired.

5. Once complete, have the adolescent take the straw out of the tube and put the cap back on tube. The data collector will collect each tube and put it in the specimen box. The data collector will need to push down firmly on the cap so the cap does not come off causing leakage.

6. Once all the tubes have been collected, label the box and put the box into the cooler and put the chemical ice packs around the box.

7. Take the specimen box to the lab and put on the assigned shelf in the freezer. Store specimens in -20°C freezer until samples ready to be shipped.


Adapted from the PASS program, Dr. Marti Rice, PI, The University of Alabama at Birmingham. The data collector should use a cuff bladder width that is approximately 40% of the circumference of the arm measured at the point midway between the olecranon a
Protocol for Blood Pressure Measurement

Equipment:

Oscillometric blood pressure device

Selection of arm cuffs

Chair

1. The participant should not have ingested caffeine (or nicotine) for 30 minutes prior to measurement.

2. Inform the adolescent that the arm cuff will squeeze for a short period of time during the blood pressure measurement.

3. The participant should rest for 5 minutes in a chair, with feet on the floor, back supported and right arm supported at heart level before measurement.

4. The Dinamap should be calibrated before use and numbers on the screen should all be zero.

5. The data collector should use the appropriate cuff size for the patient; the cuff bladder length should encircle at least 80% of the circumference of the arm between the acromion (lateral, triangular projection of the scapula, forming the point of the shoulder) and olecranon (bony prominence of the elbow) on the upper right arm.

6. The data collector should use a cuff bladder width that is approximately 40% of the circumference of the arm measured at the point midway between the olecranon and acromion.

7. With the arm supported at heart level, place the blood pressure cuff so that the cuff is located over the artery on the right arm just above the bend of the elbow.

8. Press the start button on the Dinamap machine.

9. Wait until the numbers remain on the screen and the indicator sounds. Record the blood pressure measurement on the data collection form.

10. Wait 2 minutes. Do not remove the cuff.

11. The numbers will zero out. Press the start button and wait until the numbers remain on the screen.
12. Record the blood pressure reading on the data collection sheet.

13. Record the higher of the two blood pressure readings on the sheet for the parent and/or guardian on the blood pressure form.


Adapted from the PASS program, Dr. Marti Rice, PI, The University of Alabama at Birmingham.
Appendix E

Self-Administered Rating Scale for Pubertal Development

Blood Pressure and Tobacco Exposures among Rural Adolescents

Thank you for taking the time to complete this questionnaire. The information that you provide is important to this study. This particular questionnaire should only take about 5 minutes of your time. Please remember your answers will be confidential (private).

1. Would you say that your growth in height...
   
   a. has not yet begun to spurt
   b. has barely started
   c. is definitely underway
   d. seems completed
   e. I don’t know.

2. How about the growth of your body hair? Would you say that your body hair growth...
   
   a. has not yet begun to grow
   b. has barely started to grow
   c. is definitely underway
   d. seems completed
   e. I don’t know.

3. Have you noticed any skin changes, especially pimples?
   
   a. skin has not yet started changing
   b. skin has barely started changing
   c. skin changes are definitely underway
   d. skin changes seem completed
   e. I don’t know.

FOR MALE PARTICIPANTS (BOYS):

4. Have you noticed a deepening of your voice?
a. voice has not yet started changing  
b. voice has barely started changing  
c. voice changes are definitely underway  
d. voice changes seem complete  
e. I don’t know.

5. Have you begun to grow hair on your face?

a. facial hair has not yet started growing  
b. facial hair has barely started growing  
c. facial hair has definitely started  
d. facial hair growth seems complete  
e. I don’t know.

FOR FEMALE PARTICIPANTS (GIRLS):

6. Have you noticed that your breasts have begun to grow?

a. have not yet started growing  
b. have barely started growing  
c. breast growth is definitely underway  
d. breast growth seems complete  
e. I don’t know.

7. Have you begun to menstruate (started to have your period)?

a. Yes  
b. No  

8. If yes, how old were you when you started to menstruate?

AGE________

Thank You!
Appendix F

Smoking Uptake Continuum

Blood Pressure and Tobacco Exposures among Rural Adolescents

Thank you for taking the time to complete this questionnaire. The information that you provide is important to this study. This particular questionnaire should only take about 5 minutes of your time. Please remember your answers will be confidential (private).

1. Do you think you will smoke a cigarette in the next year? Would you say...
   a. Definitely yes
   b. Probably yes
   c. Probably not
   d. I don’t know

2. Do you think that in the future you might experiment with cigarettes?
   a. Definitely yes
   b. Probably yes
   c. Probably not
   d. I don’t know

3. If one of your best friends were to offer you a cigarette, would you smoke it? Would you say...
   a. Definitely yes
   b. Probably yes
   c. Probably not
   d. I don’t know

4. Have you ever smoked a cigarette?
   a. Yes
   b. No
   c. I don’t know

5. Have you ever tried or experimented with cigarette smoking, even a few puffs?
   a. Yes
   b. No
   c. I don’t know
6. How old were you when you smoked your first whole cigarette?  
   a. Age________  
   b. I never have smoked a whole cigarette.  
   c. I don’t know  

7. Have you smoked at least 100 cigarettes in your life?  
   a. Yes  
   b. No  
   c. I don’t know  

8. Have you ever smoked a cigarette every day for at least a month?  
   a. Yes  
   b. No  
   c. I don’t know  

9. How old were you when you started smoking regularly?  
   a. Age________  
   b. I am not a regular smoker.  
   c. I don’t know  

10. Think about the last 30 days. On how many of these days did you smoke?  
   a. Number of Days_______  
   b. None  
   c. All  
   d. I do not smoke  
   e. I don’t know  

11. Was it more or less than 15 days?  
   a. Exactly 15 days  
   b. Less than 15 days  
   c. More than 15 days  
   d. I do not smoke  
   e. I don’t know  

12. Was it more or less than 10 days?  
   a. Exactly 10 days  
   b. Less than 10 days  
   c. More than 10 days  
   d. I do not smoke  
   e. I don’t know
13. Was it more or less than 20 days?
   a. Exactly 20 days
   b. Less than 20 days
   c. More than 20 days
   d. I do not smoke
   e. I don’t know

Thank You!
Appendix G

Peer and Family Smoking

Blood Pressure and Tobacco Exposures among Rural Adolescents

Thank you for taking the time to complete this questionnaire. The information that you provide is important to this study. This particular questionnaire should only take about 5 minutes of your time. Please remember your answers will be confidential (private).

1. Do any of your parents, step-parents, or guardians now smoke cigarettes?
   a. Yes
   b. No
   c. I don’t know

2. Do you have any brothers or sisters?
   a. Yes
   b. No
   c. I don’t know

3. Do your brothers or sisters smoke cigarettes?
   a. Yes
   b. No
   c. I don’t know

4. Of your best friends who are male, how many of them smoke?
   a. Number______
   b. None of my male friends smoke.
   c. I don’t know

5. Of your best friends who are female, how many of them smoke?
   a. Number______
   b. None of my female friends smoke.
   c. I don’t know

Thank You!
Appendix H

Parent Letter

Dear Parent or Guardian,

Your adolescent has been invited to participate in a research study, taking place at your local high school. The study will evaluate the effects of tobacco exposures on blood pressure in adolescents, ages 15 to 18 years. The goal of this study is to better understand if our rural adolescents are at greater risk for exposure to tobacco and high blood pressures compared to youth who live in large cities and urban centers. This study will enroll a total of 150 adolescents who are interested in participating. These adolescents must be in the 9th through 12th grades and must be 15-18 years old. The first 150 adolescents who turn in a completed informed consent document with their parent/guardian signature will be enrolled in this study.

The procedures for this study are described within the enclosed informed consent document. The informed consent document will be reviewed individually with each adolescent interested in participating. If an adolescent wants to participate in this study, the informed consent document must be completed. If you support your child’s participation in this study, please sign the informed consent document on the line for the parent/guardian signature and write in your adolescent’s name on the HIPAA Authorization on final page of the consent. Once the informed consent document is signed and sealed in the enclosed envelope, please return the document to the designated, sealed box in the high school main office.

It is important to note that any adolescent involved in this study may choose to stop participating at any time. This will not affect their class standing or grades. Please note that once the participating adolescent has completed the entire data collection (including saliva collection, blood pressure, height, weight, waist circumference and four short questionnaires), they will receive a $10 gift card from Wal-Mart.

I want to thank you in advance for your time and consideration. If you would like to review the study materials firsthand, a binder containing all study questionnaires is available to you in the high school main office. Please feel free to contact me with any questions or concerns regarding this study. I may be easily reached at (XXX) XXX-XXXX or via email at XXXX@uab.edu. As a resident of XXXX County since 2003, I am pleased to have the opportunity to work with youth in southern Indiana. It is my hope that this study will ultimately contribute to improving health in our region.

Sincerely,

Luz Huntington-Moskos, MS, RN, CPN
Appendix I
Follow-up Letter

Dear Parent or Guardian,

Your adolescent participated in a recent study at Perry Central High School conducted to learn more about tobacco exposures and blood pressure in adolescents, ages 15 to 18 years. With the help of 148 adolescent participants, I am pleased to report that the study data collection was successfully completed.

I have made an initial review of the blood pressure readings collected during this study. I am writing this letter to inform you that your adolescent had a blood pressure reading that was not within normal limits. Please see the information below:

<table>
<thead>
<tr>
<th>Student Name</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
</tr>
<tr>
<td>1st Blood Pressure Reading</td>
<td></td>
</tr>
<tr>
<td>2nd Blood Pressure Reading</td>
<td></td>
</tr>
<tr>
<td>Date Taken</td>
<td></td>
</tr>
</tbody>
</table>

With this letter, I have enclosed a pocket guide on blood pressure measurement from the National Institute of Health. Please take a moment to look over the blood pressure classifications and the recommendations for follow up presented. Please review this information and feel free to contact me with any questions or concerns. I may be easily reached at (XXX) XXX-XXXX or via email at XXXX@uab.edu. I would like to encourage you to share these blood pressure readings with your adolescent’s health care provider at their next health-related visit.

Thank you,

Luz Huntington-Moskos, MS, RN, CPN