Early Pubertal Development and Cardiometabolic Risk: a Life Course Approach

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Dedication

This thesis is dedicated to Todd, Anna, and Nate for their unwavering support and encouragement.

Abstract

A number of reports have linked early pubertal development to adverse health conditions among women. Despite race disparities in pubertal timing and later cardiovascular disease (CVD)-related health in adulthood, few studies have investigated if associations of early maturation with cardiometabolic risk factors differ for African-American and white women. Furthermore, there are limited longitudinal data tracking the trajectory of change in cardiometabolic risk factors after puberty and into adolescence and adulthood. The three primary aims of this thesis were to 1) examine the relationship between earlier age at menarche and CVD risk factors at different points in the life course, 2) account for the role of adiposity in associations, and 3) explore possible race differences in associations. These aims were explored using data from three prospective cohort studies that included different age groups: the National Growth and Health Study (NGHS) for ages 9-19 years, the Coronary Artery Risk Development in Young Adults (CARDIA) study for ages 18-54 years, and the Atherosclerosis Risk in Communities (ARIC) study for ages 45-75 years. The results from the three manuscripts included in this thesis suggested that earlier age at menarche was associated with a more adverse cardiometabolic profile over the life course, but higher lifetime accumulated adiposity among early maturing women explained most associations. Earlier menarche was more strongly related to adulthood adiposity and metabolic syndrome among white compared with African-American women, but there were no other differences by race. Targeted efforts to prevent

obesity starting before puberty, as well as among early maturing girls even if normal weight, might be helpful for primordial prevention of cardiovascular disease.

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Chapter 1: Introduction and Specific Aims

A number of reports have linked early pubertal development to long-term health, including cardiometabolic disorders such as obesity, type 2 diabetes, and cardiovascular disease [3, 8-12]. At the same time, there is a documented trend toward earlier maturation among girls in developed countries, most dramatically for African-American women, which may have important implications for women's health [13-15]. Racial differences in pubertal timing coincide with well-documented health disparities in the prevalence of cardiometabolic disorders that are associated with obesity [16-19]. Although declines in the average age at menarche correspond with increases in childhood obesity in the United States, it remains unclear if obesity is the sole driving force behind the trend [18, 20]. Independent of adiposity, hormonal changes and hyperinsulinemia associated with early onset of puberty may affect future disease risk [3, 21-23].

A recent National Heart, Lung, and Blood Institute (NHLBI) task force set forth a priority to reduce cardiovascular disease (CVD) risk starting in childhood and adolescence, and they published risk reduction guidelines with the goal of delaying progression to clinical CVD until later in life [24]. The task force noted that ethnic disparities in CVD risk begin in childhood, and they emphasized the importance of prevention of these disparities early in life [25]. Identifying which children and adolescents are most at risk for future metabolic disorders makes it

possible to prevent or delay future CVD through comprehensive programs that address nutrition, physical activity, and prevention of smoking [26].

Despite disparities in pubertal timing and later CVD-related health, few studies have investigated if the cardiometabolic consequences of early maturation differ for African-American and white women. One study showed that the effect of early menarche (age at first menstruation) on accrual of adiposity during adolescence was similar for African-American and white girls, although African-American girls had significantly higher adiposity after age 12 overall [20]. Another study noted that timing of puberty was associated with adulthood adiposity among white but not African-American women after adjusting for childhood adiposity [27]. Our own analysis of ARIC data showed that early age at menarche (8-11 years vs. 13 years) was associated with prevalent diabetes at baseline, and incident diabetes during 9 years of follow-up for white women, but not for African-American women [6]. At baseline, we found a significant (p-value=0.04) race*menarche age interaction for odds of prevalent diabetes. However, there were no differences in age at menarche between African-American and white women in the ARIC cohort, and so relationships might be different in more contemporary, younger cohorts in which the current disparities in pubertal timing are evident. A recent review paper of studies on pubertal timing and CVD and its risk factors noted that although current data are scarce, the balance of evidence is that early puberty is associated with greater risk for CVD

and some of its risk factors, but generalizability beyond populations of European decent is limited because of the lack of ethnic diversity in available studies [28].

This dissertation addresses the NHLBI priorities and gaps in the literature by investigating the role of early age at menarche, which is a discrete and objective marker of reproductive maturation, in cardiometabolic risk in childhood and adulthood among African-American and white women. The primary objectives of this thesis are to 1) comprehensively examine the relationship between earlier age at menarche and a wide variety of inter-related CVD risk factors at different points in the life course, 2) account for the role of adiposity in associations, and 3) to explore possible race differences in associations. The hypotheses are that 1) earlier age at menarche will be associated with a more adverse cardiometabolic profile, 2) lifetime accumulated adiposity will largely explain the associations, and 3) there will be differences by race in the association of earlier menarche with the cardiometabolic risk factors. The goal is to help determine if puberty might be a sensitive time point in the life course for interventions to prevent overt disease in adulthood, as well as race disparities in cardiometabolic diseases. The results may also clarify mechanisms underlying the relationship between early pubertal maturation and cardiometabolic diseases, such as type 2 diabetes.

The objectives of this thesis will be addressed in three distinct manuscripts with the following primary aims.

Manuscript #1: Examine the cardiometabolic consequences of early pubertal development among African-American and white adolescents.

Manuscript #2: Describe race/ethnic specific associations of age at menarche with type 2 diabetes, prediabetes, and metabolic syndrome, as well as with other cardiometabolic risk factors such as lipids, adiposity, insulin, and blood pressure in early- and mid-adulthood.

Manuscript #3: Use a Mendelian randomization approach to explore the causal effect of age at menarche on risk for type 2 diabetes in later adulthood.

Given race disparities in pubertal timing, as well as in adulthood cardiometabolic disorders, Manuscript #1 investigates the longitudinal changes in cardiometabolic risk factors by age at menarche, comparing African-American and white girls using data from the National Growth and Health Study (NGHS). Manuscript #2 describes the association of age at menarche and cardiometabolic risk factors by race among young adults in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Next, because menarche takes place early in the life course, and there are likely many confounding and mediating factors between menarche and onset of chronic diseases in adulthood, a Mendelian randomization approach and Atherosclerosis Risk in Communities (ARIC) Study data will be used to evaluate the association of early menarche and one specific risk factor for CVD, type 2 diabetes, using a genetic risk score (GRS) as a proxy for age at menarche (Manuscript #3). The Mendelian

randomization method helps clarify a causal role of early maturation in risk for type 2 diabetes.

Chapter 2. Background

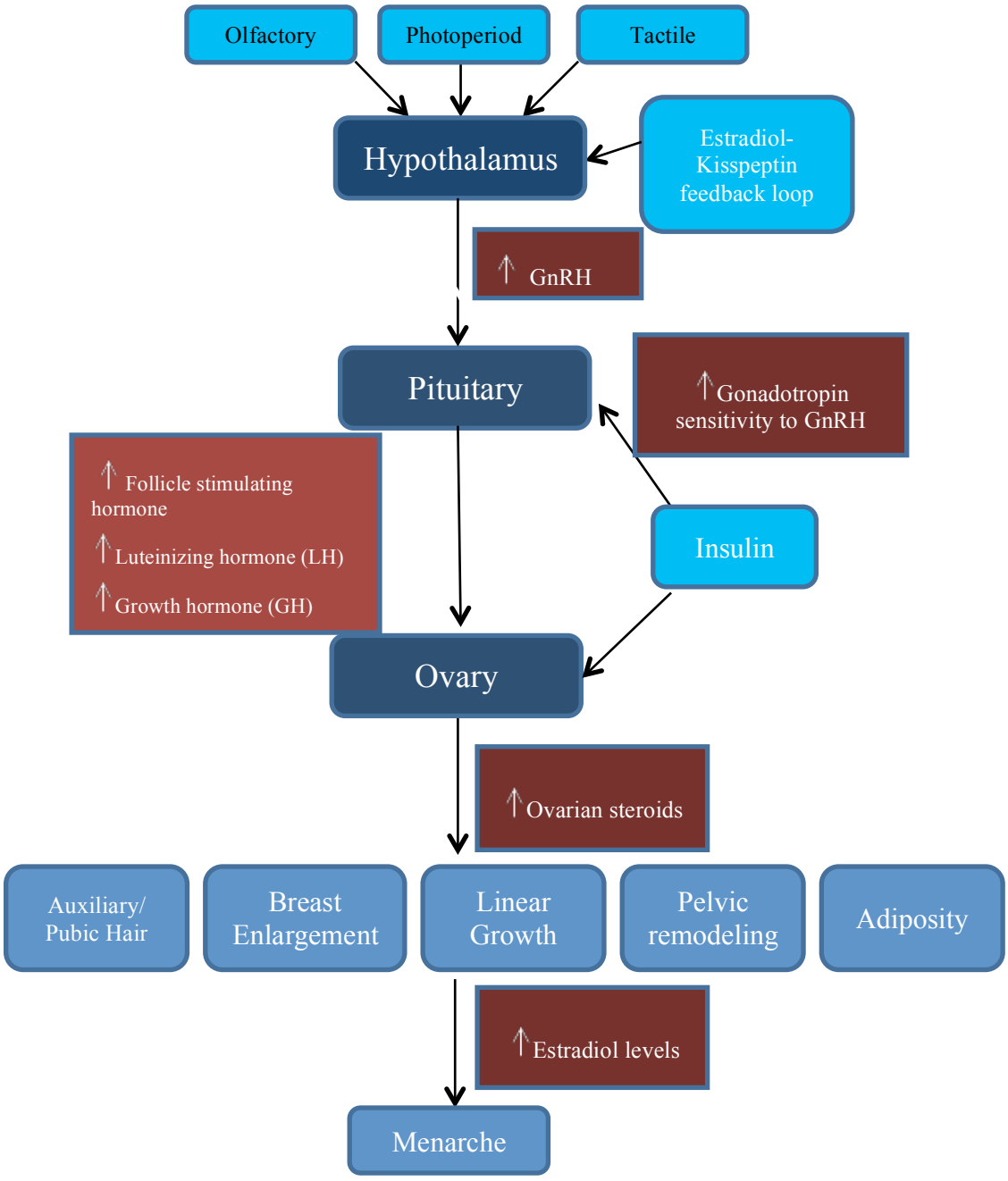
Overview

This chapter begins with a brief summary of the neuroendocrinology, stages, and other correlates of puberty to provide a foundation for understanding how early pubertal development might relate to later cardiometabolic conditions. Next, there is a summary of the literature on the descriptive epidemiology and risk factors for early menarche, and detail about what is known about race differences in these factors. In the following section, definitions used throughout this dissertation for cardiometabolic risk factors are presented, along with the descriptive epidemiology for these risk factors. Finally, there is a review of the literature that links early menarche with later cardiometabolic disease, and the possible mechanisms underlying associations.

Neuroendocrinology of Puberty

Along with fetal development and pregnancy, the process of puberty is one of the most rapid and profound developmental transitions in life [7]. Central to the process of puberty is the hypothalamic-pituitary-gonadal (HPG) axis, or specifically the hypothalamic-pituitary- ovarian (HPO) axis in females. The HPO axis regulates hormone signaling, eventually resulting in ovulation and menarche **(Figure 1)** [7, 29].

Figure 1. The hypothalamic-pituitary- ovarian (HPO) axis



Adapted from reference [7]

The hypothalamus is the main enabling center for the HPO axis [7]. The HPO axis receives input from neural centers, and the hypothalamus is susceptible to olfactory, photoperiod, and tactile stimuli [7]. Signals from the hypothalamus result in increased frequency and amplitude of the gonadotropin-releasing hormone (GnRH) pulse generator during puberty [30]. GnRH in turn stimulates the production and release of reproductive hormones, follicle stimulating hormone (FSH), and luteinizing hormone (LH), in the pituitary. The whole process takes place in a pulsatile fashion, regulated by levels of circulating steroid hormones.

The feedback system that controls the HPO axis is determined by genetics and established during gestation [7]. Pulsatile gonadotropin is detectable in prepubertal children at very low levels. Changes in the HPO axis are first detectable on average around 8-9 years in girls, when there is a sleep-associated increase in LH pulse frequency and amplitude [31]. As puberty advances, the pulsatile pattern of LH secretion extends over 24 hours. Increased LH stimulation at the beginning of puberty results in increased production of ovarian steroids, which in turn results in the development of pubic and axillary hair, breast enlargement, linear growth acceleration, pelvic remodeling, and increasing adiposity. In response to increasing estradiol level, menarche is initiated.

The exact process that leads to puberty by disinhibiting pulsatile GnRH release in the hypothalamus remains unclear [32]. Several factors are likely

involved in the timing and progression of puberty, including neuroregulation of the hypothalamo-gonadotropic and -leptinergic axes [32], and genetics have a strong influence [7, 33]. Twin and family studies have reported high heritability for one stage of pubertal development, age at menarche [34], and recent advances in genetics using genome-wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) in genes associated with both age of menarche and body composition [35].

Emerging factors in the regulation of reproductive maturation are the *Kiss1* gene, which produces Kisspeptin, and its receptor, G Protein Coupled Receptor 54 (GPR54) or Kiss1r [30, 36]. Kisspeptin and GPR54 are expressed in hypothalamic GnRH neurons, suggesting that they may act as regulators of GnRH neuronal function [30, 37]. A recent study showed that estradiol is essential for kisspeptin expression in GnRH neurons during the prepubertal period [38]. The results imply that a estradiol-kisspeptin positive feedback relationship leads to the GnRH neuron amplification, enabling the emergence of pulsatile gonadotropin secretion that spurs onset of puberty [30].

Stages of Puberty

Adrenal development, or adrenarche, precedes the pubertal changes in the HPO axis by about 1-2 years [7]. Adrenal androgens help convert estrogens in peripheral tissues and hypothalamus. This in turn contributes to the maturation of the HPO axis by desensitizing the hypothalamus via rising levels of steroids.

Among girls, the earliest sign of puberty is usually the development of a palpable breast bud (thelarche), although the development of pubic hair (pubarche) may sometimes precede thelarche [39]. Menarche occurs after thelarche and pubarche, relatively late in the pubertal process. Although there is only a moderate correlation between timing of menarche and onset of puberty (.37-.38) [40], individuals with early or late timing of secondary sexual characteristics at one point of time are likely to maintain the same relative timing throughout puberty, so early menarche probably signals earlier onset of other pubertal markers [41].

The timing of puberty and changes in body composition are well coordinated. The HPO axis works in a synergistic fashion with the somatotropic axis to stimulate the adolescent growth spurt associated with puberty [7]. The somatotropic axis is involved with linear acceleration in skeletal growth, the accumulation of lean body mass, and maintenance of many tissues. The hypothalamus secretes hormones that control the release of growth hormone (GH) from the pituitary. Similar to GnRH, GH is secreted in a pulsatile fashion. The effects of GH on skeletal growth are mediated by production of insulin-like growth factors (IGF) in target tissues, and IGF-1 levels correlate well with Tanner stage and gonadal steroid levels [42]. Higher levels of IGF-1 and adrenal androgens at age 8 years are also associated with earlier menarche, independent of body size, supporting the functional roles of these hormones in regulating puberty timing in girls [42].

Gonadal steroid production is important for regulating the adolescent growth spurt [7]. Adrenal androgens regulate acceleration in growth, while estrogens regulate deceleration [43]. The secular trends in age at menarche coincide with trends in height, and advances in pubertal timing are correlated more closely with accelerated skeletal development and attainment of adult pelvic size, rather than earlier accumulation of adipose tissue [44, 45]. The end of puberty is marked by the attainment of the final adult height and menarche. However, ovarian function continues to mature in women until the early to mid-twenties [46].

Other Correlates of HPO maturation

Insulin acts on the HPO axis and affects the timing of puberty. During mid-puberty, insulin levels rise as a consequence of a decrease in insulin sensitivity and then return to normal by the end of puberty [47, 48]. Insulin exhibits a stimulatory effect on ovarian cells resulting in an increased production of reproductive hormone steroid activity in the ovaries [49]. It also suppresses IGF binding proteins, raising levels of IGF-1. Insulin may act on the pituitary to increase the sensitivity of gonadotropins to GnRH [50].

Changes in insulin sensitivity are closely related to Tanner stage, and are correlated with pubertal timing. Data suggest that higher insulin levels are associated with higher estradiol levels [50]. In addition, hyperinsulemic states are associated with early puberty, while hypoinsulemic states are associated with delayed puberty [7]. It is possible that insulin resistance (IR) could support rapid

transit through puberty, as obese girls with IR and hyperleptinemia are reported to experience earlier onset of puberty and more rapid progression through puberty [32].

Leptin is a hormone produced in adipocytes of subcutaneous adipose tissue and is involved in energy balance, including appetite and metabolism [7]. Leptin receptors are found in the hypothalamus and in various peripheral tissues, including the ovary. Insulin regulates leptin production, and leptin levels are correlated with fat mass. Cross-sectional and longitudinal data show that leptin levels increase with increasing adiposity in girls during puberty [51, 52]. However, the role of leptin in HPO maturation has been debated, and there is currently little evidence that leptin is involved in initiating puberty and directly influencing the HPO axis [7]. The pubertal increase in leptin is likely a consequence, and not a precursor, of increased fat mass, as well as gonadal and insulin activation.

Early Menarche

Definition

Menarche (first appearance of menstrual bleeding) is an important reproductive event in a woman's life. It signals the beginning of reproductive capacity, and its timing has a number of health and psychosocial consequences. Most retrospective epidemiologic studies use age at menarche as a proxy to estimate the timing of pubertal development in females. Even prospective studies sometimes rely on self- or parental-reported age at menarche as a measure of

pubertal timing because it is an objective measure and may be less prone to observational error than other, more subjective measures such as Tanner staging, especially when truncal obesity is present [10]. Furthermore, women recall age at menarche fairly accurately into adulthood [53, 54]. In one longitudinal study, approximately 84% of women, mean age 50, were able to recall their age at menarche to within one year of the actual date [53, 54]. Therefore, self-reported age at menarche as a marker for pubertal development is used in this dissertation. To be consistent with other epidemiological studies of the association of pubertal timing and cardiometabolic risk factors [3, 55], early menarche will be defined as before 12 years of age throughout this dissertation.

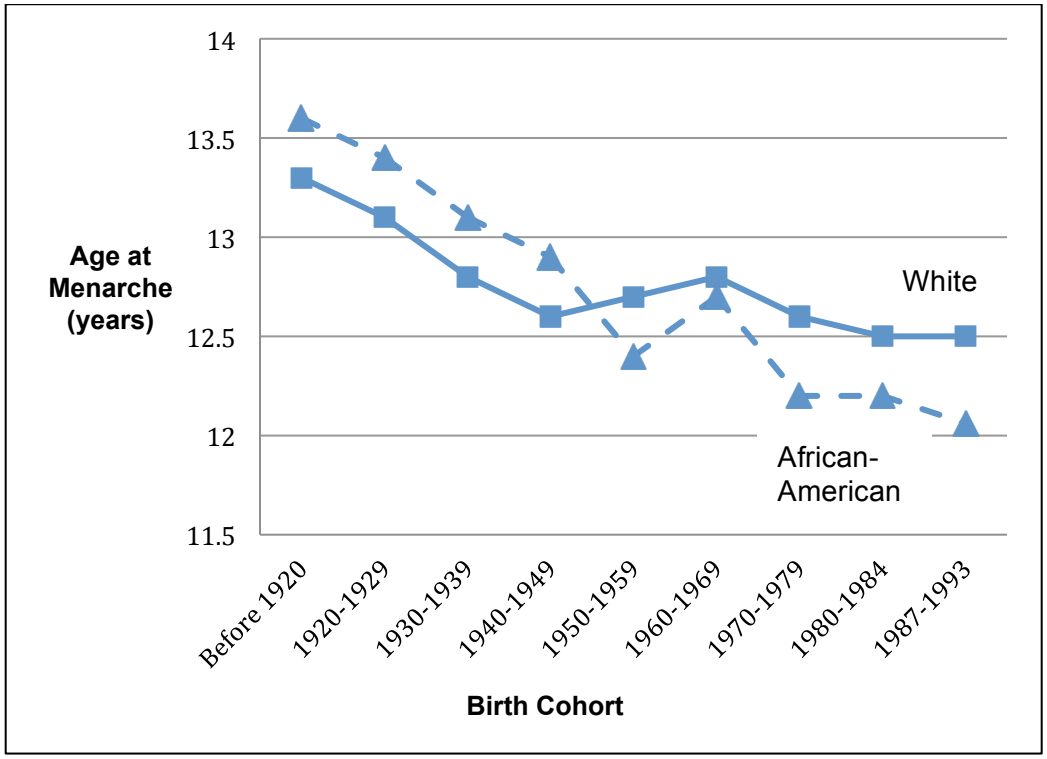
Epidemiology

Currently in the United States, the mean age at menarche is 12.3 years [56], although there are variations by race/ethnicity and geographic location. African-American girls tend to undergo menarche 2 to 3 months earlier than white girls [14], and girls in urban areas experience earlier menarche than those raised in rural areas [57, 58].

One main feature of the epidemiology of menarche is that the average age has declined over time (**Figure 2**) [15, 59, 60]. Cross-sectional and longitudinal data suggest that average age at menarche in the general population of the United States decreased from approximately 14.8 years in the late 1800's to 13 years by 1950 (approximately the 1930-1940 birth cohort in Figure 2), probably as a result of improvements in nutrition and overall health [15, 60-62]. Less

healthy lifestyle and environmental factors likely fueled the further decline [15]. According to one estimate, there has been a statistically significant one month per decade drop in average age at menarche over the last 30 years in the United States [63].

Figure 2. Mean age at menarche by birth cohort, NHANES 1999-2004



Adapted from data in references [15, 64]

Race/Ethnic Differences

Up until the mid-1900's, average age at menarche was similar for African-American and white women in the United States, with some evidence that age at menarche was slightly older for African-American females (**Figure 2**) [15].

Beginning around the 1960's-1970's, average age at menarche declined more quickly for African-American compared with white women [15]. According to NHANES data, average age at menarche declined from 13.6 years among African-American women born prior to 1920, to 12.0 years for the 1987-1993 cohort, while among white women, the decline was from 13.3 to 12.5 years, respectively [15, 64]. Supporting the NHANES data, data from the Bogalusa Heart Survey [14] show that average age at menarche in the early 1970's survey was not statistically different by race: 12.7 years for white and 12.9 years for African-American females. However, over the 20-year study period, average age at menarche declined for all race groups, most notably for African-American girls. As early menarche has been associated with a number of deleterious outcomes, it is important to investigate possible reasons for the downward trend in maturational timing in the population as a whole, as well as for race/ethnic differences in trends.

It remains unclear if race/ethnic differences in pubertal timing are the result of childhood race/ethnic differences in fat mass, or if differences in pubertal onset drive later disparities in adiposity across race and ethnicity [19]. Some have suggested that African-American girls experience earlier onset of puberty on average because they tend to be heavier, taller, and have more advanced skeletal maturation after age 9 [14, 60, 65-67]. However, other studies have noted that these anthropometric characteristics do not fully explain racial differences in pubertal timing. Ethnic differences in adiposity are not present in

prepubescent white and African-American girls, but become apparent during adolescence and early adulthood [50]. For example, in the NGHS, African-American girls were at a more advanced pubertal stage vs. white girls for each age, and also had higher adiposity [20]. However, African-American girls were not significantly more obese than white girls at age 9-10 when adjusting for maturity level [68]. In the Bogalusa Heart Survey (1973-1994), the rate of early menarche remained 1.4-fold higher among African-American girls than among white girls, even after adjustment for weight, height, and other characteristics [14]. This suggests that race is an independent risk factor for early menarche [30].

Data also suggest that differences in insulin response and action may affect the HPO axis so that GnRH is stimulated earlier in African-American girls [50]. Compared with white girls, African-American girls have higher insulin response to glucose challenge, and subsequent higher levels of IGF-1, which is associated with skeletal and sexual maturation [69]. Despite comparable BMI and fat mass, Casazza et al reported that African-American girls have lower insulin sensitivity, but higher follicle-stimulating hormone and estradiol levels at all pubertal stages compared with white girls [19, 50]. The correlation between acute insulin response to glucose and estradiol among African-American girls led the authors to conclude that postprandial insulin concentrations may stimulate estradiol and contribute to earlier menarche in African-American children. Another study reported that beta cell function decreased across puberty in

African-American ($p = 0.001$) but not in white ($p = 0.20$) adolescents [70]. The progressive decline in beta cell function among African-American versus white adolescents may reflect a unique effect of puberty on beta-cell compensation in African-Americans [70], and so it is possible that earlier pubertal onset, measured according to timing of menarche, might have differential effects on risk of diabetes and other cardiometablic outcomes by race.

The differences in pubertal timing and growth for African-American and white girls might partly explain important differences in health between African-American and white women [71]. Overall, current data are extremely limited regarding the reasons for racial differences in the age of menarche, and results from existing studies are not conclusive. Some possible areas for further research into these differences are highlighted in the next section on risk factors for earlier menarche.

Risk factors

Normal variation in menarcheal age may be the result of a female's response to environmental (including diet, physical activity, family structure, endocrine disrupters, and other pollutants), socioeconomic, climatic, genetic, and other pre- and-postnatal factors [63]. The timing of menarche is set early in life by genetics, but it is likely modified by changes in body size and composition in childhood [72], as well as other environmental factors. Since genetics have not changed over the past century, it is clear that environmental factors, and their

possible interaction with genetics, must be responsible for downward trend in average menarche age, as well as disparities by race.

Because timing of puberty is a complex interaction between factors, it is difficult to single out the effect of one factor on timing of menarche. It is widely recognized that physical activity and body composition are strong predictors of the initiation of menses [73]. Menarche tends to be delayed in circumstances of under nutrition or extreme physical activity, such as for athletes [61], via the negative hypothalamic effect on GnRH pulsatile exerted by intensive exercise [30]. The relatively recent changes in the nutritional and physical activity status of mothers and their offspring may be associated not only with obesity, but they could also alter timing of puberty in offspring, and their cardiometabolic risk in adulthood [74].

Findings from a randomized group trial of an intervention to reduce obesity lend evidence to the role of the complex interaction of various lifestyle factors in the timing of menarche [73]. The Planet Health study was a school-based intervention designed to reduce obesity in adolescents aged 10-13 years by decreasing television viewing and consumption of high-fat foods, while increasing physical activity levels and consumption of fruits and vegetables. Researchers randomized schools to either a modified curriculum or no curricular changes for two school years, and found that girls at the schools with the modified curriculum experienced menarche at a later age [73]. The delay appeared to result from

increased physical activity, reduced television viewing, and changes in body composition (BMI and fat distribution).

The role of other factors such as environmental pollutants, SES, stress, and specific dietary components require more research and replication, as findings have been mixed across studies [59, 63]. The following section briefly reviews the major factors known to be associated with timing of menarche, but with the caveat that these factors are likely interrelated. As mentioned earlier, there are few published studies looking at race/ethnic differences in risk factors for early menarche, other than for BMI and SES.

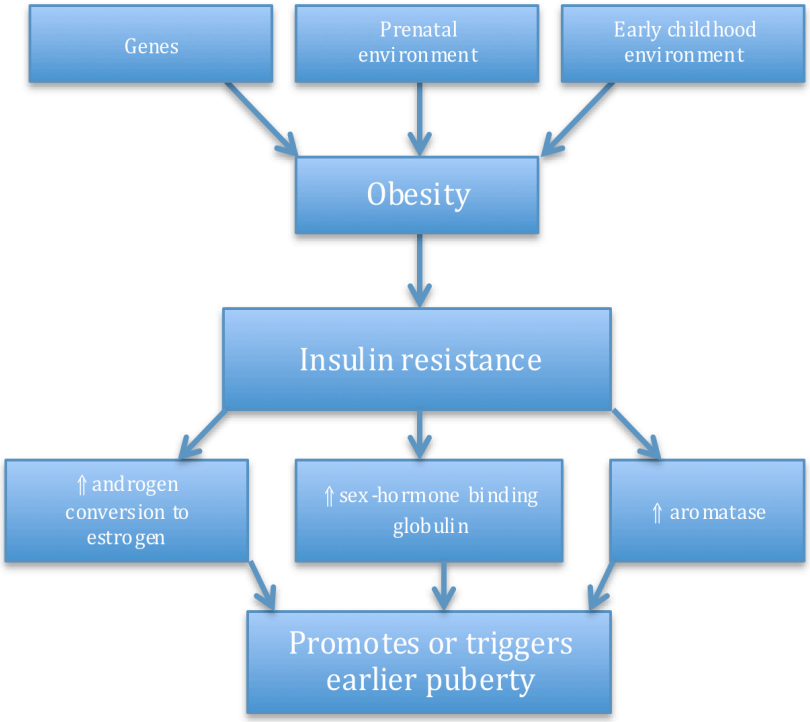
Adiposity and Growth

Hundreds of studies have noted an association between overweight and earlier onset of puberty and menarche [63]. Although adiposity in adolescence and adulthood is widely accepted to be associated with early menarche, studies are conflicting about the causal role of early childhood adiposity on timing of menarche. Some evidence suggests that higher weight gain in infancy and higher levels of adiposity levels in childhood initiate estrogen production and cause earlier onset of puberty in girls [15, 75, 76]. Gynoid fat mass in particular is thought to be associated with onset of menarche [19]. Early work by Frisch and colleagues suggests the importance of the accrual of sufficient total body fat stores to support the energetic costs of pregnancy and lactation [77, 78].

One proposed mechanism behind the causal role of adiposity in triggering puberty is shown in **Figure 3**. Underlying genetic and environmental factors may

lead to increased pre-pubertal adiposity resulting in early menarche, shorter stature, as well as increased weight gain in post-pubertal women [10]. Insulin resistance and the combination of various hormonal stimuli related to increased body fat, such as increased conversion of androgens to estrogen and increased bioavailability of circulating sex steroids, are thought to lead to earlier activation of the HPG axis [10]. It is also possible that leptin, which is secreted from body fat, acts on the hypothalamus to stimulate gonadotropin releasing hormone, luteinizing hormone, and follicle-stimulating hormone from the pituitary gland. It may also stimulate enzymes that act on androgen synthesis in adrenal glands, increasing the secretion of sex hormones [79-82]. However, others have noted the possibility that both adiposity and early menarche have a common antecedent, such as relatively high circulating insulin [50].

Figure 3. Possible pathway linking obesity to earlier puberty



Adapted from reference [10]

Other evidence suggests that adiposity may be a downstream consequence of pubertal maturation [7, 83]. Earlier pubertal onset may initiate adipose tissue accumulation during later stages of development, making temporality of the association between pubertal and adiposity development unclear [83, 84]. Secular trends in childhood obesity and timing of menarche in European countries also do not support adiposity as the main driver behind earlier age at menarche [85]. For example, a study in Copenhagen found that although the heaviest girls at age seven entered puberty earlier than thinner girls, there was a downward temporal trend in age at menarche in all BMI categories,

suggesting that the obesity epidemic is not completely responsible for earlier onset of puberty in the population [86]. Further supporting that adiposity itself does not accelerate age at menarche is that the inverse association between BMI and age at menarche is apparent in only white girls in some studies [23, 87]. Evidence suggests that among African-American girls, age at menarche is independent of adiposity [50, 60, 87]. Furthermore, it is unclear if prepubertal body composition is more relevant to the actual onset of puberty or to the duration of puberty [85].

Despite questions about the biologic mechanisms behind the relationship of increased childhood adiposity, younger age at menarche, and higher adult obesity [10, 88], there is strong evidence for genetic influences on both characteristics [89]. Genome-wide association studies (GWAS) have identified several loci for age at menarche which are also associated with measures of adult obesity and height. A study by Elks et al [35] described 11 obesity-related and 11-height related loci that are also associated with age at menarche in women of European ancestry.

Of note is that studies of the role of adiposity and growth on timing of puberty all have limitations [71]. Several began measurements around the time of puberty [14, 90-92] or lacked multiple measures prior to puberty [93]. Nonetheless, patterns have emerged. Studies have consistently reported that there is greater adiposity both immediately pre-menarche and post- menarche, and shorter attained adult height in girls with earlier menarche [91]. One study

also found significant differences in the BMI trajectories beginning around 9 years for early-, mid-, and late-onset of menarche [91], and several studies reported that accelerated height velocity begins at an earlier age among early versus late maturers [71, 94]

No matter the timing of development during the life course, adiposity increases circulating levels of insulin, testosterone, and IGF-1, and higher adiposity is a well-known risk factor for cardiometabolic conditions [30]. Therefore, it is difficult to disentangle the effects of early maturation from adiposity on adulthood disease risk, especially in studies that rely on measures of adiposity in later adolescence or adulthood only. Clearly, any investigation of the association of age at menarche and cardiometabolic risk needs to consider the role of adiposity at various points in the life course.

Fetal and Infant Growth

Size at birth, as a marker for the intrauterine environment, and rapid infant weight gain, particularly between birth and 2 months of age, are reported to be factors in the timing of menarche [27, 58, 95-99]. However, there is currently no consensus about the independent role of birth weight on the timing of menarche. Four studies report that lower birth weight infants have higher risk for earlier menarche [58, 65, 100, 101], while four studies reported that higher birth weight was associated with earlier menarche [95, 99, 102]. One other study reported that birth weight, standardized for gestational age, had different relationships with age at menarche depending upon how heavy or light a girl was at age 9 [103].

Comparisons between studies of the role of birth weight and infant growth are limited because of variations in study populations, growth measurements, and analytic approaches [65]. Taken together, existing studies suggest that menarche timing may be influenced by exposures in utero and during early infancy, likely via programmed release of gonadotrophins, and then subsequently childhood growth, lifestyle, and environmental factors may mediate the effects of these factors [104].

Genetic

There is a strong genetic influence on timing of menarche. Data from twin and family studies show that age at menarche is 49% heritable [34]. Furthermore, a recent GWAS by the CHARGE-ReproGen consortium identified 42 single nucleotide polymorphisms (SNPs) in genes associated with age of menarche [35]. The results from the GWAS studies suggest that genetic regulation of energy homeostasis and pubertal timing are closely related, and that there are likely other diverse pathways for pubertal timing and growth [35]. As mentioned earlier, genes probably interact with environmental and lifestyle factors to determine the age at menarche for individuals.

Socioeconomic Status

The role of socioeconomic status (SES) in pubertal timing remains unclear, and data suggest race differences. One issue with existing studies is that researchers use various methods to operationalize SES, for example,

parental education, parental home ownership, income level, and poverty level. Further complicating things is that SES is often a proxy for many other factors, including diet, physical activity, family environment, and access to healthcare [104]. Therefore, few studies are able to separate out the effects of specific factors underlying the observed relationships between SES and timing of menarche [104].

Despite these limitations, there are some patterns that have emerged regarding the role of SES on the timing of menarche. One author that looked at the association of SES and early menarche in the NGHS found different associations of income and early menarche by race [105]. For white girls, those in the highest income category were at lower risk for early menarche, while the opposite was found for African-American girls (i.e. highest category associated with highest early menarche risk). The paper adjusted for BMI, but did not look for interaction by BMI, or at other adiposity measures. Another study in which maternal education was categorized according to whether or not the mother was a high school dropout reported that there was no significant effect, individually or in combination with child poverty, on age of menarche for African-American girls [65]. However, for white girls, having a mother who dropped out of high school was associated with earlier menarche. The opposite effects of SES on age at menarche might be mediated by differences in growth among African-American and white children, however, few studies have looked into this question [65, 104].

Stress

Studies have found that high levels of stress associated with illness or war conditions tend to delay menarche [104, 106, 107], while stressful family environment, including absence of the biological father or presence of a step-father, are associated with earlier onset of menarche [30, 108, 109]. One explanation for why different types of stress might have different effects on pubertal timing comes from Boyce and Ellis, who proposed the “stress reactivity theory” to explain both the delaying and accelerating effects of psychosocial stress on the timing of menarche [110]. Both highly protective and highly stressful childhood environments may trigger stress reactivity systems. If this triggering inhibits maturation of the HPO axis, then both conditions may be associated with later timing of menarche in some populations [104, 110].

Additionally, the effects of a stressor might depend on the timing of exposure; exposure early in life appears to result in earlier menarche, while exposure later in childhood is associated with later menarche [104]. One theory is that girls who are exposed to chronic stressful environments may have chronic activation of the HPA axis, which in turn could produce increased body fat, early adrenarche, and puberty [111-114].

As with SES, there seems to be race/ethnic differences in the effects of stress on timing of menarche. In one study, father absence was not associated with age at menarche for African-American girls, individually or in combination with child poverty, but for white girls having a father who was absent more during

childhood was associated with a younger age of menarche [65]. It is unclear why less stressful environments are less protective on health for African-American girls [65].

Diet

Age at menarche is dependent on nutritional status, and girls with severe dietary restriction tend to experience later menarche [115]. Other than in conditions of extreme restriction, studies of the effect of diet on maturational timing are somewhat limited, possibly because it is difficult to measure diet during childhood [104].

Although very important for overall health, the role of diet in infancy on timing of puberty is unclear. Breastfeeding is known to be associated with lower risk of childhood obesity, but it is not consistently found to be associated with timing of menarche [85]. One study reported that formula feeding was associated with earlier menarche compared with breast-feeding among girls enrolled in Kaiser Permanente Oahu (Honolulu) [116]. In the Avon Longitudinal Study of Parents and Children (ALSPAC), use of soy formula was associated with early menarche compared with use of milk-based formula [29], although effects were subtle, confidence intervals crossed unity, and not many children were fed soy formula in the study.

A review of the current evidence for the association of dietary factors in mid-childhood and timing of puberty [85] did not find support that total energy intake influences the timing of puberty. However, fat intake appeared to be

associated with timing of menarche. Higher intake of total fat or polyunsaturated fatty acids (PUFA) are associated with earlier menarche [117-119], while intake of saturated fatty acids, monounsaturated fatty acids (MUFA), or animal fat have been associated with later menarche [117, 120]. The mechanisms for the association between fat intake and menarche timing remain a mystery, however.

The same review pointed out that animal protein intake, including from cow milk and dairy products, during prepuberty may be of particular relevance to timing of menarche due to the stimulatory effect on IGF-1 secretion [85, 121, 122]. In fact, higher intake of animal protein is associated with earlier menarche after controlling for BMI [119, 123]. In the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study, which was a prospective study of 227 German boys and girls, the consumption of animal protein in the highest tertile was found to be associated with pubertal onset 7 months earlier compared with animal protein consumption in the lowest tertile [85].

In contrast with animal protein, vegetable protein appears to be associated with later menarche. In the DONALD study, children with the highest intakes of vegetable protein experienced pubertal onset up to 7 months later than those in the lowest tertile [85]. However, studies looking at vegetable protein intake in childhood may indirectly have addressed the effect of prepubertal dietary isoflavone/fiber/animal protein intake on pubertal timing because there is a high content of dietary isoflavone and fiber in plant-based foods, and there tends to be

a negative association of vegetable protein intake with animal protein intake [85]. Girls with high isoflavone intakes in the DONALD study experienced the onset of breast development and peak height velocity approximately 7-8 months later compared with girls who had low isoflavone intake [85]. The authors noted that this effect size was similar to that found in studies of potentially neuroactive steroid hormones, suggesting that diet may be an important modifiable risk factor for early onset of puberty among girls.

Data on the influence of micronutrients such as vitamin C, beta-carotene, vitamin A, zinc, magnesium, thiamine, and iron, on the timing of puberty are controversial [85]. Vitamin A has been reported to be associated with both earlier [117], and later menarche [120]. One micronutrient that may merit more research is vitamin D. A prospective study in Bogota, Columbia reported that girls with low levels of vitamin D (< 50 nmol/L) had twice the risk of earlier menarche (HR: 2.05; 95% CI: 1.03, 4.07; p-value = 0.04) compared with girls in the sufficient range (> or = 75 nmol/L) [124]. There is growing evidence that there are racial disparities in consumption of vitamin D, possibly because lactose intolerance is more common among the African-American population [125]. In the NGHS, white girls consumed significantly higher amounts of vitamin D than African-American girls, and so it might be worth investigating if differences in vitamin D consumption are associated with differences in pubertal timing by race [125]. Before stronger conclusions can be drawn on micronutrients, research is needed

on the possible mechanisms mediating the associations of micronutrients with the timing of puberty [85].

Rather than studying individual micronutrients, some have looked at the association of dietary patterns in timing of puberty because of the possible combined effect of macronutrients, micronutrients, and food groups. The DONALD study found that higher dietary quality (lower intake of total fat and higher intakes of carbohydrates, fiber, and micronutrients) was associated with later puberty in girls [126]. Additionally, independent of BMI, children who had a higher dietary quality experienced their pubertal growth spurt 5 months later than children with poorer dietary quality.

Eating patterns may also be of relevance to racial differences in pubertal timing because breakfast skipping is more common in African-American than in white adolescents [125, 127]. Breakfast frequency and quality are proposed to be related to appetite controls and blood sugar control in children and adults [128], and so it is possible that meal patterns may be related to both pubertal timing and later risk for cardiometabolic diseases.

The role of sugar-sweetened beverages (SSBs) and early menarche has not been explored, but an association is mechanistically possible. Soft drinks are characterized by hyperglycemia, and are associated with higher risk for obesity in adults and children [129] as well as with elevated blood pressure and type 2 diabetes [130, 131]. In a study of 12-19 years-olds who participated in the 1999-2004 NHANES, the association of SSB intake and certain cardiometabolic

characteristics varied by race/ethnicity and gender [132]. This raises concern about soft drink intake early in life and later health disparities, particularly considering the rise in type 2 diabetes among children and adolescents [133, 134]. The adverse effects of SSB intake may be of most concern during certain periods of life, such as puberty, which are characterized by physiological insulin resistance [135]. Because African-American adolescents were found in one study to be more likely to consume SSBs compared with white adolescents [136], SSBs are one dietary factor that may merit further investigation regarding its role in differences in the timing of puberty and/or cardiometabolic conditions.

Physical activity

Menarche tends to occur later in athletes compared with the general population, suggesting that intense activity delays menarche [30, 137, 138]. Less intense levels of activity might also affect timing of menarche, possibly through decreased melatonin levels [30, 139]. A cross sectional study that included Colombian university women demonstrated that at least two hours of daily activity was associated with later age at menarche [140].

A study that began to follow girls at age 6-8 years to examine neighborhood characteristics and timing of menarche reported that availability of recreational outlets, such as parks, walking or hiking trails, sports/playing field, basketball courts, or tennis courts, was associated with later timing of thelarche and pubarche among African-American girls, but not white girls [141]. There is also research showing that African-American adolescents are less physically

active than white adolescents in the United States [142], and so physical activity and sedentary behavior is one area for further research into racial differences in pubertal timing.

Environmental pollutants

Tens of thousands of manufactured compounds, many for which the toxicity remains unknown, are commonly found in our food supply, cosmetics, and surrounding environment [19]. Several studies have investigated the role of environmental pollutants in timing of menarche, although most have been cross-sectional. One analysis of multichemical exposure among Akwesasne Mohawk Nation adolescent girls suggested that PCBs (polychlorinated biphenyls) and levels of lead were associated with early menarche [143]. Polybrominated diphenyl ethers (PBDEs), which have been widely used as flame retardants since the 1970s, have exhibited endocrine disruption properties in experimental studies [144], as well as antiandrogenic activity. Cross-sectional NHANES data suggest high concentrations of serum PBDEs during adolescence are associated with a younger age of menarche [144]. Data from animals suggest that bisphenol-A (BPA) may also be associated with earlier puberty [145]. Therefore, it is possible that environmental endocrine disruptors may accelerate the timing puberty, and contribute to racial disparities if certain racial groups are more likely to be exposed to these chemicals [19]. More work is needed regarding environmental pollutants and their influence on puberty. The recently initiated National Children's Study (NCS) should provide some clarification because one aim is to

examine the role of environmental pollutants in child growth, adiposity, and developmental timing (National Children's Health Study. http://www.nationalchildrensstudy.gov/Pages/default.aspx Accessed July 21, 2013).

Sleep

Data suggest that the onset of menarche is affected by hours of sleep, possibly mediated by body weight and height [146]. The proposed mechanism is that lighting signals influence the HPO axis, and are mediated through the melatonin circuit. Supporting this theory is that girls who are blind have been shown to have earlier menarche [147], and several studies have shown that menarche is more frequent in winter than summer months, suggesting an inhibitory effect of photo stimulation [30]. Because racial differences have been noted in sleep patterns as early as ages 3-8 years [148], independent of demographic factors, differences in sleep patterns is another possible underexplored reason for the racial disparities in timing of menarche.

Summary

Although adiposity is a well-studied risk factor for early menarche, it remains unclear if adiposity is a precursor or a consequence of the initiation of puberty, and it is possible that it is both of these situations. It is also unclear how race differences in adiposity, diet quality, physical activity, and sleep contribute to race differences in pubertal timing. The opposing role of SES by race on timing

of puberty, and possible interactions with adiposity and other factors, also requires more investigation.

Cardiometabolic Outcomes

Definitions

The cardiometabolic risk factors included in this dissertation include obesity, type 2 diabetes, prediabetes (impaired fasting glucose), metabolic syndrome (a.k.a. insulin resistance syndrome), blood pressure, blood lipids, and insulin resistance. According to the American Diabetes Association [149], these factors, in addition to age, race, gender, genetics, family history, and unhealthy behaviors such as smoking, physical inactivity, and unhealthy diet, contribute to cardiometabolic risk (global diabetes/CVD risk). **Table 1** outlines the definitions used in this dissertation for cardiometabolic risk factors for women (18 years or older) and girls. Whenever possible, risk factors are modeled as continuous variables in analyses, and any deviations from the definitions described in this table are noted.

Table 1. Definitions for cardiometabolic risk factors for women and children

Risk Factor	Definition for Woman (18+ years)	Definition for Children/Adolescents
Adiposity		
Body Mass Index (BMI)	Obese: ≥30 kg/m^{2} [(a)] Overweight: 25-29.9 kg/m^{2} [(a)]	Obese: >95 percentile for age and sex[b] Overweight: >85 percentile for age and sex[b]
Waist Circumference	Obese: >88 cm[c]	Obese: >90th percentile for age and sex [d]
Prediabetes (Impaired Fasting Glucose)[e]	100 - <126 mg/dl	100 - <126 mg/dl
Type 2 Diabetes[e]	Non-pregnant, fasting blood glucose ≥ 126 mg/dl, A1C ≥ 6.5%, 2-hour oral glucose tolerance ≥ 200 mg/dl, or use of diabetes medication.	Non-pregnant, fasting blood glucose ≥ 126 mg/dl, A1C ≥ 6.5%, 2-hour oral glucose tolerance ≥ 200 mg/dl, or use of non-insulin diabetes medication.
Hypertension[f]	>140/90 mmHg	>95th percentile
Lipids		
Total Cholesterol	≥ 240 mg/dl[g]	>150 mg/dl[h]
HDL-C	< 40 mg/dl[g]	<35 mg/dl[h]
LDL	≥160 mg/dl[g]	>160 mg/dl[h]
Metabolic Syndrome		
Categorical Definition	3 or more of the following[i]: 1) Waist circumference >88 cm 2) Blood pressure >130/85 mmHg 3) HDL-C < 50 mg/dl 4) Triglycerides ≥150 mg/dl 5) Fasting glucose ≥ 100 mg/dl or known T2DM	3 or more of the following[j]: 1. Abdominal obesity (≥85 percentile for race/age) 2. Triglycerides ≤ 110 mg/dl 1) HDL-C ≤ 50 mg/dl 2) Systolic or diastolic BP ≥90% for age and height 3) Fasting glucose ≥ 100 mg/dl or known T2DM
Cluster Score Definition	Sum of the z-scores of all metabolic syndrome components (reference values from specific study population)	Sum of the z-scores of all metabolic syndrome components (reference values from specific study population)

[a] CDC. "About BMI for Adults". Available at: http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html. Accessed July 10, 2011.

[b] CDC. "Body Mass Index: BMI for Children and Teens". Available at: http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html. Accessed July 10, 2012.
[c] National Cholesterol Education Program (2002). *Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III Final Report)*. National Institutes of Health. p. II–17.
[d] Fernández JR, Redden DT, Pietrobelli A, Allison DB. Waist Circumference Percentiles in Nationally Representative Samples of African-American, European-American, and Mexican-American Children and Adolescents J Pediatr 2004;145:439-44.
[e] The American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2004;27(suppl 1):S5–S10
[f] Urbina E. Ambulatory Blood Pressure Monitoring in Children and Adolescents: Recommendations for Standard Assessment. (Hypertension. 2008;52:433-451.)
[g] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) JAMA 2001, 285:2486-249.
[h] Kavey RE. American Heart Association Guidelines for Primary Prevention of Atherosclerotic Cardiovascular Disease Beginning in Childhood. Circulation. 2003;107:1562-1566.
[I] Expert Panel On Detection, Evaluation, And Treatment Of High Blood Cholesterol In Adults (May 2001). "Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)". *JAMA: the Journal of the* American Medical Association 285 (19): 2486–97. DOI:10.1001/jama.285.19.2486. PMID 11368702.
[j] Cook S, Weitzman M, Auinger P, et al. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med* 2003;157:821-7.

Epidemiology

Cardiometabolic risk factors are highly prevalent in the United States. They are associated with high levels of morbidity, as well as substantial health care costs, and tend to affect African-American women more often than white women. Cardiometabolic risk factors tend to cluster together and have a shared contribution to risk for CVD [150]. Obesity and hyperinsulinemia are the main characteristics of cardiometabolic diseases, and they link hypertension with dyslipidemia [150]. Impaired glucose regulation (i.e. prediabetes and type 2 diabetes) is closely linked to the metabolic syndrome and its components beginning in early life [150]. The following section summarizes the descriptive epidemiology of obesity, hypertension, dyslipidemia, impaired glucose regulation (including prediabetes and type 2 diabetes), and metabolic syndrome.

Obesity

Obesity has been linked to a number of health conditions, including heart disease, stroke, and type 2 diabetes. There have been dramatic increases in prevalence of obesity over the past 20 years, although the rate of increase appears to be leveling off in recent years [151]. In the United States, approximately 36% of adults, and 17% of children 2-17 years of age, are currently obese according to 2009-2010 NHANES data [152, 153]. There are large disparities in the prevalence of obesity by race and geographic location. The prevalence of obesity is twice as high among African-American girls compared with white girls (29% vs 14%, respectively) [153]. In the United States,

prevalence is highest in the South and lowest in the West. Obesity takes a large economic toll on this country, with medical costs estimated at $147 billion in the United States, or 10% of all medical spending [154].

Hypertension

Hypertension increases the risk for heart disease and stroke [155]. Currently, 33% of adults have hypertension, and only about 50% of those with hypertension have it under control [156]. The prevalence of hypertension is higher among African-American women than white women (45.7% vs. 31.3%), and African-American women tend to develop hypertension at a younger age [157]. Costs from hypertension-related health care, medications, and missed work days were projected to reach $93.5 billion in 2010 in the United States [157].

High Total Serum Cholesterol

High total cholesterol increases the risk for heart disease. Approximately 16.3% of adults and 8.5% of children currently have high cholesterol [158, 159]. The prevalence of high total cholesterol is higher among women (16.2%) than men (13.5%) in the United States, and it is the only cardiometabolic risk factor covered in this dissertation for which the prevalence of is higher in white women (16.9%) compared with African-American women (13.3%) [160]. There has been a large decline over time in the prevalence of high cholesterol, with prevalence falling by half from 33% in 1960-1962 to the current level of 16.3% [160].

Type 2 Diabetes and Prediabetes

Type 2 diabetes is a leading cause of kidney failure, heart disease, and stroke in the United States. In 2010, 25.8 million adults age 20 years or older had type 2 diabetes (prevalence=8.3%), and 79 million had prediabetes (prevalence=35%) [161]. The prevalence of diabetes is slightly higher for men (11.8%) vs. women (10.8%), and much higher for African-American (18.7%) vs. white (10.2%) adults in the United States. Among adolescents aged 12-19, the prevalence of prediabetes or diabetes increased from 9% in 1999-2000 to 23% in 2007-2008 [162]. In 2007, the direct and indirect cost of diagnosed diabetes was estimated at $174 billion in the United States ($116 billion for direct medical costs, $58 billion for indirect costs [disability, work loss, premature mortality]) [163]. That cost is expected to reach $3.4 trillion by 2020.

Metabolic Syndrome

Metabolic syndrome is a highly prevalent disorder in the United States and includes a clustering of related factors that increase the risk for CVD and type 2 diabetes [164]. There is currently debate not only about the best definition for metabolic syndrome, particularly for children, but also about the merits of this syndrome as a distinct condition [164]. The controversy is fueled by the fact that there remains no clear definition or pathogenic mechanism for the syndrome. Furthermore, changing levels of risk factors with growth and maturation complicate the diagnosis of metabolic syndrome in pediatric populations. Some have proposed that metabolic syndrome is simply a surrogate for a combination

of risk factors that increase risk for type 2 diabetes and CVD [164]. Gerald Reavan, who first proposed the syndrome in the 1980s and called it “Syndrome X”, argues that there is an underlying mechanism to the mutual involvement of the various risk factors [165]. However, he states that Syndrome X is not a disease, but rather that insulin resistant/hyperinsulemic individuals are at higher risk for a whole host of factors (e.g. dyslipidemia, high blood pressure, glucose intolerance) that in turn increase the risk for CHD.

The prevalence of metabolic syndrome among the U.S. population varies depending on the definition used, but it is estimated at approximately 34% among adults according to NHANES 2003-2006 data using the *National Cholesterol Education Program's* Adult Treatment Panel III report (NCEP: ATPIII) revised criteria [166]. Prevalence is 1.5 times higher among African-American females compared with white females [166]. Among children and adolescents aged 2-19, prevalence ranges from 1.2% to 22.6% in the general population, depending on the definition used and population characteristics [167].

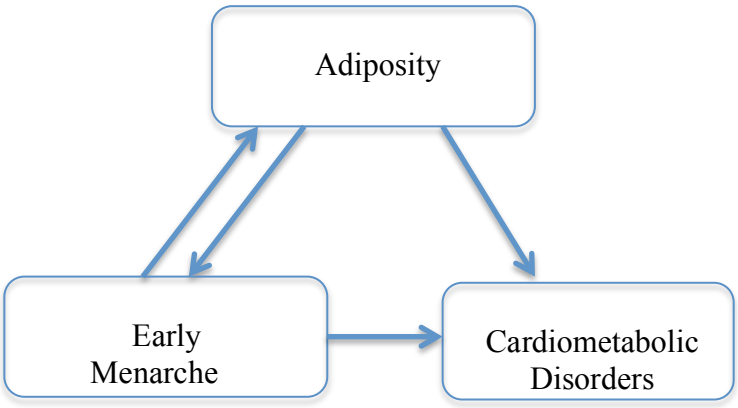
Previous Studies on Early Menarche and Cardiometabolic Risk

Overview

Existing studies conflict about the independent association of early menarche and cardiometabolic risk [168]. Estimating the association of early pubertal development and later disease in adulthood is complicated by the fact that timing of menarche is associated with higher BMI and waist circumference in adolescence and adulthood **(Figure 4)** [83, 92, 169-171]. Some studies suggest

that the mechanisms behind early puberty itself, such as changes to the HPO axis and metabolism, apart from adiposity, might play a role in risk for cardiometabolic disorders later in life [8].

Figure 4. Role of adiposity in the association of age at menarche and cardiometabolic disease

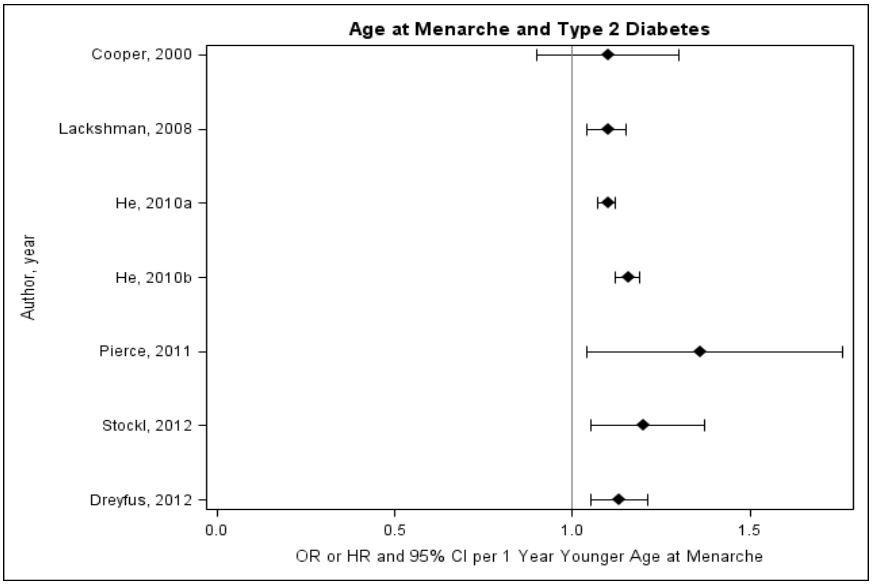


One study that followed women from age 9 to 39 years to investigate the role of BMI in the association of age at menarche with a number of CVD risk factors concluded that age at menarche is risk marker, not an independent risk factor, for CVD [168]. In that study, BMI at age 9 appeared to lead to earlier menarche and to correspondingly higher adult BMI and greater CVD risk. However, childhood BMI was measured close to puberty in that study, and another group subsequently found that BMI measured earlier, between 4 and 6 years of age, did not explain the relationship between age at menarche and adulthood BMI, type 2 diabetes, or lipids [4, 172, 173]. In the Fels Longitudinal Study, girls with early menarche (<12 years) had a more negative CVD risk profile during adolescence compared with girls with menarche 12 or older, even after adjustment for childhood and adolescent adiposity [174]. Therefore, it

appears that the role of adiposity in the association between early menarche and later cardiometabolic conditions may differ depending on when in the life course adiposity and cardiometabolic risk factors are measured, and possibly which adiposity measure is included (i.e. BMI, waist circumference, percent body fat). It is also not clear if the role of adiposity in these associations differ by race/ethnicity.

Type 2 diabetes is a well-studied cardiometabolic outcome with regard to pubertal timing, with some studies reporting an inverse association, while others finding no statistically significant association **(Figure 5)** [1-6, 175].

Figure 5. Summary of studies of the association of earlier age at menarche and type 2 diabetes



References [1-6]

Studies reporting inverse associations tended to be large and to include both pre- and post-menopausal women [2-6], while those that did not find an association included only post-menopausal women and might have been underpowered [1, 175]. In all studies, associations were attenuated by adiposity to varying degrees. Women in the U.S. Nurses' Health Study (NHS) I who reported menarche at age 11 or younger (vs. age 13) had a 34% increased risk for diagnosed type 2 diabetes (HR=1.34, 95%: CI 1.26-1.42) [1]. After adjustment for BMI and other social and lifestyle factors, the risk was 18% higher (HR=1.18, 95% CI: 1.10-1.27). In the NHS II, which followed a younger cohort of women, those with menarche at or before 11 years (vs. age 13) had 98% increased risk for type 2 diabetes (HR=1.98, 95% CI: 1.79-2.20) before adjustment for adult BMI, and 40% (HR=1.40, 95% CI: 1.24-1.57) increased risk after adjustment for adiposity and other potential confounders [3]. In the European Prospective Investigation of Cancer (EPIC)-Norfolk study, the odds for diagnosed diabetes at baseline or during 8-years of follow-up was reduced by 10% (OR=0.90, 95% CI: 0.95-0.86) for each 1 year delay in menarche [2]. The association was completely attenuated and no longer significant after adjustment for adult BMI (2% reduced odds for each year delay in menarche [OR=0.98, 95% CI: 0.97-1.03]). In our own analysis of ARIC data, early menarche (<12 years of age vs. 13 years) was associated with an increased odds of diabetes at baseline (OR 1.72, 95% CI: 1.32-2.25) and increased risk for diabetes over follow-up (HR 1.43, 95% CI: 1.08-1.89) for White women only, and that the association was

attenuated after adjustment for BMI [6]. The German Cooperative Health Research in the Augsburg Region (KORA) study found that the RR for diabetes per one-year delay in menarche was nearly the same before and after adjustment for BMI and other confounders (unadjusted: RR=0.83, 95% CI 0.73-0.95; BMI-adjusted: RR=0.84, 95% CI 0.73-0.98) [5]. Finally, a study that followed women from birth to age 53 found that age at menarche was associated with diabetes before, but not after, adjustment for adulthood BMI (unadjusted: RR per year delay in menarche=0.73, 95% CI 0.56–0.96; adjusted for BMI: RR=0.85, 95% CI 0.65–1.10) [4].

A number of studies have reported on the association of early menarche and other cardiometabolic risk factors in various age groups and populations. One recent review paper found that although data on cardiometabolic risk factors and puberty were too scarce for meta-analysis, existing studies suggest that earlier puberty is associated with hypertension, metabolic syndrome, and abnormal glycaemia, independent of childhood adiposity [28]. Although most studies have found that earlier age at menarche is associated with hypertension or raised blood pressure [8, 28, 55, 174, 176], at least one found no association [177] , and the association was retained after adjustment for adulthood BMI in only about half of the positive studies [28]. Studies have also reported that earlier menarche is associated with higher HOMA-IR [177, 178], higher risk for metabolic syndrome or higher number of metabolic syndrome components [176, 177, 179], higher fasting insulin [8, 177, 178], and glucose intolerance [174, 176,

177]. Results on lipids have been mixed, with some reporting associations with higher triglycerides [176, 179] and/or lower HDL [8, 179], and others finding no association [174, 177]. In the Cardiovascular Risk in Young Finns Study, each 1-year younger age at menarche was associated with elevated systolic blood pressure, higher insulin resistance, and greater risk of metabolic syndrome before, but not after adjustment for adiposity [168]. One recent study also reported that earlier menarche is associated with hyperuricemia [5]. Studies have not found associations with C-reactive protein (CRP) [144].

Some studies have reported that earlier menarche increases the risk for CVD outcomes, but others have found no association. The review paper by Prentice et al included a meta-analysis of the existing data and estimated that menarche prior to age 12 increases the risk of CVD by 15% (RR 1.15, 95% CI 1.02, 1.28) compared with menarche at or after age 12 years [28]. In a prospective study of college-educated white women, the RR of ischemic heart disease (IHD) through age 73 was 0.76 (95% CI 0.60-0.95) for each one year later age at menarche [180]. In the EPIC-Norfolk study, which included over 15,000 women, young age at menarche (< 12 years vs. >=12 years) was associated incident CVD [HR 1.17 (95% CI 1.07, 1.27)], incident CHD [HR 1.23 (95% CI 1.06, 1.43)], all-cause mortality [HR 1.22 (95% CI 1.07, 1.39)], and CVD mortality [HR 1.28 (1.02–1.62)] [55]. In the Korean Kangwha Cohort Study, risk of CHD mortality was 51% lower (95% CI, 0.25%-0.95%) among women who reported menarche at age 17-18 years versus women who were younger than 17

years at menarche [181]. The Seventh Day Adventist study reported that each 1-year delay in menarche was associated with 4.5% (95% CI 2.3%–6.7%) lower total mortality; the association was stronger for mortality from ischemic heart disease [6.0% (95% CI 1.2%–10.6%)] and stroke [8.6% (95% CI 1.6%–15.1%)] [182]. However, there were also a number of studies that did not find associations with CVD-related outcomes [183-187]. For example, two studies found no statistically significant association with myocardial infarction [184, 185], and another found no association with coronary heart disease [183].

Although race appears to be an independent risk factor for early menarche, as well as for cardiometablic conditions such as type 2 diabetes, the relationship of age at menarche with cardiometabolic risk among African-American women has not been well studied. The review by Prentice et al [28] noted this lack of ethnic-specific information on the association of pubertal timing and CVD risk factors. Most studies of the association of early menarche with cardiometabolic risk have included women of European descent only [1, 2, 4, 8, 9, 168, 182], a few included women from Asian countries [28], and those that included a more diverse study population did not look at risk of disease separately for white and African-American women [3, 175, 183]. We previously showed that in ARIC, age at menarche was associated with prevalent diabetes at baseline and incident diabetes during follow-up for white, but not African-American women [6]. Building on that work, we examine more closely the race-

specific associations of early menarche and cardiometabolic risk factors earlier in life in Manuscripts #1 and #2 of this dissertation.

In summary, there is evidence that early menarche is associated with cardiometabolic risk factors and cardiovascular disease outcomes, suggesting shared mechanisms between earlier pubertal development and cardiometabolic diseases [8]. However, there remain gaps in the literature regarding the role of adiposity, the differing influence of pubertal timing on cardiometabolic risk at different points in the life course, and race/ethnic differences.

Mechanisms

Relevant to the study of pubertal timing and cardiometabolic risk are a number of regulators of metabolism that affect gonadal function, including insulin, growth hormone, and IGF-1 [50]. During puberty, there are pro-inflammatory and pro-oxidative changes, and relative insulin resistance, which also play a role development of cardiometabolic conditions [188]. Early age at menarche is preceded by hyperinsulemia [23], and puberty is a critical period for adiposity development.

In adulthood, gonadal steroids are known to influence the cardiovascular system and lipid metabolism [189]. A large increase in risk for CHD after both natural and surgical menopause points to the cardioprotective properties of endogenous estrogens. Androgens are known to decrease HDL and increase LDL, but estrogen has the opposite effect on lipids, while also exhibiting antioxidant and anti-inflammatory properties. Estrogen also works to vasodialate

the vascular endothelium and boost insulin sensitivity, while endogenous androgen levels appear to contribute to type 2 diabetes risk [189]. The process of puberty has been found to modulate endothelial function and antioxidant mechanisms [190], and oxidative stress is thought to play a role in insulin resistance [191].

Data from an ancillary study to the NGHS indicate an important role for sex hormones, oligomenorrhea (menstrual cyclicity > or = 42 days), polycystic ovarian syndrome (PCOS) (a hyperandrogenic sub cohort of oligomenorrhea), and insulin at age 14 years in both metabolic syndrome and severe obesity in young adulthood [192]. One proposed link between early puberty and type 2 diabetes in particular is that girls who mature earlier have an exaggerated insulin response that is disproportionate to their insulin resistance [23, 50]. Ibanez et al. demonstrated that both premature pubarche and premature adrenarche were associated with hyperinsulinemia in girls [193]. Although data have not yet supported this, one theory is that an exaggerated insulin response is causally related to earlier puberty [50], and also related to later risk of diabetes.

Menarche and cardiometabolic conditions all have strong genetic underpinnings, and so it is possible that there is a common genetic basis for early menarche, higher adiposity, and later disease. Alternatively, it is possible that early menarche is simply a surrogate marker for obesity in childhood, which subsequently increases the risk for cardiometabolic conditions in adulthood [9, 168]. Therefore, careful consideration for various measures of adiposity (e.g.

BMI, waist, percent body fat) is needed to clarify the confounding and/or mediating role of adiposity in associations between pubertal development and cardiometabolic risk.

CHAPTER 3. MANUSCRIPT #1 - AGE AT MENARCHE AND CARDIOVASCULAR DISEASE RISK FACTORS AMONG AFRICAN-AMERICAN AND WHITE ADOLESCENTS

Introduction

Earlier pubertal development among girls is associated with increased risk for adverse health conditions in adulthood including cardiovascular disease (CVD), metabolic syndrome, and type 2 diabetes [3, 11, 12, 28]. A recent meta-analysis estimated that menarche prior to 12 years of age increased the risk for CVD in adulthood by 15% compared with menarche at or after 12 years of age [28]. The proposed mechanisms behind this association include increased body fatness among earlier maturing girls, as well as neuroendocrine hormonal changes that negatively impact metabolism [84, 177, 194]. The association of earlier maturation with long-term CVD health is relevant for public health because the average age at both breast development (thelarche) and first menstruation (menarche) have declined in recent decades in the United States for all race/ethnic groups, although most dramatically for African-American girls [15, 64, 115, 195]. The racial differences in pubertal timing coincide with well-documented health disparities in the prevalence of CVD risk factors such as obesity, type 2 diabetes, and metabolic syndrome among women [16-18].

Although several studies have examined the role of earlier menarche on levels of risk factors for CVD at various points across the life course [3, 6, 8, 9, 55, 168, 172, 174, 180, 182], most were conducted in adult populations and/or included participants of primarily European descent. Longitudinal studies of the

association between timing of menarche and cardiometabolic risk during adolescence are limited [174, 177], especially for African-American girls. Existing studies have reported somewhat mixed results regarding the relation of menarche timing with CVD risk factors, and they vary according to how early menarche was defined, the demographic characteristics of the cohort, and the time period during which the data were collected [28]. The secular trend towards earlier pubertal onset in the general population, and widening racial disparities in the timing of puberty in recent decades, suggest that a contemporary, large, racially diverse cohort of girls may help clarify the pattern and consequences of these trends on risk for CVD among women in adulthood. Studies examining the correlates of changes in CVD risk factors during adolescence are particularly important because this is a sensitive developmental stage for both adiposity accumulation [20] and formation of lifestyle habits [196] that are amenable to interventions that may delay or prevent development of CVD and its risk factors in adulthood.

Using data from the NHLBI National Growth and Health Study (NGHS), this paper assesses race-specific associations of earlier age at menarche with 1) overall levels and age-related changes in blood lipids, systolic blood pressure, and adiposity from age 9 to19 years, and 2) levels of blood lipids, systolic blood pressure, adiposity, fasting glucose, insulin, and a metabolic syndrome cluster score at mean age 19. It also examines the potential confounding or mediating role of adiposity in the relationship between earlier pubertal maturation and other

cardiometabilic risk factors. The longitudinal nature of the NGHS and its rich annual follow-up data offer a unique opportunity to examine the changes in CVD risk factors for African-American and white girls according to timing of menarche, thus helping to clarify if earlier maturation might contribute to racial disparities in cardiometabolic health later on in the life course.

Materials and Methods

Study Design and Population

This is a secondary analysis of publically available data from the NGHS, accessed with permission from the National Heart Blood and Lung Institute's Biologic Specimen and Data Repository Information Coordinating Center (NHBLI BioLINCC) [197]. The goal of the multicenter NGHS was to study the development of obesity among African-American and white adolescent girls, and to examine the effects of obesity on cardiovascular disease risk factors [197]. The three field centers participating in the study were: 1) University of California-Berkeley, 2) Children's Hospital Medical Center, Cincinnati, OH, and 3) WESTAT, Rockville, MD [198]. A total of 2,379 girls aged 9-10 years enrolled in the NGHS (1,213 African-American and 1,166 white) during the baseline visit in 1987-1988 and were followed annually for 10 years. Participants were recruited from schools in Richmond, CA and Cincinnati, OH, and from families enrolled in a health maintenance organization (HMO) in the Washington, DC area. Sampling was designed to ensure a study population that represented the general 9-10-year-old African-American and white female population in each of study

communities. Participation was limited to girls self-identifying as African-American or white race and who were living in racially concordant households [198]. The number of participants varied from visit to visit because of the longitudinal nature of the study. Participation rates were high at visits two, three, and four (96%, 94%, and 91%, respectively), then declined to a low of 82% at visit seven, but then increased to 89% at visit 10 [127]. More details of the study design and population have been reported elsewhere [199].

The NGHS was approved by the institutional review boards (IRBs) of the University of California at Berkeley, University of Cincinnati and Cincinnati Children's Hospital Medical Center, Cincinnati, OH, and Westat/Group Health Association in Rockville, MD. All parents/legal guardians gave informed consent, and all participants over the age of 12 provided assent. Use of the publically available data in this secondary data analysis was approved by the IRB at the University of Minnesota.

For all analyses, girls who were missing age at menarche (n=22), clinic location (n=1), parental smoking status (n=227), or who reported menarche less than 8 years of age (n=2) or diabetes (i.e. assumed type 1 diabetes, n=5) were excluded. After these exclusions, there were 2,122 girls (African-American=1,046; white=1,076) who had valid measures available for any of the CVD risk factor outcomes. Subsets of this dataset were analyzed for each cardiometabolic outcome, and visits in which participants were missing any of the covariates were excluded. **Table 2** shows the number of participants included in

the analyses at each visit, and **Table 3** shows how the sample size varied for each outcome. For the cross-sectional analyses at mean age 19 years, we additionally excluded participants who were missing percent body fat (n=40) at visit 10, for a final year 10 sample size of 2,082 (African-American=1,030; white=1,052). Models that were stratified by pre-menarche BMI excluded girls who reported menarche at the baseline visit (n=69 for the longitudinal models; n=67 for the mean age 19 models).

Clinical Measures and Data Collection

Age at menarche

Information on menarche was collected during the annual pubertal stage assessment by centrally trained and certified female examiners who asked girls whether their menstrual periods had started, and if so, to specify the date [200]. The reliability of self-reported age at menarche is well established [12, 201]. For participants who provided conflicting age at menarche responses at different visits, we used the first age that was reported in response to the question regarding the onset of menses.

Cardiometabolic outcome variables

The outcome variables included total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides, systolic blood pressure, percent body fat, body mass index (BMI), glucose, insulin, and a metabolic syndrome cluster score (z-score). Complete lipid profiles were measured in the morning after an overnight fast (8 hours) at visits 1 (mean

age 10), 3 (mean age 12), 5 (mean age 14), 7 (mean age 16), and 10 (mean age 19) as previously described in detail [199]. Total cholesterol, HDL-C, and triglyceride concentrations were analyzed enzymatically with a commercially available method, and LDL-C was calculated by using a Friedewald formula that was modified based on the Lipids Research Clinics data as follows [202, 203]:

LDL Cholesterol = Total Cholesterol – HDL Cholesterol – (Triglycerides/6.5)

Triplicate blood pressure measurements were taken with a standard mercury sphygmomanometer and cuff sizes appropriate to upper arm circumferences at each annual visit while subjects were seated with feet resting flat and the right arm resting at heart level. The average systolic blood pressure from the three measures at each visit was used in the blood pressure analyses for this study.

During annual visits, trained study staff obtained anthropometric measures that included height, weight, waist circumference, and the sum of skinfold (SSF) thickness at triceps, subscapular, and suprailiac sites [41]. Measures were repeated twice for all anthropometric measures, and a third measure was taken if the two measures differed by more than a preset amount. The percent body fat was calculated according to the formulae of Slaughter et al [31], using the triceps and suscapular skinfolds. BMI was calculated using the average height in meters and weight in kg (weight/height^2). Waist circumference (cm) was measured at the narrowest part of the torso [204].

Fasting glucose was measured at year 10 (mean age 19) using the glucose oxidase method with the Hitachi 704 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) [205]. Coefficients of variation for glucose ranged from 2% to 7%. Fasting insulin was measured at visit 10 by the Endocrine Laboratory at the University of Cincinnati/ Children's Medical Center using competitive protein-binding radioimmunoassay [205]. Coefficients of variation for insulin measurement ranged from 5% to 11% [205].

As done in other studies, we considered fasting insulin as the measure of insulin resistance [205, 206], instead of the homeostasis model assessment of insulin resistance (HOMA-IR). Huang et al [206] compared estimates of insulin resistance from HOMA-IR to results from a frequently sampled oral glucose tolerance test and concluded that the utility of HOMA-IR in accurately predicting insulin sensitivity is similar to using fasting insulin alone. A study that included African-American and white adolescents and compared fasting insulin and other surrogates of insulin resistance to the gold standard euglycemic-hyperinsulinemic clamp reported that the correlation between fasting insulin and HOMA-IR was very high (r=0.99) [207]. However, the study also showed that although insulin and the surrogates that included this measure might be a marker of CVD risk, they were imprecise representations of insulin resistance based on the gold standard. As euglycemic-hyperinsulinemic clamp measures were not part of the NGHS, we rely on insulin as the measure of insulin resistance despite its shortcomings.

In addition, we created a metabolic syndrome cluster score [208] at visit 10 (mean age 19) for those participants who had measurements available for all five components of the metabolic syndrome. The cluster score consisted of the sum of the z-scores of waist circumference (cm), systolic blood pressure (mmHg), triglycerides (mg/dl), inverse HDL cholesterol (mg/dl), and fasting glucose (mg/dl) divided by 5 at mean age 19 in the NGHS. The score was calculated as 1/5*([waist circumference-85.6]/15.0 - [HDL-cholesterol-53.6]/11.8 + [triglycerides-80.8]/40.8 + [systolic blood pressure-108.5]/9.2 + [fasting glucose-86.6]/7.8). A higher z-score represents higher overall risk.

Covariates

Demographic information for participants and their parents or guardians was obtained at enrollment. Self-reported data included age, race/ethnicity (non-Hispanic African-American or white according to United States census categories), parental education level, and parental smoking. Age was coded in whole years. Parental education was categorized into high school degree or less, some college, and college graduate. Parental smoking was categorized into ever smoking (yes/no). Female examiners performed pubertal maturation assessment at each visit according to Tanner for pubic hair stages and by the system of Garn and Falkner for areolar stages [200]. Pubertal stage was categorized into 6 categories at each annual visit, ranging from stage 1=prepubertal to stage 5-6=post menarche.

Statistical Methods

Descriptive statistics for the baseline visit were calculated as means (SD) for continuous variables and percent for categorical variables according to the following categories of age at menarche: early=8.0-11.9, mid=12.0-13.9, late=14.0-17.0. Fasting insulin and triglycerides were not normally distributed and were therefore log-transformed for analyses. However, there were no qualitative differences between the transformed and non-transformed values for triglycerides and so the non-transformed data are presented and used in the metabolic syndrome cluster score for simplicity and ease of interpretation. Log-transformed insulin values were used in all analyses.

To account for correlated observations for lipids, blood pressure, and adiposity measures within participants over the study period, we used repeated measures mixed linear regression models (PROC MIXED in SAS) to examine how associations of age at menarche with the cardiometabolic risk factor measures changed over time (i.e. with age). Age at menarche was modeled as a continuous variable in whole years for results in the tables, and as a categorical variable (early, mid, and late) for ease of interpretation in **Figures 6 and 7.** Each of the cardiometabolic risk factors was included as the dependent continuous variable in separate models. Higher order time variables were included in models to determine if there was a non-linear change in cardiometabolic risk factors during adolescence and retained only if statistically significant. Based on the best fit of predicted values over time, we modeled linear and quadratic slopes for

changes in total cholesterol, LDL-C, HDL-C, blood pressure, percent body fat, and BMI in separate models. Linear, quadratic, and cubic slopes best fit the pattern of change for triglycerides. The main menarche coefficients measured the effect of age at menarche on each risk factor overall during adolescence (menarche main effect). The coefficients for the menarche*time interactions measured the linear, quadratic, or cubic age-related change per year younger age at menarche.

Models were run in a series, adding covariates only if they were associated with age at menarche category and CVD risk, but not likely to be intermediate variables between menarche timing and the outcomes. Model 1 included terms for continuous age at menarche, follow-up visit number 1-10 (the time variable), age at each visit (time varying as the difference between current age at the visit and the mean age at each visit), parental education level, parental smoking, study center, and the products of the time variable with age at menarche. Models were further adjusted (Model 2) for percent body fat at each visit (except for the adiposity outcomes) to account for the role of adiposity in both earlier pubertal maturation and in the development of cardiometabolic risk factors. Adjusted mean levels of the cardiometabolic risk factors at each time point by age at menarche category (early, mid, late) for the **Figures 6 and 7** were derived from mixed models in which follow-up visit year was included as a categorical variable. The adjusted means provided goodness of fit information regarding the linear, quadratic, or cubic slopes.

For cross-sectional analyses at visit year 10 (mean age 19), multiple linear regression was used to test if there were differences in the mean values of continuous total cholesterol, LDL-C, HDL-C, triglycerides, systolic blood pressure, percent body fat, BMI, glucose, insulin, and the metabolic syndrome cluster score by age at menarche. Age at menarche was modeled as a continuous variable in whole years. As with the longitudinal models, Model 1 covariates included age (at visit 10), center, parental education level, and parental smoking, and then percent body fat at visit 10 was added in Model 2 (except for the adiposity outcomes).

A formal test for statistical interaction by race in each of the longitudinal and cross-sectional models assessed differences in the influence of the timing of menarche on the CVD outcomes. Even if the formal interaction test was not significant, separate models were built for African-American and white girls as our *a priori* research question related to race-specific changes in CVD risk factors over time, and given the race differences in age at menarche and age-related growth in adiposity in the cohort. In addition, because adiposity is strongly associated with both age at menarche and CVD risk factors, the longitudinal and cross-sectional models were stratified by pre-menarche BMI category ($<85^{th}$ percentile vs. $\geq 85^{th}$ percentile for age), with a formal BMI category*menarche interaction test for differences in the associations between categories. These models were adjusted for age, clinical center, parental education, parental

smoking status, BMI at baseline (or at visit for the cross-sectional analysis at mean age 19), pubertal stage at baseline, and race.

All analyses were conducted in SAS v9.2 (Cary, NC). The alpha error value was set at 0.05 and all p-values were two-sided. Because of the multiple comparisons included in these analyses, Bonferonni corrected p-values <0.005 (p=0.05/10 outcomes) were considered to be statistically significant for the purposes of these analyses.

Results

The overall cohort of 2,122 girls had a mean age of 10.0 years at baseline (range=9.0-11.0 years) and 19.0 years at the final visit (range=17.6-21.5 years). The mean age at menarche was 12.3 years (SD=1.2) for the total sample, and was younger for African-American (12.0 years, SD=1.1) compared with white (12.6 years, SD=1.2) girls (t=12.2, p-value<0.0001). At baseline, mean BMI was 18.5 kg/m^2 (SD=3.8) and mean percent body fat was 21.2% (SD=8.3). Baseline adiposity was significantly higher among African-American (BMI: 19.2 kg/m^2, SD=3.2; percent body fat: 21.6%, SD=9.1) compared with white (BMI: 17.9 kg/m^2, SD=3.2; percent body fat: 20.7%, SD=7.5) girls (BMI: t=-8.08, p<.0001; percent body fat: t=-2.41, p=0.02).

Table 4 shows the baseline characteristics of the study population by age at menarche category. For both African-American and white girls, earlier menarche was associated with greater BMI, percent body fat, and height at baseline. A lower level of parental education and higher percentage of parental

smoking were more frequent in the early menarche group for white girls, but not for African-American girls.

Longitudinal Analysis

In longitudinal analyses, there were 20,609 visits for these 2,122 girls, with an average of 9.7 visits per participant. **Figures 6 and 7** display the cross-sectional means for percent body fat, BMI, total cholesterol, LDL-C, HDL-C, triglycerides, and systolic blood pressure for African-American and white girls at each visit by menarche group (early, average, and late), as well as the main effect for menarche category over the entire study period, and the influence of menarche on changes in each risk factor over time (menarche*time interactions). The mean values in **Figure 6** are adjusted for age, clinical center, parental education, and parental smoking, while means in **Figure 7**are additionally adjusted for percent body fat. For all groups, we found a dip in total cholesterol and LDL-C, but an increase in HDL-C during mid-adolescence. The pattern in triglycerides was more complex, but levels were generally higher in early adolescence compared with late adolescence after adjusting for percent body fat (**Figure 7**). Systolic blood pressure, percent body fat, and BMI all increased during adolescence.

Table 5 shows the results (beta+/-SE and p-value) of the repeated measures mixed regression models, which tested whether each one year earlier age at menarche was a predictor of the overall main effect values and the age-related change in each risk factor during adolescence after adjustment for age,

race, clinical center, parental education, and parental smoking (Model 1), and then additionally adjusted for percent body fat (Model 2, except percent body fat and BMI). For simplicity, only the menarche main effect and menarche*time slope terms are reported in the table as these coefficients best captured the story of the overall levels of risk factors and pattern of change during adolescence. The quadratic and cubic time interaction terms indicated that there were no meaningful differences by age at menarche category in the concave or convex pattern of change in the risk factors with age during adolescence.

Overall during adolescence, each one year earlier age at menarche was associated with lower mean total cholesterol and HDL-C, but higher body fat, BMI, and systolic blood pressure (**Table 5**, Model 1 main menarche effects). After adjustment for percent body fat, total cholesterol and LDL-C were lower and systolic blood pressure was higher per one-year earlier age at menarche (**Table 5,** Model 2 main menarche effects**)**. There were no race differences in the influence of age at menarche on any of the CVD risk factors.

When examining age at menarche in categories of early, mid, and late in **Figure 6,** girls in the early menarche category had higher percent body fat, BMI, and systolic blood pressure compared with later maturing girls overall during adolescence. After adjustment for percent body fat (**Figure 7**), girls with early menarche had higher systolic blood pressure, and African-American girls with early menarche had lower HDL-C, compared with the later menarche categories.

As shown by the menarche*age interaction terms in **Table 5**, there was an age-related pattern of convergence in the levels of total cholesterol, LDL-C, HDL-C, and systolic blood pressure, but a divergence in percent body fat, (menarche*age interactions $p<0.005$) per one-year earlier age at menarche over the course of adolescence. There were no race differences with regard to the influence of age at menarche on the age-related rate of change in the other CVD risk factors.

When the participants were stratified by pre-menarche BMI category **(Table 6),** each one-year earlier age at menarche was associated with lower total and LDL cholesterol and higher systolic blood pressure, percent body fat, and BMI among girls with pre-menarche BMI <85th percentile only. The BMI category*menarche interaction p-values for systolic blood pressure, percent body fat, and BMI all reached statistical significance at the $p<0.005$ level. There were no differences by race in the influence of age at menarche on the CVD risk factors within in BMI category.

Cross-Sectional Analyses

Table 7 shows the cross-sectional associations of each one-year younger age at menarche with each risk factor at mean age 19 years, and **Table 8** shows the BMI-stratified results. Each one-year earlier age at menarche was associated with higher percent body fat, BMI, systolic blood pressure, and insulin resistance at age 19 (**Table 7**). After adjustment for percent body fat at mean age 19 years, age at menarche was no longer associated with any of the CVD risk factors. Age

at menarche was associated with higher percent body fat and BMI among girls who had pre-menarche BMI <85th percentile only (**Table 8**), and there were no other statistically significant associations in the BMI-stratified models. The influence of the timing of menarche did not differ by race for any of the risk factors.

Discussion

This study extends the findings from past studies by looking at race-specific, longitudinal changes in a number of CVD risk factors by menarche status during a time in which the obesity epidemic was taking hold in the population, and race disparities in pubertal timing and adiposity were becoming more evident. The study results show that earlier menarche was associated with greater adiposity throughout adolescence. There were also some weak associations of earlier menarche with lower total cholesterol, LDL-C, and HDL-C, but higher systolic blood pressure overall during adolescence before considering the greater degree of adiposity among earlier maturing girls. By the end of the 10-year follow-up, early menarche was associated with higher systolic blood pressure and insulin, but all associations were attenuated and no longer statistically significant after adjustment for percent body fat. There was no indication of an association of early menarche with triglycerides or glucose. Examination of associations by pre-menarcheal BMI category suggested that earlier menarche might have a greater influence more adverse levels of systolic blood pressure and adiposity accumulation post-menarche among normal vs. obese girls. Contrary to

expectations, there were no differences by race in the influence of age at menarche in the levels or change in the CVD risk factors despite higher average levels of obesity and earlier age at menarche among African-American girls.

As in other studies, this study demonstrated that levels of CVD risk factors change during adolescence with growth and development [202, 209-212]. This study showed a mid-adolescent decline in total cholesterol, LDL-C, and triglycerides, but an increase in HDL-C, systolic blood pressure, percent body fat, and BMI with advancing pubertal stage. The tendency toward a more healthy lipid profile during mid-puberty may be the result of increased oestradiol and growth hormone concentrations during this developmental stage [213]. In late adolescence, total cholesterol, LDL-C, and triglycerides increased, while HDL-C and blood pressure tended to decrease, all while adiposity continued its upward climb.

Also in agreement with past studies [91, 92, 174, 177] was the finding that earlier menarche was associated with higher levels of adiposity for both African-American and white girls during adolescence. A study that also used NGHS data previously reported on changes in a wide variety of body composition measures by age at menarche, including BMI and percent body fat, but the authors did not present results adjusted for potential confounding factors and used different menarche categories by race, which made comparisons difficult [91]. New in the current analysis regarding adiposity was that earlier menarche was more strongly associated with greater percent body fat and BMI overall during adolescence

among girls who were normal vs. overweight prior to menarche; earlier menarche did not appear to influence adiposity levels among girls who were already overweight prior to menarche even after adjusting for overall pubertal status at baseline. Furthermore, the current study showed that although adiposity remained higher overall for African-American girls, there were not any meaningful race differences in the relationship between earlier menarche and adiposity.

Earlier studies are mixed with regard to the role of early maturation on blood lipid levels during adolescence or adulthood, but like us, most have found that earlier menarche is not detrimental for blood lipids [173, 174, 177]. For example, in the Bogalusa Heart Study, mean levels of lipoproteins were similar for girls with menarche <12 and 12+ years [177]. However, the authors noted that even through the girls reporting later menarche had relatively less adiposity compared to those reporting early menarche, the later maturing girls were not lean, and so incomplete adiposity adjustment may have masked subtle differences in lipid levels. There was also no association between earlier menarche and lipids in the Fels Longitudinal Study [174], but as pointed out in reference [168], findings might have been subject to a cohort effect due to the variation in the timing of baseline measurements. In the current study, which was not subject to cohort effects, we found that earlier menarche was associated with lower total cholesterol and LDL-C overall during adolescence for African-American and white girls, but by mean age 19, total cholesterol and LDL-C did not differ by menarche groups. We found a significant main effect of earlier

menarche with lower HDL-C in the longitudinal models before but not after adjustment for percent body fat. Previous studies are mixed with regard to HDL-C, with at least three studies reporting no association of earlier menarche with HDL-C levels during adolescence or adulthood [173, 174, 177], and one reporting that early menarche was associated with lower HDL-C in adulthood [179].

Insulin and glucose were not measured throughout adolescence in the overall NGHS, and so we examined cross-sectional associations at age 19 only. Similar to other studies that have reported that earlier menarche is associated with higher insulin levels or insulin resistance during adolescence and adulthood [168, 174, 177-179], we found that early menarche was associated with higher mean insulin before adiposity adjustment. The association was completely attenuated after adjustment for adiposity, which has also been found by others [168].

Adverse levels of metabolic factors tend to cluster together (blood pressure, insulin, obesity, and blood lipids) [150]. This clustering changes with growth and development, and levels increase during preadolescence, decrease over the course of puberty, and increase again in young adulthood, independently of race or timing of pubertal maturation [212]. The clustering of metabolic risk factors has also been shown to track into adulthood, so adverse levels during adolescence have implications for long-term health [214]. Therefore, we examined the association of earlier age at menarche and the clustering of these risk factors using a metabolic syndrome cluster score. Before adjusting for percent body fat,

there was an inverse association between age at menarche and the metabolic syndrome cluster score (Beta=0.10 μU/ml [SE 0.04], p=0.01) that did not quite meet the Bonferroni-adjusted threshold, but the association was completely attenuated after adjustment for percent body fat. Other studies have reported that age at menarche was inversely associated with metabolic syndrome in adolescence [177] and adulthood [168, 176, 177, 179], although associations were attenuated, sometimes completely (as in [168]) after adjustment for adiposity.

Methodological Considerations

Our study has certain limitations worth noting. First, age at menarche was based on self-report. However, the validity and reliability of self-report is well established, especially in the short term [12, 201]. We expect that any misclassification of age at menarche would be random with respect to the outcomes, which would tend to underestimate the true differences between groups. The publically available NGHS dataset also lacks serial measurements of insulin and glucose, so we were unable to estimate the change during adolescence for these measures, or for the metabolic syndrome cluster score that incorporates glucose.

Additionally, measurements in the NGHS began at mean age 10 and some girls (close to 50% of the cohort) had already started puberty. Therefore, our study is unable to examine if higher adiposity preceded the onset of puberty, or vice versa, making it unclear if adiposity should be considered a confounder or a

mediator in the relationship between early menarche and later CVD risk. Some evidence suggests that higher weight gain in infancy and higher levels of adiposity levels in childhood initiate estrogen production and cause earlier onset of puberty in girls [15, 75, 76]. Other evidence suggests that adiposity may be a downstream consequence of pubertal maturation [7, 83]. Earlier pubertal onset may initiate adipose tissue accumulation during later stages of development, making temporality of the association between pubertal and adiposity development unclear [83, 84]. Furthermore, it is unclear if prepubertal body composition is more relevant to the actual onset of puberty or to the duration of puberty [85].

Lastly, several outcomes were included in our analyses, raising concern over multiple comparisons and an increased risk for false-positive (type 1 error) results. Although we based our hypotheses on *a priori* evidence, we present both the Bonferroni adjusted and traditional significance levels to account for multiple comparisons.

The strengths of the study include the use of both cross-sectional and longitudinal methods to examine the influence of earlier menarche on CVD risk factor levels and changes during adolescence. The cohort included a large sample size of roughly equal numbers of African-American and white girls, making race-specific analyses possible. Furthermore, the data were collected from a relatively recent cohort of girls during a period of time in which the prevalence of obesity was increasing and age at menarche was decreasing. In

addition, the study collected information on a large number of CVD risk factors, allowing us to look not only at individual factors, but to calculate a metabolic syndrome cluster score for a more comprehensive look at CVD risk during late adolescence. Finally, we adjusted directly for adiposity using percent body fat, instead of BMI or waist circumference, both of which might be influenced by body composition factors other than adiposity during adolescence.

Conclusion

In summary, the association of early maturation with CVD risk factors during adolescence was subtle and appeared to be driven primarily by higher adiposity levels among earlier maturing girls (as found in reference [168]). Even among girls who were normal weight prior to menarche, earlier age at menarche was associated with greater adiposity overall during adolescence. Age at menarche did not seem to influence adiposity levels during adolescence among girls who were already overweight prior to menarche. There were no important differences in the influence of early menarche on CVD risk factors by race. A focus on healthy lifestyle and prevention of adiposity accumulation starting early in childhood and prior to menarche may be helpful in the primordial prevention of CVD into adulthood.

Table 2. Number of participants included in analyses for each follow-up visit in the NGHS

Visit year	Baseline	2	3	4	5	6	7	8	9	10
Mean age (yrs)	10	11	12	13	14	15	16	17	18	19
N*	2,101	2,113	2,102	2,098	2,070	2,059	2,020	1,989	1,975	2,067

*N=number of participants who reported menarche 8-17 years, did not have diagnosed diabetes, had data available for all covariates, and who had measurements available for at least one cardiometabolic outcome at that visit

Table 3. Number of measurements for each cardiometabolic outcome in the NGHS

Variable	N*	Mean # of measures	Std Dev	Minimum	Maximum
Total cholesterol	2,087	3.6	1.1	1	5
LDL-C	2,087	3.6	1.1	1	5
HDL-C	2,087	3.6	1.1	1	5
Triglycerides	2,087	3.6	1.1	1	5
Systolic blood pressure	2,122	9.7	0.7	5	10
BMI	2,122	9.7	0.7	5	10
Percent body fat	2,122	9.7	0.7	5	10
Insulin	1,542	1	NA	1	1
Glucose	1,588	1	NA	1	1
Metabolic syndrome	1,352	1	NA	1	1

*N=participants with at least one measurement for the outcome over the study period after exclusions

Table 4. Baseline characteristics by age at menarche category in the NGHS

	Menarche Category			
	8.0-11.9 years	12.0-13.9 years	14.0-17.0 years	
	Mean (SD) or (%)	Mean (SD) or (%)	Mean (SD) or (%)	p-trend
African-American N (%) (n=1,046)	511 (48.9)	493 (47.1)	42 (4.0)	
Age at menarche (yrs)	10.6 (0.7)	12.3 (0.5)	14.2 (0.4)	
Age (years)	10.1 (0.6)	10.1 (0.6)	10.2 (0.5)	0.09
BMI (kg/m^2)	19.9 (4.1)	18.6 (4.2)	17.8 (4.1)	<.0001
Percent body fat (%)	22.7 (8.9)	20.8 (9.1)	18.6 (9.4)	<.0001
Height (cm)	145.5 (8.0)	140.8 (6.9)	137.2 (5.7)	<.0001
Parental smoking (ever=yes)	52.3	57.4	52.4	0.21
Parent Education				0.27
HS or less	31.3	28.0	16.7	
Total calories (kcal/day)	1868.3 (562.8)	1867.5 (637.5)	1791.8 (683.9)	0.70
Screen time (h/week)	36.2 (16.9)	37.2 (18.4)	36.1 (20.1)	0.54
Physical activity (MET-h/week)	29.5 (19.8)	31.6 (19.4)	30.3 (15.2)	0.14
White N (%) (n=1,076)	304 (28.3)	648 (60.2)	124 (11.5)	
Age at menarche (yrs)	10.7 (0.6)	12.4 (0.5)	14.3 (0.6)	
Age (years)	9.9 (0.5)	10.0 (0.6)	10.0 (0.6)	0.16
BMI (kg/m^2)	18.8 (3.3)	17.7 (3.2)	16.5 (2.6)	<.0001
Percent body fat (%)	22.2 (7.1)	20.6 (7.6)	17.6 (7.0)	<.0001
Height (cm)	142.0 (7.6)	139.1 (6.8)	137.1 (6.2)	<.0001
Parental smoking (ever yes)	58.9	57.9	42.7	0.01
Parent Education				0.005
HS or less	21.1	18.2	12.1	
Total calories (kcal/day)	1837.9 (504.6)	1796.1 (428.9)	1803.3 (421.2)	0.30
Screen time (h/week)	25.5 (14.3)	25.0 (14.5)	22.5 (13.7)	0.08
Physical activity (MET-hr/week)	32.7 (19.2)	33.5 (19.1)	34.4 (20.1)	0.38

Table 5. Main effect and annual age-related change in levels of cardiometabolic risk factors during adolescence according to each 1-year younger age at menarche in the NGHS

	All girls	African-American	White	
	beta+/-SE (P)	beta+/-SE (P)	beta+/-SE (P)	p-interaction (race)
Total Cholesterol (mg/dl)				
Model 1				
Main menarche effect	-1.61+/-0.47 (0.0006)	-2.13+/-0.71 (0.003)	-1.24+/-0.62 (0.05)	0.39
*Menarche*age*	0.30+/-0.06 (<.0001)	0.30+/-0.10 (0.002)	0.38+/-0.09 (<.0001)	0.57
Model 2				
Main menarche effect	-2.08+/-0.47 (<.0001)	-2.45+/-0.71 (0.0005)	-1.87+/-0.62 (0.003)	0.38
*Menarche*age*	0.28/-0.06 (<.0001)	0.28+/-0.10 (0.002)	0.30+/-0.08 (<.0001)	0.49
LDL-C (mg/dl)				
Model 1				
Main menarche effect	-0.96+/- 0.43 (0.02)	-1.39+/-0.67 (0.04)	-0.65+/-0.57 (0.26)	0.41
*Menarche*age*	0.22+/-0.06 (0.002)	0.17+/-0.09 (0.06)	0.30+/-0.09 (0.0004)	0.25
Model 2				
Main menarche effect	-1.58+/-0.42 (0.0002)	-1.94+/-0.66 (0.003)	-1.30+/-0.57 (0.02)	0.40
*Menarche*age*	0.20+/-0.06 (0.0009)	0.15+/-0.09 (0.10)	0.30+/-0.08 (0.0004)	0.18
HDL-C (mg/dl)				
Model 1				
Main menarche effect	-0.63+/-0.18 (0.0004)	-0.66+/-0.28 (0.02)	-0.58+/-0.23 (0.01)	0.93
*Menarche*age*	0.10+/-0.03 (0.0003)	0.13+/-0.04 (0.002)	0.09+/-0.04 (0.01)	0.46
Model 2				
Main menarche effect	-0.31+/-0.17 (0.07)	-0.29+/-0.27 (0.28)	-0.32+/-0.22 (0.15)	0.96
*Menarche*age*	0.11+/-0.03	0.15+/-0.04 (0.0004)	0.10+/-0.04 (0.008)	0.33

	(<.0001)			
Triglycerides (mg/dl)				
Model 1				
Main menarche effect	0.27+/-0.60 (0.65)	-0.13+/-0.64 (0.84)	0.30+/-1.00 (0.77)	0.70
*Menarche*age*	-0.25+/-0.11 (0.02)	-0.13+/-0.12 (0.29)	-0.21+/-17 (0.21)	0.73
Model 2				
Main menarche effect	-0.95+/-0.05 (0.11)	-0.97+/-0.62 (0.12)	-1.36+/-0.96 (0.16)	0.66
*Menarche*age*	-0.30+/-0.10 (0.003)	-0.17+/-0.12 (0.16)	-0.22+/-0.16 (0.18)	0.89
Systolic Blood Pressure (mmHg)				
Model 1				
Main menarche effect	0.76+/-0.11 (<.0001)	0.61+/-0.16 (0.0002)	0.90+/-0.14 (<.0001)	0.19
*Menarche*age*	-0.07+/-0.02 (<.0001)	-0.07+/-0.08 (0.01)	-0.09+/-0.02 (0.0001)	0.49
Model 2				
Main menarche effect	0.45+/-0.10 (<.0001)	0.30+/-0.15 (0.05)	0.61+/-0.14 (<.0001)	0.14
*Menarche*age*	-0.09+/-0.2 (<.0001)	-0.09+/-0.02 (0.0004)	-0.16+/-0.08 (0.04)	0.78
Percent Body Fat (%)				
Model 1				
Main menarche effect	1.35+/-0.15 (<.0001)	1.40+/-0.25 (<.0001)	1.24+/-0.18 (<.0001)	0.82
*Menarche*age*	0.07+/-0.02 (<.0001)	0.07+/-0.03 (0.01)	0.02+/-0.02 (0.44)	0.12
BMI (kg/m^2)				
Model 1				
Main menarche effect	0.77+/-0.08 (<.0001)	0.87+/-0.14 (<.0001)	0.69+/-0.09 (<.0001)	0.34
*Menarche*age*	0.02+/-0.01 (0.01)	0.02+/-0.02 (0.12)	-0.01+/-0.01 (0.44)	0.07

Notes:
Main menarche effect is the average effect per one year younger age at menarche on the outcomes over the entire study period.

Menarche*age estimates are interpreted as the predicted change per year of age in an outcome variable assuming a 1-year decrease in age at menarche while the other predictor variables are held constant.

Model 1 adjusted for age, race, study year, parental education, parental smoking, and study center.

Model 2 additionally adjusted for percent body fat.

Year (the time variable) modeled as linear and quadratic terms in the main effect models, except for triglycerides, where a cubic time term was the best fit for the change over time.

Table 6. Main effect of each 1-year younger age at menarche on cardiometabolic risk factors from mean age 10 through 19 year in the NGHS, by pre-menarche BMI category* at baseline

Outcome	BMI<85th Percentile (n=1,431)		BMI>=85th Percentile (n=622)		P-Interaction BMI*Age at Menarche
	Beta (SE)	P-value	Beta (SE)	P-value	
All girls (n=2,053)					
Total Cholesterol (mg/dl)	**-2.11 (0.62)**	**0.0006**	-0.59 (1.02)	0.56	0.06
LDL-C (mg/dl)	**-1.56 (0.56)**	**0.005**	-0.21 (0.94)	0.82	0.11
HDL-C (mg/dl)	-0.56 (0.23)	0.02	0.12 (0.37)	0.73	0.009
Triglycerides (mg/dl)	-0.30 (0.61)	0.62	-2.45 (1.75)	0.16	0.006
Systolic Blood Pressure (mmHg)	**0.67 (0.14)**	**<.0001**	0.12 (0.22)	0.58	**0.0002**
Percent Body Fat (%)	**0.99 (0.12)**	**<.0001**	-0.64 (0.28)	0.02	**<.0001**
BMI (kg/m^2)	**0.49 (0.05)**	**<.0001**	-0.48 (0.29)	0.09	**0.0009**

Notes:

*Analysis excludes girls who reported reaching menarche at the baseline visit (n=69)

Models adjusted for age, clinical center, parental education, parental smoking status, BMI at baseline, pubertal stage at baseline, and race.

BMI cut at 85th percentile for age 9-10 according to CDC guideline: http://www.cdc.gov/growthcharts/percentile_data_files.htm

No significant interactions by race

Table 7. Cross-sectional associations of each 1-year younger age at menarche with cardiometabolic risk factors at mean age 19 years, the NGHS

MODEL 1	Beta	SE	p-value
All girls (n=2,082)			
Total Cholesterol (mg/dl)	0.31	0.77	0.69
LDL-C (mg/dl)	0.44	0.67	0.51
HDL-C (mg/dl)	-0.23	0.26	0.37
Triglycerides (mg/dl)	-0.04	0.91	0.97
Systolic blood pressure (mmHg)	0.46	0.17	0.007
Insulin (μ U/ml)*	**0.05**	**0.02**	**0.003**
Glucose(mg/dl)	0.36	0.46	0.45
Metabolic syndrome cluster score (z-score)	0.10	0.04	0.01
Adiposity			
Percent body fat (%)	**1.63**	**0.22**	**<.0001**
BMI (kg/m^2)	**0.84**	**0.12**	**<.0001**
African-American (n=1,030)			
Total Cholesterol (mg/dl)	-0.44	1.07	0.68
LDL-C (mg/dl)	-0.54	0.95	0.57
HDL-C (mg/dl)	-0.04	0.39	0.92
Triglycerides (mg/dl)	-0.17	1.10	0.88
Systolic blood pressure (mmHg)	0.32	0.25	0.21
Insulin (μ U/ml)*	0.06	0.03	0.01
Glucose(mg/dl)	-0.23	0.76	0.77
Metabolic syndrome cluster score (z-score)	0.12	0.05	0.04
Adiposity			
Percent body fat (%)	**1.83**	**0.36**	**<.0001**
BMI (kg/m^2)	**0.99**	**0.20**	**<.0001**
White (n=1,052)			
Total Cholesterol (mg/dl)	1.00	1.12	0.38
LDL-C (mg/dl)	1.30	0.95	0.17
HDL-C (mg/dl)	-0.40	0.36	0.27
Triglycerides (mg/dl)	-0.02	1.46	0.99
Systolic blood pressure (mmHg)	0.57	0.22	0.01
Insulin (μ U/ml)*	0.03	0.02	0.12
Glucose(mg/dl)	0.76	0.56	0.17
Metabolic syndrome cluster score (z-score)	0.07	0.05	0.15
Adiposity			
Percent body fat (%)	**1.39**	**0.26**	**<.0001**
BMI (kg/m^2)	**0.67**	**0.13**	**<.0001**
MODEL 2			
All girls (n=2,067)			
Total Cholesterol (mg/dl)	-0.48	0.77	0.54
LDL-C (mg/dl)	-0.46	0.66	0.48

HDL-C (mg/dl)	0.11	0.28	0.65
Triglycerides (mg/dl)	-1.09	0.90	0.23
Systolic blood pressure (mmHg)	0.05	0.16	0.74
Insulin (μ U/ml)*	-0.004	0.02	0.80
Glucose(mg/dl)	-0.18	0.46	0.70
Metabolic syndrome cluster score (z-score)	-0.01	0.03	0.72
African-American (n=1,021)			
Total Cholesterol (mg/dl)	-1.25	1.06	0.24
LDL-C (mg/dl)	-1.52	0.94	0.11
HDL-C (mg/dl)	0.37	0.39	0.34
Triglycerides (mg/dl)	-1.21	1.09	0.27
Systolic blood pressure (mmHg)	-0.13	0.24	0.58
Insulin (μ U/ml)*	0.003	0.02	0.90
Glucose(mg/dl)	-0.89	0.75	0.24
Metabolic syndrome cluster score (z-score)	0.02	0.05	0.73
White (n=1,046)			
Total Cholesterol (mg/dl)	0.19	1.12	0.86
LDL-C (mg/dl)	0.45	0.94	0.62
HDL-C (mg/dl)	-0.12	0.35	0.74
Triglycerides (mg/dl)	-1.10	1.45	0.45
Systolic blood pressure (mmHg)	0.22	0.22	0.32
Insulin (μ U/ml)*	-0.01	0.02	0.69
Glucose(mg/dl)	0.41	0.57	0.47
Metabolic syndrome cluster score (z-score)	0.01	0.05	0.85

Notes:

Model 1 adjusted for age, center, parental education, parental smoking

Model 2 additionally adjusted for percent body fat.

*Log-transformed value

Table 8. Cross-sectional associations of each 1-year younger age at menarche with cardiometabolic risk factors at mean age 19 years in the NGHS by pre-menarche BMI category* at baseline

	BMI <85th Percentile(n=1,414)		BMI >=85th Percentile (n=601)		P-Interaction BMI*Age at Menarche
	Beta (SE)	P-value	Beta (SE)	P-value	
All girls (n=2,015)					
Total Cholesterol (mg/dl)	-1.72 (1.02)	0.27	1.27 (1.67)	0.44	0.11
LDL-C (mg/dl)	-0.89 (0.88)	0.31	1.17 (1.50)	0.43	0.13
HDL-C (mg/dl)	-0.17 (0.36)	0.64	0.68 (0.54)	0.21	0.05
Triglycerides (mg/dl)	-0.89 (1.20)	0.46	-1.88 (2.05)	0.36	0.33
Systolic blood pressure (mmHg)	0.37 (0.21)	0.07	-0.01 (0.35)	0.98	0.18
Insulin (μ U/ml)**	0.01 (0.02)	0.44	0.03 (0.03)	0.25	0.72
Glucose(mg/dl)	0.28 (0.57)	0.62	-0.42 (1.20)	0.72	0.27
Metabolic syndrome cluster score (z-score)	0.06 (0.05)	0.19	-0.02 (0.07)	0.81	0.21
Adiposity					
Percent body fat (%)	**1.06 (0.21)**	**<.0001**	-0.19 (0.50)	0.71	0.07
BMI (kg/m^2)	**0.45 (0.10)**	**<.0001**	-0.12 (0.28)	0.67	0.28

Notes:

*Analysis excludes girls who reported menarche at the baseline visit (n=67)

**Log transformed

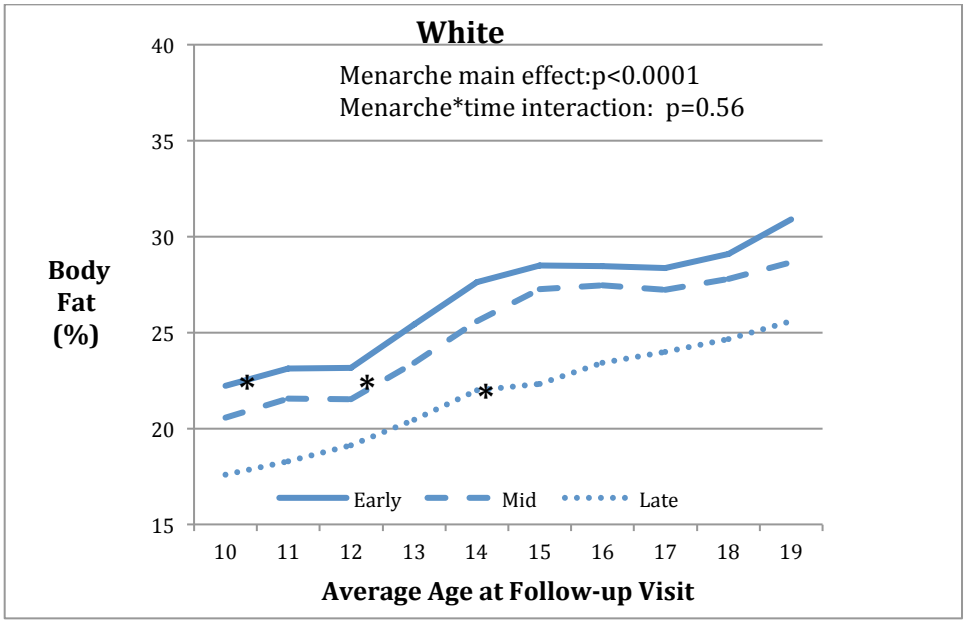
Adjusted for age at year 10, center, race, parental education, parental smoking, pubertal stage at baseline, and BMI at year 10 (except for adiposity measures)

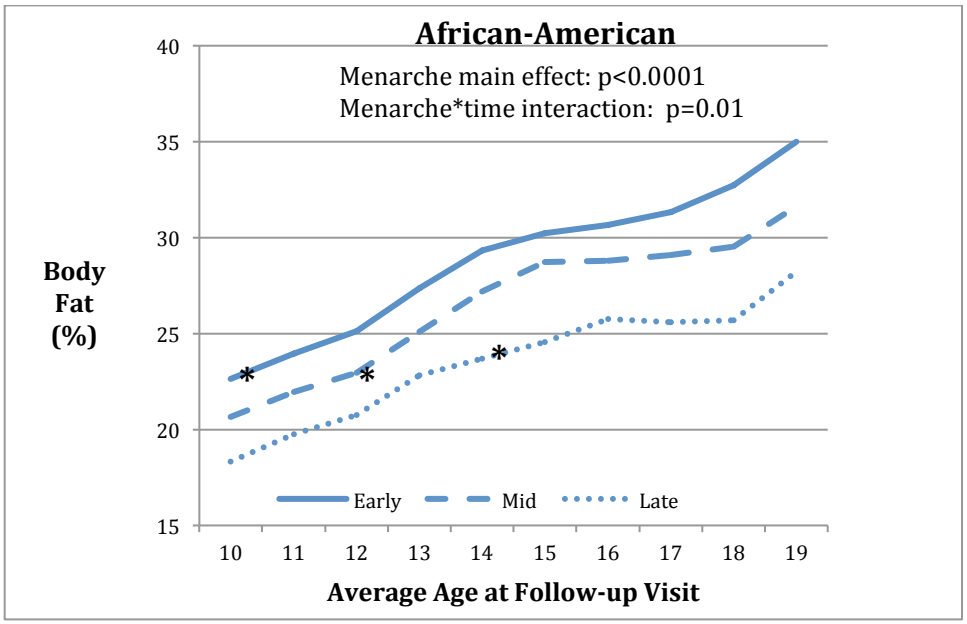
BMI cut at 85th percentile for age 9-10 according to CDC guideline:

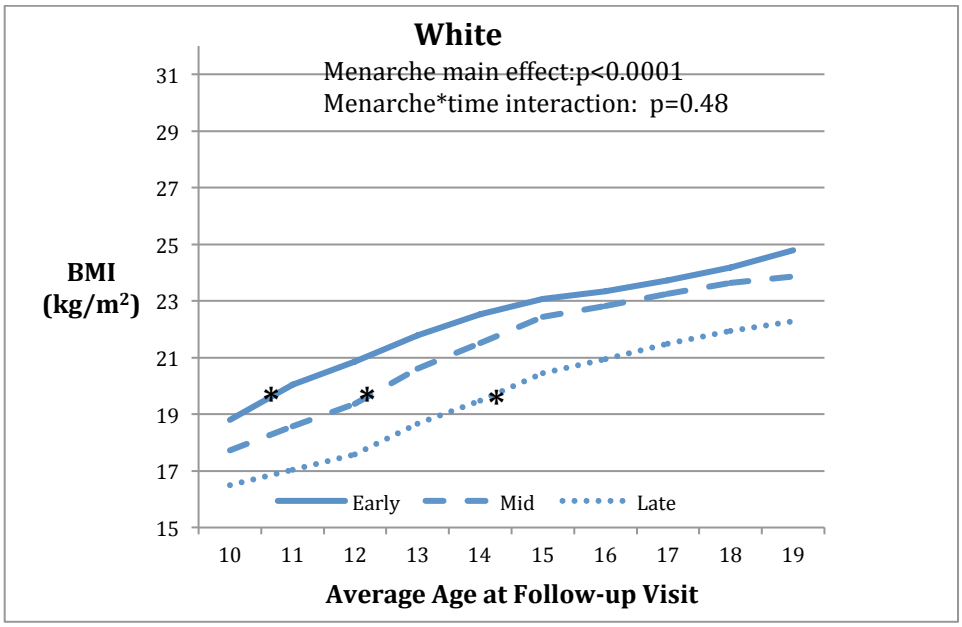
No significant interactions by race

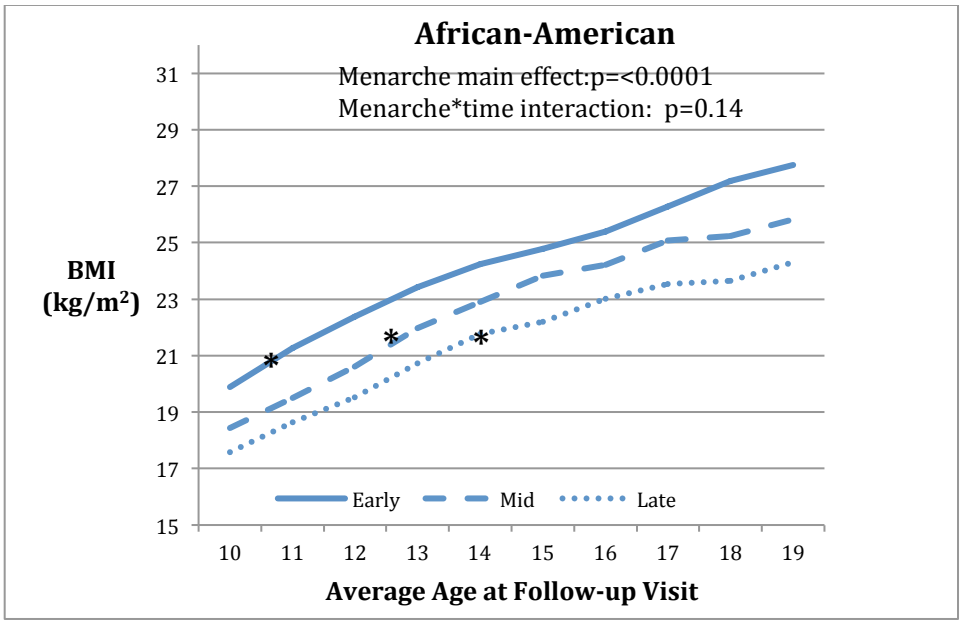
Figure 6. Longitudinal age-related changes in cardiovascular disease risk factors during adolescence by age at menarche category in the NGHS

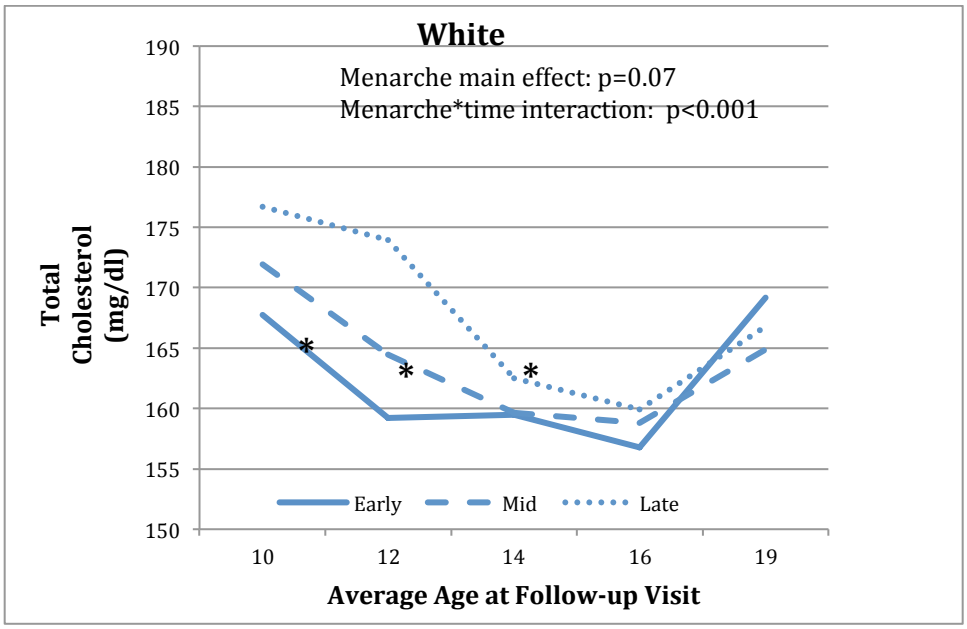
*=mean age at menarche for each category

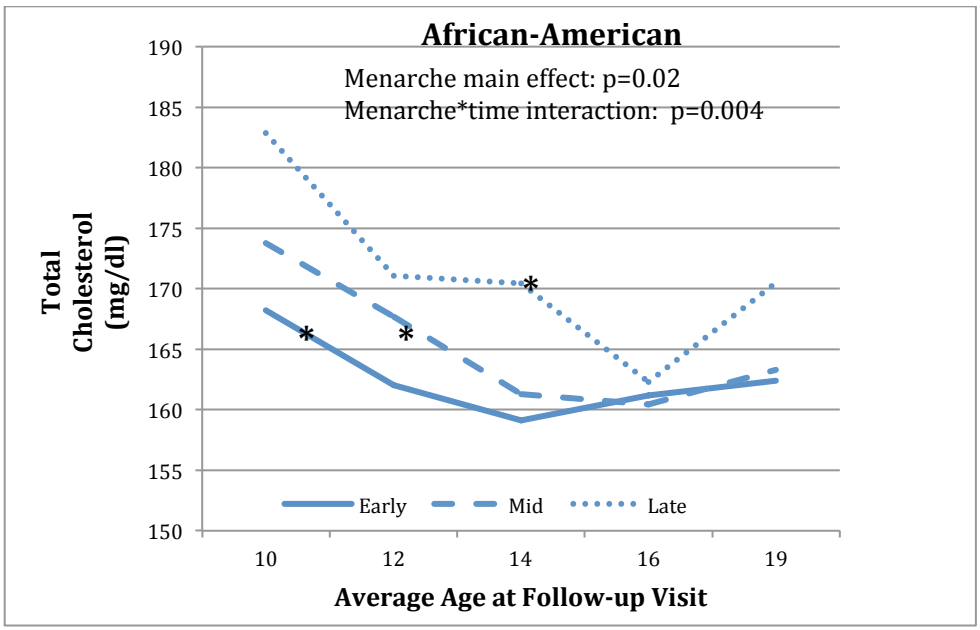


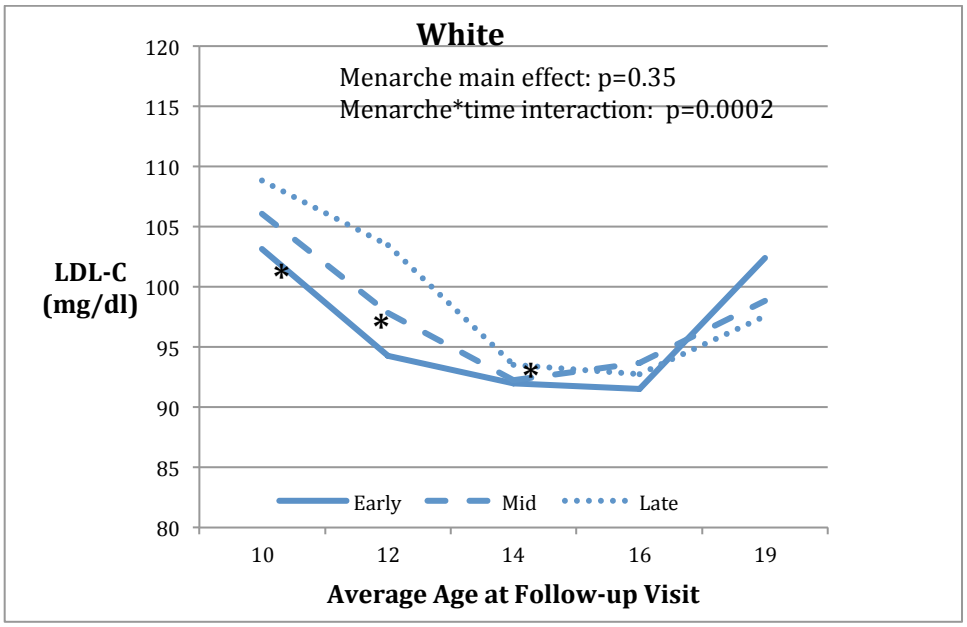


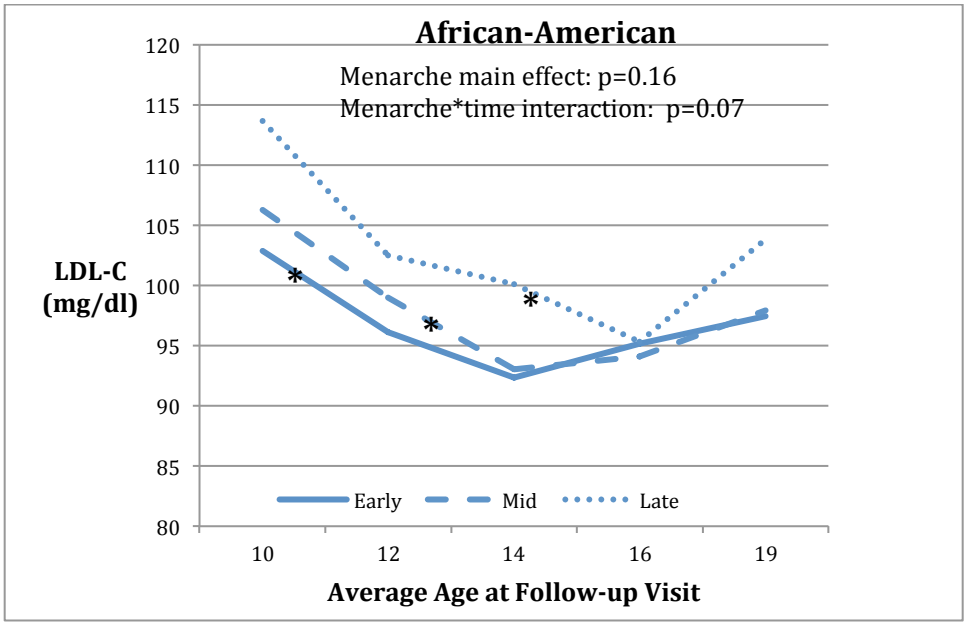

White
Menarche main effect:p<0.0001
Menarche*time interaction: p=0.48
BMI (kg/m²)
31
29
27
25
23
21
19
17
15
*
*
*
Early
Mid
Late
10
11
12
13
14
15
16
17
18
19
Average Age at Follow-up Visit

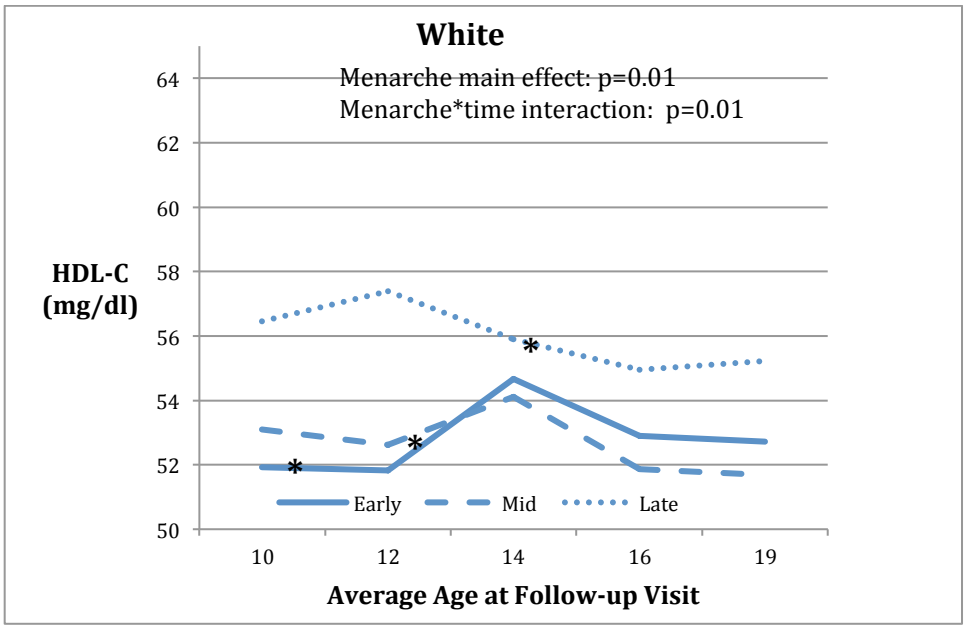

African-American
Menarche main effect:p=<0.0001
Menarche*time interaction: p=0.14
BMI (kg/m²)
31
29
27
25
23
21
19
17
15
*
*
*
Early
Mid
Late
10
11
12
13
14
15
16
17
18
19
Average Age at Follow-up Visit

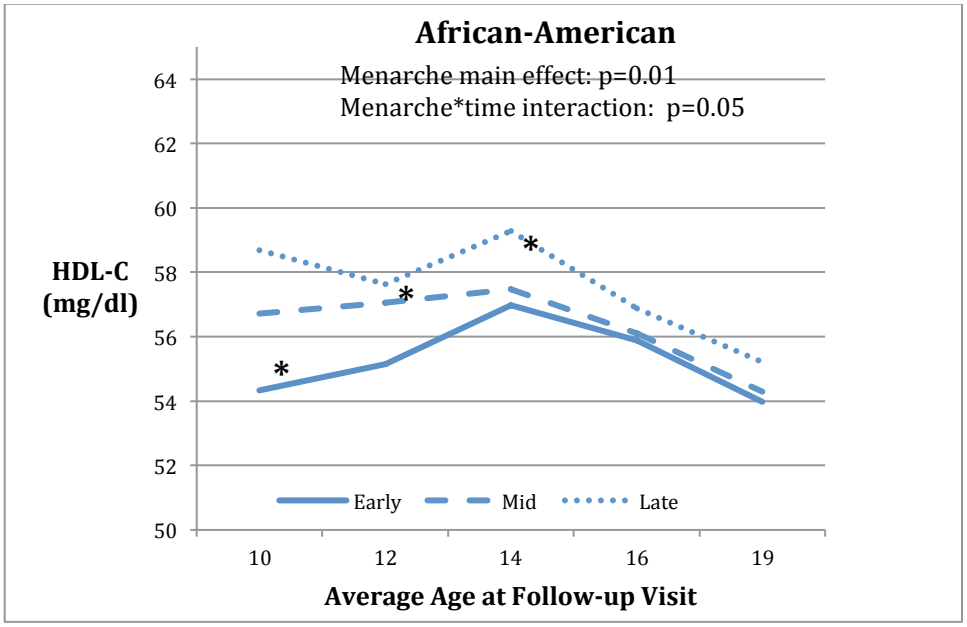

White
Menarche main effect: p=0.07
Menarche*time interaction: p<0.001
Total Cholesterol (mg/dl)
190
185
180
175
170
165
160
155
150
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

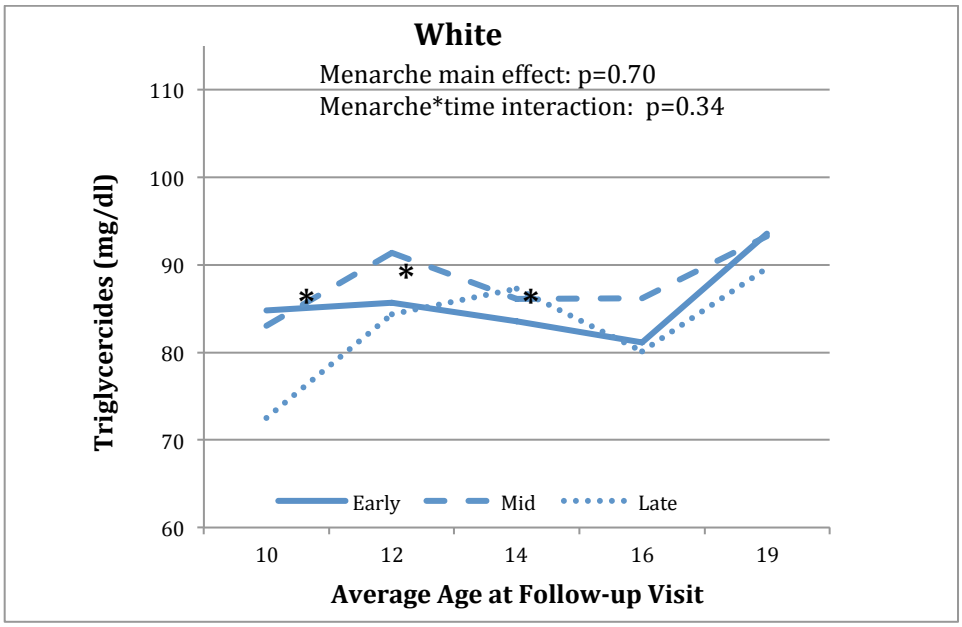

African-American
Menarche main effect: p=0.02
Menarche*time interaction: p=0.004
Total Cholesterol (mg/dl)
190
185
180
175
170
165
160
155
150
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

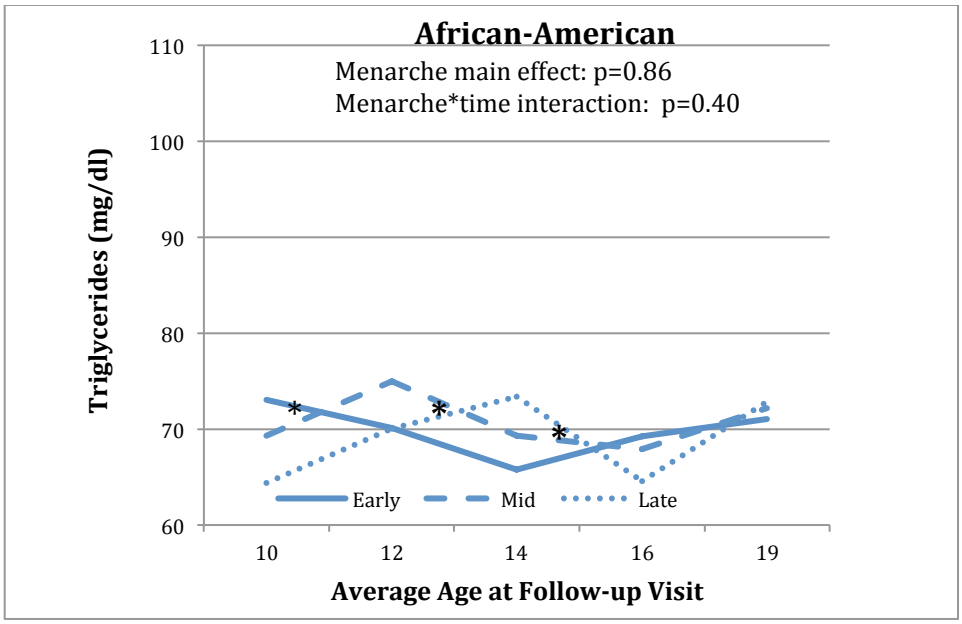

White
Menarche main effect: p=0.35
Menarche*time interaction: p=0.0002
LDL-C (mg/dl)
120
115
110
105
100
95
90
85
80
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

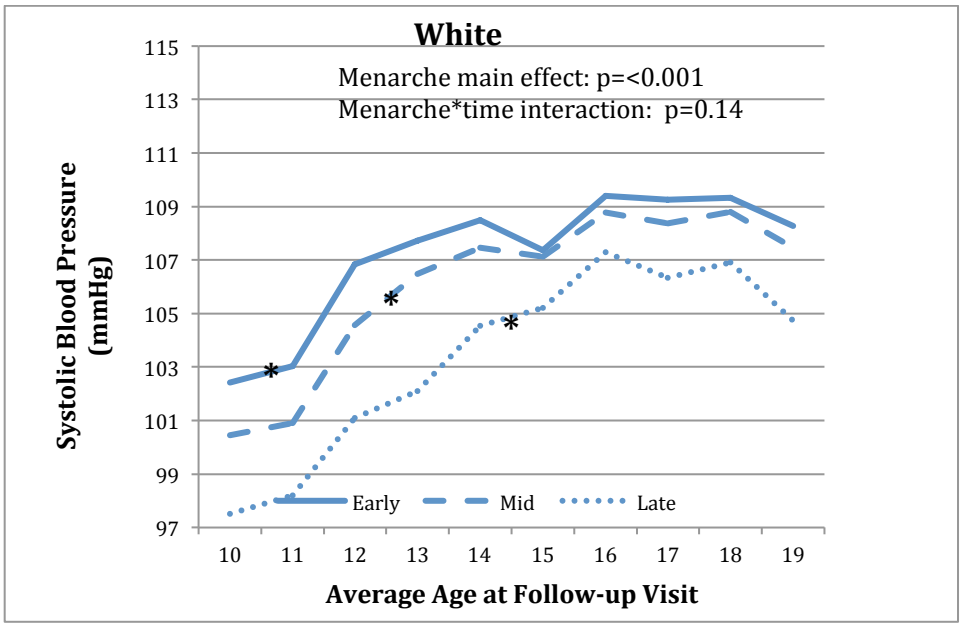

African-American
Menarche main effect: p=0.16
Menarche*time interaction: p=0.07
LDL-C (mg/dl)
120
115
110
105
100
95
90
85
80
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

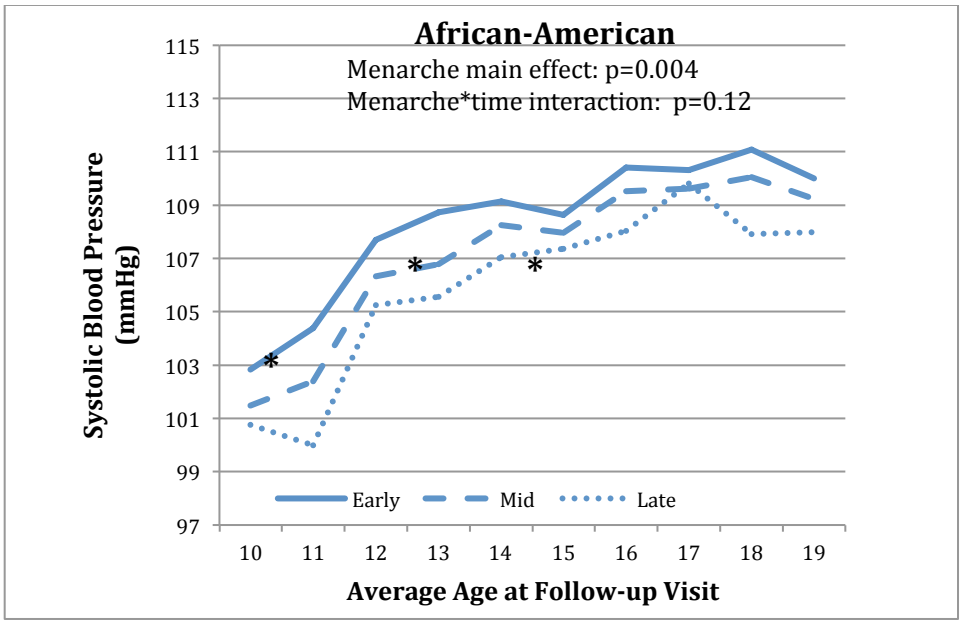

White
Menarche main effect: p=0.01
Menarche*time interaction: p=0.01
HDL-C (mg/dl)
64
62
60
58
56
54
52
50
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


African-American
Menarche main effect: p=0.01
Menarche*time interaction: p=0.05
HDL-C (mg/dl)
64
62
60
58
56
54
52
50
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


White
Menarche main effect: p=0.70
Menarche*time interaction: p=0.34
Triglycercides (mg/dl)
110
100
90
80
70
60
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


African-American
Menarche main effect: p=0.86
Menarche*time interaction: p=0.40
Triglycerides (mg/dl)
110
100
90
80
70
60
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

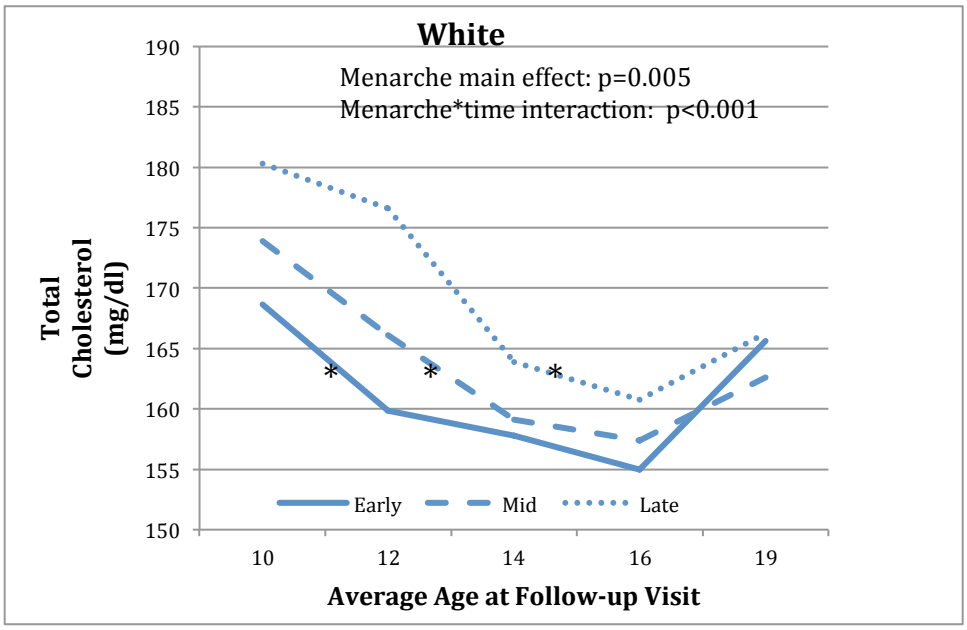


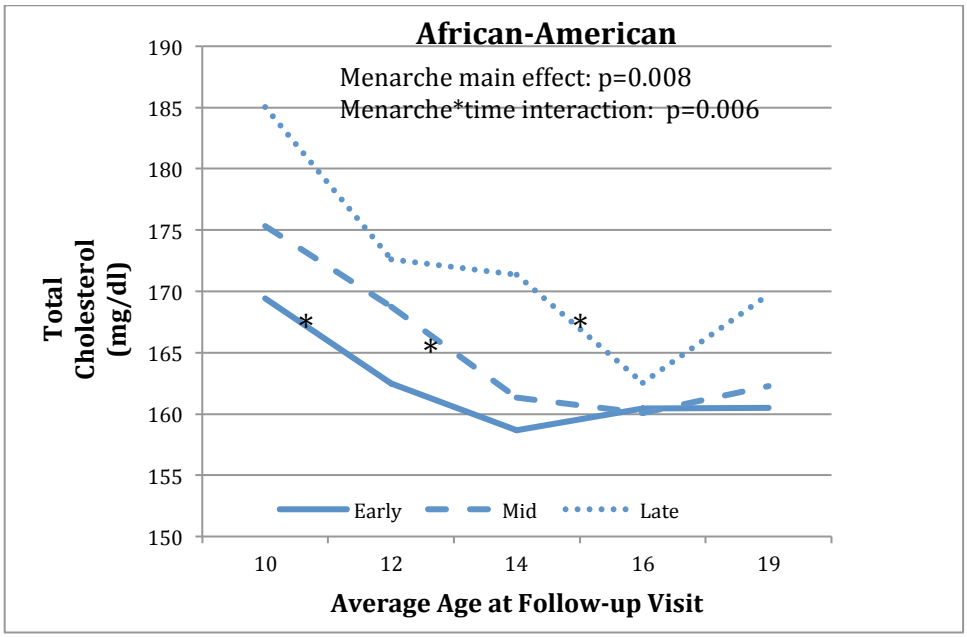


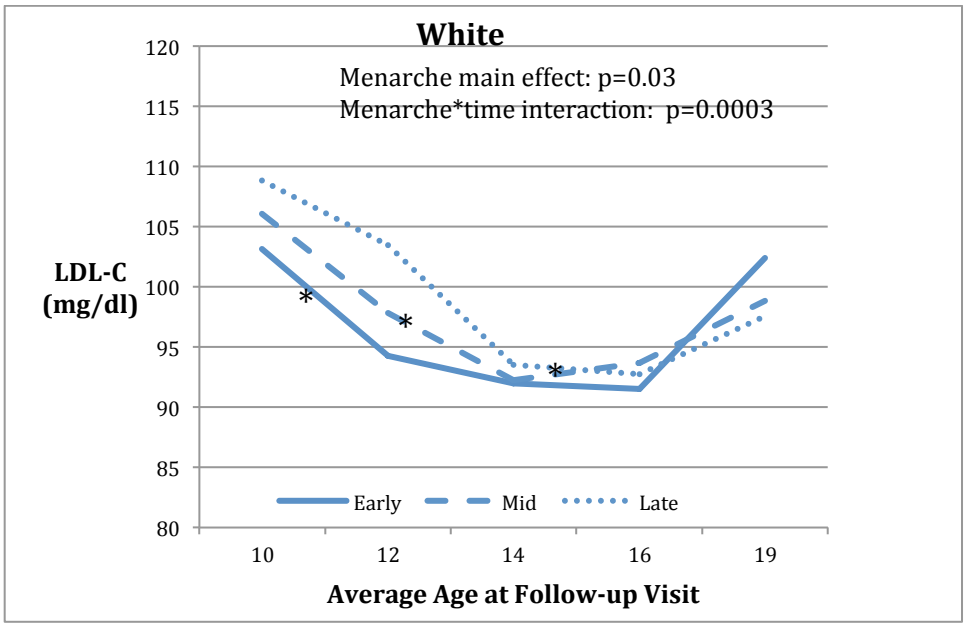
Notes:
Adjusted for age, clinical center, parental education, and parental smoking.
Menarche categories: early= 8.0-11.9 years, mid=12.0-13.9 years, late=14.0-17.0

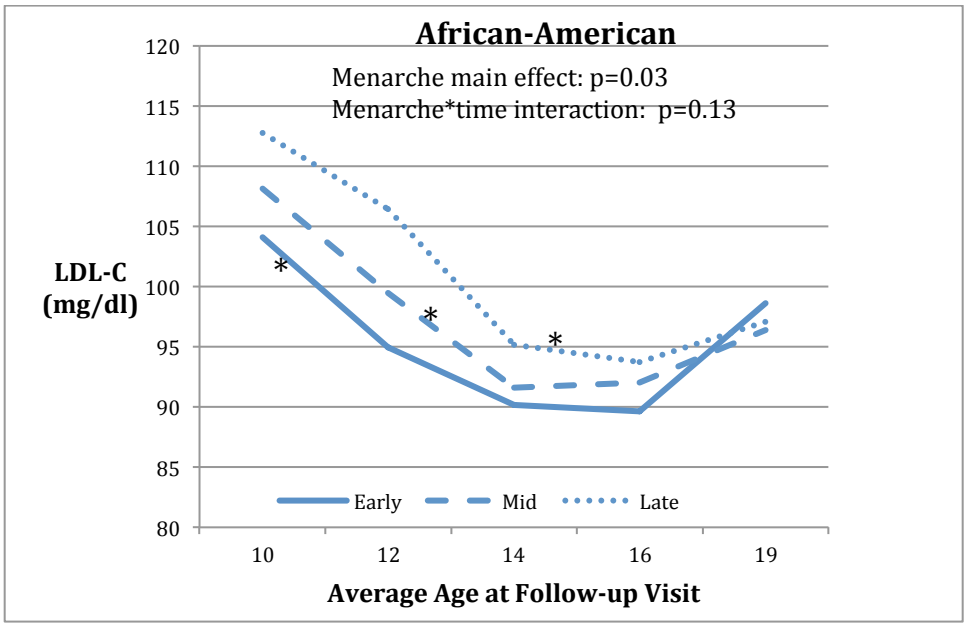
Figure 7. Longitudinal age-related changes in cardiovascular disease risk factors during adolescence by age at menarche category in the NGHS, adjusted for percent body fat

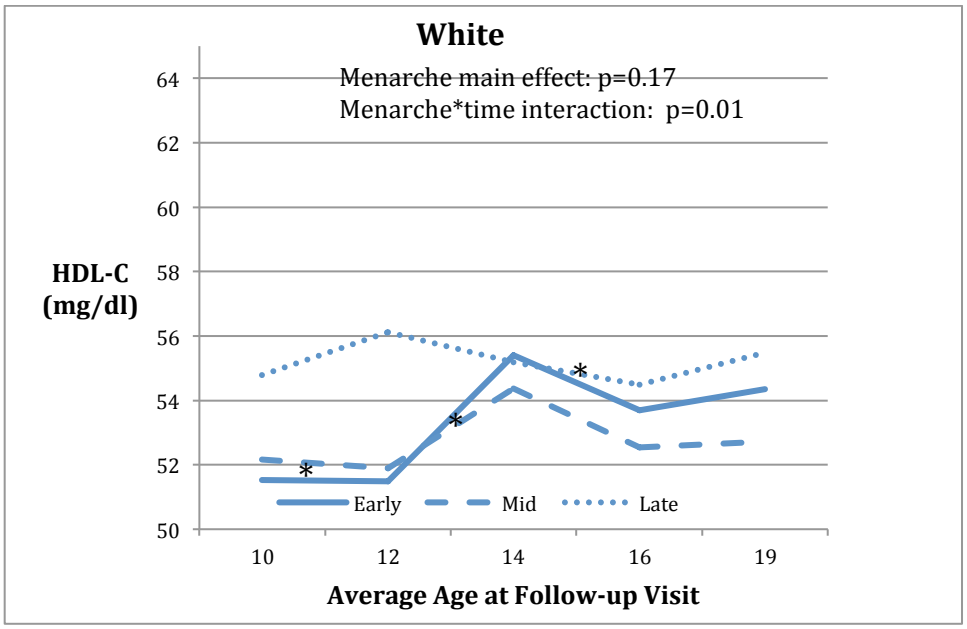
*=mean age at menarche for each category

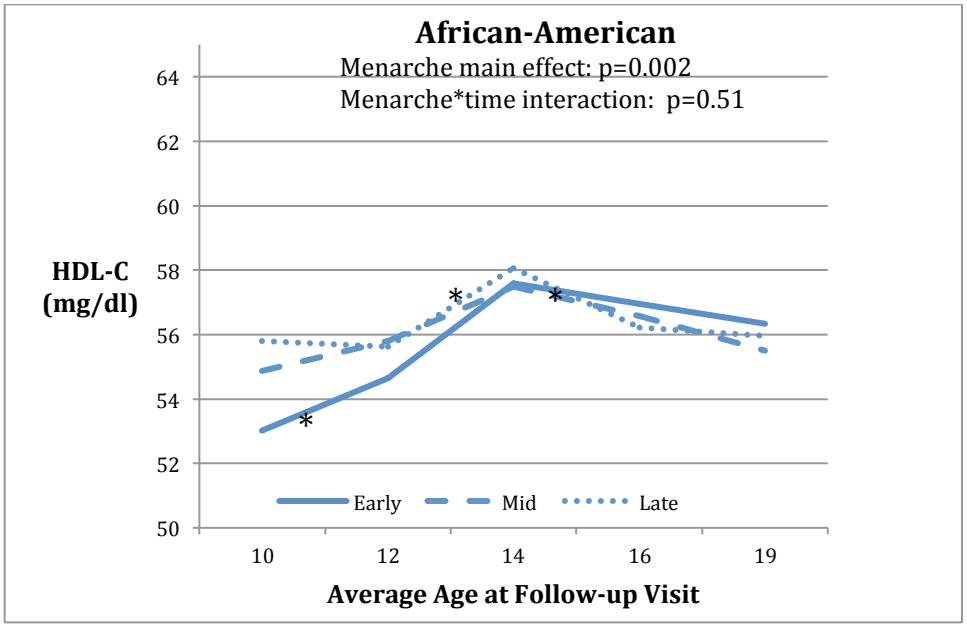


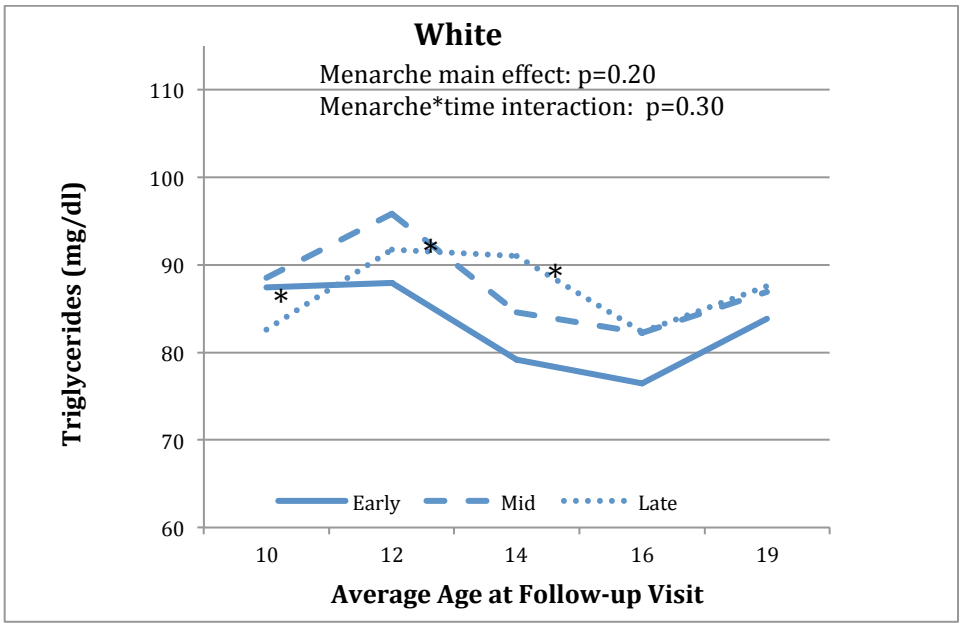


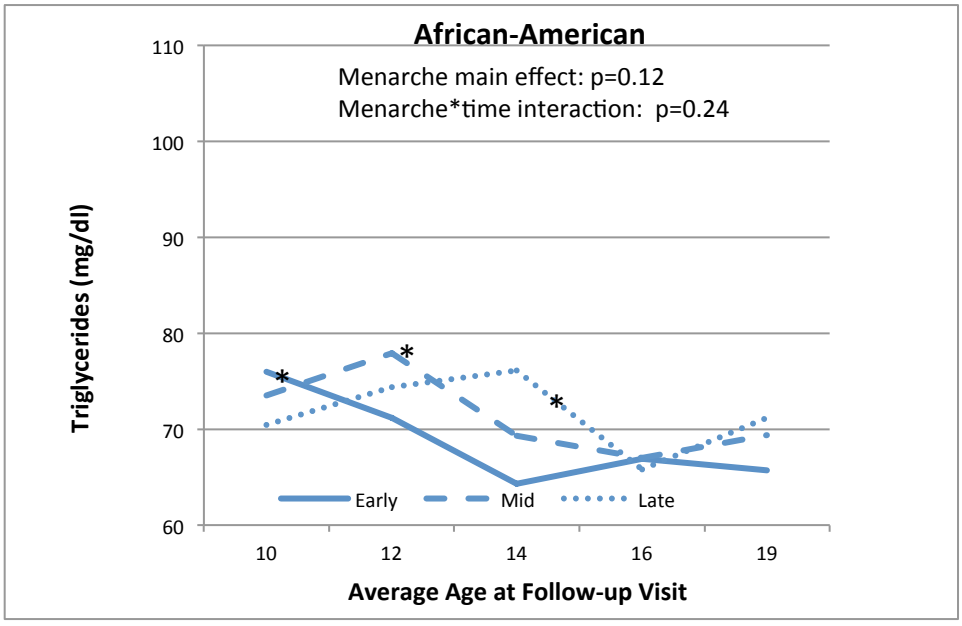

White
Menarche main effect: p=0.03
Menarche*time interaction: p=0.0003
LDL-C (mg/dl)
120
115
110
105
100
95
90
85
80
*
*
*
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

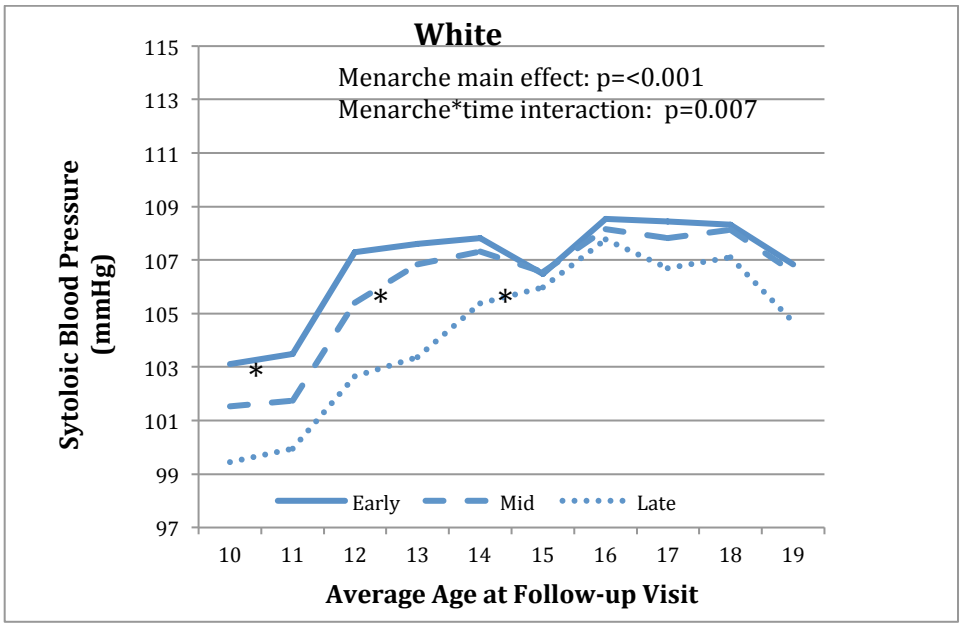

African-American
Menarche main effect: p=0.03
Menarche*time interaction: p=0.13
LDL-C (mg/dl)
120
115
110
105
100
95
90
85
80
*
*
*
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit

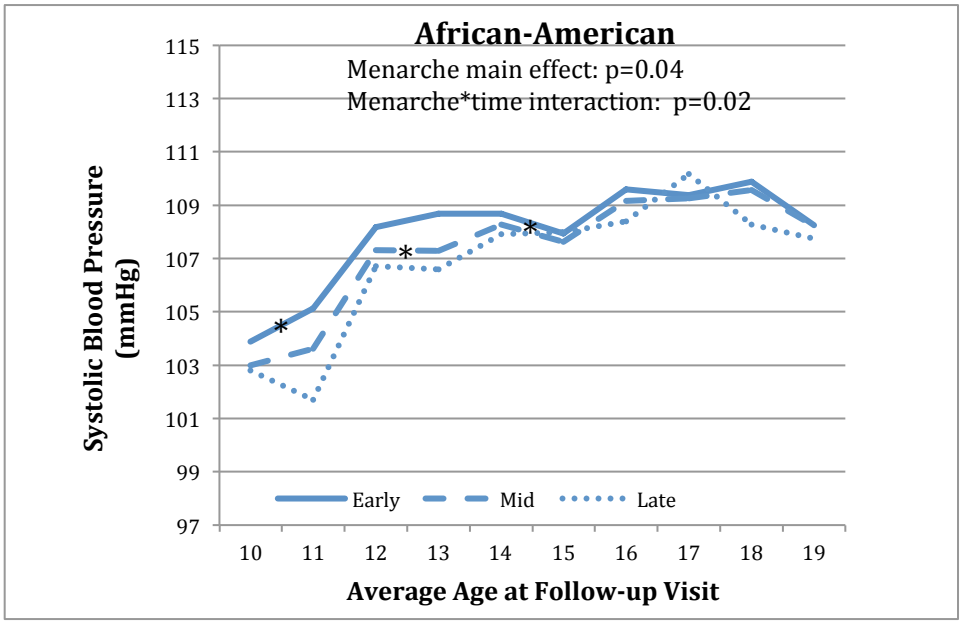

White
Menarche main effect: p=0.17
Menarche*time interaction: p=0.01
HDL-C (mg/dl)
64
62
60
58
56
54
52
50
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


African-American
Menarche main effect: p=0.002
Menarche*time interaction: p=0.51
HDL-C (mg/dl)
64
62
60
58
56
54
52
50
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


White
Menarche main effect: p=0.20
Menarche*time interaction: p=0.30
Triglycerides (mg/dl)
110
100
90
80
70
60
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit


African-American
Menarche main effect: p=0.12
Menarche*time interaction: p=0.24
Triglycerides (mg/dl)
110
100
90
80
70
60
Early
Mid
Late
10
12
14
16
19
Average Age at Follow-up Visit





Notes:
Adjusted for age, clinical center, parental education, parental smoking, and percent body fat.
Menarche categories: early= <12.0 years, mid=12.0-13.9 years, late=14.0-17.0.

CHAPTER 4. MANUSCRIPT #2 - AGE AT MENARCHE AND CARDIOMETABOLIC RISK IN ADULTHOOD: THE CARDIA STUDY

Introduction

A number of studies have demonstrated an association between early pubertal development among girls, measured using age at menarche, and increased risk for cardiovascular disease (CVD) and some of its risk factors, such as type 2 diabetes, high blood pressure, and metabolic syndrome [3, 5, 6, 28, 176]. However, early menarche is also strongly associated with obesity, which increases the risk for these adulthood diseases [60, 97]. Recent studies conflict about the confounding or mediating role of childhood and adulthood obesity in the relationship between earlier puberty and CVD risk [2-6]. One potential reason for the uncertainty is that childhood obesity and puberty likely influence each other through certain common pathways such as hormonal changes, insulin resistance, and hyperinsulinemia, and pubertal timing may have an additional subtle influence on adult obesity and CVD risk among women [28, 139]. Another reason is that multiple factors (e.g. infant weight gain and nutrition) likely work together in the timing of puberty [28], simultaneously influencing accumulation of adiposity in childhood, the onset of puberty, and later CVD risk [28, 85, 139]. Additionally, the influence of obesity in driving the relationship between pubertal timing and cardiometabolic risk may differ depending on when in the life course adiposity and risk factors are measured. A recent meta-analysis [28] concluded that the balance of evidence suggests that early puberty is associated with

increased risk of adult obesity and some cardiometabolic disorders (e.g. higher blood pressure, abnormal glycemia, and metabolic syndrome) independently of childhood obesity, but that it is not clear if the relationships are causal. Furthermore, longitudinal data are limited for the CVD risk factors outside of obesity.

Although race appears to be an independent risk factor for early menarche [14, 30], as well as for certain cardiometablic conditions [215], the relationship of age at menarche with cardiometabolic risk among African-American women has not been well studied. Most reports have included women of European descent only [1, 2, 4, 8, 9, 168, 182], or did not look at risk of disease separately for African-American and white women [3, 175, 183]. We previously showed that in the Atherosclerosis Risk in Communities (ARIC) study, age at menarche was associated with prevalent diabetes at baseline and incident diabetes during follow-up for white, but not African-American women during mid to late-adulthood [6]. Manuscript # I of this dissertation showed that age at menarche was inversely associated with adiposity throughout adolescence, as well as with systolic blood pressure and insulin levels before, but not after, adjustment for percent body fat. There were no meaningful differences between African-American and white girls in the association of age at menarche with any of the CVD risk factors.

Building on that work, this study evaluates the association between age at menarche and cardiovascular risk factors in the longitudinal CARDIA cohort of

young to middle-aged women. The specific aims of this study were to 1) assess race-specific associations between age at menarche and incident type 2 diabetes, impaired fasting glucose (IFG), and metabolic syndrome over 25 years of follow-up in the CARDIA study; and 2) examine how race-specific associations of age at menarche with fasting glucose, insulin, and additional components of cardiometabolic risk (adiposity, blood pressure, and blood lipids) change with age. We also considered if associations were independent of adiposity. The hypothesis is that earlier age at menarche will be associated with adverse levels of CVD risk factors among white and African-American women. Our analysis adds to the literature because CARDIA includes a biracial cohort of women with serial measurements available for a variety of cardiometabolic outcomes starting in early adulthood through middle-age. The results might help clarify the timing and mechanisms underlying the relationship between pubertal timing and cardiometabolic conditions.

Materials and Methods

Study Design and Population

The CARDIA study is a prospective cohort of 5,115 African-American and white men (n=2,328) and women (n=2,787) aged 18-30 years at baseline, originally designed to examine the development of heart disease in adults. The details of the study cohort, including eligibility criteria, sources and methods of recruitment and follow-up have been described in detail elsewhere [216]. Briefly, participants were recruited from four communities in the USA: Birmingham, AL;

Chicago, IL; Minneapolis, MN; Oakland, CA. Data for our analyses were collected during home interviews and clinic visits at the baseline and follow-up exams over 25 years (1985-2011). The institutional review boards at each of the study sites approved the study, and participants provided informed consent.

All analyses excluded women with missing age at menarche (n=118), or who reported their age at menarche as <8 (n=1) or >17 (n=9) years as we are interested in studying women within the normal range of menarche timing. Other exclusions were those with diabetes (n=20), missing diabetes status (n=45), or missing BMI (n=11) at the baseline visit, for an overall sample size of 2,583 women (African-American=1,333, white=1,250). For incident impaired fasting glucose and metabolic syndrome, women with those conditions at baseline were also excluded (IFG: n=38; metabolic syndrome: n=49). The final sample for the IFG analysis was 2,545, and for metabolic syndrome the sample size was 2,534. For the longitudinal analysis of cardiometabolic risk factors during follow-up, time points were excluded for patients who were pregnant or missing an outcome variable, or who had developed diabetes at any time prior to the visit.

Clinical Measures and Data Collection

Age at menarche

Age at menarche was assessed at baseline via self-report on a questionnaire by asking “How old where you when you began menstruating?”. For this manuscript, age at menarche is defined as the age in whole years at the

first menstrual period. The same question was asked at the second visit and reliability was high (r=0.84). We included age at menarche as both continuous and categorical variables in separate models, using the categories of early (8-11 years), average (12-13 years), and late (14-17 years) menarche.

Outcomes

Diabetes status was assessed at baseline and at each CARDIA clinic visit. All participants were asked to fast for 12 hours before the clinical examination. Type 2 diabetes was defined using blood glucose measurements and self-reported medication use at each visit. Specifically, diabetes was defined among non-pregnant women as fasting blood glucose ≥ 126 mg/dl, HbA1c ≥ 6.5%, 2-hour oral glucose tolerance ≥ 200 mg/dl, or use of diabetes medication. A 2-hour oral glucose tolerance test (OGTT) was administered at visit years 10, 20, and 25 only, and HbA1c was measured at years 20 and 25 only.

Impaired fasting glucose was defined as fasting blood glucose (FPG) ≥ 100 mg/dl but <126 mg/dl and not taking diabetes medication. Metabolic syndrome was defined according to the NCEP ATP-III revised criteria of 3 or more of the following factors: waist circumference >88 cm, systolic blood pressure ≥130/85 mmHg (or use of anti-hypertensive medication), HDL-C < 50 mg/dL, triglycerides ≥150 mg/dL (or use of lipid-lowering medication), or fasting glucose ≥ 100 mg/dL (or use of hypoglycemic medication) [217].

Measurements

Blood pressure was measured three times at each visit after a 5-minute rest, and the mean of the last two measurements were used in this analysis. Waist circumference was measured laterally at the point midway between the iliac crest and the lowest lateral portion of the rib cage and anteriorly at the point midway between the xiphoid process of the sternum and the umbilicus [218]. An enzymatic method was used to measure plasma concentrations of total cholesterol, HDL cholesterol, and triglycerides. HDL cholesterol was measured after dextran-magnesium precipitation [219], and LDL cholesterol was calculated by using the Friedewald equation [220]. The test-retest reliability coefficients for split specimens of total, HDL cholesterol, LDL cholesterol, and triglycerides were high, at >0.98 [221]. A radioimmunoassay (Linco Research, St Charles, MO) was used to measure fasting insulin. Additional details of the CARDIA examination procedures were published previously [221, 222].

Covariates

The covariates of age at baseline (years), race (African-American or white), parental history of diabetes (yes/no), oral contraceptive use (yes/no), smoking status (never, former, current), physical activity level (MET-hours/week), alcohol consumption (g/day), and level of education (<high school, high school, >high school) were all measured via self-report at baseline. Physical activity prior to high school (or 14 years of age) and during high school (or 14-18 years of age) was measured using an activity scale of 1 (physically inactive) to 5 (very active) and assessed via recall at the baseline visit. Anti-hypertensive and

hypoglycemic medication use was assessed at all visits, and lipid-lowering medication use beginning at exam year 5. Menopause status (yes/no) was obtained at visit years 15, 20, and 25. Weight (kg) and height (cm) were measured at each of the clinic visits while participants dressed in scrub suits and removed shoes. Body height (cm) and weight (kg) were measured with a calibrated scale and a vertical ruler. BMI was calculated as weight (kg)/baseline height^2 (m^2).

Statistical Methods

Baseline characteristics of the study population by race and by age at menarche category (early menarche [8-11 years], average menarche [12-13 years], and late menarche [14-17 years]) were summarized using mean (SD) for continuous variables or n (%) for categorical variables. The linear trend was tested using the likelihood ratio test by modeling the value in each menarche category as a continuous variable. All outcome variables were assessed for normality. Triglycerides and insulin were skewed and therefore log transformed for analyses. However, there were no qualitative differences between the associations for transformed and non-transformed values and so the non-transformed data are presented for simplicity.

Cox proportional models were used to estimate hazard ratios (HR) for incident diabetes (diabetes yes/no), impaired fasting glucose (yes/no), or metabolic syndrome (yes/no) according to continuous and categorical age at menarche. Estimates (hazard ratio [HR] and 95% CI) were calculated for all

women, and stratified by race as our *a priori* research question was about race differences in associations. The proportional hazards assumption was tested and verified by generating a time dependent predictor and creating interactions of age at menarche and a log function of survival time, and then the Wald chi-square procedure tested whether the coefficients were equal to 0.

Separate repeated measures linear regression models (PROC MIXED in SAS) were used to examine the association of age at menarche with each cardiometabolic risk factor over 25 years of follow-up. Participants with at least one outcome measurement were included in the analysis for each risk factor. We used the compound symmetry (CS) covariance structure because the models did not converge with the unstructured covariance structure, and CS fit the data better than other, more complex structures that were appropriate for unequally spaced measurements.

All mixed models included the independent variables of age at menarche, follow-up year (the time variable), current age and age^2, and clinical center. The cardiometabolic risk factors were the dependent continuous variable in each of the separate models. Higher order time variables were included to determine if there was a non-linear change in cardiometabolic risk factors during adulthood and retained only if statistically significant. Models were run in a series by adding covariates, starting with minimally adjusted **Model 1** (age, age^2, center, and time). **Model 2** also included the following covariates that were chosen *a priori* and have been found to be associated with age at menarche and/or

cardiometabolic risk factors: race (other than for race-stratified models), alcohol use, education level, smoking status, physical activity prior to high school, physical activity during high school, baseline physical activity, parental history of diabetes, oral contraceptive use, and menopause status. Blood pressure models were also adjusted for anti-hypertensive medication use and lipid models for cholesterol-lowering medications use. Finally, to test whether associations were independent of body composition, the models were further adjusted by the addition of baseline BMI (kg/m^2) in **Model 3**. Estimated adjusted means were calculated and plotted in **Figure 8** for the minimally adjusted Model 1 and in **Figure 9** for the fully adjusted Model 3 for each risk factor at each CARDIA visit**.**

For most outcomes, the models indicated linear, dose-response effects of age at menarche. Therefore, age at menarche was included as a continuous variable in the longitudinal and Cox models. Age at menarche was also included as a categorical variable (early, average, late) to calculate adjusted means for ease of interpretation in figures, and for comparison with other studies. To test for differences by race, adjusted models were run with an interaction term (race*menarche age). Even if the interactions are not statistically significant, race-stratified results were presented, as this was an *a priori* study objective.

All analyses were run in SAS version 9.2. The Cox proportional hazard models were assessed with the statistical significance level for alpha set at $p<0.05$. Because of the multiple comparisons included in our longitudinal analyses, Bonferroni corrected p-values <0.005 ($p=0.05/10$ outcomes) were

considered to be statistically significant for the purposes of the longitudinal analyses.

Results

The overall cohort of 2,583 women had a mean age of 26.9 years at baseline (range=18-35 years) and 50.2 years at the final visit (range=42-59 years). The mean age at menarche was 12.6 years (SD=1.5) for the total sample, and was younger for African-American (12.5 years, SD=1.5) compared with white (12.7 years, SD=1.5) women (t=-3.66, p-value=0.0003). At baseline, mean BMI was 24.4 kg/m^2 (SD=5.7) and mean waist circumference was 74.1 cm (SD=11.6). Baseline adiposity was significantly higher among African-American (BMI: 25.8 kg/m^2, SD=6.4; waist: 76.4 cm, SD=13.1) compared with white (BMI: 23.1 kg/m^2, SD=4.3; waist cm: 71.8, SD=9.2) women (BMI: t=12.5, p<.0001; waist: t=10.4, p<.0001).

Table 9 shows the unadjusted baseline characteristics of the study sample by age at menarche category. For both African-American and white women, earlier menarche was associated with lower physical activity level during high school, as well as greater BMI, waist circumference, and insulin at baseline. Glucose and systolic blood pressure were higher, and physical activity level prior to higher school was lower among African-American women with early vs. late menarche. Shorter stature, less alcohol consumption, more frequent parental history of diabetes, and lower HDL-C were more frequent among white women with early menarche.

Age at Menarche and Type 2 Diabetes

We identified 271 incident cases of type 2 diabetes (n=193 African-American, n=78 white) over 25 years of follow-up (**Table 10**). Each one-year earlier age at menarche was associated with an increased risk for type 2 diabetes in Models 1 and 2 among all women, but the association was completely attenuated after adjustment for baseline BMI (Model 3). Examining menarche in categories, early age at menarche (8-11 years) was associated with greater risk of diabetes during follow-up compared with menarche at age 14-17 years in Models 1 and 2, but not in fully adjusted Model 3. For white women, early age at menarche was associated with greater risk for type 2 diabetes in the minimally adjusted Model 1 only. Age at menarche was not associated with type 2 diabetes among African-American women in any of the models. There was no evidence of interaction between age at menarche and race for type 2 diabetes.

Age at Menarche and Impaired Fasting Glucose

There were a total of 613 incident cases of IFG over 25 years of follow-up (n= 348 African-American, n=265 white) (**Table 10**). Continuous age at menarche was inversely associated with risk for IFG over follow-up among all women, even after adjustment for BMI. When examining menarche in categories, early menarche (8-11 years) was associated with greater risk of IFG before (Models 1 and 2) but not after adjustment for BMI (Model 3). For white women, there was an inverse association between age at menarche and IFG that was independent of all covariates including baseline BMI. There was also an inverse

association of continuous age menarche with IFG in Models 1 and 2 for African-American women, but the association was attenuated after adjustment for baseline BMI. There were no associations with IFG for African-American women when considering menarche in categories.

Age at Menarche and Metabolic Syndrome

There were a total of 629 incident cases of metabolic syndrome over 25 years of follow-up (n= 388 African-American, n=241 white) (**Table 10**). Age at menarche was inversely associated with risk for metabolic syndrome over follow-up, and associations were approximately two to three times as strong among white compared with African-American women. There was strong evidence for interaction between age at menarche and race both before and after adjustment for adiposity (Model 1 p-interaction for race = 0.0002, Model 3 p-interaction for race <.0001). Associations were independent of BMI for white but not African-American women. The results after adjustment for waist circumference (which is one component of metabolic syndrome) at baseline instead of BMI at baseline in Model 3 (all women: HR=1.10, 95% CI 1.05, 1.15; white: 1.20, 95% CI 1.12, 1.27; African-American: 1.05, 95% CI 0.98, 1.10) were nearly identical to the Model 3 results that adjusted for baseline BMI (all women: HR=1.09, 95% CI 1.04, 1.14; white: HR=1.19, 95% CI 1.11, 1.26; African-American: HR 1.03, 95% CI 0.97, 1.09).

Longitudinal Analysis of Continuous Cardiometabolic Risk Factor Measures

After exclusions, there were 16,078 observations for the 2,583 women included in the longitudinal analyses, with a mean of 6.2 visits per participant. The mean number of measurements was 6.1 for lipids, 6.2 for blood pressure, 5.6 for insulin, 6.2 for glucose, and 6.2 for BMI and waist circumference. **Figures 8 and 9** display the cross-sectional adjusted means for BMI, waist circumference, total cholesterol, LDL-C, HDL-C, triglycerides, diastolic blood pressure, systolic blood pressure, glucose, and insulin for African-American and white women at each visit by categorical menarche group (early, average, and late). The mean values in **Figure 8** are adjusted for age, age^2, and clinical center only, while means in **Figure 9** are fully adjusted for lifestyle factors, BMI, and height. **Tables 11 and 12** show the main effect and linear age-related change in the risk factors per each one-year younger age at menarche. The quadratic or cubic time terms were included in the main effects models, but the higher order time coefficients are not reported in the tables or figures unless they reached the Bonferroni-adjusted threshold for significance and there were meaningful differences in the concave or convex shapes of the curves upon visual inspection of the figures.

Main effect of age at menarche on CVD risk factors

The figures and tables show that women with earlier menarche had greater overall mean BMI and waist circumference throughout the 25 year follow-up period (menarche main effect $p<.0001$ in all models). Among white women specifically, earlier age at menarche was also associated with higher triglycerides, diastolic blood pressure, systolic blood pressure, glucose, and

insulin, but lower HDL-C in the minimally adjusted models (**Figure 8 and Table 11).** For African-American women, age at menarche was inversely associated with insulin in the continuous, unadjusted model only (**Table 11**). The inverse association between continuous age at menarche and triglycerides was stronger for white compared with African-American women (p-race interaction=0.0003), but otherwise there were no statistically significant differences in the association of age at menarche with other CVD risk factors by race. After adjusting for BMI, the main effects of menarche timing on all risk factors were attenuated and no longer statistically significant (**Figure 9 and Table 12).**

Change over time in the association of age at menarche with CVD risk factors

Earlier menarche was associated with steeper increases in BMI and waist circumference over the study period for white women only (**Figure 8 and Table 11),** and the slopes indicated a strengthening of the association between early menarche and adiposity over time. In fact, there were essentially no changes in waist circumference or BMI over the study period for white women with late menarche (**Figure 8**), but those with early menarche continued to accumulate adiposity until leveling off around visit year 15. Earlier menarche did not appear to influence the slope in adiposity measures for African-American women, and there was a significant menarche*race*time interaction for both BMI and waist (p-interaction<.0001).

In minimally adjusted models, each one-year earlier age at menarche was associated with steeper increases in insulin for both white and African-American

women (**Table 11**), but when considering menarche in categories (**Figure 8),** early menarche (8-11 years) was associated with greater increases in insulin compared with later menarche for white women only. Among white women only, age at menarche was negatively associated with change in HDL-C, but inversely associated with change in triglycerides, systolic blood pressure, diastolic blood pressure, and glucose (**Figure 8 and Table 11**) over 25 years.

After adjustment for BMI, each one year earlier age at menarche was associated with steeper increases in triglycerides, diastolic blood pressure, and insulin for white women only (**Table 12**). For African-American women, earlier menarche was associated with a faster decline in LDL-C over follow-up (**Table 12** and **Figure 9**). The only significant difference by race was for diastolic blood pressure; earlier menarche was associated with steeper increases over time for white compared with African-American women (p-race interaction=0.001).

Discussion

The findings from this biethnic cohort study of young to middle-aged women suggest that each one year earlier age at menarche is associated with increased risk for metabolic syndrome, type 2 diabetes, and IFG, as well as greater adiposity. The associations with IFG and metabolic syndrome remained statistically significant after adjusting for baseline BMI for white women only. Each one year earlier age at menarche was also associated with lower mean HDL-C and higher mean triglycerides, blood pressure, insulin, and glucose, but these associations were driven by higher BMI among earlier maturing women.

Earlier age at menarche was also associated with greater increases in triglycerides and insulin over time after adjustment for adiposity, specifically among white women. Earlier menarche did not appear to be detrimental for change over time in CVD risk factors for African-American women.

There were a few differences by race in the relationship between age at menarche and CVD risk factors. Associations were stronger between early menarche and risk for metabolic syndrome, higher mean triglycerides, greater accumulation of adiposity, and increases in blood pressure and insulin during adulthood among white compared with African-American women. After adjustment for adiposity, the association of earlier menarche with increases over time in blood pressure was stronger for white compared with African-American women.

Our finding that early age at menarche was associated with greater waist circumference and BMI over 25 years for both African-American and white women is supported by numerous other studies [28, 168, 176-178]. Accumulation of adiposity with age was faster for earlier vs. later maturing white women in our study, while African-American women in all menarche categories experienced near parallel increases in BMI and waist. Reports about race differences in the role of pubertal timing in adulthood adiposity are limited [28]. As far as we are aware, only the Bogalusa Heart Study reported findings separately for African-American and white women [27]. In that study the significant association between early menarche and higher adulthood BMI disappeared when childhood BMI was

accounted for in African-Americans, and early menarche remained an independent predictor of adulthood BMI for white women. Furthermore, early menarche was more strongly associated with body fatness among white compared with African-American adolescents in that study, which is in agreement with our findings for adults [177]. New in our study was the finding that white, but not African-American women, reporting menarche between 14-17 years of age did not experience the increases in adiposity over time that was evident for the earlier menarche groups or for African-American women in general. These later maturing white women subsequently had lower risk for type 2 diabetes, IFG, and metabolic syndrome compared with the earlier maturing women. Others have also reported a potentially protective role of later pubertal onset against obesity and cardiometabolic risk [223].

The associations of early menarche with IFG and metabolic syndrome remained after adjusting for BMI among white women only. Others have also found independent associations between earlier menarche and these factors [5, 9, 28, 176, 177, 179]. An independent association between earlier pubertal onset and glucose dysregulation and overall metabolic syndrome is biologically plausible through increased hormone exposure and metabolic changes among earlier maturing girls during a window of susceptibility, but these mechanisms are not necessarily causal for overall cardiometabolic risk in adulthood [8, 28]. Residual confounding by adiposity in childhood or at other points in the life course might also explain the higher risk for metabolic syndrome and IFG since

obesity is one of the strongest risk factors for these conditions. The recent genome-wide association studies [35, 223] that have found genetic variants related to timing of menarche and obesity might help elucidate possible causal pathway in these relationships [28].

The results from examining the individual components of metabolic syndrome (i.e. waist circumference, lipids, insulin, glucose, and blood pressure) in CARDIA suggest that insulin resistance and greater waist circumference probably drive the metabolic syndrome associations, but synergy resulting from weak associations between early menarche and the other risk factors might also contribute to the overall metabolic syndrome risk. Of note is that results did not change materially when waist circumference was included in the models instead of, or along with, BMI. Studies have reported that a clustering of metabolic risk factors increases the risk for CVD over and above the risk conferred by each of the individual risk factors, with the clustering of risk factors tending to have an additive effect on CVD risk [150, 217]. Therefore our findings related to metabolic syndrome have public health implications, even if they are explained largely by adiposity.

The reason for the stronger associations for metabolic syndrome and some of its components among white compared with African-American women is unclear, but might relate to the fact that African-American women in all menarche categories had high adiposity overall during adulthood. By year 25, the adjusted mean BMI in all three menarche categories for African-American women reached

obese status (≥30 kg/m^2). Therefore, any independent influence of pubertal timing (i.e. outside of body composition) on metabolic syndrome risk was probably less relevant in comparison to the influence of adult obesity on overall cardiometabolic risk for African-American women.

As far as we are aware, this is the first study to examine both the level and serial changes in individual cardiometabolic risk factors by age at menarche over the long-term in young to mid-adulthood in biracial cohort. Although early menarche had strong associations with several of the risk factors in the minimally adjusted models, it did not have any meaningful, independent influence on lipids, blood pressure, insulin, or glucose among non-diabetic women outside of its influence on BMI and waist circumference. Others have found associations primarily with insulin, glucose, systolic blood pressure, triglycerides, and HDL in various age groups and populations [28, 176, 224], but in most cases the relationships were attenuated after adjusting for adiposity.

Methodological Considerations

One of the main limitations of this study is that age at menarche was recalled, and is therefore subject to misclassification. The validity of recall for age at menarche in middle age is moderate (r=0.67) [53]. In one longitudinal study, approximately 84% of women, mean age 50, were able to recall their age at menarche to within one year of the actual date [53, 54]. Reliability between reported age at menarche at baseline and at the second visit was high in this study (r=0.84), and because women recalled age at menarche in early adulthood

(18-30 years), we expect that validity was higher than what was reported in these studies for middle-age women. Another limitation is that power is low to detect interactions by race/ethnicity at modest effect levels. Because our *a priori* research question was related to associations by race, race-specific results were provided even when interactions are not statistically significant. Finally, we were not able to adjust for potential early life factors, which might confound the association between early pubertal development and later disease risk. CARDIA did not include measures of growth rate or adiposity prior to age 18, and so we are unable to investigate whether observed observations between early pubertal onset and adulthood adiposity and CVD risk were independent of childhood BMI.

We investigated several outcomes, raising concern about multiple testing. Although we based our hypotheses on *a priori* evidence, both the Bonferroni adjusted and traditional significance levels are presented to decrease the risk of false-positive (type 1 error) results.

Bias in this study was possible if error in the recall of menarche age was differential according to cardiometabolic condition status. There is no reason to believe that women with diabetes or other cardiometabolic conditions recalled age at menarche any differently than those without these conditions, and such bias would be unlikely in our analysis of incident diabetes, impaired fasting glucose, or metabolic syndrome. Nondifferential misclassification of menarche age and covariates might bias associations, likely toward the null. It is also possible that there was misclassification of diabetes cases because our definition

for diabetes was less stringent than that used in clinical practice, which requires confirmation of elevated glucose or presence of symptoms [225, 226]. Such misclassification will likely be nondifferential and unlikely to impact HR estimates. There is also potential for selection bias if women with younger age at menarche were less likely to enroll in CARDIA compared to their later-maturing counterparts**.**

There are several strengths in our study design. First, CARDIA included a relatively large, biethnic sample, which allowed investigation of race-specific associations. Furthermore, the prospective design of CARDIA permitted determination of the temporality of associations and reduced several sources of bias. There were also robust, serial measures of exposures and outcomes, including objective measures for diabetes, body composition, blood pressure, and cholesterol.

Conclusion

The results from this study suggest that early menarche is associated with higher adiposity in adulthood, as well as the related conditions of metabolic syndrome, type 2 diabetes, and IFG. Associations with accumulation of adiposity and metabolic syndrome were stronger among white compared with African-American women. Among white women, associations with metabolic syndrome and IFG remained after adjustment for BMI. It is not clear if there is an underlying causal mechanism between early menarche and these conditions, but residual confounding by adiposity, especially during childhood and adolescence, could

not be ruled out in this study. Earlier age at menarche was also associated with deleterious levels of other CVD risk factors (i.e. HDL-C, triglycerides, blood pressure, insulin, and glucose) during early and middle adulthood, but the greater adiposity that developed earlier in life for earlier maturing women largely explained those relationships. Our findings suggest that targeted nutrition, lifestyle counseling, and obesity prevention efforts focusing on girls prior to menarche might help to reduce the burden of obesity and its sequelae in the population.

Table 9. Baseline characteristics of the study population by age at menarche category: the CARDIA Study

	Age at Menarche (years)			
	8-11	**12-13**	**14-17**	
African-American (n=1,333)	Mean (SD) or n (%)	Mean (SD) or n (%)	Mean (SD) or n (%)	**p-trend**
n (%)	294 (22.1)	749 (56.2)	290 (21.8)	
Age at menarche (years)	10.5 (0.8)	12.5 (0.5)	14.7 (0.8)	
Age at baseline (years)	24.3 (3.8)	24.6 (3.8)	23.9 (4.0)	0.35
BMI (kg/m^2)	27.6 (6.6)	25.8 (6.4)	24.0 (5.7)	<.0001
Waist circumference (cm)	79.4 (13.7)	76.4 (13.1)	73.5 (11.7)	<.0001
Height (cm)	163.0 (7.0)	163.8 (6.5)	163.8 (7.3)	0.14
Physical activity at baseline (MET h/week)	283.4 (206.5)	270.3 (234.2)	294.1 (231.5)	0.60
Physical activity prior to high school (activity index)	3.5 (1.2)	3.7 (1.2)	3.9 (1.2)	0.0005
Physical activity during high school (activity index)	3.7 (1.2)	3.9 (1.1)	3.9 (1.1)	0.01
Smoking status				0.97
Never	177 (60.2)	461 (61.6)	173 (59.9)	
Former	25 (8.5)	67 (9.0)	26 (9.0)	
Current	92 (31.3)	221 (29.5)	90 (31.1)	
Alcohol (g/day)	5.1 (11.1)	4.9 (10.7)	6.5 (16.5)	0.15
Ever used oral contraceptives (yes)	240 (82.0)	590 (78.8)	225 (77.6)	0.20
Education				0.21
<High school	27 (9.2)	70 (9.3)	28 (9.7)	
High school	200 (68.0)	537 (71.6)	209 (72.1)	
>High school	67 (22.8)	143 (19.1)	53 (18.2)	
Parental history of diabetes (yes)	83 (28.1)	186 (24.7)	63 (21.7)	0.07
Impaired fasting glucose (yes)	4 (1.4)	16 (2.1)	3 (1.0)	0.77

Glucose (mg/dl)	80.3 (7.5)	79.3 (8.5)	79.0 (8.0)	0.05
Insulin (uU/ml)	14.6 (7.6)	13.0 (6.3)	12.8 (5.5)	0.004
LDL-C (mg/dl)	110.0 (31.8)	110.9 (31.5)	110.4 (33.0)	0.87
HDL-C (mg/dl)	54.8 (13.4)	55.4 (12.7)	55.7 (13.0)	0.33
Triglycerides (mg/dl)	62.8 (30.5)	63.1 (30.8)	63.2 (37.6)	0.92
Total cholesterol (md/dl)	177.3 (34.7)	178.9 (33.3)	178.7 (34.8)	0.58
Systolic blood pressure (mmHg)	109.0 (10.3)	108.4 (10.1)	106.6 (9,1)	0.003
Diastolic blood pressure (mmHg)	68.2 (9.6)	67.5 (9,3)	66.6 (9.4)	0.03

White (n=1,250)	Mean (SD) or n (%)	Mean (SD) or n (%)	Mean (SD) or n (%)	**p-trend**
n (%)	213 (17.0)	736 (58.9)	301 (24.1)	
Age at menarche (years)	10.6 (0.7)	12.5 (0.5)	14.7 (0.9)	
Age at baseline (years)	25.4 (3.6)	25.5 (3.4)	25.4 (3.3)	0.99
BMI (kg/m^2)	24.4 (5.4)	23.1 (4.3)	22.0 (3.3)	<.0001
Waist circumference (cm)	73.8 (11.1)	71.9 (9.1)	70.0 (7.4)	<.0001
Height (cm)	164.0 (6.2)	165.4 (6.4)	165.4 (6.5)	0.02
Physical activity (MET-h/week)	385.9 (237.9)	395.8 (261.8)	417.9 (271.2)	0.15
Physical activity prior to high school	3.5 (1.1)	3.6 (1.1)	3.7 (1.1)	0.19
Physical activity in high school	3.5 (1.1)	3.6 (1.1)	3.8 (1.0)	0.0001
Current smoker				0.35
Never	126 (59.2)	378 (51.4)	162 (53.8)	
Former	37 (17.5)	160 (21.7)	55 (18.3)	
Current	50 (23.5)	198 (26.9)	84 (27.9)	
Alcohol (g/day)	6.7 (12.3)	10.2 (4.6)	10.5 (17.1)	0.01
Ever used oral contraceptives	158 (74.2)	560 (75.6)	218 (72.4)	0.55
Education				0.90
<High school	10 (4.7)	27 (3.7)	14 (4.7)	

High school	112 (52.2)	344 (46.7)	152 (50.5)	
>High school	91 (43.0)	365 (49.6)	135 (44.9)	
Parental history of diabetes (yes)	38 (17.8)	112 (15.1)	35 (11.6)	0.05
Impaired fasting glucose (yes)	6 (2.8)	10 (1.4)	3 (1.0)	0.12
Glucose (mg/dl)	81.4 (8.1)	80.6 (7.4)	80.2 (7.0)	0.08
Insulin (uU/ml)	11.1 (5.6)	10.2 (4.6)	10.1 (4.6)	0.04
LDL-C (mg/dl)	106.0 (29.7)	106.6 (29.2)	104.0 (27.7)	0.41
HDL-C (mg/dl)	55.0 (13.4)	55.7 (12.8)	58.3 (13.1)	0.002
Triglycerides (mg/dl)	71.9 (36.0)	69.2 (39.4)	67.4 (35.4)	0.19
Total cholesterol (md/dl)	175.3 (31.2)	176.1 (31.3)	175.8 (30.4)	0.84
Systolic blood pressure (mmHg)	105.1 (9.3)	105.1 (9.4)	104.2 (9,2)	0.24
Diastolic blood pressure (mmHg)	66.9 (8.1)	66.1 (8.7)	65.9 (8.2)	0.26

Table 10. Adjusted HRs for incident type 2 diabetes, impaired fasting glucose, and metabolic syndrome according to age at menarche: The CARDIA Study

	Categorical Age at Menarche								
	8-11 years		12-13 years		14-17 years		Continuous Age at Manarche		
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	p-interaction (race)
All women									
Type 2 Diabetes (n=271)	n=69		n=159		n=43				
Model 1	1.69	1.16, 2.29	1.38	0.99, 1.94	REF		1.10	1.02, 1.17	0.20
Model 2	1.61	1.09, 2.37	1.35	0.96, 1.89	REF		1.08	1.00, 1.16	0.31
Model 3	1.33	0.90, 1.96	1.24	0.88, 1.75	REF		1.04	0.95, 1.12	0.37
IFG (n=613)	n=146		n=355		n=112				
Model 1	1.49	1.16, 1.91	1.29	1.05, 1.60	REF		1.11	1.07, 1.16	0.08
Model 2	1.50	1.17, 1.94	1.31	1.06, 1.62	REF		1.11	1.06, 1.16	0.17
Model 3	1.28	0.99, 1.62	1.22	0.98, 1.51	REF		1.08	1.02, 1.13	0.05
Metabolic syndrome (n=629)	n=158		n=368		n=103				
Model 1	1.96	1.54, 2.48	1.50	1.22, 1.85	REF		1.15	1.10, 1.20	0.0002
Model 2	1.95	1.53, 2.47	1.49	1.20, 1.84	REF		1.15	1.10, 1.19	0.0005
Model 3	1.45	1.14, 1.85	1.31	1.06, 1.62	REF		1.09	1.04, 1.14	<.0001
African-American									
Type 2 Diabetes (n=193)	n=48		n=114		n=31				
Model 1	1.50	0.96, 2.36	1.33	0.90, 1.98	REF		1.07	0.97, 1.15	
Model 2	1.47	0.93, 2.33	1.29	0.87, 1.93	REF		1.06	0.96, 1.15	
Model 3	1.28	0.80, 2.04	1.28	0.86, 1.92	REF		1.03	0.92, 1.12	
IFG (n=348)	n=96		n=182		n=70				
Model 1	1.30	0.96, 1.77	1.03	0.78, 1.35	REF		1.08	1.01, 1.14	
Model 2	1.29	0.94, 1.77	1.02	0.77, 1.34	REF		1.07	1.01, 1.14	
Model 3	1.12	0.81, 1.54	0.97	0.73, 1.28	REF		1.04	0.97, 1.11	
Metabolic syndrome (n=388)	n=101		n=212		n=75				
Model 1	1.58	1.18, 2.09	1.23	0.95, 1.58	REF		1.09	1.03, 1.14	
Model 2	1.55	1.17, 2.08	1.20	0.93, 1.55	REF		1.09	1.03, 1.15	
Model 3	1.17	0.87, 1.57	1.07	0.83, 1.38	REF		1.03	0.97, 1.09	
White									
Type 2 Diabetes (N=78)	n=21		n=45		n=12				
Model 1	2.31	1.13, 4.71	1.48	0.78, 2.81	REF		1.17	1.03, 1.29	
Model 2	1.98	0.96, 4.13	1.41	0.74, 2.70	REF		1.12	0.97, 1.24	
Model 3	1.14	0.53, 2.46	1.00	0.51, 1.96	REF		1.00	0.82, 1.15	
IFG (n=265)	n=50		n=173		n=42				
Model 1	1.79	1.18, 2.69	1.75	1.25, 2.46	REF		1.16	1.09, 1.23	
Model 2	1.84	1.21, 2.80	1.89	1.21, 2.66	REF		1.16	1.08, 1.23	
Model 3	1.52	0.99, 2.33	1.63	1.16, 2.31	REF		1.13	1.04, 1.20	
Metabolic syndrome (n=241)	n=57		n=156		n=28				
Model 1	3.04	1.97, 4.69	2.26	1.53, 3.32	REF		1.26	1.19, 1.32	
Model 2	3.04	1.96, 4.72	2.35	1.59, 3.46	REF		1.25	1.18, 1.32	
Model 3	2.15	1.36, 3.39	2.02	1.37, 2.98	REF		1.19	1.11, 1.26	

Notes:

Model 1 = age, center, and race adjusted

Model 2 = Model 1+parental history of diabetes (Y/N), educational status (<HS, HS, >HS), pre-high school physical activity, high school physical activity,smoking status (current, former, never), oral contraceptive use (yes/no), physical activity level (METS), and alcohol intake (g/day).

Model 3 = Model 2+BMI and height

Diabetes models exclude women with diabetes at baseline.

IFG models exclude women with diabetes or IFG at baseline.

Metabolic syndrome models exclude women with diabetes or metabolic syndrome at baseline.

Table 11. Main effect and per year change in cardiometabolic risk factors among women without diabetes over 25 years according to each 1-year earlier age at menarche in CARDIA, age and center adjusted models.

	All women			African-American			White			
	Beta	**se**	**p-value**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**p-race interaction**
BMI (kg/m^2)										
Menarche main effect	**0.88**	**0.08**	**<.0001**	**0.88**	**0.12**	**<.0001**	**0.89**	**0.10**	**<.0001**	0.99
Menarche*time	**0.02**	**0.002**	**<.0001**	0.004	0.003	0.18	**0.03**	**0.002**	**<.0001**	**<.0001**
Waist (cm)										
Menarche main effect	**1.52**	**0.16**	**<.0001**	**1.44**	**0.23**	**<.0001**	**1.60**	**0.21**	**<.0001**	0.68
Menarche*time	**0.04**	**0.004**	**<.0001**	0.01	0.01	0.27	**0.06**	**0.01**	**<.0001**	**<.0001**
Total cholesterol (mg/dl)										
Menarche main effect	0.04	0.35	0.90	-0.48	0.52	0.35	0.58	0.5	0.25	0.13
Menarche*time	-0.01	0.01	0.43	-0.02	0.02	0.28	0.01	0.02	0.81	0.34
LDL-C (mg/dl)										
Menarche main effect	0.34	0.34	0.32	-0.25	0.49	0.61	0.94	0.48	0.05	0.07
Menarche*time	-0.02	0.01	0.14	-0.04	0.02	0.02	0.00	0.02	0.80	0.06
HDL-C (mg/dl)										
Menarche main effect	**-0.72**	**0.16**	**<.0001**	-0.35	0.21	0.10	**-1.09**	**0.24**	**<.0001**	0.02
Menarche*time	**-0.02**	**0.01**	**0.0003**	0.004	0.01	0.62	**-0.04**	**0.01**	**<.0001**	**<.0001**
Triglycerides (mg/dl)										
Menarche main effect	**2.12**	**0.45**	**<.0001**	0.56	0.58	0.34	**3.71**	**0.7**	**<.0001**	**0.0003**
Menarche*time	**0.90**	**0.5**	**<.0001**	0.07	0.03	0.02	**0.20**	**0.03**	**<.0001**	0.007
Systolic blood pressure (mmHg)										
Menarche main effect	**0.42**	**0.12**	**0.001**	0.36	0.18	0.05	**0.49**	**0.16**	**0.002**	0.57
Menarche*time	0.01	0.01	0.07	-0.02	0.01	0.05	**0.02**	**0.01**	**0.002**	**0.0005**
Diastolic blood pressure (mmHg)										
Menarche main effect	**0.37**	**0.09**	**0.0001**	0.31	0.14	0.03	**0.46**	**0.13**	**0.0004**	0.43
Menarche*time	**0.02**	**0.01**	**0.0005**	-0.01	0.01	0.18	**0.04**	**0.01**	**<.0001**	**<.0001**
Insulin (μ U/ml)										
Menarche main effect	**0.35**	**0.06**	**<.0001**	**0.29**	**0.1**	**0.003**	**0.43**	**0.07**	**<.0001**	0.26
Menarche*time	**0.02**	**0.003**	**<.0001**	0.001	0.01	0.89	**0.03**	**0.002**	**<.0001**	**0.002**
Glucose (mg/dl)										
Menarche main effect	**0.42**	**0.08**	**<.0001**	0.32	0.12	0.01	**0.55**	**0.11**	**<.0001**	0.19
Menarche*time	0.01	0.004	0.01	0.001	0.01	0.91	**0.02**	**0.01**	**0.002**	0.06

*Adjusted for age and center

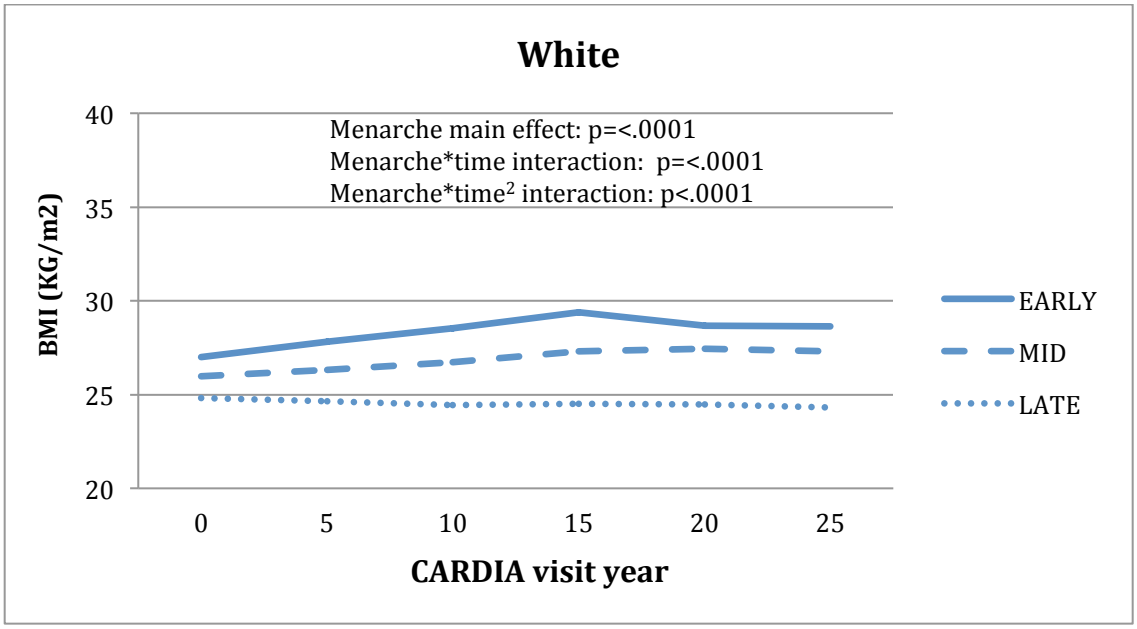
Statistical significance assessed at the Bonferonni corrected threshold of p<.0005.

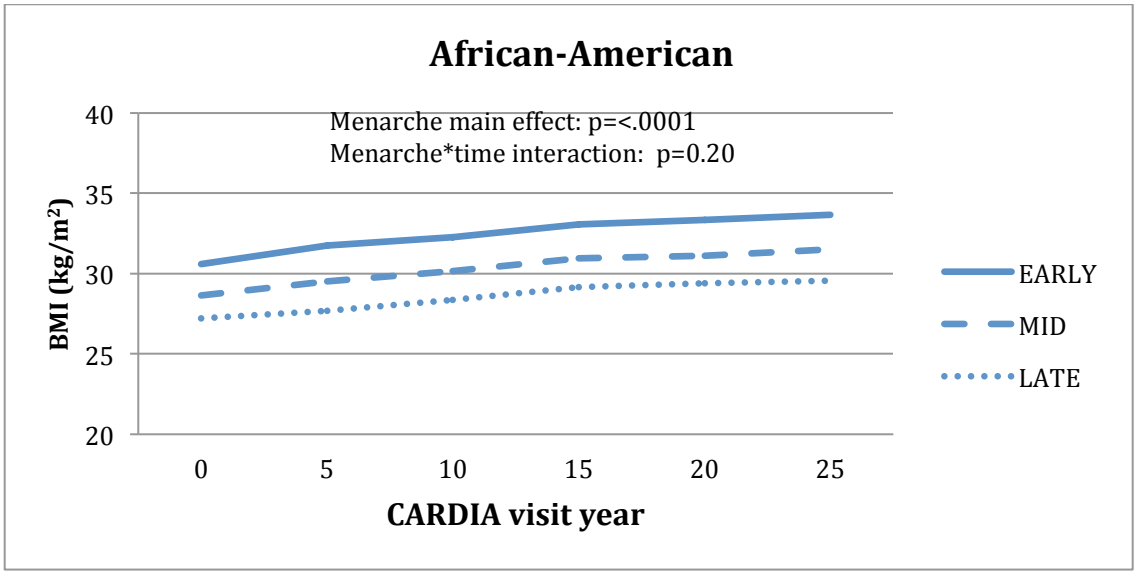
Table 12. Main effect and per year change in cardiometabolic risk factors among women without diabetes over 25 years according to each 1-year earlier age at menarche in CARDIA, fully adjusted models

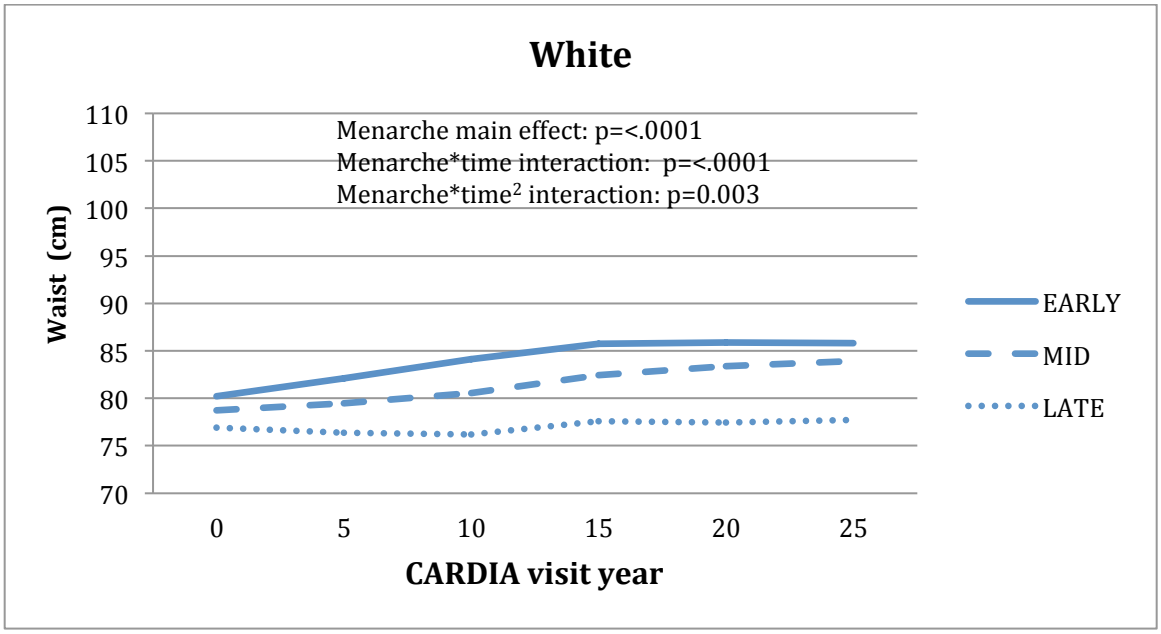
	All women			African-American			White			
Outcome	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**Beta**	**se**	**p-value**	**p-race interaction**
Total cholesterol (mg/dl)										
Menarche main effect	-0.69	0.37	0.06	-0.93	0.53	0.08	-0.67	0.51	0.19	0.14
Menarche*time	-0.03	0.01	0.03	-0.04	0.02	0.03	0.01	0.06	0.24	0.06
LDL-C (mg/dl)										
Menarche main effect	-0.83	0.35	0.02	-1.26	0.49	0.01	-0.57	0.48	0.24	0.09
Menarche*time	**-0.05**	**0.01**	**0.0001**	**-0.06**	**0.02**	**0.0002**	-0.03	0.02	0.08	0.06
HDL-C (mg/dl)										
Menarche main effect	0.12	0.15	0.43	0.42	0.21	0.04	-0.17	0.22	0.45	0.01
Menarche*time	-0.01	0.01	0.16	0.01	0.01	0.54	-0.02	0.01	0.02	0.03
Triglycerides (mg/dl)										
Menarche main effect	0.33	0.44	0.47	-0.25	0.57	0.66	0.48	0.67	0.47	0.0009
Menarche*time	**0.11**	**0.02**	**<.0001**	0.08	0.03	0.02	**0.11**	**0.03**	**0.0003**	0.12
Systolic blood pressure (mmHg)										
Menarche main effect	-0.01	0.11	0.92	0.08	0.16	0.63	-0.07	0.14	0.62	0.55
Menarche*time	0.01	0.01	0.32	-0.02	0.01	0.07	0.01	0.01	0.13	0.007
Diastolic blood pressure (mmHg)										
Menarche main effect	-0.02	0.09	0.81	-0.04	0.14	0.97	0.01	0.13	0.92	0.29
Menarche*time	0.01	0.01	0.06	-0.01	0.01	0.13	**0.02**	**0.01**	**0.004**	**0.001**
Insulin (μ U/ml)										
Menarche main effect	-0.04	0.05	0.43	-0.07	0.09	0.41	0.01	0.05	0.87	0.49
Menarche*time	**0.01**	**0**	**0.0004**	0.01	0.01	0.34	**0.01**	**0**	**<.0001**	0.16
Glucose (mg/dl)										
Menarche main effect	0.07	0.08	0.38	0.001	0.12	0.99	0.19	0.11	0.07	0.17
Menarche*time	0.01	0.26	0.30	0.0004	0.01	0.95	0.01	0.01	0.24	0.44

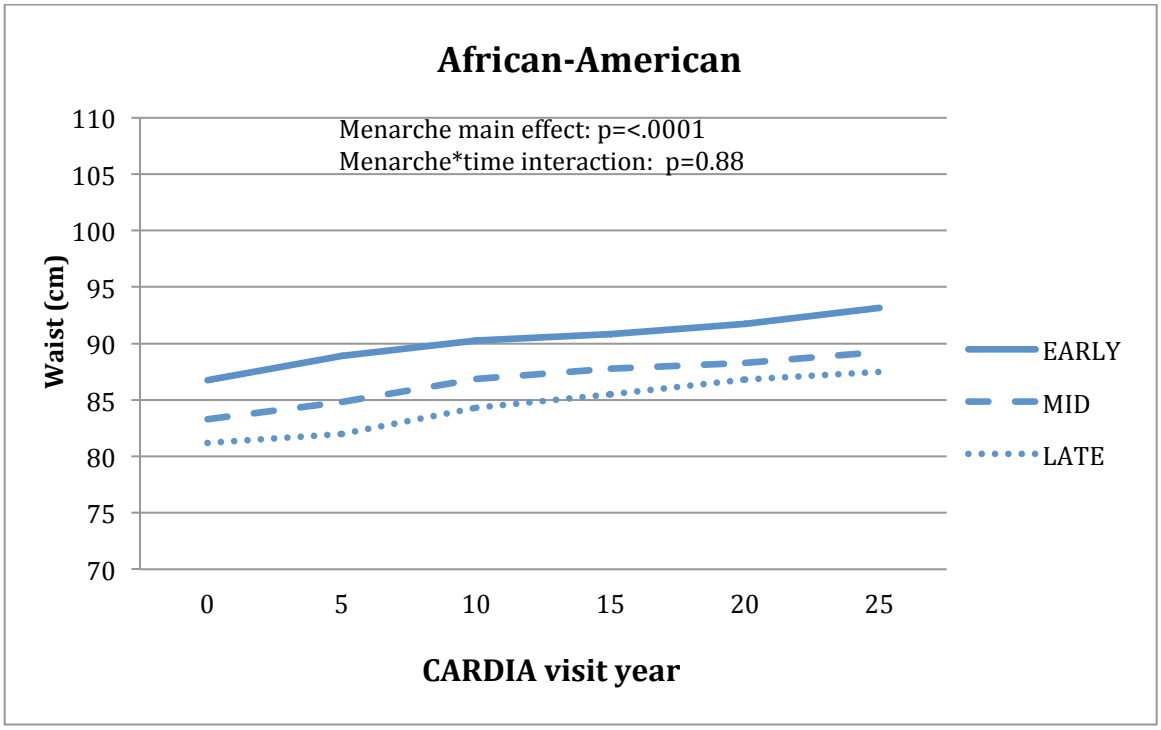
Adjusted for age, center, race, parental history of diabetes, education, smoking, physical activity, oral contraceptives, menopause status, alcohol intake, BMI, height, blood pressure lowering medications, and lipid-lowering medications
Statistical significance assessed at the Bonferonni corrected threshold of p<.0005.

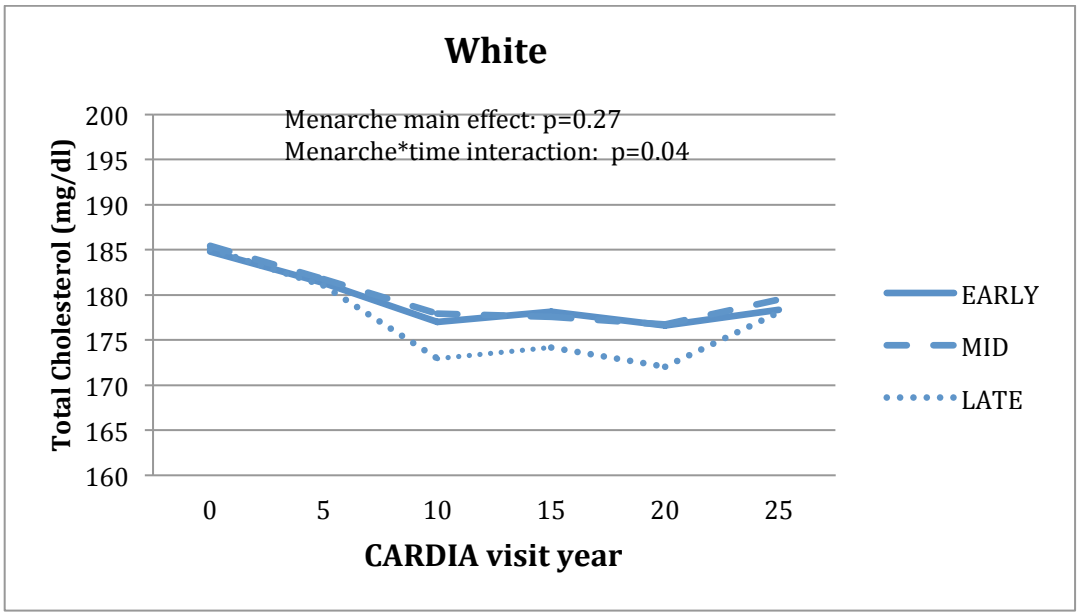
Figure 8. Age- and center-adjusted longitudinal changes in cardiovascular disease risk factors during adulthood by age at menarche category in the CARDIA Study

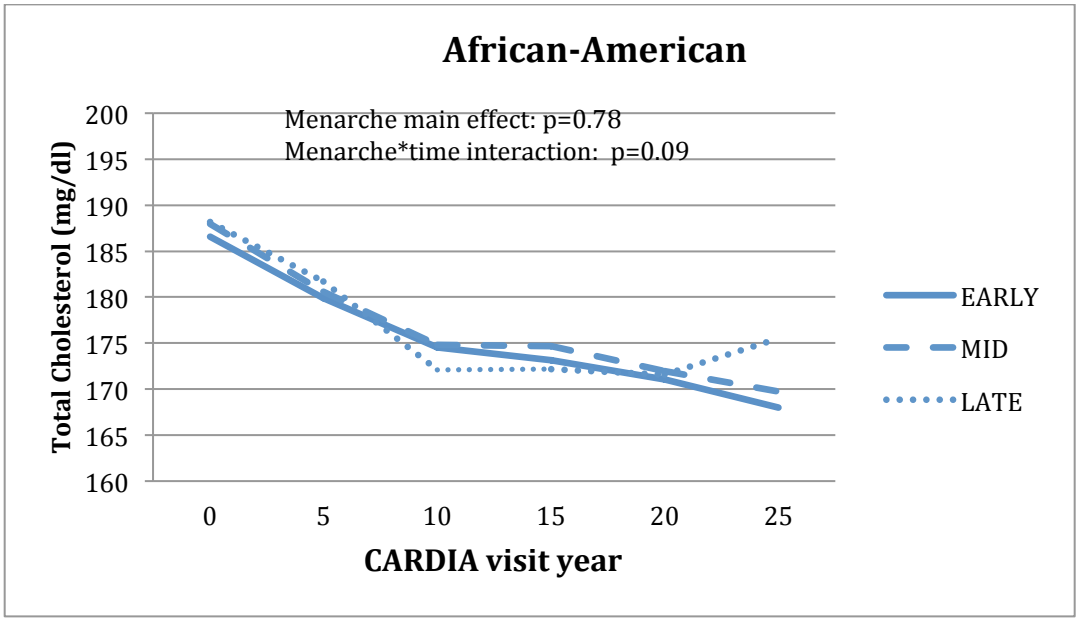


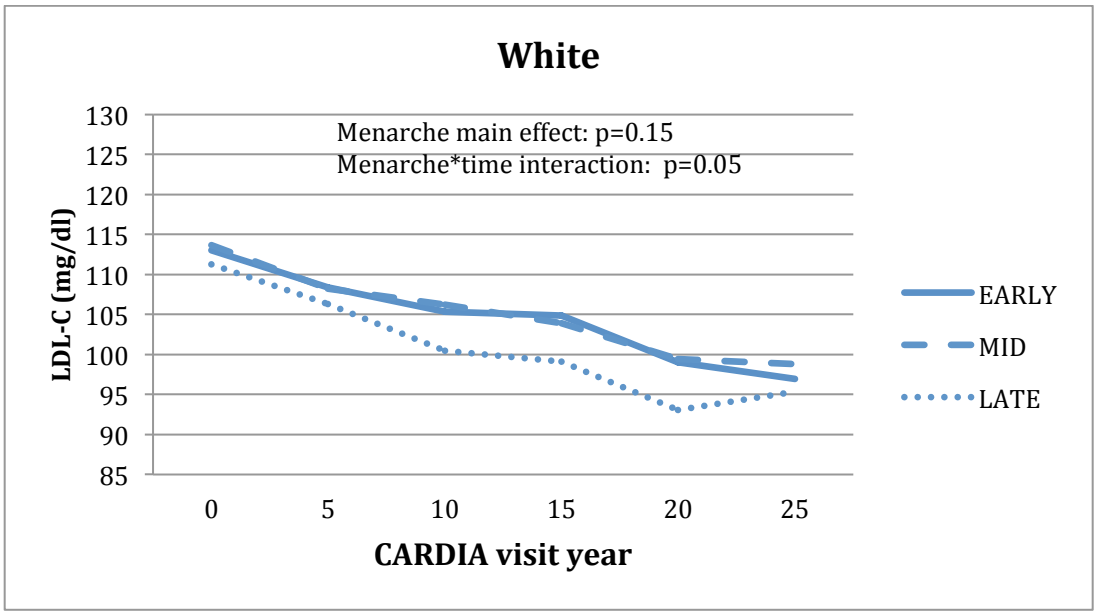


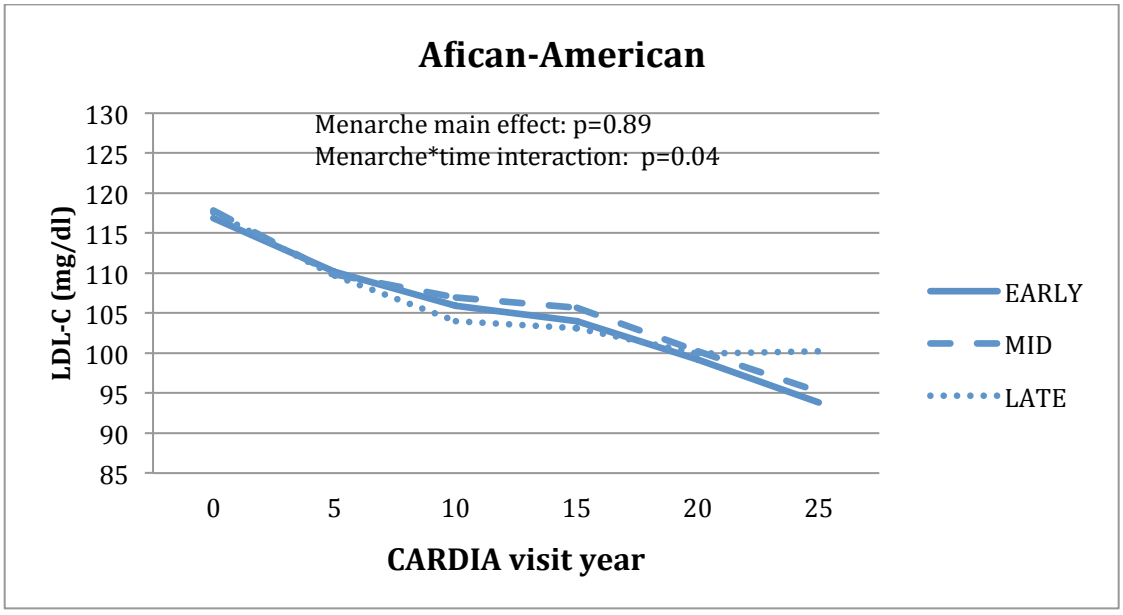

White
Menarche main effect: p=<.0001
Menarche*time interaction: p=<.0001
Menarche*time² interaction: p=0.003
Waist (cm)
110
105
100
95
90
85
80
75
70
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

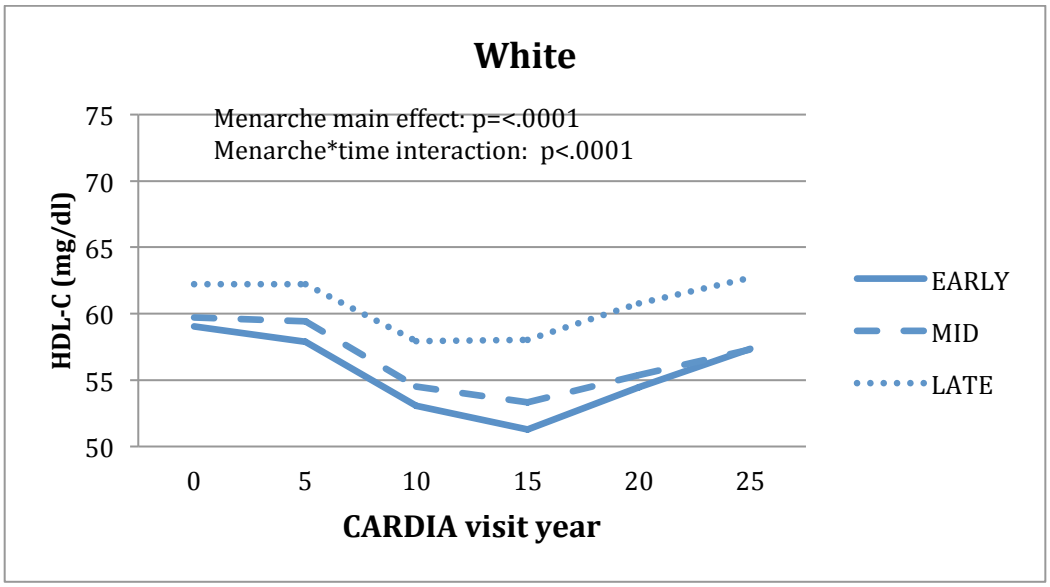

African-American
Menarche main effect: p=<.0001
Menarche*time interaction: p=0.88
Waist (cm)
110
105
100
95
90
85
80
75
70
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

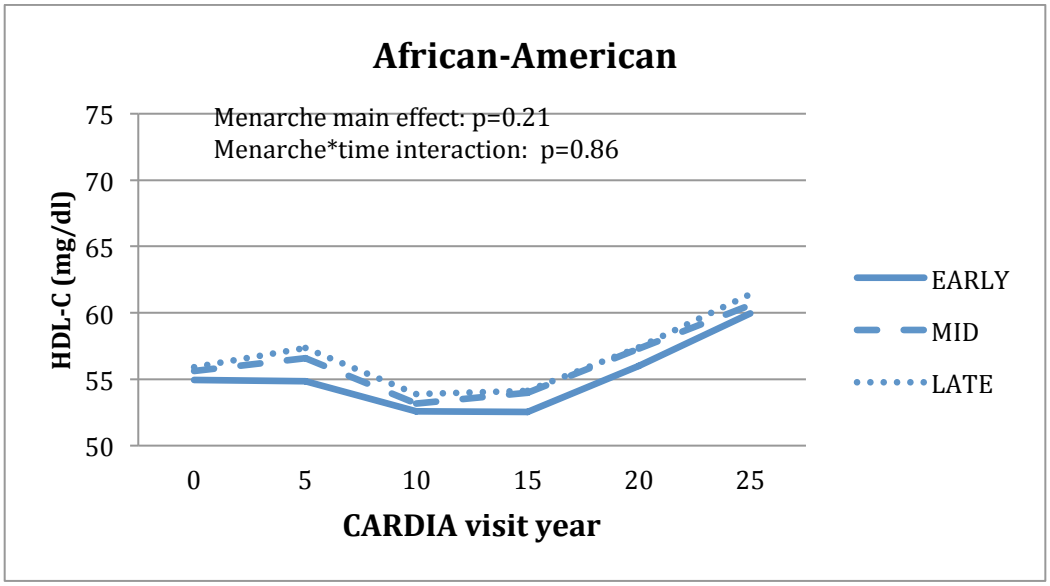

White
Menarche main effect: p=0.27
Menarche*time interaction: p=0.04
Total Cholesterol (mg/dl)
200
195
190
185
180
175
170
165
160
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

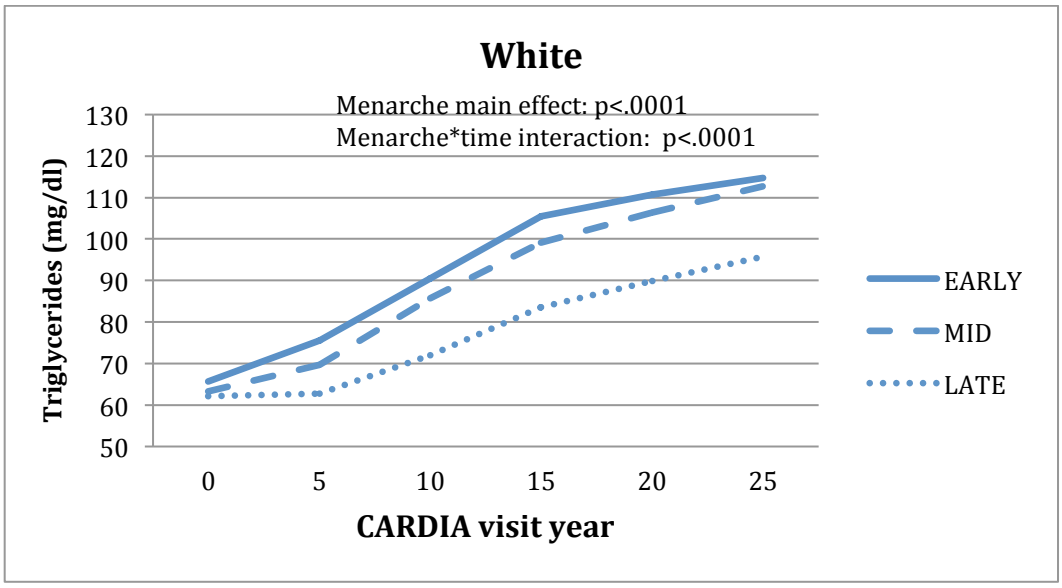

African-American
Menarche main effect: p=0.78
Menarche*time interaction: p=0.09
Total Cholesterol (mg/dl)
200
195
190
185
180
175
170
165
160
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

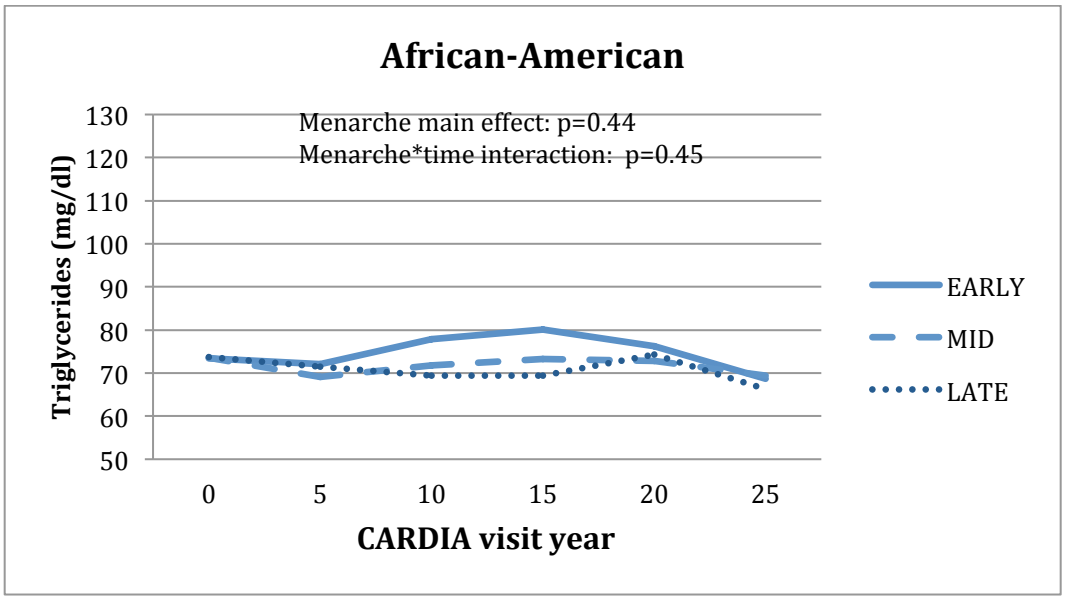

White
Menarche main effect: p=0.15
Menarche*time interaction: p=0.05
LDL-C (mg/dl)
130
125
120
115
110
105
100
95
90
85
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

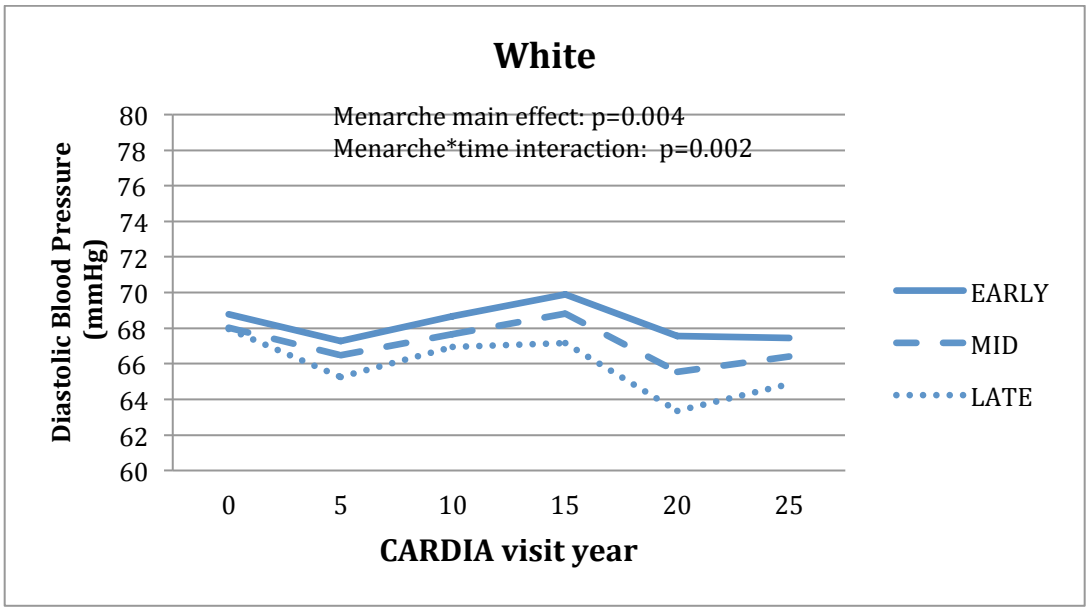

Afican-American
Menarche main effect: p=0.89
Menarche*time interaction: p=0.04
LDL-C (mg/dl)
130
125
120
115
110
105
100
95
90
85
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

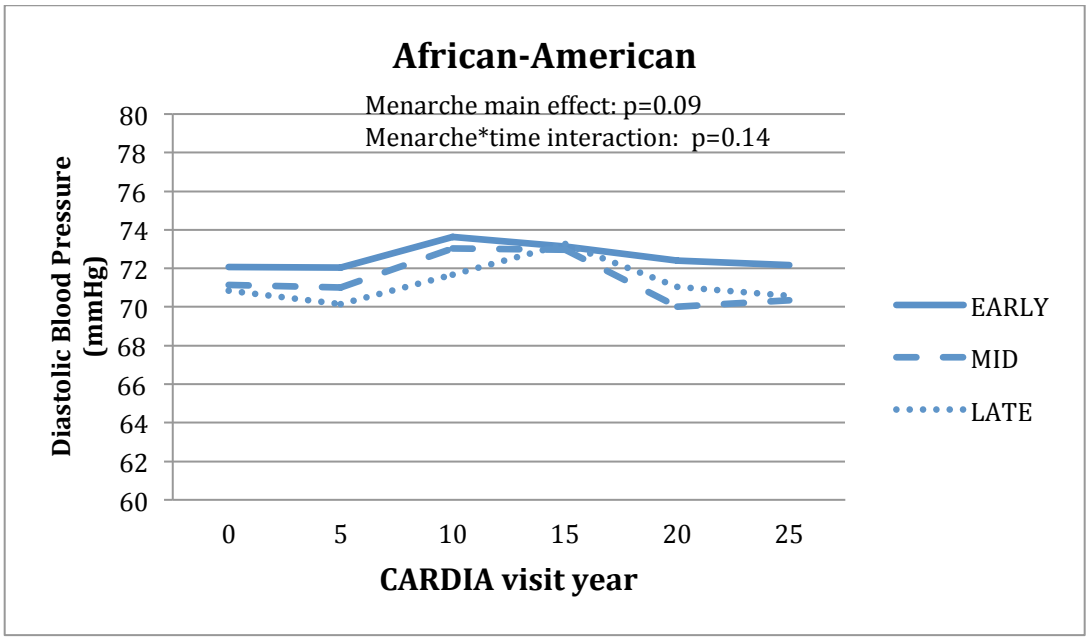

White
Menarche main effect: p=<.0001
Menarche*time interaction: p<.0001
HDL-C (mg/dl)
75
70
65
60
55
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

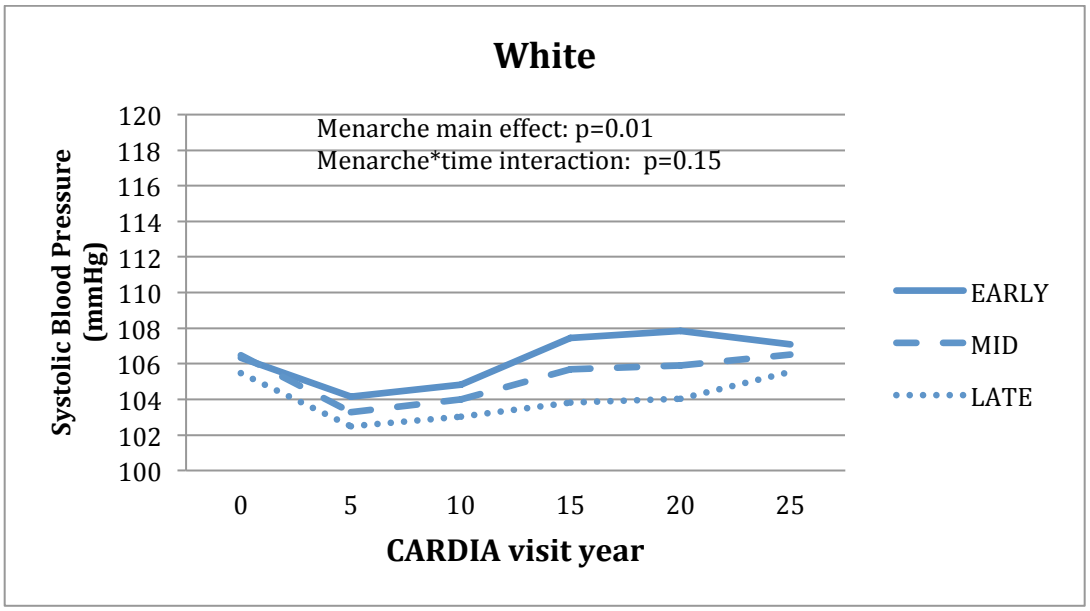

African-American
Menarche main effect: p=0.21
Menarche*time interaction: p=0.86
HDL-C (mg/dl)
75
70
65
60
55
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

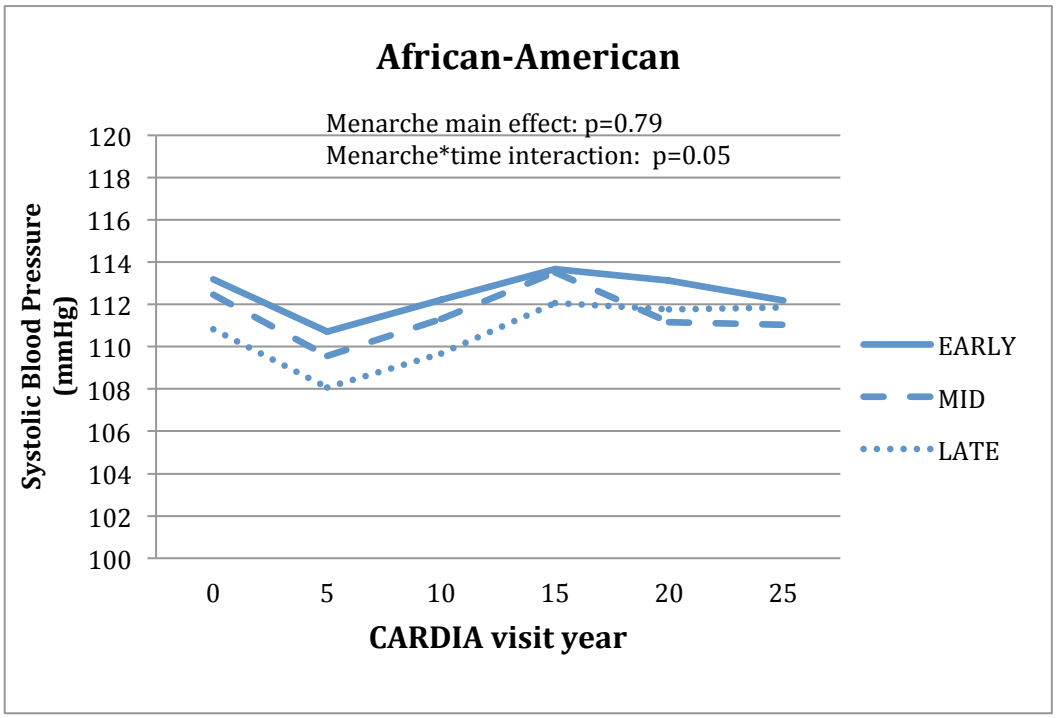

White
Menarche main effect: p<.0001
Menarche*time interaction: p<.0001
Triglycerides (mg/dl)
130
120
110
100
90
80
70
60
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

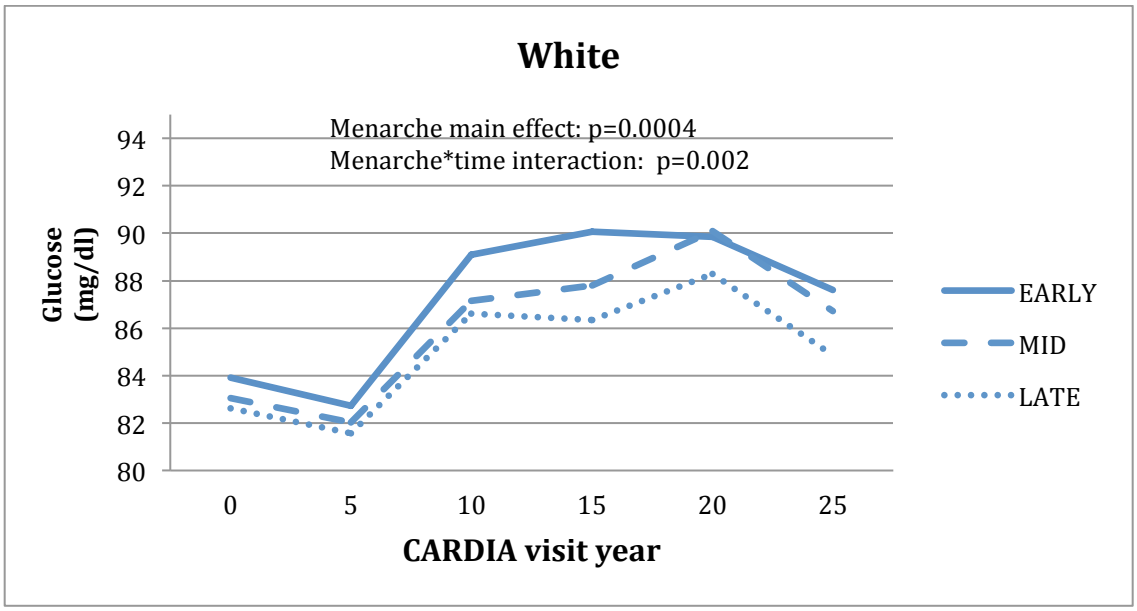

African-American
Menarche main effect: p=0.44
Menarche*time interaction: p=0.45
Triglycerides (mg/dl)
130
120
110
100
90
80
70
60
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

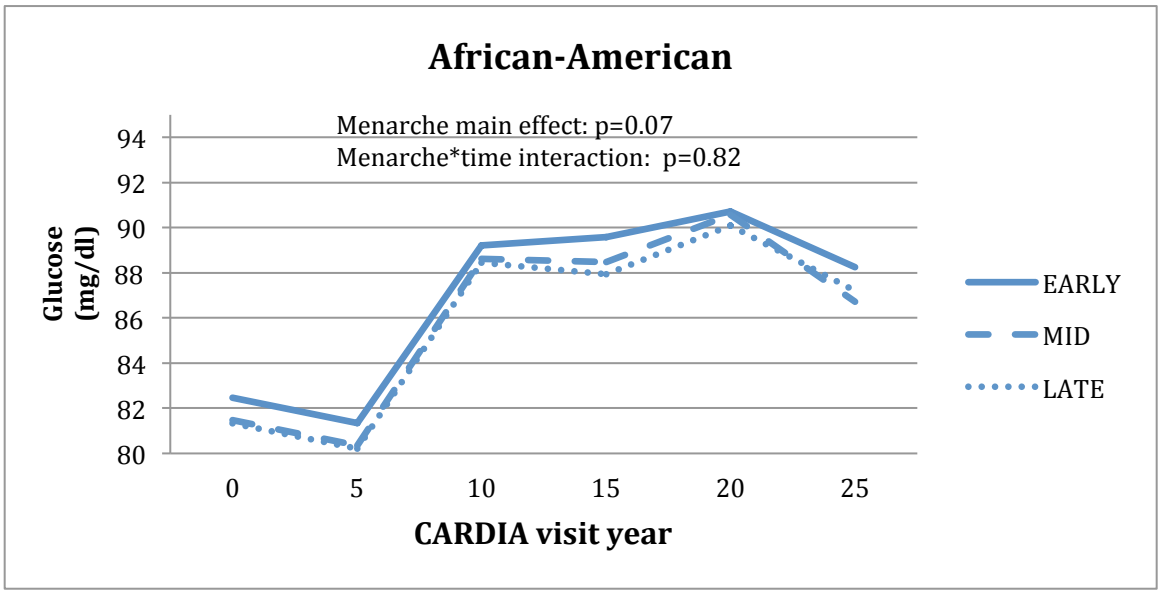

White
Menarche main effect: p=0.004
Menarche*time interaction: p=0.002
Diastolic Blood Pressure (mmHg)
80
78
76
74
72
70
68
66
64
62
60
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

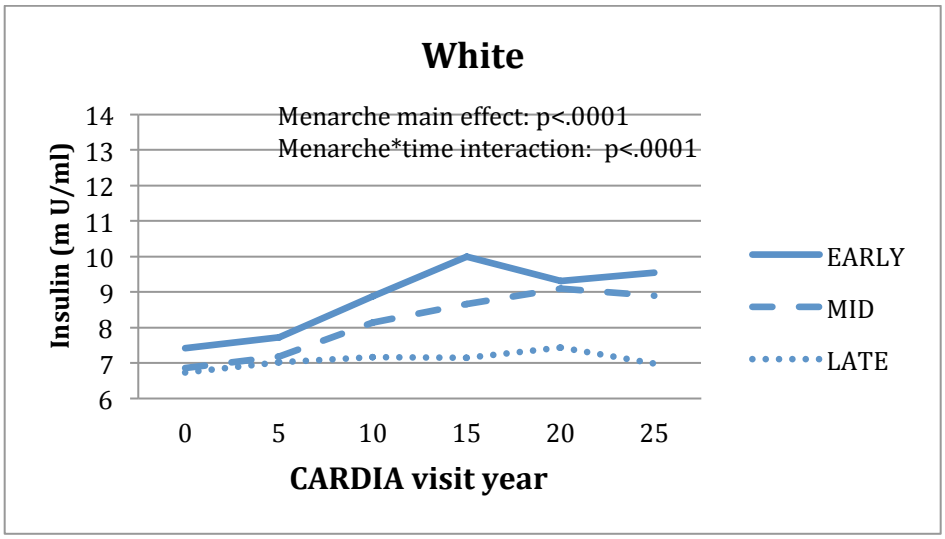

African-American
Menarche main effect: p=0.09
Menarche*time interaction: p=0.14
Diastolic Blood Pressure (mmHg)
80
78
76
74
72
70
68
66
64
62
60
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

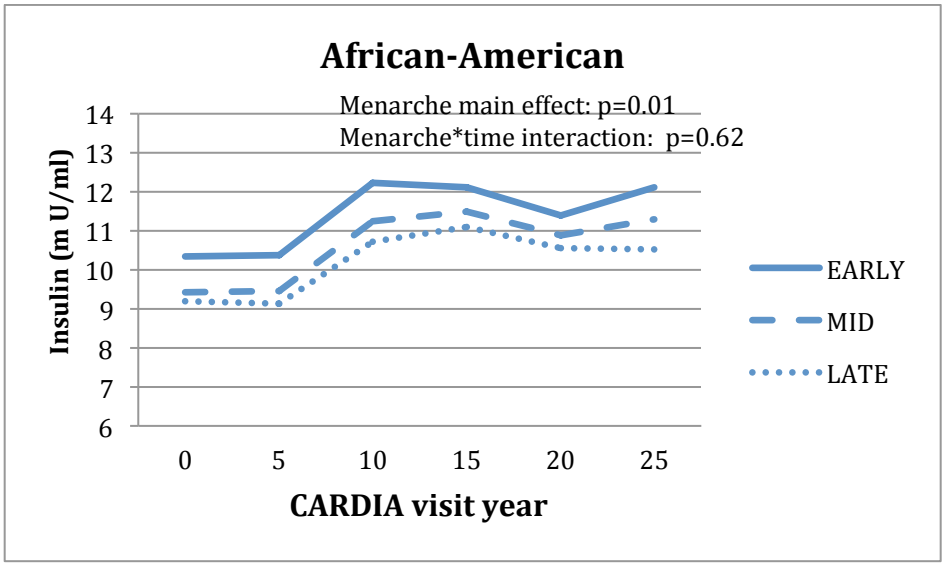

White
Menarche main effect: p=0.01
Menarche*time interaction: p=0.15
Systolic Blood Pressure (mmHg)
120
118
116
114
112
110
108
106
104
102
100
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE


African-American
Menarche main effect: p=0.79
Menarche*time interaction: p=0.05
Systolic Blood Pressure (mmHg)
120
118
116
114
112
110
108
106
104
102
100
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE


White
Menarche main effect: p=0.0004
Menarche*time interaction: p=0.002
Glucose (mg/dl)
94
92
90
88
86
84
82
80
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

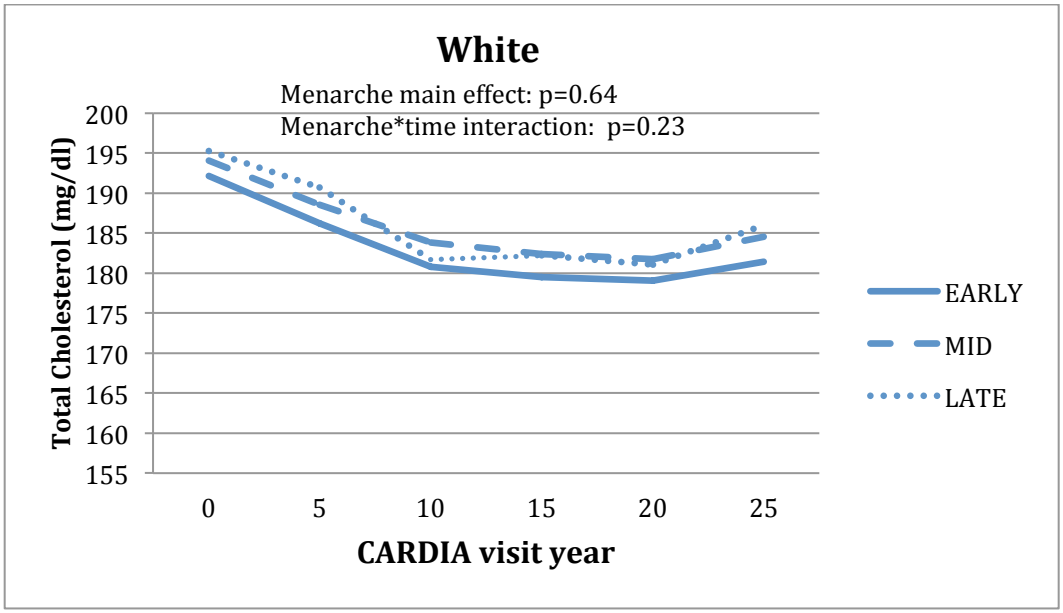

African-American
Menarche main effect: p=0.07
Menarche*time interaction: p=0.82
Glucose (mg/dl)
94
92
90
88
86
84
82
80
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

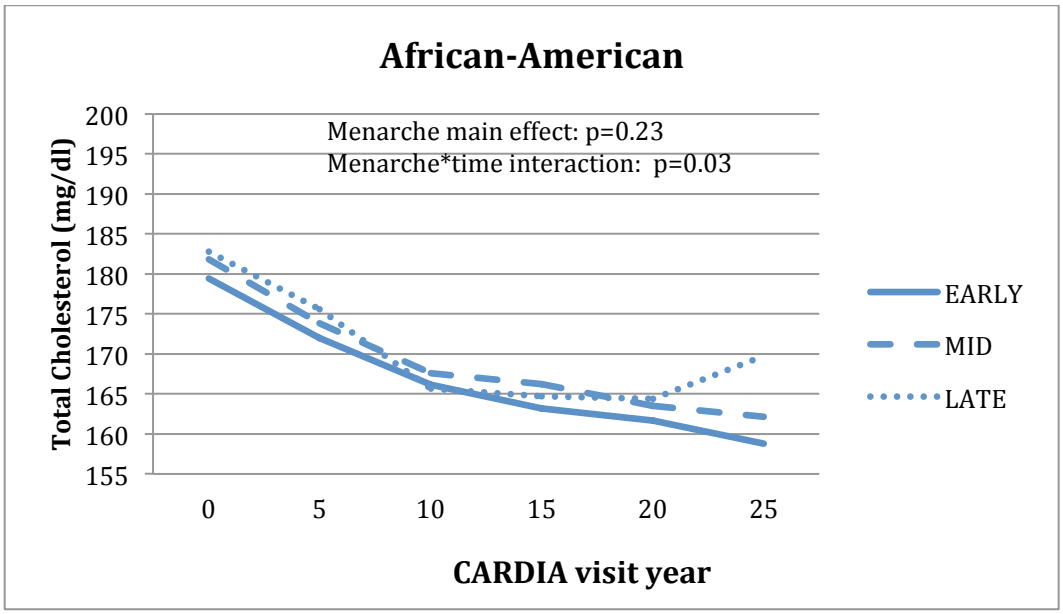


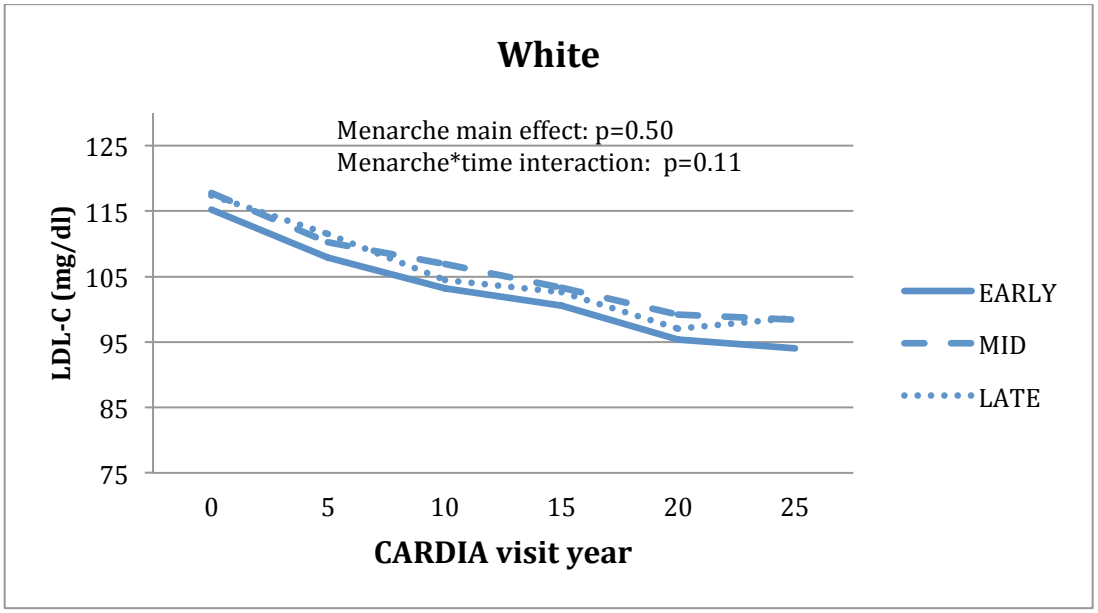


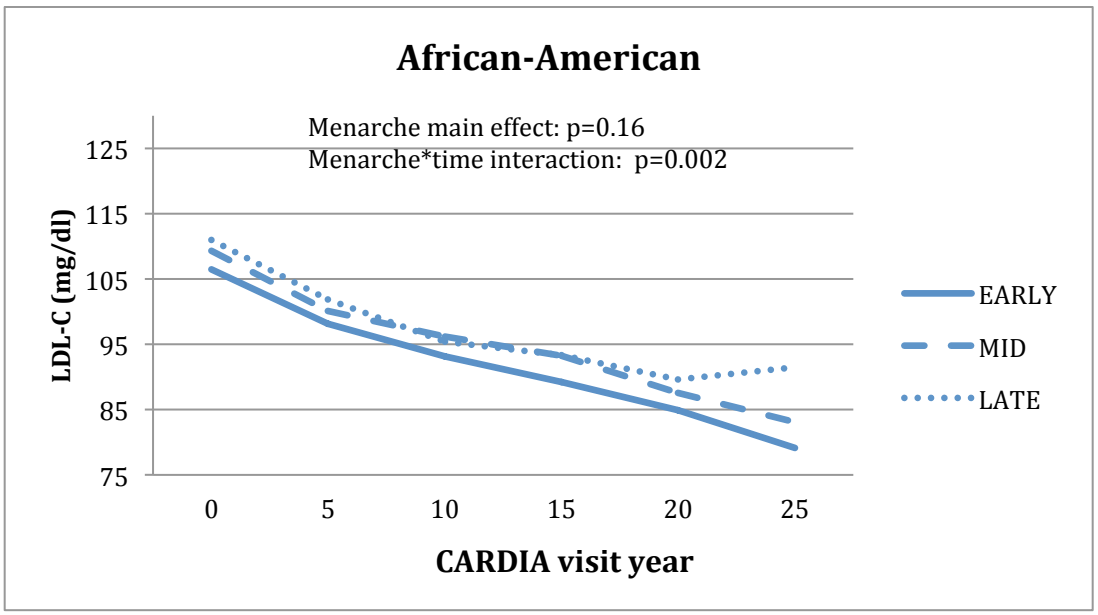
Notes:
Includes women who reported menarche 8-17 years, were non-diabetic at baseline, and who had data available on covariates.
Excludes the visits for women who were pregnant or developed diabetes.
Adjusted for age, age^2, and clinical center.

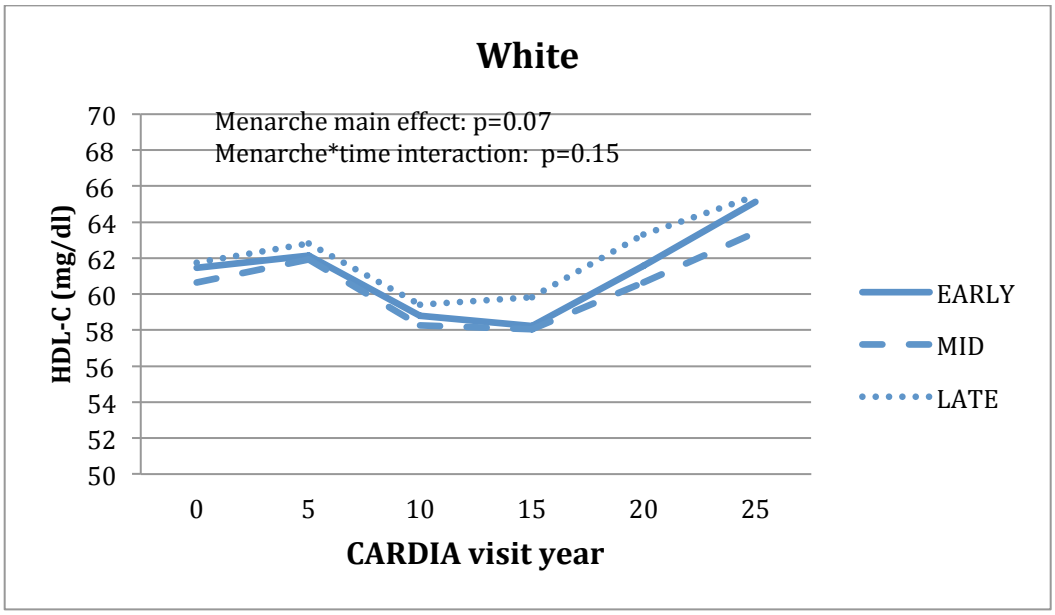
Figure 9. Fully adjusted longitudinal changes in cardiovascular disease risk factors during adulthood by age at menarche category in the CARDIA Study

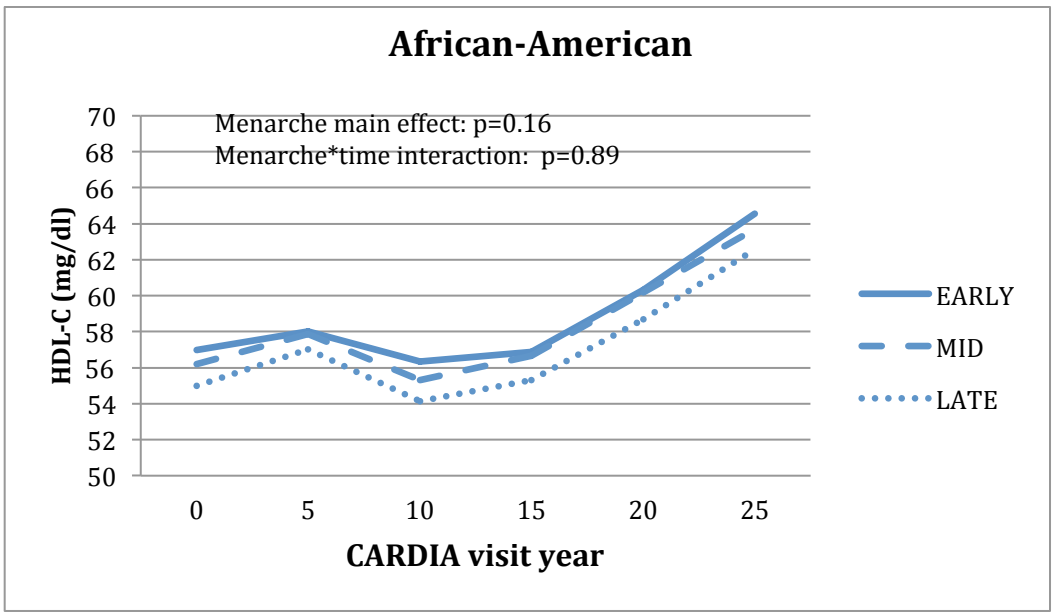


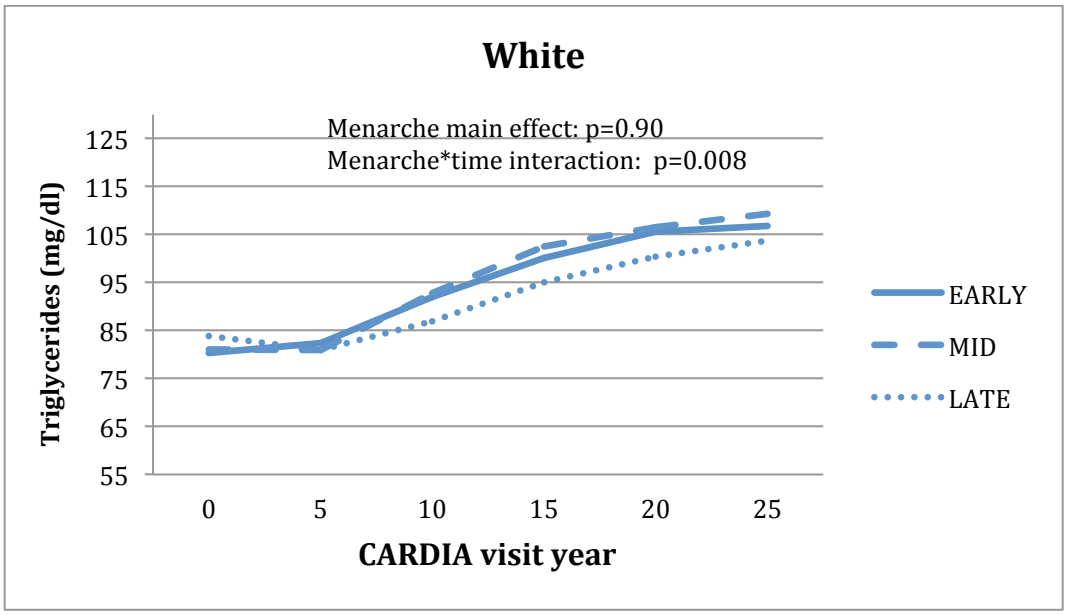


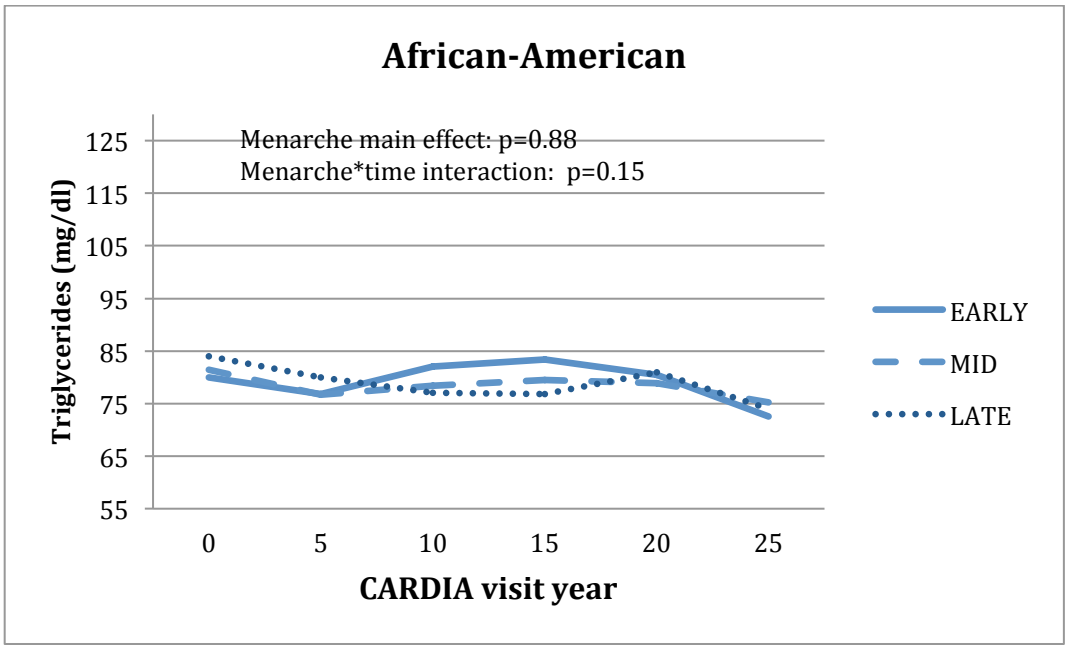

White
Menarche main effect: p=0.50
Menarche*time interaction: p=0.11
LDL-C (mg/dl)
125
115
105
95
85
75
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

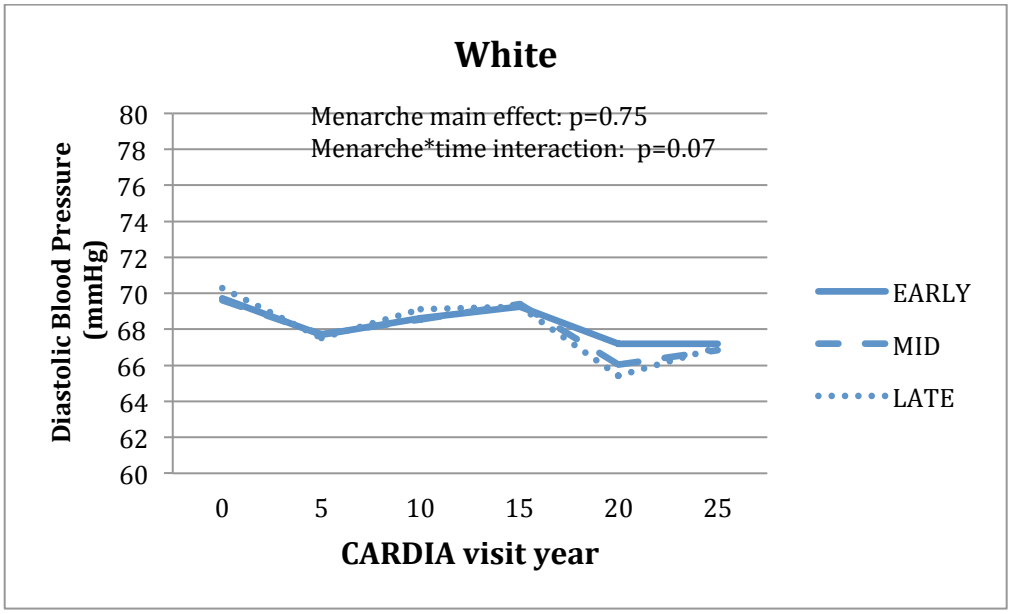

African-American
Menarche main effect: p=0.16
Menarche*time interaction: p=0.002
LDL-C (mg/dl)
125
115
105
95
85
75
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

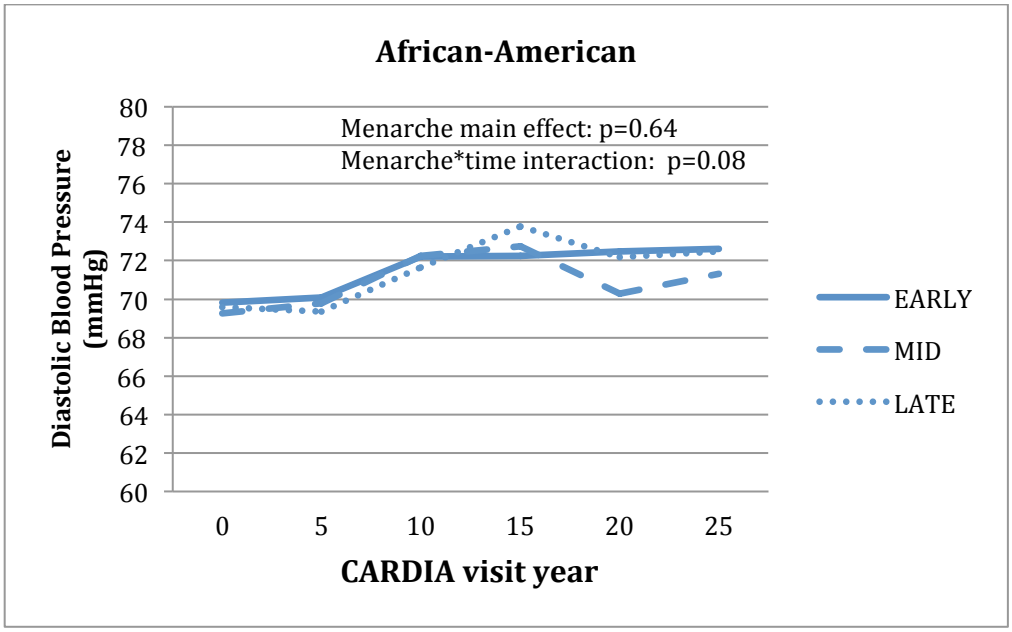

White
Menarche main effect: p=0.07
Menarche*time interaction: p=0.15
HDL-C (mg/dl)
70
68
66
64
62
60
58
56
54
52
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

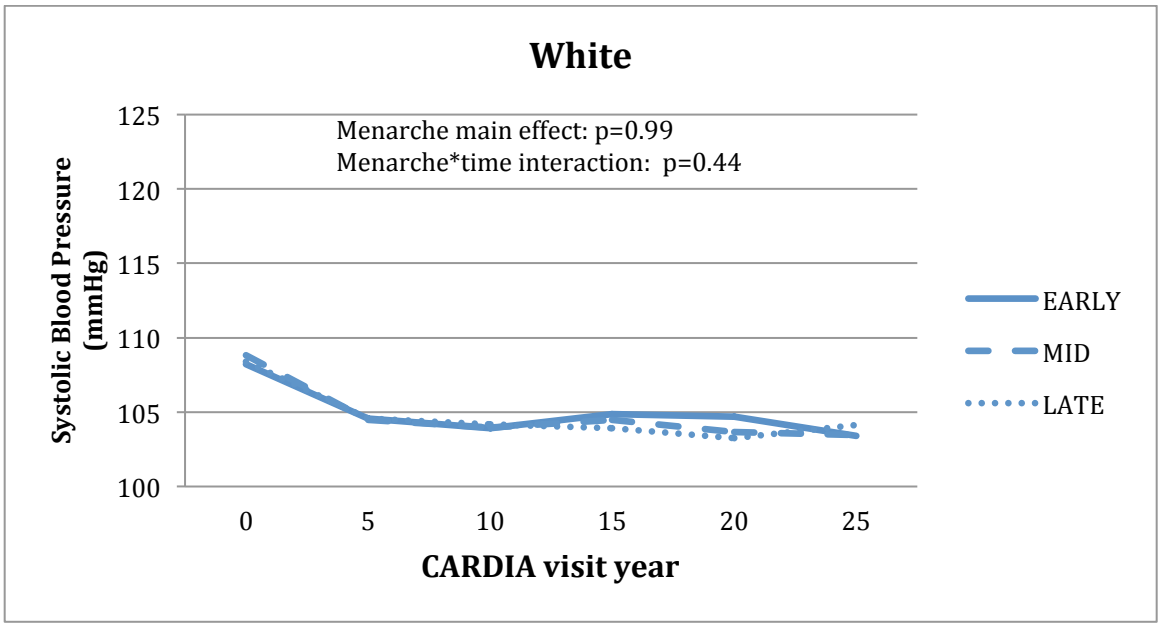

African-American
Menarche main effect: p=0.16
Menarche*time interaction: p=0.89
HDL-C (mg/dl)
70
68
66
64
62
60
58
56
54
52
50
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

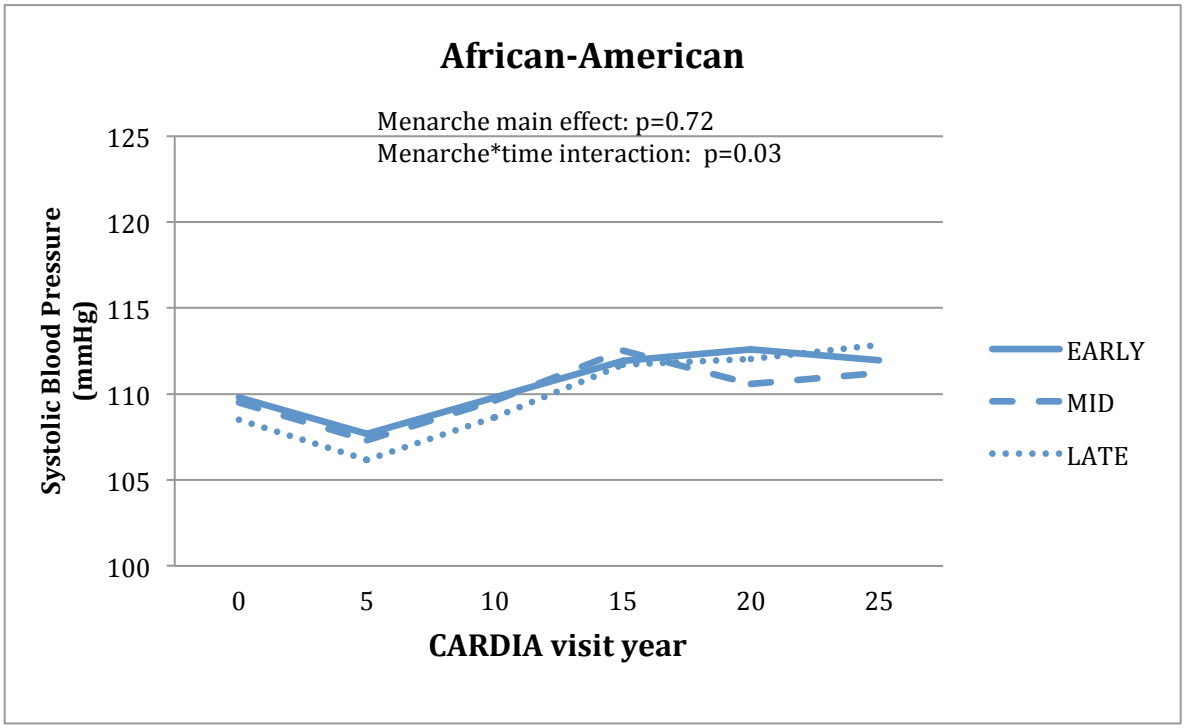

White
Menarche main effect: p=0.90
Menarche*time interaction: p=0.008
Triglycerides (mg/dl)
125
115
105
95
85
75
65
55
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

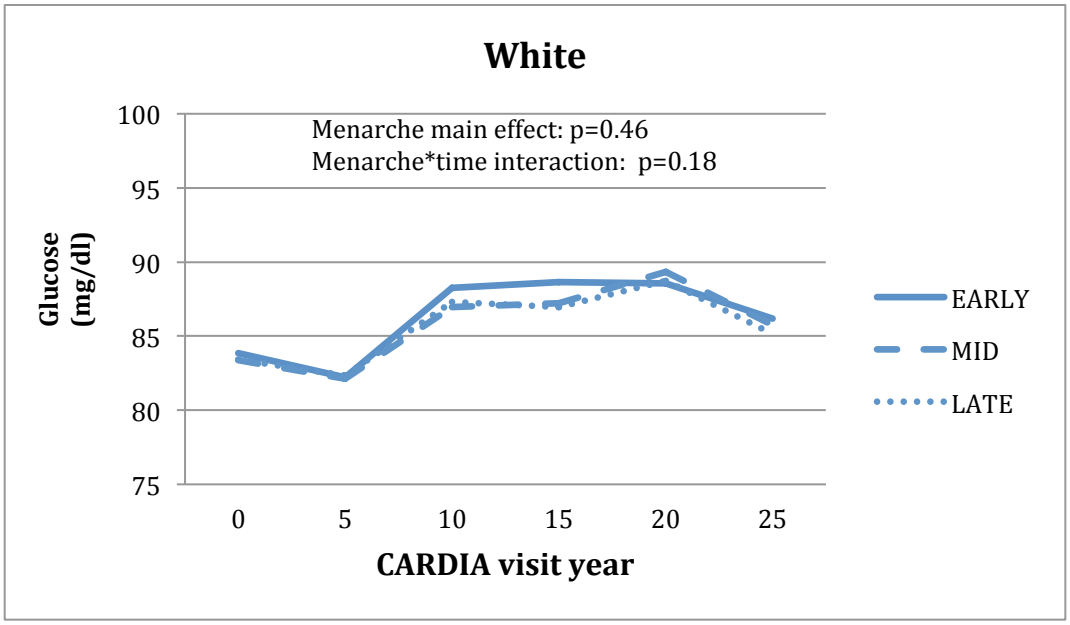

African-American
Menarche main effect: p=0.88
Menarche*time interaction: p=0.15
Triglycerides (mg/dl)
125
115
105
95
85
75
65
55
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

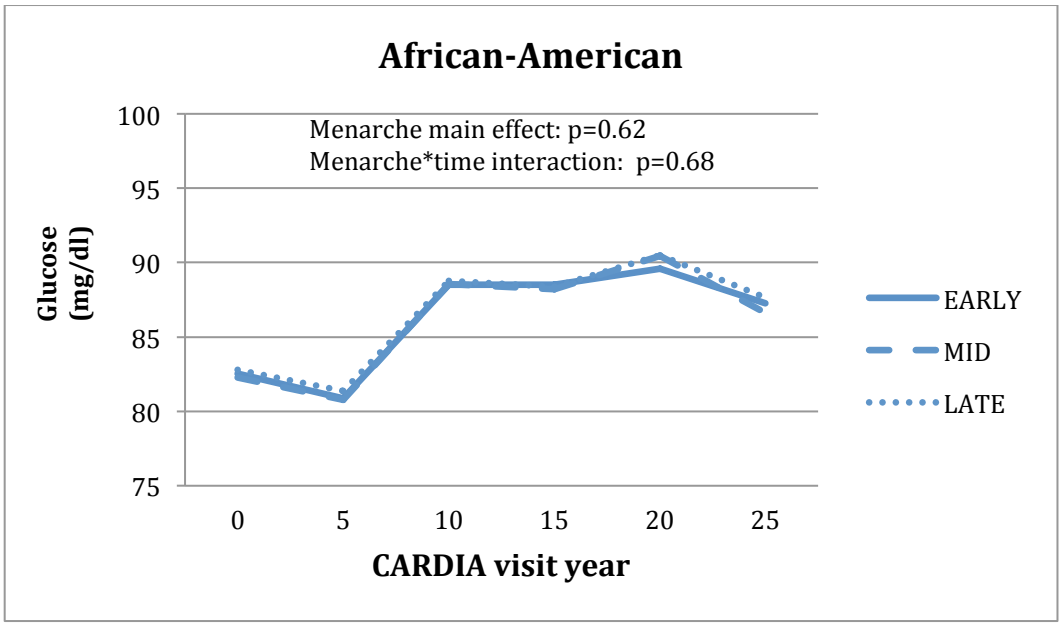

White
Menarche main effect: p=0.75
Menarche*time interaction: p=0.07
Diastolic Blood Pressure (mmHg)
80
78
76
74
72
70
68
66
64
62
60
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

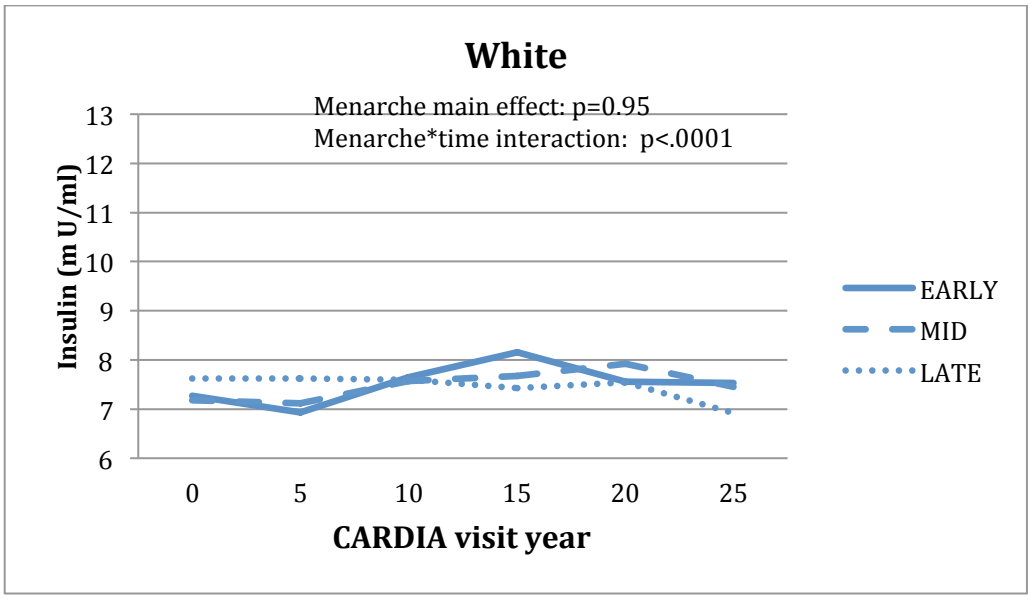

African-American
Menarche main effect: p=0.64
Menarche*time interaction: p=0.08
Diastolic Blood Pressure (mmHg)
80
78
76
74
72
70
68
66
64
62
60
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE

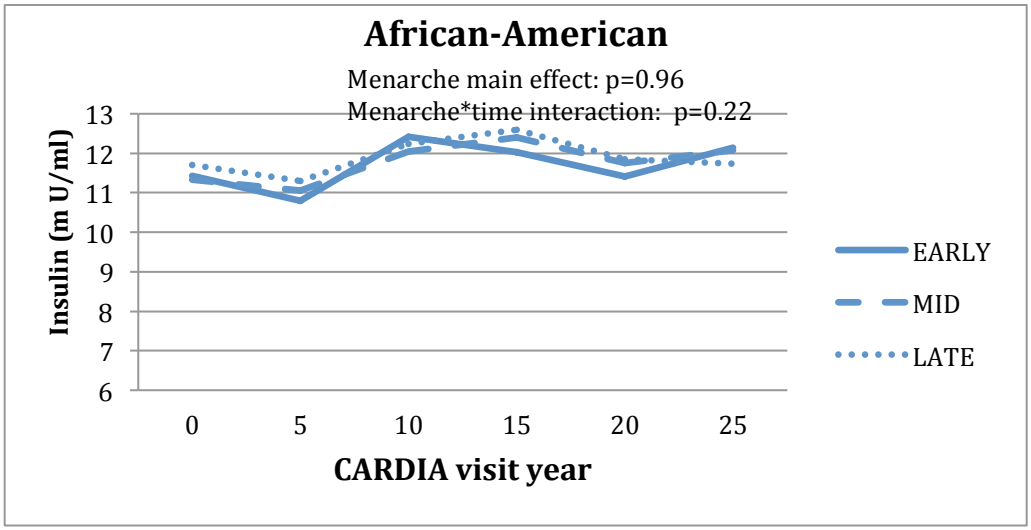

White
Menarche main effect: p=0.99
Menarche*time interaction: p=0.44
Systolic Blood Pressure (mmHg)
125
120
115
110
105
100
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE


African-American
Menarche main effect: p=0.72
Menarche*time interaction: p=0.03
Systolic Blood Pressure (mmHg)
125
120
115
110
105
100
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE


White
Menarche main effect: p=0.46
Menarche*time interaction: p=0.18
Glucose (mg/dl)
100
95
90
85
80
75
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE


African-American
Menarche main effect: p=0.62
Menarche*time interaction: p=0.68
Glucose (mg/dl)
100
95
90
85
80
75
0
5
10
15
20
25
CARDIA visit year
EARLY
MID
LATE





Notes:
Includes women who reported menarche 8-17 years, were non-diabetic at baseline, and who had data available on covariates.
Excluded the visits for women who were pregnant or developed diabetes.
Adjusted for age, age^2, center, smoking (never, former, current), parental diabetes (yes/no), education (<HS, HS, >HS), alcohol use (g/day), physical activity (METS), physical activity prior to high school, physical activity during high school, use of oral contraceptives (yes/no), menopause status (yes/no), and BMI. Lipid models additionally adjusted for cholesterol lowering medications.

CHAPTER 5. MANUSCRIPT #3 - MENDELIAN RANDOMIZATION STUDY OF THE ASSOCIATION OF AGE AT MENARCHE AND TYPE 2 DIABETES

Introduction

Recent studies have reported that early age at menarche increases the risk of type 2 diabetes [2-6]. However, early menarche is also strongly related to higher childhood and adulthood adiposity [60, 97], prompting uncertainty regarding the independent role of the timing of menarche in the risk of type 2 diabetes. In the CARDIA study, we found that each 1-year younger age at menarche was associated with 17% (HR=1.17, 95% CI 1.03, 1.29) greater risk of type 2 diabetes over 25 years of follow-up among white women; however, the results were attenuated and no longer statistically significant after adjustment for baseline BMI (see Manuscript #2 of this dissertation). Others have also reported that adiposity completely attenuates the association between menarche and diabetes [2, 4, 175]. However, data from some large studies such as the German KORA study [5], Atherosclerosis Risk in Communities (ARIC) study [6], and the Nurses Health Study (NHS) [3], suggest that the association between earlier menarche and type 2 diabetes may be independent of adiposity in populations of primarily European ancestry, and in the case of ARIC and NHS, might be stronger among younger versus older cohorts of women. Due to concern about the declining age at menarche in the population [15, 64], as well as increasing prevalence of type 2 diabetes and the need to intervene in the disease process earlier in the life course, it seems important to determine if early puberty itself

might be a causal factor for diabetes, or if associations are purely the result of other confounding factors among early maturing girls.

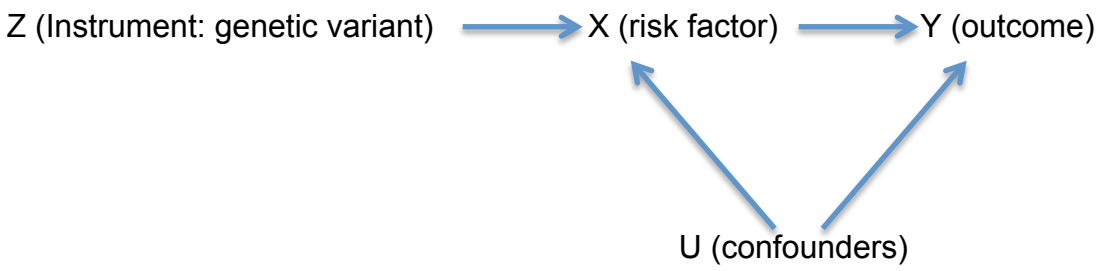
One possible reason for the uncertainty about the independent role of the timing of menarche in the risk of type 2 diabetes is that menarche occurs early in the life course, while type 2 diabetes onset typically occurs decades later. This lag between exposure and outcome leaves open the possibility for a number of factors, including adiposity, during childhood and adulthood to confound or mediate the association. Most studies have not included measures of early-life factors that are potential confounders in the association, and residual confounding could result from unobserved or imprecisely measured variables.

As more and more genetic variants for common diseases are identified, Mendelian randomization has emerged as a tool to strengthen causal inference and to help overcome the issues of confounding and selection bias inherent in observational research [227]. Mendelian randomization takes advantage of the theory that genetic alleles are randomly distributed at gamete formation, and relies on the assumption that genetic variants are distributed independently with respect to behavioral and environmental factors [227]. Therefore, the association of the genetic variants with an outcome should not be subject to traditional epidemiologic biases and confounding. Under certain circumstances, Mendelian randomization is akin to a natural randomized controlled trial [228].

Mendelian randomization has its roots in the econometric method of instrumental variables (IV) analysis, wherein genetic variants are considered to

be instruments for an intermediate risk factor (or phenotype), and thus can be used to distinguish between a causal and non-causal association of that intermediate variable with an outcome. **Figure 10** illustrates how a genetic variant (Z) is related to the outcome (Y), through an intermediate exposure variable (X) in Mendelian randomization [228, 229]. U represents confounders, which should be unrelated to the genetic variant. Note that Z (the instrument) should not directly cause Y (the outcome), but rather Z is correlated with Y via an indirect path through X (the risk factor).

Figure 10. Relationship of the instrumental variable with an intermediate risk factor and outcome in Mendelian Randomization



The question about an independent role of the timing of menarche in the risk for type 2 diabetes is a suitable candidate for a Mendelian randomization study because twin and family studies have reported high heritability for menarcheal age [34, 230-232]. Furthermore, a recent genome-wide association study (GWAS) by the CHARGE-ReproGen consortium identified numerous independent single nucleotide polymorphisms (SNPs) in genes associated with age of menarche [35].

Given that 1) several studies, including our own analyses of ARIC and CARDIA data, have replicated the association of early menarche and type 2 diabetes, 2) there is uncertainty regarding the role of adiposity (e.g. mediating or confounding), and other childhood factors in the association, and 3) age at menarche has strong genetic underpinnings, the objective of this study was to use a Mendelian randomization approach to explore the association between age at menarche and type 2 diabetes using a genetic risk (GRS) as an instrumental

variable and uconfounded proxy for age at menarche. The GRS includes the set of SNPs that were associated with younger age at menarche in a published GWAS study [35]. The hypothesis is that younger age at menarche will be associated with a greater probability of prevalent type 2 diabetes in IV regression models employing the GRS as an instrument for age at menarche, and that the association will be attenuated after considering adiposity as a mediator. This analysis might help to clarify if early age at menarche is an independent risk factor for type 2 diabetes, and it might contribute to the understanding of mechanisms underlying the association of growth and pubertal timing with risk of diabetes.

Materials and Methods

Study Design and Population

This study involved analyses of baseline and visit 4 data from the ARIC Study. The ARIC Study is a prospective cohort of 15,792 participants (8,710 women) aged 45-65 at baseline, originally designed to identify risk factors for atherosclerosis and cardiovascular disease. The details of the study cohort, including eligibility criteria, sources and methods of recruitment, and methods for follow-up have been described in detail elsewhere [216]. Briefly, participants were selected from four communities: Forsyth County, NC (n=4,035); Jackson, MS (African-American only, n=3,728); northwest suburbs of Minneapolis, MN (n=4,009); and Washington County, MD (n=4,020). Data for these analyses were collected during home interviews and clinic visits at the baseline examination

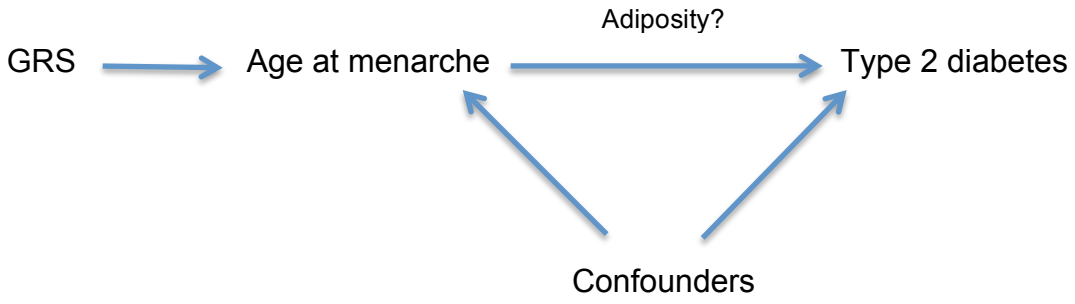
(1987-1989) and three follow-up visits (1990-1992, 1993-1995, and 1996-1998). The response rate was 60% to the baseline exam in the four communities, and ranged from 80%-93% for follow-up of the cohort over four visits. The ARIC study was approved by the institutional review boards at each of the study sites and informed consent was obtained from each participant.

The current analysis included all women of European ancestry with genetic, age at menarche, and diabetes status information at baseline. In ARIC, a total of 4,939 white women had genetic information for the SNPs of interest in this analysis. Those with missing age at menarche (n=23) or reporting age at menarche <8 or >17 years at the baseline visit (n=21) were excluded as we were interested in women within the normal range of pubertal development. Also excluded were those missing diabetes status at baseline (n=3) or with diabetes diagnosed prior to age 30 years (n=13), as these women were assumed to have type 1 diabetes. For the analysis of prevalent diabetes at visit 4, those with missing information at visit 4 (n=894) were also excluded. Participants from race/ethnic groups other than white were not included in this analysis because the SNPs for early menarche have only been replicated in non-Hispanic white women. Furthermore, there was not a statistically significant association between age at menarche and diabetes risk among African-American women in our published analysis that used ARIC data [6] or in Manuscript #2 of this dissertation that used CARDIA data. The final sample size for the analysis of prevalent diabetes at baseline was 4,879, and for prevalent diabetes at visit 4 n=3,985.

Clinical Measures and Data Collection

This section summarizes the definitions used for the instrumental variable (GRS), intermediate risk factor (age at menarche), outcome (type 2 diabetes), and covariates in the IV analyses. **Figure 11** shows the relationships of interest in this analysis.

Figure 11. Relationship of the GRS to age at menarche and type 2 diabetes



The use of Mendelian randomization to estimate the causal effect of age at menarche on type 2 diabetes using the menarche-GRS as an instrument relies on the following three main assumptions [227, 229].

1. The GRS must be robustly associated with age at menarche, also known as the “relevance assumption” in IV analysis.
2. The GRS must not be associated with confounders that may bias the association between age at menarche and type 2 diabetes.

3. The GRS must exert its effect on type 2 diabetes only through age at menarche and not through other pathways. This is known as the "exclusion restriction assumption".

These three assumptions formed the framework for the data that were chosen for inclusion in this study, and were addressed as part of the analyses.

Genetic Risk Score (Instrumental Variable)

The GRS included the SNPs that are positively associated with early menarche among cases and noncases of diabetes in ARIC. **Appendix 1** shows the list of the 42 SNPs that were included in the GRS. The information for the GRS comes from the ReproGen consortium [35] and includes the SNPs that are associated with age at menarche at p-values $<5X10^{-8}$ from stage 1 and replication meta-analysis. Genotyping in ARIC was performed using an Affymetrix 6.0 Array and genetic variants with a call rate ≤90%, minor allele frequency ≤0.01, or Hardy-Weinberg Equilibrium p-value $\leq 1x10^{-6}$ were excluded. Imputation and analysis were performed with MACH and ProbABEL software.

First, a "GRS-42" was calculated for each participant by summing the number of risk alleles they had for each of the 42 SNPs (0, 1, 2). The risk allele is that which was associated with younger age at menarche, regardless of which allele was the minor allele or the effect allele in the ReproGen analysis. Following is the equation for the GRS-42:

GRS-42 = (# SNP1 risk alleles) + (# SNP2 risk alleles) + … + (# SNP42 risk alleles)

The minimum possible value for the GRS-42 would be 0 risk alleles, and the maximum possible value would be 84 risk alleles (42 x 2). Some SNPs in the GRS are located in/near genes that are also hypothesized to regulate BMI, energy homeostasis, adult height, eating behavior, pituitary regulation, and estrogen encoding [233]. As mentioned, adiposity is an outcome (and possibly a predictor) of early menarche, and it is also a strong risk factor for type 2 diabetes. Furthermore, several of the SNPs in the menarche GRS-42 are associated with BMI during various points in the life course. This pleiotropy would violate the third key condition of IV analyses (i.e. the instrument exerts it effect on the outcome only through the intermediate risk factor and not through other pathways). Therefore, the association of a reduced GRS that excluded 13 SNPs with possible pleiotropic effects on childhood or adulthood adiposity ("GRS-29") was also examined, defined as in the paper by W. Johnson et al (2013) [234]. The excluded SNPs were those previously reported to be associated with adulthood or childhood BMI [35, 235, 236], and those located in or near genes involved in body weight and energy homeostasis in animal models [237-240]. See appendix A for the list of SNPs included in the GRS-29. The GRS-29 variable was calculated using the same method as described for the GRS-42 variable. The IV analyses that use the GRS-29 as an instrument for age at menarche provided a sensitivity test of whether associations that included the GRS-42 as an instrument were the result of the pleiotropic effects of menarcheal genes on adiposity.

Age at Menarche (Intermediate Risk Factor/Phenotype)

Age at menarche was measured via self-report in whole years at the baseline visit in ARIC by asking women at what age their menstrual periods began**.** The validity of recall for age at menarche in middle age is moderate. For example, in one retrospective study, approximately 84% of women who were mean age 50 years, recalled their age at menarche to within one year of the actual date based on prior records [53, 54]. Age at menarche was modeled as a continuous variable in whole years in all models.

Type 2 Diabetes (Outcome)

Diabetes status was assessed during each clinic visit. As mentioned, there appears to be a stronger association between early menarche and diabetes diagnosed at younger vs. older ages [3, 6], possibly because the outcome is closer to the timing of exposure or different physiological pathways to diabetes. Therefore, we first evaluated the association of age at menarche with prevalent diabetes at the baseline visit (i.e. the time during which the cohort was the youngest) and then with prevalent diabetes at visit 4 to obtain the largest number of diabetes cases that were objectively assessed in ARIC. Diabetes was defined by either self-reported history of diabetes diagnosis outside of pregnancy, blood glucose measurements (fasting glucose ≥ 7.0 mmol/l [126 mg/dl], non-fasting glucose >11.1 mmol/l [200 mg/ dl]), or self-reported use of hypoglycemic medication in the 2 weeks prior to the visit. Fasting glucose was measured by a hexokinase/glucose-6-phosphate dehydrogenase method at the Central Clinical

Chemistry Laboratory in Minneapolis, Minnesota, and the Central Lipid Laboratory in Houston, Texas.

Covariates

Although Mendelian randomization is based on the theory that genetic variants are distributed randomly with regard to potential environmental and behavior confounders, the use of covariates can increase the efficiency of IV estimators [241]. Furthermore, adjustment for covariates in Mendelian randomization analyses is relevant when a variable is both a confounder and suspected of modifying the effect of phenotype on outcome [241], which is the case for BMI. The covariates in adjusted models included age at baseline (years), field center, baseline BMI, BMI at age 25 (kg), and height. Weight (kg) and height (cm) were measured at the clinic visits with participants dressed in scrub suits and shoeless. Baseline BMI was calculated as baseline weight (kg)/baseline height^2 (m^2). BMI at age 25 was calculated as self-reported weight at age 25 (kg)/baseline height^2 (m^2). Because weight at age 25 was based on self-reported recall, it may be subject to misclassification. However, in the Nurses' Health Study II Cohort, the correlation between recalled weight in adulthood and measured weight at age 18 years was high at 0.87 [242].

Statistical Methods

Statistical analyses were performed with STATA, version SE 12 (College Station, TX) and SAS version 9.2 (Cary, NC). All statistical tests were 2-sided

and significance was defined as $p<0.05$. As done in some IV analyses in the field of health economics [243], marginal significance was set at a threshold of $p<0.10$ for the IV estimates only. Means (SD) for continuous variables, and n (%) for categorical variables (**Table 13)** summarized the characteristics of the study populations by age at menarche category (8-11, 12-13, 14-17 years). P-trend was calculated using chi-square for categorical variables or chi-squared test for Pearson's correlation coefficient for continuous variables.

To explore the causal role of age at menarche and type 2 diabetes, a series of Mendelian randomization IV-regression analyses were estimated as described below. By using the GRS in place of age at menarche, the IV statistical method relates the variation in age at menarche (the risk factor) that is influenced by the GRS (the instrument) to type 2 diabetes (the outcome). The method produces an estimate of the causal effect of the exposure on the outcome that is similar to an intention to treat analysis in a randomized controlled trial, assuming that the three assumptions of IV analyses are met [244, 245].

Traditionally, IV analysis is performed using a 2-stage least squares (2SLS) model that predicts risk differences. However, economists often use probit structural models for dichotomous outcomes because 2SLS is analogous to linear regression and is more appropriate for continuous outcomes [246] [247]. Of note is that standard errors in all IV models are larger (i.e. point estimates are less precise) than in their corresponding standard regression approaches [246].

We started by testing the relevance assumption of Mendelian randomization (i.e. the GRS is associated with age at menarche) by evaluating the basic associations of the GRS-42 and GRS-29 with age at menarche using linear regression (beta [SE] and 95% CI per 1-SD increase in the GRS) (**Table 14**). The F statistics from this linear regression were used to evaluate the strength of the association of the instrument (GRS-42 or GRS-29) with age at menarche, with values greater than the conventional threshold of 10 indicating sufficient strength of the instrument to ensure the validity of IV methods (Staiger-Stock rule) [248, 249]. The F-statistic is considered an indicator of instrument strength because in the presence of X-Y confounding, the IV estimates will be biased towards the confounded X-Y association, and the magnitude of that bias is inverse to F [250]. The ratio of the IV bias to the bias in the X-Y association is referred to as 'relative bias'. The threshold of F>10 to indicate a strong instrument in MR analysis is based on the observation that values greater than this threshold ensures a relative bias of <10% at least 95% of the time [250].

To test the assumption that the GRS is not associated with potential confounders, linear or logistic regression evaluated the associations (beta [SE] or OR, 95% CI per 1-SD increase in the GRS) of the GRS-42 and GRS-29 with potential confounding characteristics **(Table 15).** While it is not possible to test for the association of the GRS with unobserved confounders, guidelines for IV studies state that associations with observed factors should be tested and reported [251].

Next, IV probit regression analysis with a maximum likelihood estimator explored the causal role of age at menarche in type 2 diabetes by examining whether 1-SD increases in the GRS-42 or GRS-29 predicted absolute risk probabilities for prevalent type 2 diabetes at baseline or prevalent diabetes at visit 4 through their association with age at menarche. First, a standard probit model without the instrumental variable (GRS) was estimated using the "probit" function in STATA. Logistic regression models were also run so that the standard odds ratios (ORs) could be compared with other epidemiologic studies of the association of age at menarche and type 2 diabetes. Then, the IV probit regression models with robust standard errors (to account for heteroscedasticity of the outcome, as recommended in [251]) were estimated using the "ivprobit" command. The probit model is defined as $Pr(y=1|x) = \mathbf{\Phi}(xb)$, where $\mathbf{\Phi}$ is the standard cumulative normal probability distribution and xb is the probit coefficient [252]. Therefore, the probit model provided the predicted probability of diabetes based on all predictors, using the cumulative distribution function of the standard normal. The Mendelian randomization analyses were repeated with covariates for adjusted estimates.

Logistic regression models, in which the β_i's are interpretable as log (odds ratios), are usually preferred in the field of epidemiology over probit regression because the β_i's in probit are models somewhat more difficult to interpret [246]. With logistic regression, a one-unit change in X_1 corresponds with a β_1 change in the log odds of disease, assuming all else is equal. With probit, the one unit

change in X_1 corresponds with a 1-unit change of β_1 z's [246]. To help with interpretation of probit coefficients, the marginal effect of age at menarche evaluated at the mean values of covariates are reported. To obtain the postestimation marginal values, "*margins, dydx(menage) predict(pr)"* was added to the STATA code. The marginal effect is interpreted as the change in the slope of the probability function, and is comparable to a risk difference from a 2SLS model [246]. In the tables, each 1-year decrease in age at menarche changes the absolute risk of diabetes by the marginal value when all other independent variables are held constant at their mean level.

The standard and IV regression models were run in a series, starting with an unadjusted model, then adding baseline age and BMI at age 25 (Model 1), and then baseline age and BMI at baseline in place of BMI at age 25 (Model 2) to examine the potential mediating role of BMI at different times in the life course. In standard regression models, interaction terms including age at menarche and age at baseline tested if the association of age at menarche with diabetes differed by age at the baseline visit. Interaction terms including age at menarche and BMI at baseline tested if associations differed by BMI.

Results

Table 13 shows the characteristics of the 4,879 study participants included in the baseline analysis by age at menarche category. Women with earlier menarche were younger, shorter, had greater adiposity, higher GRS-42 and GRS-29 scores, and greater prevalences of type 2 diabetes at baseline and

visit 4 compared with women reporting later menarche. The mean age of the cohort was 53.9 years (SD=5.7, range 44-66) at the baseline visit, and 62.8 years (SD=5.6, range 53-75) at visit 4. The mean age at menarche in the entire cohort was 12.9 years (SD=1.6). Menarche before the age of 12 years (the age used in many studies to define early menarche) was reported by 17.5% of the participants (n=857).

There were no meaningful differences in the baseline characteristics by menarche category for the subsample (n=3,985) used for the analysis of prevalent diabetes at visit 4, and the mean age at menarche was the same for women who did vs. did not return at visit 4 (12.9 years). However, the prevalence of diabetes at baseline was higher among women who did not return (12.5%) versus those who did return (6.5%) at visit 4. There were 371 cases of type 2 diabetes identified at the baseline visit (mean age at diagnosis=51 years), 302 incident cases identified between baseline and follow-up year 9 (mean age at diagnosis=58 years), and 562 prevalent cases remaining at visit 4 (260 of which were from baseline; mean age at diagnosis=53 years).

Genetic Risk Score

The mean GRS-42 was 41 risk alleles (SD 3.9, range 27-55 risk alleles), and the mean GRS-29 was 35 risk alleles (SD 3.5, range 22-47). **Figure 12** shows the inverse association between the GRS-42 and age at menarche. As shown in **Table 14,** the F-statistics from the first stage regression of the instrumental variable analyses were 96.2 for the GRS-42 (R^2=0.02) and 85.4 for

the GRS-29 (R^2=0.02), suggesting that these were credible instrumental variables for age at menarche according to conventional standards in IV analyses. Each 1-SD increase in the GRS-42 or GRS-29 was associated with approximately 11 weeks younger age at menarche.

Table 15 shows that the GRS was not associated with any baseline behavioral characteristics, as expected because it is a randomly allocated variable. However, the GRS was associated with body composition characteristics. Each 1-SD increase in the GRS-42 was associated with 0.24 cm (SD=0.08, p=0.004) shorter stature at baseline, and 0.11 kg/m^2 (SD=0.05, p=0.01) greater BMI at age 25. Each 1-SD increase in the GRS-29 was associated with 0.21 cm (SD=0.08, p=0.01) shorter stature, but not with any adiposity variables.

Standard Regression for Age at Menarche and Type 2 Diabetes

The standard probit regression analyses indicated increased probability of type 2 diabetes per 1-year younger age at menarche at both baseline and at visit 4 in unadjusted models and in models adjusted for BMI at age 25 (**Table 16).** The association remained only for prevalent diabetes at baseline after adjusting for baseline BMI (Model 2). According to the marginal values in the standard probit regression models, each 1-year earlier age at menarche increased the risk of type 2 diabetes at baseline and at visit by approximately 1% in all of the standard regression models.

The interaction term between age at menarche and age at baseline suggested that the association of younger age at menarche with diabetes at visit 4 was weaker among women who were older at baseline (p-interaction=0.05). There was no indication of interaction between menarche age and baseline age for prevalent diabetes at the baseline visit (p>0.10). There were also no differences in the association of age at menarche with type 2 diabetes at baseline or at visit 4 by BMI at baseline (p-interaction>0.29).

Instrumental Variables Regression for Age at Menarche and Type 2 Diabetes

As shown in **Table 16**, the IV probit estimates for the effect of age at menarche on type 2 diabetes at baseline were three to five times stronger using the GRS-42 or GRS-29 as instruments compared with the standard probit regression estimates for diabetes at baseline. However, only the unadjusted estimate of diabetes at baseline using the GRS-42 reached statistical significance at conventional p<0.05 level (coefficient=0.23, 95% CI 0.01, 0.42). IV estimates using the GRS-29 before adjustment for baseline BMI were suggestive of an association at the p<0.1 level. According to the marginal IV probit values, each 1-year earlier age at menarche increased the risk of type 2 diabetes at baseline by 3% in all of the IV models. Overall, the GRS-42 and GRS-29 instrumented coefficients were similar.

None of the IV estimates for prevalent diabetes at visit 4 were not statistically significant. Furthermore, the confidence intervals for the IV models

fully included the confidence intervals from standard probit models, indicating that the standard and IV estimates were not different.

Discussion

The IV methods applied in the current study were used to estimate the causal effect of age at menarche on type 2 diabetes using Mendelian randomization to avoid uncontrolled confounding and selection bias. Looking strictly at the point estimates, the results provide some suggestion for a causal role for earlier age at menarche in the risk of type 2 diabetes at the baseline visit, as well as mediation by adiposity. However, the standard and instrumented regression results for diabetes at visit 4 do not support a causal role of earlier menarche in the probability for diabetes overall in later life. There was a significant interaction between age at baseline and age at menarche for prevalent diabetes at visit 4, indicating that the association of age at menarche with type 2 diabetes might differ by age. However, larger sample sizes would be required to further investigate this age interaction with IV analyses. The standard regression estimates had narrow confidence intervals compared with the wide confidence intervals for the IV estimates, which was the result of the small proportion of the variation in age at menarche explained by the GRS (~2%) and the weak effect sizes for age at menarche on type 2 diabetes.

The estimates from the IV analyses for diabetes at baseline were substantially higher than the estimates from the standard probit regression, suggesting that standard observational studies might underestimate the

association between early age at menarche and type 2 diabetes at the baseline visit. Mendelian randomization might better characterize the effects of menarche on diabetes risk because it reflects lifetime exposure to hormones and metabolic changes associated with earlier maturation, including a lifetime with greater adiposity. Also, Mendelian randomization avoids the underestimation of risk associations caused by regression dilution in traditional prospective studies [253].

However, the influence of an exposure on an outcome might lessen with time, and so the lack of any association at visit 4 in the IV estimates might reflect less influence of the weak association of age at menarche and type 2 diabetes risk as women age, possibly because of the decline in beta cell function and the increase in insulin resistance that occur naturally as part of the aging process. Furthermore, genetic associations with health outcomes tend to weaken with age as the overall aging process takes over [254]. The finding in the current study corresponds with results from the NHS I and II, which reported stronger associations in the younger versus older cohorts [3]. Finally, the lack of association at visit 4 might simply support the null hypothesis of no causal effect of age at menarche on type 2 diabetes.

Methodological Considerations

The use of Mendelian Randomization and IV methods for causal inference relies on a number of assumptions [229, 251]. Unfortunately, it is not possible to directly test some of these conditions, such as the key assumption of no

pleiotropic effects of the genetic variants on multiple phenotypic traits. If the menarche SNPs have other effects (such as increasing or decreasing childhood or adult adiposity outside of the influence of age at menarche on these characteristics) which in turn affect risk of diabetes, then we cannot necessarily infer that the Mendelian Randomization models are measuring the causal association of early menarche with diabetes. The findings that the GRS-42 was associated with BMI at age 25, and both the GRS-42 and GRS-29 were associated with height, suggests possible pleiotropy by these characteristics and reinforces the shared biological underpinnings of age at menarche and body composition. Height is closely tied to pubertal timing [255], and girls who reach menarche early tend to be taller in earlier adolescence, but then attain shorter stature in adulthood [91]. Although shorter stature is associated with type 2 diabetes, one possible pathway is through early childhood environment, such as intrauterine and early childhood nutrition, or levels of insulin like growth factor-I, instead of height itself being on the causal pathway to diabetes [256].

In contrast, greater adiposity is on the causal pathway to type 2 diabetes. Consequently, the GRS might independently exert its effect on diabetes via an adiposity pathway that is separate from age at menarche. Although the GRS-42 was not associated with weight at age 25 or with waist or BMI at baseline (and the associations with BMI at age 25 could have been driven by height), in an attempt to address possible pleiotropy with adiposity, we analyzed the GRS with and without adiposity-related SNPs. The point estimates were similar using both

the GRS-42 and GRS-29 as instruments. This suggests limited pleiotropic effects of the GRS on type 2 diabetes directly through adiposity, although there is no way to rule out possible pleiotropic effects of the GRS-29. Nonetheless, the GRS-29 excludes those adiposity SNPs that have been replicated in a number of large GWAS studies of obesity with extremely large sample sizes, and so any effect of the remaining SNPs in the GRS-29 on adiposity would be tiny.

Another key assumption that is unverifiable is that the GRS is not associated with unmeasured confounders between age at menarche and type 2 diabetes. We examined associations with known lifestyle confounders and found that the GRS was not associated with some behavioral factors that might affect diabetes risk, providing some reassurance that that the randomly assigned GRS is unlikely to be associated with other unmeasured behavioral confounders. However, the associations of the GRS-42 and GRS-29 with shorter stature and the GRS-42 with higher BMI at age 25 do not clarify if these factors should be considered confounders or mediators of the association. Therefore, models were adjusted for these covariates to try to parse out the independent influence of menarche on diabetes. After controlling for body composition, effect sizes were only slightly attenuated in the IV models. This suggests a small amount of confounding or, more likely, mediation given the lack of evidence of pleiotropy by adiposity.

As is usual with Mendelian randomization studies, power was limited to detect a true effect, if one existed, and therefore the results should be considered

as preliminary and replicated with a larger sample size. Reasons for low power included the small R^2 (~2%) for the association between the GRS and age at menarche, and the weak to moderate effect size for age at menarche and type 2 diabetes. However, our R^2 was well with the range found in other Mendelian randomization studies [250]. The imprecision in the IV models provides low confidence in conclusions of Mendelian randomization studies such as ours without a very large N (in the realm of 20k to 40k participants to increase power above 80%). Nonetheless, the IV point estimates from this study are of value to compare with the standard regression estimates, and the results from this study provide data for better power calculations for further study [250, 257]. This study also demonstrated how Mendelian randomization might be used in studies with larger sample sizes to evaluate the effect of pubertal timing on other disease outcomes with stronger effect sizes.

The main strength of the Mendelian randomization approach is that the method is not subject to traditional epidemiologic biases, such as selection or information bias, under the plausible assumption that the genotype is randomly allocated before the exposure of interest, in this case menarche. Other strengths of this study include the objective measurement of diabetes to avoid misclassification and robust measurement of a number of possible confounders. Finally, combining the genetic alleles into a GRS as we did decreases bias and helps to alleviate weak IV problems [250].

Conclusions

Use of a GRS as an instrument for age at menarche supported observational studies that have found a stronger role for earlier age at menarche in the risk of type 2 diabetes in younger versus older cohorts of women, with possible mediation of the association by adiposity. However, the lack of association in the IV estimates at visit 4 suggests that early menarche may not be a causal factor for type 2 diabetes overall in adulthood. From an etiologic point of view, although the overall IV findings were not statistically significant, their direction and magnitude and the possible differences by age might warrant further study with Mendelian randomization in larger samples with improved statistical power and a wider age range to replicate findings.

Table 13. Characteristics[a] of the sample participants (n=4,879) according to age at menarche category: the ARIC Study

	Age at Menarche (years)			
	8-11	**12-13**	**14-17**	
	% or Mean (SD)	% or Mean (SD)	% or Mean (SD)	p-trend[b]
n (%)	857 (17.6)	2,592 (53.1)	1,430 (29.3)	
Baseline age (years)	53.2 (5.5)	53.8 (5.6)	54.6 (5.8)	<.0001
Baseline BMI (kg/m^2)	27.9 (6.0)	26.6 (5.4)	25.9 (5.1)	<.0001
BMI at age 25 (kg/m2)	22.7 (3.6)	21.9 (3.2)	21.4 (3.0)	<.0001
Waist circumference (cm)	95.9 (16.0)	92.8 (14.5)	91.6 (14.3)	<.0001
Height (cm)	161.1 (5.9)	162.3 (5.8)	162.2 (5.9)	<.0001
Current smoking (yes)	5.1	12.4	7.8	0.26
Physical activity (leisure index)	2.5 (0.6)	2.5 (0.5)	2.5 (0.5)	0.57
Alcohol consumption (g/day)	3.3 (10.0)	3.2 (7.3)	3.0 (7.1)	0.89
Education (<HS)	18.3	13.4	17.2	0.95
Glucose (mg/dl)	105.1 (33.9)	102. 6 (30.3)	101.3 (26.0)	0.04
Diabetes at baseline (yes)	10.4	7.5	6.2	0.001
Diabetes at visit 4 (yes)	19.5	13.9	13.8	0.001
GRS-42 (# risk alleles)	42.2 (3.7)	41.4 (3.9)	40.6 (3.9)	<.0001
GRS-29 (# risk alleles)	35.6 (3.4)	35.1 (3.5)	34.4 (3.5)	<.0001

Notes:

[a]Characteristics are from baseline unless otherwise noted.

[b]p-trend from chi-square for categorical variables, or chi-squared test for Pearson's correlation coefficient for continuous variables

Table 14. Testing the strength of the IV: associations of GRS-42 and GRS-29 with age at menarche: the ARIC Study

	β (SE) per 1-SD increase in GRS*	95% CI	F-statistic	R^2
GRS-42	-0.22 (0.02)	-0.26, -0.18	96.2	0.02
GRS-29	-0.20 (0.02)	-0.25, -0.16	85.4	0.02

Notes: β, standardized linear regression coefficient; CI, confidence interval; R^2, fraction of explained variance (%).

Table 15. Associations of GRS-42 and GRS-29 with potential confounders: the ARIC Study

Covariate	β (SE) or OR per 1-SD increase in GRS	95% CI	p-value
GRS-42			
Age (years)	0.09 (0.08)	-0.07, 0.25	0.25
Education (<HS)*	1.06 (0.04)	0.98, 1.14	0.15
Current smoking (yes)*	1.01 (0.03)	0.95, 1.08	0.67
Alcohol consumption (g/day)	-0.09 (0.12)	-0.31, 0.14	0.46
Baseline BMI (kg/m^2)	0.05 (0.07)	-0.10, 0.21	0.50
Baseline weight (lbs)	-0.18 (0.47)	-1.09, 0.73	0.69
Baseline waist circumference (cm)	0.07 (0.21)	-0.34, 0.49	0.73
Baseline height (cm)	-0.24 (0.08)	-0.41, -0.08	0.004
BMI at age 25 (kg/m^2)	0.11 (0.05)	0.02, 0.21	0.01
Weight at age 25 (lbs)	0.28 (0.28)	-0.28, 0.83	0.33
Physical activity (leisure index)	-0.002 (0.008)	-0.02, 0.01	0.83
GRS-29			
Age (years)	0.09 (0.08)	-0.06, 0.25	0.24
Education (<HS)*	1.05 (0.04)	0.97, 1.14	0.21
Current smoking (yes)*	1.00 (0.03)	0.94, 1.07	0.90
Alcohol consumption (g/day)	-0.003 (0.12)	-0.23, 0.23	0.98
Baseline BMI (kg/m^2)	-0.02 (0.08)	-0.17, 0.14	0.82
Baseline weight (lbs)	-0.50 (0.47)	-1.41, 0.41	0.28
Baseline waist circumference (cm)	-0.009 (0.21)	-0.42, 0.41	0.96
Baseline height (cm)	-0.21 (0.08)	-0.38 -0.05	0.01
BMI at age 25 (kg/m^2)	0.03 (0.05)	-0.06, 0.13	0.46
Weight at age 25 (lbs)	-0.13 (0.28)	-0.68, 0.43	0.65
Physical activity (leisure index)	-0.003 (0.01)	-0.02, 0.01	0.68

Notes: β, standardized linear regression coefficient; CI, confidence interval.

*Regression coefficient expressed as odds ratios.

Table 16. Results from standard and GRS-instrumented analysis of the association of age at menarche (per year younger) and type 2 diabetes: the ARIC Study

Outcome	Standard Logistic Regression			Standard Probit Regression				IV Regression: GRS-42[b]				IV Regression: GRS-29[b]			
	OR	95% CI	p-value	Probit coefficient[a]	95% CI	p-value	Marginal value[c]	Probit coefficient[a]	95% CI	p-value	Marginal value[c]	Probit coefficient[a]	95% CI	p-value	Marginal value[c]
Prevalent diabetes at Baseline (n=371)															
Unadjusted	1.12	1.06, 1.18	<.0001	0.06	0.03, 0.10	<.001	0.01	0.23	0.01, 0.42	0.04	0.03	0.19	-0.01, 0.37	0.06	0.03
Model 1	1.11	1.04, 1.17	0.002	0.05	0.02, 0.09	0.004	0.01	0.19	-0.01, 0.39	0.07	0.03	0.19	-0.02, 0.40	0.08	0.03
Model 2	1.09	1.02, 1.15	0.02	0.04	0.01, 0.08	0.03	0.01	0.20	-0.05, 0.45	0.11	0.03	0.20	-0.12, 0.51	0.22	0.03
Prevalent diabetes at visit 4 (n=562)															
Unadjusted	1.11	1.05, 1.16	<.0001	0.06	0.03, 0.09	<.0001	0.01	-0.03	-0.26, 0.20	0.77	-0.01	-0.08	-0.31, 0.15	0.50	-0.02
Model 1	1.08	1.02, 1.13	0.01	0.04	0.01, 0.07	0.01	0.01	-0.11	-0.35, 0.12	0.35	-0.03	-0.11	-0.35, 0.12	0.35	-0.02
Model 2	1.05	0.99, 1.11	0.11	0.03	-0.01, 0.06	0.11	0.01	-0.08	-0.32, 0.17	0.54	-0.02	-0.08	-0.33, 0.17	0.52	-0.02

[a]Change per unit increase in the predictor variable. The marginal value is a risk difference

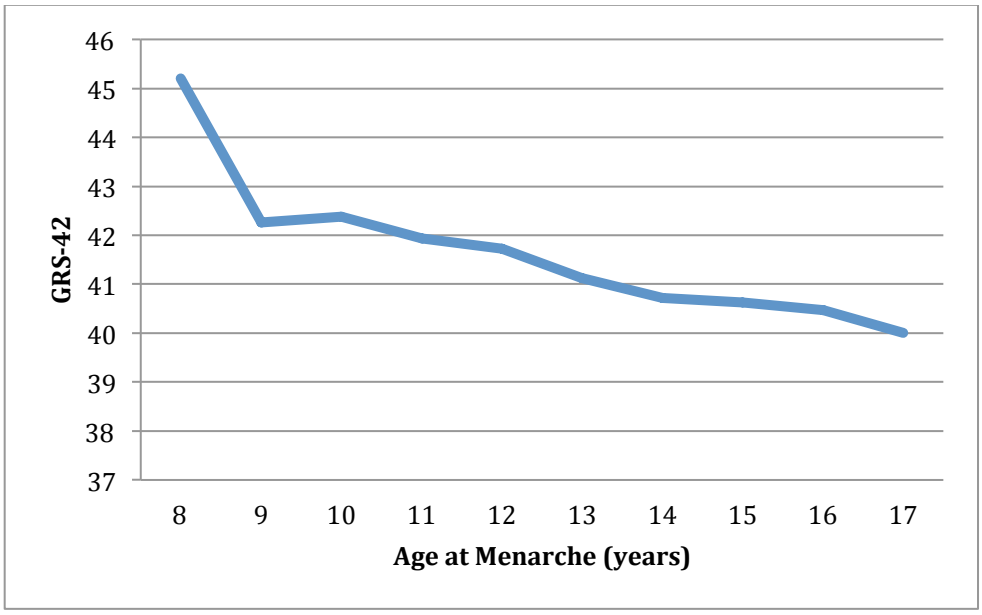
[b]In the instrumental variables regression, the 42-SNP or 29-SNP genetic risk scores for age at menarche were used as instruments for age at menarche

[c]Marginal value: each 1-year decrease in age at menarche changes the probability of diabetes by this value when all other variable are held constant at their mean level.

Model 1 adjusted for age and BMI at age 25

Model 2 adjusetd for age and baseline BMI

Figure 12. Mean GRS-42 by age at menarche in the ARIC study



Chapter 6. Summary and Conclusions

Summary of Manuscript Results

The trend toward earlier age at menarche in recent decades, as well as widening race disparities in the timing of puberty, has raised concern in the public because of reports of the link between earlier maturation and a number of adverse health outcomes. This dissertation focused on the cardiometabolic health consequences at various points in the life course of earlier age at menarche using three different cohorts. Although previous studies have investigated the association of early age at menarche with several of the CVD risk factors included in this thesis, results conflict about independence of associations from adiposity, and data for ethnic minority groups are limited.

Therefore, the objectives of the manuscripts in this thesis were to 1) comprehensively examine the relationship between earlier age at menarche and a wide variety of inter-related CVD risk factors at different points in the life course, 2) account for the role of adiposity in associations, and 3) explore possible race differences in associations. The hypotheses were that 1) earlier age at menarche would be associated with a more adverse cardiometabolic profile, 2) lifetime accumulated adiposity would largely explain the associations, and 3) there would be differences by race in associations. The public health related goal was to identify if puberty might be a sensitive time in the life course for targeted lifestyle interventions to prevent overt disease in adulthood.

The first manuscript examined the longitudinal and cross-sectional associations of age at menarche with CVD risk factors from age 9-19 years in the NGHS, comparing African-American and white girls. The main finding was that there were no differences by race in the influence of earlier menarche with levels or change in the CVD risk factors during adolescence, despite higher levels of adiposity and earlier menarche among African-American compared with white girls. As expected, age at menarche was associated with greater adiposity during adolescence, but the association was stronger and statistically significant specifically only among girls who were normal weight prior to menarche. Earlier menarche was not associated with level of adiposity overall during adolescence or at age 19 among girls who were already overweight prior to menarche even after considering pubertal stage at the baseline visit. In addition, earlier menarche was associated with adverse levels of systolic blood pressure and insulin during adolescence, but there were no clinically meaningful associations between earlier menarche and other CVD risk factors by average age 19 after adjusting for age 19 adiposity.

The second manuscript compared African-American and white women and examined the association of the timing of menarche with cardiometabolic risk factors during early to mid-adulthood in CARDIA, picking up at the age where the NGHS left off (18-30 years). Early menarche continued to be associated with greater adiposity throughout adulthood, as well as with the related conditions of metabolic syndrome, type 2 diabetes, and IFG. Each one year earlier age at

menarche was also associated with more adverse levels of HDL-C, triglycerides, blood pressure, insulin, and glucose before adjustment for BMI. Greater adiposity explained the associations with adverse levels of CVD risk factors, except for metabolic syndrome and IFG, which remained statistically significant for white women even after adjustment for BMI at the baseline visit. Contrary to the findings from the NGHS, some differences by race emerged in the adult cohort. Specifically, the associations of early menarche with accumulation of adiposity and risk for metabolic syndrome were stronger among white compared with African-American women.

The third manuscript addressed the uncertainty about an independent role of early maturation in the risk for the increasingly prevalent cardiometabolic condition of type 2 diabetes, and the potential for unmeasured confounding in the relationship. A Mendelian randomization approach was employed to explore a potential causal association between age at menarche and type 2 diabetes using a genetic risk score (GRS) as an instrumental variable and uconfounded proxy for age at menarche in the ARIC study, which included women who were aged 45-65 years at baseline. This study showed that earlier age at menarche was associated with prevalent type 2 diabetes at the baseline visit, but not at visit 4, when the cohort was approximately a decade older. There was a significant interaction between age at baseline and age at menarche in the odds for type 2 diabetes at visit 4, which might merit further study. The standard and IV regression results supported findings from the Nurse's Health Study that showed

a stronger association of earlier age at menarche with diabetes in younger versus older cohorts. However, given the lack of association at visit 4 and wide confidence intervals for the IV estimates, it remains unclear if earlier age at menarche plays a role in diabetes risk overall during adulthood. From an etiologic viewpoint, more may be learned about the relationship of earlier pubertal development with type 2 diabetes at various points in the life course from a Mendelian randomization study with a larger sample size, perhaps including cohorts of both younger and older women.

Limitations and Strengths

There are number of strengths and limitations in the three studies included in this thesis, and those for the specific studies were outlined in the individual manuscripts. In general, the observational nature of the three studies had certain limitations, such as selection bias, information bias, and residual confounding. We were also unable to establish temporality of some risk factors such as adiposity and insulin levels with the timing of menarche. Furthermore, none of the studies had information available on early life factors or measurements of body composition that preceded the onset of puberty. Therefore, important body composition and lifestyle factors could not be adjusted for prior to puberty.

Another limitation of all of three studies was that misclassification of age at menarche was possible because they all relied on self-reported recall of menarche. However, this misclassification would have been minimal in the NGHS as menarche status was ascertained annually, and in CARDIA

ascertainment took place during early adulthood. Furthermore, even studies that included women of middle age have shown high validity and reliability for self-reported age at menarche.

The strengths of the studies used in this thesis included their prospective design and that each study included a cohort of women from different points in the life course, starting at age 9 years in the NGHS. This provided a life course perspective of the association of age at menarche with various related cardiometabolic risk factors and confounders. The longitudinal nature of the analyses for the NGHS and CARDIA studies allowed examination of the trajectories of CVD risk factors according to menarche timing. Another strength of all three studies was that they included both African-American and white participants from several different communities around the United States, providing some generalizability of the results.

Future Directions

Examining the association between earlier pubertal development and cardiometabolic risk factors is challenging because puberty and body composition are so closely tied together. It is difficult to say what came first in the cohorts included in this thesis, greater adiposity or onset of puberty, without measures of body composition prior to the onset of puberty. Therefore, future studies might include measurements starting in infancy to measure body composition and possibly hormones, insulin, and other early cardiometabolic changes related to changes in the HPO axis to assess when greater adiposity

first develops and at what point during development cardiometabolic changes begin. Furthermore, this thesis included girls only, and so future studies might look at the cardiometabolic consequences of altered pubertal timing among boys. The Mendelian randomization analysis had limited power to detect true associations, and so future studies should include larger sample sizes and possibly outcomes with stronger associations with age at menarche. As age at menarche appears to be more strongly associated with type 2 diabetes in younger cohorts, any future Mendelian randomization study of this association should include a younger age-range than ARIC. One possible future analysis might be a Mendelian randomization study of age at menarche with metabolic syndrome given the moderate association we saw in CARDIA. Another possible future direction might be a clinical trial of lifestyle interventions prior to menarche that follows girls after menarche to see if changes early in life might beneficially delay menarche, subsequent adiposity accumulation, and onset of CVD risk factors.

Conclusions

Considering the results from all three manuscripts, this thesis largely supports the hypothesis that earlier age at menarche is associated with a more adverse cardiometabolic profile over the life course, but that increased adiposity among early maturing girls explains most associations. The only associations that were not explained by adiposity in the manuscripts were with metabolic syndrome and impaired fasting glucose (which is part of metabolic syndrome)

among white women in CARDIA. Given that this clustering of metabolic factors has been shown to have a synergistic effect on CVD risk over and above the risk from each of the individual risk factors included in the metabolic syndrome, this finding points to a potential role of earlier maturation in overall cardiometabolic risk for white women, but not for an important role of early maturation in the individual risk factors.

There were very few race differences in the association of earlier menarche with cardiometabolic outcomes. Earlier age at menarche was more strongly associated with accumulation of adiposity and metabolic syndrome in adulthood among white women despite higher prevalence of obesity and average earlier age at menarche among African-American girls. This finding, along with the general overall weak to null associations for the other outcomes among African-American women, suggest that earlier maturation *per se* is unlikely to be contributing to racial disparities in the prevalence of cardiometabolic conditions such as type 2 diabetes and metabolic syndrome in adulthood. One would have expected associations at least as strong, or stronger, between early menarche and these outcomes among African-American compared with white women if early maturation itself was a factor in racial disparities in cardiometabolic health. Therefore, with regard to cardiometabolic outcomes, focus should probably be on the racial disparities in obesity starting early in life rather than on racial disparities in the timing of puberty. However, early maturation has other negative health consequences, such as greater risk for breast cancer and more risky health

behaviors, and so disparities in menarche timing may still warrant public health concern for other reasons.

Results from the three studies included in this thesis replicate findings from a number of past studies showing that earlier age at menarche is associated with higher adiposity in both adolescence and adulthood. The findings that earlier menarche was associated with greater adiposity accumulation in adulthood specifically among white women, as well as among girls who were normal weight, but not overweight, prior to menarche, provide further insight into the role of pubertal timing in adiposity development over the life course. The propensity for greater adiposity among earlier maturing girls had cardiometabolic consequences beginning in adolescence in the NGHS, including higher blood pressure and insulin levels, and then subsequently greater risk for type 2 diabetes, prediabetes, and metabolic syndrome in adulthood in CARDIA. Furthermore, the greater degree of adiposity earlier in life appears to set earlier maturing women up for adverse levels of blood pressure, HDL-C, triglycerides, insulin, and glucose in adulthood.

In summary, this thesis contributed to the understanding of the influence of maturational timing among women on levels and changes in CVD risk factors with age from adolescence through adulthood, taking into consideration the greater degree of adiposity among earlier maturing women. The findings suggest that puberty might be a sensitive point in the life course for interventions to reduce the burden of obesity and its sequeale among women. Therefore,

obesity-reducing strategies such as improved nutrition and increased physical activity that begin prior to menarche, and possibly also targeted at girls maturing early even if they are normal weight, might be helpful for primordial prevention of CVD disease and its risk factors in adulthood.

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Appendix A. List of 42 SNPs included in the GRS for Manuscript #3

Adapted from reference [35]

							Stage 1 + Replication			
SNP	GRS-29	Nearest gene(s)	Distance from gene (kb)	Chr.	MAF[a]	Alleles[b]	*Beta*[c]	*se*	*Direction*[d]	*p-value*[e]
rs10423674	N	*CRTC1*	intronic	*19*	0.35	A/C	2.3	0.4	+/+	8.70E-09
rs1079866	Y	*INHBA*	~250KB	*7*	0.15	G/C	4.2	0.5	+/+	2.00E-14
rs10899489	N	*GAB2*	intronic	*11*	0.15	A/C	3.2	0.6	+/+	8.40E-09
rs10980926	Y	*ZNF483*	intronic	*9*	0.36	A/G	2.6	0.4	+/+	3.90E-11
rs12472911	Y	*LRP1B*	intronic	*2*	0.20	C/T	2.5	0.5	+/+	1.50E-07
rs12617311	Y	*PLCL1*	~195kb	*2*	0.32	A/G	-3.1	0.4	-/-	1.10E-12
rs13187289	N	*PHF15*	~12kb	*5*	0.20	G/C	3.1	0.5	+/+	4.20E-10
rs1361108	Y	*C6orf173, TRMT11*	~98kb, ~407kb	*6*	0.46	C/T	2.1	0.4	+/+	2.20E-08
rs1364063	Y	*NFAT5*	~10kb	*16*	0.43	C/T	2.1	0.4	+/+	1.80E-08
rs1398217	Y	*FUSSEL18*	intron	*18*	0.43	G/C	-2.6	0.4	-/-	2.90E-12
rs1659127	Y	*MKL2*	~28kb	*16*	0.34	A/G	2.4	0.4	+/+	3.20E-08
rs16938437	Y	*PHF21A*	intronic	*11*	0.09	T/C	-3.8	0.7	-/-	6.20E-08
rs17188434	Y	*NR4A2*	~84kb	*2*	0.07	C/T	-4.7	0.8	-/-	7.30E-10
rs17268785	Y	*CCDC85A*	intronic	*2*	0.17	G/A	3.3	0.5	+/+	1.40E-10
rs1862471	Y	*OLFM2*	intronic	*19*	0.47	G/C	2.2	0.4	-/+	6.80E-08
rs2002675	N	*TRA2B, ETV5*	~4kb, ~135kb	*3*	0.42	G/A	2.3	0.4	+/+	2.80E-09
rs2090409	Y	*TMEM38B*	~400kb	*9*	0.31	A/C	-4.7	0.4	-/-	4.40E-31
rs2243803	Y	*SLC14A2*	~238kb	*18*	0.40	A/T	2.0	0.4	+/+	3.40E-07
rs2687729	Y	*EEFSEC*	intronic	*3*	0.27	G/A	2.3	0.4	+/+	1.30E-07
rs2947411	N	*TMEM18*	~53kb	*2*	0.17	A/G	2.8	0.5	+/+	1.70E-08
rs3743266	Y	*RORA*	3'UTR	*15*	0.32	C/T	-2.0	0.4	-/-	8.00E-07
rs3914188	N	*ECE2*	3'UTR	*3*	0.27	G/C	-2.5	0.4	-/-	9.60E-09
rs466639	N	*RXRG*	intronic	*1*	0.13	T/C	-4.2	0.6	-/-	5.00E-13
rs4840086	N	*PRDM13, MCHR2*	~145kb, ~160 kb	*6*	0.42	G/A	-2.1	0.4	-/-	2.40E-08
rs4929923	N	*TRIM66*	3'UTR	*11*	0.36	T/C	2.3	0.4	+/+	1.20E-08
rs633715	N	*SEC16B*	~44kb	*1*	0.46	T/C	2.7	0.5	+/+	4.70E-08
rs6438424	Y	*3q13.32*	intergenic	*3*	0.50	A/C	-2.7	0.4	-/-	1.40E-13
rs6439371	Y	*TMEM108, NPHP3*	~146kb, ~170kb	*3*	0.66	A/G	-2.3	0.4	-/-	1.30E-08
rs6575793	Y	*BEGAIN*	intronic	*14*	0.42	C/T	2.4	0.4	+/+	5.70E-09
rs6589964	N	*BSX*	~18kb	*11*	0.48	A/C	-2.7	0.4	-/-	1.10E+11
rs6762477	Y	*RBM6*	intronic	*3*	0.44	G/A	2.6	0.5	+/+	2.00E-08
rs7359257	N	*IQCH*	intronic	*15*	0.45	A/C	1.8	0.4	+/-	8.90E-07
rs757647	Y	*KDM3B*	intronic	*5*	0.22	A/G	-2.4	0.5	-/-	8.80E-08
rs7617480	Y	*KLHDC8B*	intronic	*3*	0.22	A/C	2.5	0.4	+/+	2.50E-08
rs7642134	Y	*VGLL3*	~70kb	*3*	0.38	A/G	-2.5	0.4	-/-	3.00E-10
rs7759938	Y	*LIN28B*	~26kb	*6*	0.32	C/T	6.5	0.4	+/+	8.20E-57

rs7821178	Y	*PXMP3*	~181kb	*8*	0.34	A/C	-2.5	0.4	-/-	1.90E-09
rs852069	Y	*PCSK2*	~84kb	*20*	0.37	A/G	-2.2	0.4	-/-	9.30E-09
rs900145	Y	*ARNTL*	~5kb	*11*	0.30	C/T	2.3	0.4	+/+	1.60E-08
rs9555810	Y	*C13orf16, ARHGEF7*	~185kb, ~223KB	*13*	0.28	G/C	2.5	0.4	+/+	1.80E-08
rs9635759	Y	*CA10*	~94kb	*17*	0.32	A/G	2.9	0.4	+/+	1.90E-11
rs9939609	N	*FTO*	intronic	*16*	0.40	A/T	-2.1	0.4	-/+	3.10E-08

Notes:

[a]Minor allele frequency

[b]Minor/Major allele

[c]Per allele change in age at menarche (weeks) obtained from meta analysis of Stage 1 + replication cohorts

[d]Direction of the minor allele association with age at menarche in Stage 1/replication studies

[e]P-value from meta-analysis of Stage 1 + replication cohorts

Appendix B. Abbreviations

ARIC – Atherosclerosis Risk in Communities Study
BioLINCC - Biologic Specimen and Data Repository Information Coordinating Center
BMI – Body mass index
BPA – Bisphenol-A
CARDIA - Coronary Artery Risk Development in Young Adults
CRP - C-reactive protein
CVD – Cardiovascular disease
DBP – Diastolic blood pressure
DONALD – Dortmund Nutritional and Anthropometrical Longitudinally Designed Study
EPIC - European Prospective Investigation of Cancer
FSH - Follicle stimulating hormone
GH – Growth hormone
GnRH - Gonadotropin-releasing hormone
GRS – Genetic risk score
GWAS - Genome-wide association studies
HDL – High-density lipoprotein
HOMA - Homeostasis model assessment of insulin resistance
HPG - Hypothalamic-pituitary-gonadal
HPO - Hypothalamic-pituitary- ovarian
HR – Hazard ratio
IFG – Impaired fasting glucose
IGF - Insulin-like growth factors
IR – Insulin resistance
IV – Instrumental variable
KORA - Cooperative Health Research in the Augsburg Region
LDL – Low-density lipoprotein
LH - Luteinizing hormone
MAF – Minor allele frequency
NCEP: ATP III – National Cholesterol Education Program's Adult Treatment Panel III
NCS – National Children's Study
NGHS – National Growth and Health Survey
NHANES – National Health and Nutrition Examination Survey
NHLBI – National Heart, Lung, and Blood Institute
NHS- Nurses Health Study
OR – Odds ratio
PBDE - Polybrominated diphenyl ethers
PCBs – Polychlorinated biphenyls
SD – Standard deviation
SE – Standard error
SES – Socioeconomic status
SBP – Systolic blood pressure
SNP - Single nucleotide polymorphisms
SSB – Sugar sweetened beverages
SSF – Sum of skin folds
TG - Triglycerides
2SLS – 2-stage least squares