

**Mediation of the effects of obesity on coronary heart disease in a bi-ethnic cohort:  
the Atherosclerosis Risk in Communities (ARIC) Study**

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## **ABSTRACT**

Jill McClain: Mediation of the effects of obesity on coronary heart disease in a bi-ethnic cohort: the Atherosclerosis Risk in Communities (ARIC) Study  
(Under the direction of June Stevens, PhD)

Excess adiposity is associated with physiologic risk factors for coronary heart disease (CHD), including dyslipidemia, high blood pressure and insulin resistance, however few studies have analyzed mediation of the obesity-CHD relationship by individual physiologic risk factors. Mediation can be examined using structural equation modeling (SEM), which has several advantages over standard regression for analyses of mediation, including significance-testing of the individual indirect (mediated) effects. We used data from the Atherosclerosis Risk in Communities (ARIC) Study, a longitudinal cohort of 15,792 African American and White adults aged 45-64 years at baseline (1987-1989) and followed through 2005, to examine mediation of the effects of body mass index (BMI) on CHD hazard. Physiologic risk factors examined included total and high density lipoprotein (HDL) cholesterol, triglycerides, systolic and diastolic blood pressure (SBP, DBP) and insulin resistance. The overall effect of BMI on CHD was curved, with a steeper slope at lower compared to higher BMI levels. Mediated effects of BMI on CHD tended to be strongest through HDL cholesterol and insulin resistance. Mediation was more moderate through total cholesterol and SBP, and was null through DBP and triglycerides. In models that included all 6 hypothesized mediators, BMI was not directly associated with CHD. We found no differences in mediation by race or gender. Because antihypertensive medication use is highly prevalent in this cohort (31% at baseline), we explored the impact of medication use

on our analyses involving blood pressure. The associations of BMI with blood pressure and with CHD were markedly attenuated in participants taking antihypertensive medication compared to participants not taking antihypertensive medications, though the association of blood pressure with CHD was not different between the two groups. When associations of BMI with SBP and DBP were compared in the same individuals before and after initiating treatment with antihypertensive medications, no differences were found. Overall, these results highlight the complexity of analyses of blood pressure in persons being treated with antihypertensive medication and underscore the importance of traditional physiologic risk factors, particularly HDL cholesterol and insulin resistance, as mediators of the effect of obesity on CHD.

Dedicated to the memory of my maternal grandparents,  
Dr. Charles Colvin Nimmo and Elizabeth Moore Nimmo

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## LIST OF ABBREVIATIONS

ARIC: The Atherosclerosis Risk in Communities Study

ATP III: Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)

BMI: Body mass index ( $\text{kg}/\text{m}^2$ )

BP: Blood pressure

CHD: Coronary heart disease

CVD: Cardiovascular disease

DBP: Diastolic blood pressure

ECG: Electrocardiogram

HDL: High density lipoprotein cholesterol

HR: Hazard ratio

IR: Insulin resistance

LDL: Low density lipoprotein cholesterol

JNC 7: The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure

MI: Myocardial infarction

mmHg: millimeters of mercury

NCEP: National Cholesterol Education Program

NHANES: The National Health and Nutrition Examination Survey

R: Reliability coefficient

RR: Risk ratio

SBP: Systolic blood pressure

SEM: Structural equation modeling

SES: Socio-economic status

SFR: Skinfold ratio (subscapular to triceps)

TC: Total cholesterol

TG: Triglycerides

UNC: University of North Carolina, Chapel Hill

VLDL: Very low density lipoprotein cholesterol

WC: Waist circumference

WHR: Waist-to-hip ratio

## LIST OF SYMBOLS

$x$  = observed exogenous variable

$y$  = observed endogenous variable

$\beta$  = path coefficient for association of an endogenous variable with another endogenous variable

$\gamma$  = path coefficient for association of an endogenous variable with an exogenous variable

$\Delta$  = change

$\delta$  = disturbance term

$\varepsilon$  = observed error term

$\zeta$  = latent error term

$\eta$  = latent endogenous variable

$\lambda$  = factor loading

$\xi$  = latent exogenous variable

$\varphi$  = covariance of exogenous variables



## **I. INTRODUCTION**

### **A. Background**

Excess adiposity is associated with physiologic risk factors for coronary heart disease (CHD), including high blood pressure, elevated glucose and dyslipidemia. Although many studies have examined the relationship between obesity and these risk factors, and between these risk factors and CHD events, few have analyzed mediation of the obesity-CHD relationship by individual risk factors, and even fewer have compared mediation across risk factors. The lack of studies comparing risk factors as mediators is due, in part, to the scarcity of appropriate data on both measured metabolic mediators and CHD outcomes and, in part, to the limitations of traditional epidemiologic methods for this purpose. Mediation is commonly analyzed in other fields using structural equation modeling (SEM). SEM has several advantages over standard regression for analyses of mediation, including the specification of each path (and importantly, each non-path) among all variables in a given model and the ability to explicitly model the measurement error of variables. In particular, SEM permits significance-testing of the indirect (mediated) effect.

We know of no studies that have assessed racial and gender differences in the mediation of CHD. Racial and gender differences have been found in the prevalences of obesity, CHD risk factors and CHD outcomes, as well as in the associations among these variables, but it is not clear whether risk factors mediate the adiposity-CHD relationship to the same degree across race and gender. For example, African American women have higher incidence of CHD than white women, as well as a higher average body mass index (BMI)

and higher blood pressure, but is the higher incidence of CHD due to the effects of increased obesity on blood pressure, or are these health issues less associated with each other in African American women?

A comparison of effects of obesity on CHD through blood pressure, glucose and lipids must consider how to incorporate medication use. This methodological issue is especially important for blood pressure medication because of the high prevalence of antihypertensive medication use among middle-aged Americans. Hypertension in obese individuals is known to be more resistant to antihypertensive treatment than in normal weight individuals (Pi-Sunyer 2007), but it is not known if the relationship between BMI and blood pressure in persons taking antihypertensive medication is similar to that in persons not taking medication. This issue is not only of methodological value, but of substantive interest as well. Therefore we will explore this topic in order to inform our primary analyses and to better understand the impact of medications.

## **B. Research aims**

The aims of this research were as follows.

### **1. Primary Aim – Examine and compare the role of physiologic risk factors as mediators of the relationship between body mass index and CHD in a bi-ethnic cohort**

The physiologic risk factors examined were: total and high-density lipoprotein (HDL) cholesterol, triglycerides, systolic and diastolic blood pressure, and insulin resistance.

1.a. Examine and compare physiologic risk factors individually as mediators of the effect of obesity on CHD

1.b. Examine and compare physiologic risk factors in combination as mediators of the effect of obesity on CHD

1.c. Determine if mediation of the obesity-CHD relationship differs by race and/or gender

**2. Secondary Aim – Investigate the impact of antihypertensive medication use on relationships of BMI with blood pressure and CHD**

2.a. Determine if antihypertensive medications modify the cross-sectional associations between obesity and blood pressure

2.b. Explore how antihypertensive medications affect mediation of the obesity-CHD relationship by blood pressure

Analyses used data from the Atherosclerosis Risk in Communities (ARIC) Study, a cohort of African American and White adults aged 45-64 at baseline (1987-1989). Follow-up data on fatal and non-fatal CHD events and procedures were available through 2005. The cohort at baseline comprised 15,792 subjects. To our knowledge, this is the first study to use structural equation modeling to examine physiologic risk factors as mediators of the adiposity-CHD relationship, as well as the first to systematically examine differences in mediation across race-gender groups.

## **II. LITERATURE REVIEW**

### **A. Obesity and the risk of coronary heart disease**

#### **1. *Obesity***

The prevalence of overweight and obesity (BMI  $\geq 25$  kg/m<sup>2</sup>) together (hereafter “overweight”) among U.S. adults was 66% as of 2003-2004, with 32% obese (BMI  $\geq 30$  kg/m<sup>2</sup>) and 4.8% extremely obese (BMI  $\geq 40$  kg/m<sup>2</sup>). Among non-hispanic African American adults, overweight was 76% and obesity was 45%. All of these figures represent increases from 1999-2000 (Ogden, Carroll et al. 2006).

Both general adiposity, usually measured by BMI, and body composition, measured in various ways, have been shown to adversely affect health (Pi-Sunyer 1993; Stevens 1995; Harris and Stevens 1998; Okosun, Liao et al. 2000; Kenchaiah, Evans et al. 2002; Bigaard, Tjonneland et al. 2003; Okura, Nakata et al. 2004; de Koning, Merchant et al. 2007; Janiszewski, Janssen et al. 2007). Although relative risks associated with obesity are usually modest, population attributable risks are often dramatic due to the extremely high prevalence of this disorder. Recent estimates of the deaths attributable to obesity in the U.S. range from 112,000 to 414,000 per annum (Mokdad, Marks et al. 2004; Flegal, Graubard et al. 2005; Mokdad, Marks et al. 2005).

#### **2. *Coronary heart disease***

In 2004, about 16 million U.S. adults (7.3% of the population) had prevalent Coronary heart disease (CHD). There were 865,000 incident (new and recurrent) myocardial

infarctions (MI) in the U.S. CHD is the largest single cause of death in the U.S., with 452,327 deaths in 2004 (one of every 5 deaths) (AHA 2007).

### **3. *Obesity's association with CHD***

Obesity has been shown in prospective studies to increase the risk of CHD events (Manson, Colditz et al. 1990; Pi-Sunyer 1993; Jousilahti, Tuomilehto et al. 1996; Kannel, Wilson et al. 2002; Kenchaiah, Evans et al. 2002; Bigaard, Tjonneland et al. 2003; Batty, Shipley et al. 2006; Kim, Meade et al. 2006; Mann, Lee et al. 2006). In a large prospective Danish study, BMI predicted CHD events for a given waist circumference (Bigaard, Tjonneland et al. 2003). BMI predicts CHD in the ARIC study (Folsom, Stevens et al. 1998).

### **4. *Physiologic risk factors for CHD***

The effects of overweight on CHD are understood to occur at least partly through several physiologic risk factors, especially dyslipidemia, elevated blood sugar and high blood pressure. The prevalences of obesity and selected CHD risk factors in the U.S. over the last 45 years are shown in Figure 2.1.

#### **a. Definitions and prevalence**

##### *Hypertension*

Hypertension is defined by the 7<sup>th</sup> report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) as a systolic blood pressure (SBP) of 140 mmHg or higher or a diastolic blood pressure (DBP) of 90 mmHg or higher (see Table 2.1). An SBP of 120-139 mmHg or a DBP of 80-89 mmHg is considered pre-hypertension. Thirty percent of U.S. adults 20 years and over had high blood

pressure or were on antihypertensive medication in 1999-2002; another 28% of U.S. adults are pre-hypertensive (Centers for Disease Control 2007).

Although hypertension prevalence had declined in the U.S. from the 1970s to the 1990s, it appears to be on the rise again (Figure 2.2, above) (Hajjar, Kotchen et al. 2006; Ong, Cheung et al. 2007). Antihypertensive medication use has risen markedly in the last decade, especially among overweight and obese individuals (Figure 2.2).

### *Dyslipidemia*

Dyslipidemia is defined by 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 (ATP III) as high total ( $\geq 240$  mg/dL) or LDL cholesterol ( $\geq 160$  mg/dL), low HDL cholesterol ( $< 40$  mg/dL in men;  $< 50$  mg/dL in women), and/or high triglycerides (TG) ( $\geq 200$  mg/dL) (NCEP Expert Panel 2002). There were 36.6 million U.S. adults (17% of the population) with total cholesterol over 240 mg/dL and over 100 million (48%) with total cholesterol over 200 mg/dL in 2004 (Rosamond, Flegal et al. 2007). The prevalence of high LDL cholesterol was 13%, but only 29% had LDL in the optimal range ( $< 100$  mg/dL) (Hyre, Muntner et al. 2007), and 17% of adults had HDL  $< 40$  mg/dL (Rosamond, Flegal et al. 2007). Geometric mean triglycerides rose from 114 mg/dL at the 1976-80 U.S. National Health and Nutrition Examination Survey (NHANES) survey to 122 mg/dL at the 1999-2002 survey (Carroll, Lacher et al. 2005). About 24% of men and 16% of women age  $\geq 40$  had TG  $\geq 200$  mg/dL in 1999-2002 (Kim, Alley et al. 2006).

### *Insulin resistance and diabetes mellitus*

Diabetes Mellitus occurs when pancreatic beta cells cannot provide sufficient insulin to control blood sugar. It is defined as a fasting blood glucose  $\geq 126$  mg/dL or a non-fasting glucose  $\geq 200$  mg/dL. Fifteen million U.S. adults (7.1%) had physician-diagnosed diabetes in 2004, and 5 million (2.4%) were estimated to have undiagnosed diabetes. Another 56.5 million (27.6%) were estimated to have pre-diabetes (fasting glucose  $\geq 110$  mg/dL). Type 2 diabetes is preceded by insulin resistance, which occurs when body cells become less sensitive to insulin's effects. Initially, glucose levels do not change because insulin rises sufficiently to keep glucose within a normal range. Increasing insulin resistance of the cells and eventual decrease in pancreatic function result in frank diabetes. Elevated insulin resistance therefore precedes elevated glucose in the onset of diabetes and can be a useful tool in assessing cardiovascular (CVD) risk. Insulin resistance is measured by means of the hyperglycemic euglycemic clamp (DeFronzo, Tobin et al. 1979), but can be approximated by the homeostasis model assessment, which multiplies glucose by insulin and divides by a constant (Wallace, Levy et al. 2004).

#### **5. *Body mass index predicts physiologic risk factors***

Although absolute levels of some physiologic risk factors, including cholesterol and blood pressure, have fluctuated over the past several decades, overweight and obese persons continue to have higher levels of these factors than non-overweight persons, especially diabetes and hypertension. The odds of hypertension in NHANES 2003-04 were 1.7 (1.2-2.5) for overweight (compared to normal weight) and 3.4 (2.5-4.6) for obesity, after adjustment for age, sex, race and education. The US Behavioral Risk Factor Surveillance System found an approximately 3.5-fold risk for diabetes in individuals with a BMI of

30 - < 40 kg/m<sup>2</sup> compared to normal weight and an approximately 7-fold risk in those with a BMI ≥ 40 kg/m<sup>2</sup>, with similar increased risks for hypertension; the increased risk of high cholesterol was about 1.9 in both obese groups (Mokdad, Ford et al. 2003). In a population-based sample of Canadians, BMI was associated with blood pressure, blood lipids and diabetes mellitus (Ledoux, Lambert et al. 1997). In an analysis from NHANES III, BMI was significantly correlated with serum lipids, glucose and blood pressure, and predicted odds of having one or more CVD risk factors in all race-gender groups (Zhu, Heshka et al. 2004).

## **6. *Physiologic risk factors predict CVD***

### **a. Hypertension**

There is no doubt that high blood pressure is a causal contributor to, as well as an indicator of, atherosclerosis. High blood pressure is a significant risk factor for stroke, coronary heart disease events, and cardiovascular and total mortality, independent of other risk factors (Lloyd-Jones 2007). Treatment of high blood pressure has been shown to reduce the incidence of all of these (Turnbull 2007). High blood pressure may directly damage the heart, making it less able to respond to increased demand (Labarthe 1998). Further, atherosclerosis and insulin resistance, which are independently associated with obesity, can contribute to hypertension (Pi-Sunyer 2007). In ARIC, even normal and high normal blood pressure levels are associated with increased CVD incidence, especially among obese individuals (Kshirsagar, Carpenter et al. 2006). Lowering blood pressure with antihypertensive medication reduces CVD risk (Collins 1994).

### **b. Dyslipidemia**

LDL-cholesterol is one of the better understood risk factors for CVD. Low-density lipoproteins carry cholesterol through the endothelial cells and into the intimal layer of the



arterial wall. Within the intima, byproducts of the oxidation of LDL damage endothelial cells and convert macrophages into foam cells, causing additional damage and precipitating formation of atherosclerotic plaques (Labarthe 1998). There is abundant literature associating LDL-cholesterol levels with increased risk of CVD and mortality, and elevated LDL-cholesterol is the primary target of the NCEP ATP III (NCEP 2001; Grundy, Cleeman et al. 2004). Gene-induced reductions in LDL lead to substantially lower risk of CHD (Cohen, Boerwinkle et al. 2006).

High-density lipoproteins carry cholesterol out of the intimal layer of the arterial wall, counterbalancing the effects of low-density lipoproteins (Labarthe 1998). As with high LDL-cholesterol, low HDL-cholesterol has been well documented as an independent risk factor for CVD in adults. The NCEP ATP III identifies HDL cholesterol below 40 mg/dL as a risk factor for CHD, and levels at or above 60 mg/dL as a “negative” risk factor (i.e., high HDL removes one other risk factor from an individual’s risk count) (NCEP 2001).

Triglycerides are mostly transported in the body as part of very-low-density lipoproteins. Triglyceride level has been shown to be an independent predictor of risk of myocardial infarction in men (Stampfer, Krauss et al. 1996), of accelerated atherogenesis and cardiovascular mortality in women (Tanko, Bagger et al. 2005), and of CVD in both genders (Austin 1997). The mechanism for the independent contribution of triglycerides to CVD is not fully understood. One possible mechanism is through direct accumulation in the vessel wall (Forrester 2001).

Total cholesterol is the sum of LDL, HDL and very-low-density lipoproteins (VLDL). LDL and VLDL are usually not measured directly, but VLDL can be estimated as one fifth triglycerides and LDL can then be calculated from total, HDL and estimated VLDL.

Despite being a combination of both “good” and “bad” cholesterol, total cholesterol predicts heart disease and is part of the Framingham risk score (D'Agostino, Vasan et al. 2008).

In the ARIC study, total and HDL cholesterol and triglycerides (in women) predict incident CHD (Sharrett, Ballantyne et al. 2001; Chambless, Folsom et al. 2003; McNeill, Rosamond et al. 2005).

c. Insulin resistance and diabetes

Diabetes and its precursors – glucose intolerance and hyperinsulinemia – contribute to cardiovascular disease. In addition, hyperinsulinemia, (present in type 2 diabetes as long as the pancreatic beta cells continue functioning reasonably well) affects atherogenesis directly due to effects on synthesis of lipids and connective tissue, growth and persistence of lipid lesions, and on sterol and LDL-cholesterol activity, and indirectly through effects on blood lipids and blood pressure. Mortality due to diabetes was 72.8 thousand in 2004 (Rosamond, Flegal et al. 2007).

Diabetes is associated with incident CHD in ARIC. The association is lower in African Americans, but because of the higher prevalence of diabetes in that population, the percent of CHD cases attributable to diabetes was higher (Folsom, Szklo et al. 1997).

Among diabetics in ARIC, poor glycemic control is associated with severe peripheral arterial disease (Selvin, Wattanakit et al. 2006).

**7. *Obesity, physiologic risk factors and CHD in ARIC***

Numerous studies of predictors of CHD have been published using data from the ARIC study. BMI is associated with CHD (Folsom, Stevens et al. 1998), as are diabetes (Folsom, Szklo et al. 1997), LDL, HDL and total cholesterol, triglycerides (Sharrett, Ballantyne et al. 2001; McNeill, Rosamond et al. 2005) and blood pressure (Chambless,

Folsom et al. 2003; Kshirsagar, Carpenter et al. 2006). The association with diabetes was weaker in African Americans, but because of the higher prevalence of diabetes in that population, the percent of CHD attributable to diabetes was higher.

#### **8. *Obesity, physiologic risk factors and CHD outcomes vary by race***

African Americans have a shorter life expectancy than Whites and suffer higher rates of many chronic diseases (Kochanek and Smith 2004; NCHS 2004; CDC 2005). Racial differences have been found in the prevalences of obesity (Li, Ford et al. 2007; Ogden 2007), CHD risk factors (Winkleby, Kraemer et al. 1998; Sundquist, Winkleby et al. 2001) and CHD outcomes (Jones, Chambless et al. 2002; American Heart Association 2008), as well as in the associations among these variables (Despres, Couillard et al. 2000; Harris, Stevens et al. 2000; Jones, Chambless et al. 2002; Carnethon, Lynch et al. 2006; Shai, Jiang et al. 2006; Abell, Egan et al. 2008).

Differences in body composition between White and African American populations have long been noted (Merz, Trotter et al. 1956; Wagner and Heyward 2000). Both the Charleston Heart Study (Stevens, Plankey et al. 1994) and the ARIC study (Duncan, Chambless et al. 1995) have shown that, at the same BMI, African Americans had smaller abdominal circumferences than Whites. Using computed tomography, Conway et al. found that obese African American women had 23% less visceral fat than obese White women with similar WHR measurements (Conway, Yanovski et al. 1995).

Further, there is evidence to indicate that the metabolic consequences of fat distribution may differ by ethnicity. Ethnic differences have been noted in the association of visceral adipose tissue or waist-to-hip ratio (WHR) with blood pressure, plasma lipids and glucose and insulin kinetics (Dowling, Fried et al. 1991; Conway, Yanovski et al. 1995).

Generally, the associations between the index of fat distribution and the outcome were weaker in African Americans compared to Whites.

There may also be differences in effects of treatment; among African Americans and Whites taking antihypertensive medications, African Americans have poorer blood pressure control despite being on more classes of medications (Safford, Halanych et al. 2007; Ostchega 2008). Mortality rates from CHD are much higher in African Americans than in Whites (Clark, Ferdinand et al. 2001).

Despite the many known differences, little is known about the interplay among obesity, physiologic risk factors and incident CHD. There is some evidence that the relationships may differ by race. For example, diabetes is more prevalent in African Americans than in Whites, but it is less predictive of CHD (Folsom, Szklo et al. 1997). Further, since the traditional physiologic risk factors were first identified in a largely White cohort (Framingham), it may be that these risk factors are not as relevant in other groups as in Whites. Unfortunately, few longitudinal studies with hard CVD outcomes have included sufficient African Americans for racial comparisons.

## **B. Effects of antihypertensive medication on analyses of blood pressure**

Medication use can present a methodological problem for analyses involving continuous blood pressure because the unmedicated blood pressure value is unobservable. When blood pressure is the outcome, the underlying unobserved value is likely to be the value of interest, and the reduced observed value in unmedicated individuals can attenuate the observed effects of risk factors in a population-based sample. Common approaches to this problem are adjustment for medication use as a covariate or exclusion of medicated subjects from the analysis. Exclusion will reduce power and generalizability and has the

potential to cause substantial bias because of the high prevalence of antihypertensive medication use (18% of all U.S. adults (Cutler, Sorlie et al. 2008) and 45% of those aged 60 and over (Ostchega, Dillon et al. 2007) (author's calculations from tables in cited references)). Statistical adjustment is problematic because, although medication use lowers blood pressure at the individual level, it may be correlated with increased blood pressure in a community sample. In this case, a medication use variable functions as an indicator of hypertension rather than reflecting effects of medication on blood pressure, and adjustment for the medication use variable will not correctly account for confounding (Tobin, Sheehan et al. 2005). Tobin et al. used simulated data to compare a variety of methods for handling treatment effects on blood pressure as an outcome variable (Tobin, Sheehan et al. 2005). They showed that some methods for imputing the underlying untreated value of blood pressure result in very little loss in power or bias.

When blood pressure is the exposure variable, handling of treatment depends on whether the analyst is interested in the blood pressure experienced at the time of measurement, or in some proxy of adult lifetime blood pressure. In the former case, treatment can be ignored; for the latter, a value in between the unmeasured underlying value and the measured treated value might be preferred, though such a value will be difficult to determine. Lowering blood pressure with antihypertensive medication reduces CVD risk (Collins 1994), but the treated individual is likely still at higher risk due to historical exposure to higher blood pressure, and medications may not reduce average daily blood pressure to the same degree that blood pressure measured at the physician's office is reduced (Chau, Bauduceau et al. 1992).

Since almost a third of ARIC participants were taking antihypertensive medication at baseline, effects of medication use on observed associations could be substantial for the analyses proposed here. Many factors influence the effect of treatment (Chobanian, Bakris et al. 2003), including body mass index (Cushman, Ford et al. 2002; Pi-Sunyer 2007). It will be important to understand the effects of treatment in this cohort and the association of treatment with other variables in order to determine the best methodology for handling treatment in these analyses.

### **C. Analyses of mediation of the adiposity-CVD relationship**

#### **1. Overview**

A number of cohort studies have examined mediation of the relationship between CVD and weight-related variables (Table 2.3). The majority of such studies assessed mediation simply by controlling for the hypothesized mediators in a regression model and then reporting the effect of the weight/adiposity variable with and without the mediator(s). Most papers focused on whether overweight was still significant after potential mediators were added, rather than on assessing mediation itself. In many cases mediators were added as a block, though some added mediators individually. None compared mediation in African Americans versus Whites.

The adiposity exposure variable was usually BMI. Other adiposity measures examined were waist circumference, skinfolds, waist-to-hip and skinfold ratios, and change in weight. Mediators examined were blood lipids (total, HDL and LDL cholesterol, Lipoprotein(a), triglycerides), blood pressure (SBP, DBP and hypertension status), glucose, diabetic status, and inflammatory markers (C-reactive protein, von Willebrand factor, fibrinogen, factor VII). In most studies, the overall relationship between the adiposity

variable and the outcome was positive (linear, U or J-shaped), as expected. In a few cases, the relationship was inverse, but these were in sick or older cohorts.

The majority of the papers found that the mediators assessed attenuated but did not eliminate the relationship between overweight and the outcome. A meta-analysis of 21 cohort studies (Bogers, Bemelmans et al. 2007) found a relative risk (RR) for a 5-unit change in BMI of 1.27 (95% CI: 1.21, 1.33) without and 1.15 (95% CI: 1.11, 1.19) with BP and cholesterol in the model. A minority found that the mediators eliminated the association between overweight and the outcome (this often represented a similar absolute effect on the numbers as in the studies where the effect was not eliminated, but with a wider confidence interval). Rarely, the mediators had no attenuating effect. Since mediators were generally added as a block or successively, it is difficult to conclude from these studies which were more important. There were six papers that tested the mediators individually. Of these, four had CHD as the outcome. These four papers are described in detail below.

## **2. *Papers assessing mediation of the adiposity-CHD relationship***

Manson et al (Manson, Colditz et al. 1990) used data from the Nurses' Health Study to assess mediation of the adiposity-CHD relationship. The mediators they examined were a "history of hypertension, diabetes and hypercholesterolemia". BMI was positively associated with nonfatal MI and fatal CHD, though RRs became significant only above a BMI of 25 kg/m<sup>2</sup>. A history of hypercholesterolemia slightly attenuated the relationship for BMIs  $\geq$  25 kg/m<sup>2</sup>. This study differs slightly from most others in that weight, height and history of the mediators were self-reported rather than measured. Kim et al assessed BMI and various skinfold measures as predictors of fatal CHD in the Northwick Park Heart Study (Kim, Meade et al. 2006). In separate models for each exposure (BMI and forearm, triceps,

subscapular and suprailiac skinfolds), they successively added age, smoking, total cholesterol, SBP, fibrinogen and factor VII. The addition of cholesterol to a model with age and smoking strengthened rather than attenuated the RR for the exposure. The further additions of SBP, fibrinogen and factor VII attenuated, strengthened and attenuated the RR, respectively. Jousilahti et al examined the relationship between BMI and CHD mortality in Finnish men and women aged 30-59 (Jousilahti, Tuomilehto et al. 1996). While total cholesterol was significant in a model with BMI, the addition of cholesterol did not attenuate the crude relationship between BMI and CHD mortality. The addition of blood pressure did attenuate (and inconsistently eliminated, depending on gender and age group) the RR for BMI (in models with and without smoking and cholesterol).

Tanne et al assessed hazard ratios for death from CHD and stroke in male Israeli civil servants (Tanne, Medalie et al. 2005). Exposures were BMI, subscapular skinfolds and the skinfold ratio (SFR - subscapular to triceps). Their models adjusted for A) age, B) age + SBP, and C) age, SBP, diabetes, smoking and SES. The addition of SBP attenuated and eliminated the RRs for each of the main effects on both outcomes, except for the effect of BMI on CHD. The addition of diabetes, smoking and SES (model C) resulted in little change from the model with age and SBP.

#### **D. Preliminary studies**

In an analysis of mediation, principles of causal inference dictate that the exposure should occur prior to the mediator, but an interval of several years between the measurement of exposure and mediator may be too long if the mediator responds quickly to the exposure. Many CHD risk factors respond relatively rapidly to weight change (days or weeks), but the average interval between examinations in the ARIC study is approximately 3 years. To

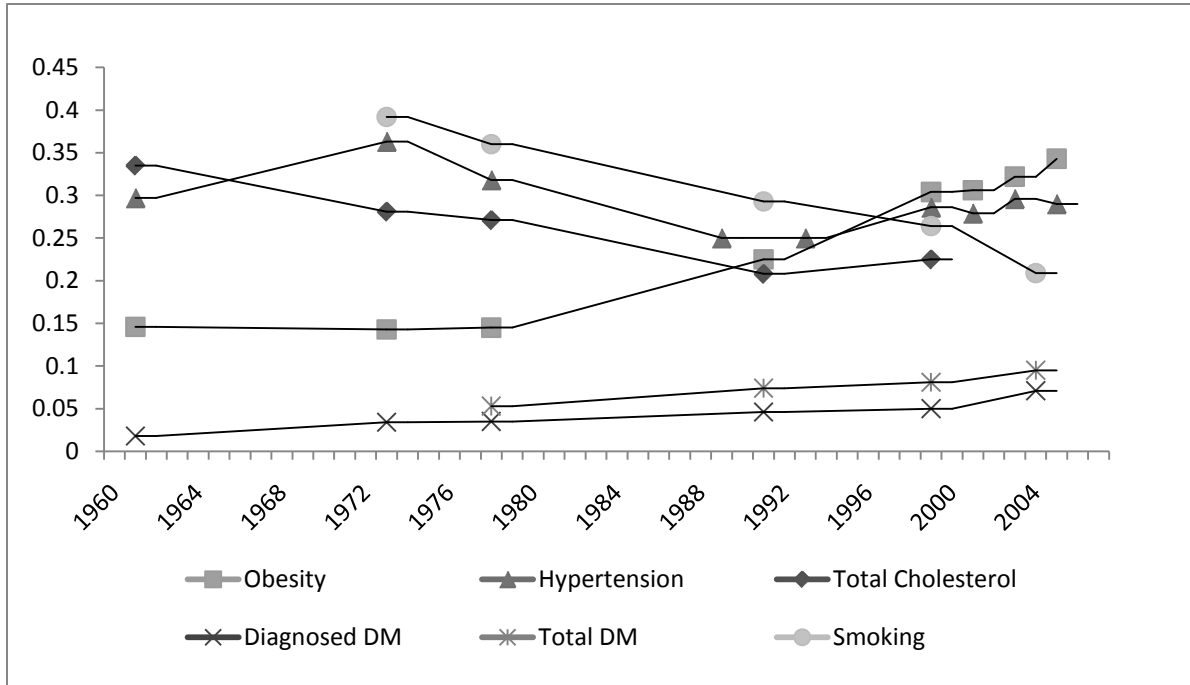


inform our decision about whether to use physiologic risk factors measured at the same or the subsequent study examination, we compared the strength of association of two anthropometric measures – BMI and waist circumference – with systolic and diastolic blood pressure, serum glucose, total, HDL and LDL cholesterol, and triglycerides (log-transformed) in cross-section and longitudinally (3-year lagged).

We created a “stacked” ARIC dataset with 27,623 observations. We ran linear mixed models using BMI or waist as the predictor, adjusting for age, sex, race, field center, education, relevant medications, smoking, and drinking. We used Wald tests to test differences between the cross-sectional and longitudinal analyses.

BMI and waist were significantly associated with all of the risk factors, except for total cholesterol in the longitudinal analysis (Table 2.4). Though differences were not always statistically significant, the associations of BMI and waist with risk factors measured in cross-section were generally larger than longitudinally. This suggests that the association between obesity and the studied risk factors may be reduced when examined across a lag time of several years. In the mediation analyses, therefore, we used risk factors measured at the same time as adiposity. We recognize that this approach has some weaknesses. For instance, associations might be more subject to reverse causation when cross sectional data are used. Nevertheless, given the available data and our knowledge of the variables, we think that contemporaneous risk factor levels are a better indicator of the effect of obesity than is the level of the risk factor 3 years later.

**Figure 2.1: Prevalence of selected CHD risk factors in the U.S. population from 1960-2006**

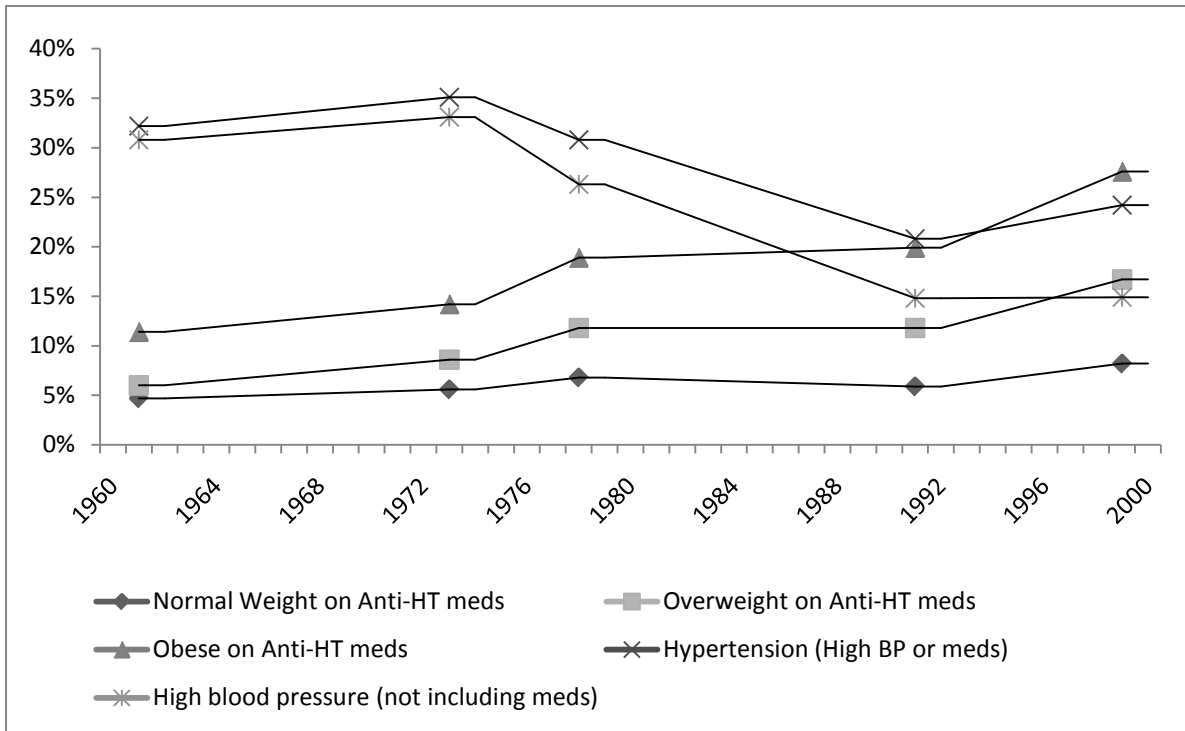


**Table 2.1. JNC 7 definitions and US prevalence of high blood pressure**

<b>JNC 7 definitions</b>	<b>SBP (mmHg)</b>		<b>DBP (mmHg)</b>	<b>U.S. Prevalence in 1999-2002</b>
Normal blood pressure	<120	and	<80	42%
Pre-hypertension	120-139	or	80-89	28%
Stage 1 Hypertension	140-159	or	90-99	30%*
Stage 2 Hypertension	≥160	or	≥100	

\*Had high blood pressure or were on medication for high blood pressure.

**Figure 2.2: Prevalence of antihypertensive medication use (by weight status), hypertension and high blood pressure in U.S. National Health Examination Surveys from 1960-2000**

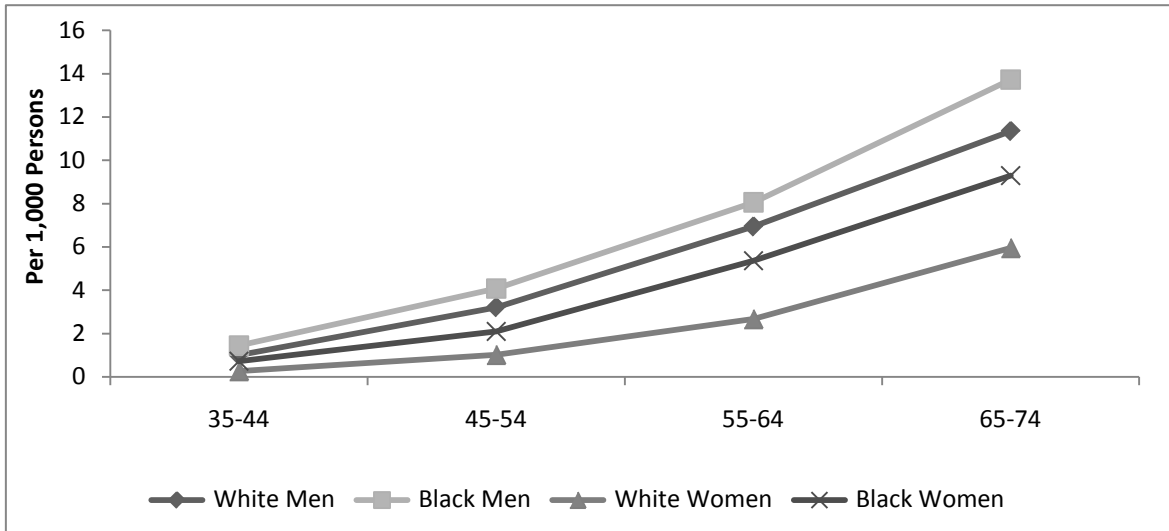


**Table 2.2. Prevalences of selected characteristics in the U.S. by race and gender (highest value in bold)\***

	Women		Men		Source
	African American	White	African American	White	
Abdominal obesity (WC >102cm in men or >88cm in women)	<b>76%</b>	58%	35%	45%	NHANES 2003-04 (Li, Ford et al. 2007)
Overweight or obese (BMI $\geq$ 25 kg/m <sup>2</sup> )	<b>80%</b>	58%	67%	71%	AHA Heart Disease and Stroke Statistics – 2008 Update (data from 2005)
Obesity (BMI $\geq$ 30 kg/m <sup>2</sup> )	<b>51%</b>	31%	31%	30%	
Diabetes	<b>13%</b>	5.6%	11%	6.7%	
Hypertension	<b>47%</b>	32%	43%	33%	
Total cholesterol $\geq$ 240 mg/dL	13%	<b>18%</b>	14%	16%	
LDL $\geq$ 130 mg/dL	30%	<b>34%</b>	32%	32%	
HDL < 40 mg/dL	6.9%	8.8%	16%	<b>26%</b>	
Prevalent CHD	7.8%	6.0%	7.1%	<b>9.4%</b>	

\*Bold is to highlight the differences across groups; it does not represent a significance test.

**Figure 2.3. Annual rate of first heart attack by age, sex and race (ARIC :1987-2004).**



Source: American Heart Association Heart Disease and Stroke Statistics 2008.

**Table 2.3. Cohort studies that examined mediation of the adiposity-CVD relationship**

Citation	Cohort	Method of testing mediation	Exposures	Mediators	Outcomes	Conclusions / comments
(Batty, Shipley et al. 2006)	Whitehall Study (London-based male govt employees)	Control for all mediators at once	• BMI	• Cholesterol • BP • glucose • diabetes	Mortality: • CHD • Stroke • CVD • Non-CVD	Depending on mortality type, attenuate but may or may not eliminate effect.
(Bogers, Bemelmans et al. 2007)	Meta-analysis of 21 cohort studies	Control for all mediators at once	• BMI	• Total cholesterol • SBP	CHD	Attenuated but did not eliminate the association of BMI with CHD.
(Dyer, Stamler et al. 2004)	Chicago Heart Assoc Detection Project in Industry Study	Control for all mediators at once (though not testing as mediators)	• BMI	• SBP • total cholesterol • diabetes	• CVD mort	Attenuate but don't eliminate, but mediators not tested/shown individually.
(Hu, Tuomilehto et al. 2005)	Finnish	Control	• BMI	• SBP • Cholesterol • diabetes	Mortality: • CVD • Cancer • All-cause	SBP, cholesterol and diabetes together attenuate but do not eliminate the effect of BMI on total and CVD mort.
(Huang, Rodreiguez et al. 1997)	3741 Japanese-American men from the Honolulu Heart Program - 71-93 y of age	Control. Added individually/successively.	• BMI • WHR • waist	• Fasting glucose • HDL	CHD	BMI & waist persist after adjustment for glucose but not after HDL. Association of BMI with CHD was not independent of abdominal adiposity. WHR & waist circumference were significant after adjustment for BMI.

Citation	Cohort	Method of testing mediation	Exposures	Mediators	Outcomes	Conclusions / comments
(Jousilahti, Tuomilehto et al. 1996)	Finnish	Control individually, but treat smoking individually also, so individual models do not control for smoking.	<ul style="list-style-type: none"> <li>• BMI</li> </ul>	<ul style="list-style-type: none"> <li>• Cholesterol</li> <li>• SBP</li> </ul>	CHD mortality	Men: Both attenuate but do not eliminate. Women: Cholesterol attenuates but does not eliminate. SBP attenuates and eliminates. Stratified by age: BMI effects were stronger in older men and in younger women.
(Kanaya, Vittinghoff et al. 2003)	Heart and Estrogen/progestin Replacement Study (HERS) trial & HERS II cohort	Time-dependent covariates in Cox model (using most recent value for the covariate).	<ul style="list-style-type: none"> <li>• BMI</li> <li>• waist</li> <li>• <math>\Delta</math> in weight</li> <li>• <math>\Delta</math> in waist</li> </ul>	<ul style="list-style-type: none"> <li>• diabetes</li> <li>• HT</li> <li>• Lipids (total cholesterol, HDL, LDL, Lp(a))</li> </ul>	Mortality: <ul style="list-style-type: none"> <li>• Total</li> <li>• CHD</li> </ul>	According to the text, the mediators attenuated the association between waist and both outcomes, but did not change the <u>inverse</u> association between BMI and either outcome when both were in the model. Diabetes was the principal mediator of waist for both outcomes.
(Kannel, Wilson et al. 2002)	Framingham	Control & stratification by number of risk factors (including age & smoking as well as BP, cholesterol, diabetes)	<ul style="list-style-type: none"> <li>• BMI</li> </ul>	<ul style="list-style-type: none"> <li>• HDL</li> <li>• Total cholesterol</li> <li>• Type 2 diabetes</li> <li>• SBP</li> <li>• heart rate</li> <li>• ECG-LVH</li> </ul>	<ul style="list-style-type: none"> <li>• MI and CHD death</li> </ul>	All but heart rate and overweight were significant in the final model. Overweight was still a substantial predictor of CHD in men, and obesity was significant and substantial in both genders.
(Kim, Meade et al. 2006)	Northwick Park heart	Control – Mediators added successively to model, so not tested independently	<ul style="list-style-type: none"> <li>• Skinfolds</li> <li>• BMI</li> </ul>	<ul style="list-style-type: none"> <li>• BP</li> <li>• Cholesterol</li> <li>• Fibrinogen</li> <li>• Factor VII</li> </ul>	<ul style="list-style-type: none"> <li>• CHD mortality</li> <li>• Total mortality</li> </ul>	The effect of obesity on CHD mortality was not mediated by BP, cholesterol, fibrin, or factor VII.



Citation	Cohort	Method of testing mediation	Exposures	Mediators	Outcomes	Conclusions / comments
(Mann, Lee et al. 2006)	NHANES I Epidemiologic Follow-up	Control and stratification	• BMI	• HT • total chol • diabetes (stratified)	• CHD mort	Compared to lean non-diabetics, OW & OB diabs and OB non-diabs have significantly higher HR
(Manson, Colditz et al. 1990)	Nurses Health	Control	• Current BMI	• History of hypertension • History of diabetes • History of high cholesterol	• Non-fatal MI and fatal CHD	Eliminated the effect of BMI 25 < 29, but only attenuated for BMI $\geq 29$ kg/m <sup>2</sup> .
(Mora, Yanek et al. 2005)	Johns Hopkins Sibling Study (families with known premature CHD)	Compared fit of model with Framingham Risk Score (FRS) & BMI to model w/o BMI. Tested for interaction between FRS & BMI.	• BMI	• (FRS = age, total chol, HDL, BP, diabetes, smkg)	CHD events	They were not really trying to test mediation. The best fitting model was the one with BMI, FRS and BMI*FRS. BMI was an independent predictor of CHD events.
(Onat, Uyarel et al. 2006)	Turkish Adult Risk Factor Study	Control	• Waist	• Cholesterol (also PA and alcohol added at same time)	incident CHD	The effect of waist was eliminated in terms of significance, but the RR was still large.
(Rosengren, Wedel et al. 1999)	Goteborg (Sweden) primary prevention trial	Control for all mediators at once	• BMI • Weight gain	• SBP • Total cholesterol • Diabetes	• Coronary mortality • Total mortality	Mediators attenuated and almost eliminated the effect of BMI. They attenuated but did not eliminate the effect of weight gain.

Citation	Cohort	Method of testing mediation	Exposures	Mediators	Outcomes	Conclusions / comments
(Song and Sung 2001)	Korean Civil Servants (males)	Control for all mediators at once	BMI	<ul style="list-style-type: none"> <li>• DBP</li> <li>• Total cholesterol</li> <li>• Glucose</li> </ul>	Mortality: <ul style="list-style-type: none"> <li>• All-cause</li> <li>• Coronary</li> <li>• Cancer</li> <li>• Cerebro-vascular</li> <li>• The rest</li> </ul>	The mediators attenuated but did not eliminate the effect of BMI for most outcomes. (Weight was not measured using one standard, though measures were done at a doctor's office)
(Tanne, Medalie et al. 2005)	Israeli Ischemic Heart Disease	Control	<ul style="list-style-type: none"> <li>• BMI</li> <li>• Sum of skinfolds</li> <li>• Skinfold ratio</li> </ul>	<ul style="list-style-type: none"> <li>• BP</li> <li>• diabetes</li> </ul>	<ul style="list-style-type: none"> <li>• Stroke mortality</li> <li>• CHD mort</li> </ul>	SBP alone attenuated and statistically eliminated the effects of adiposity, but the other mediators were not tested/shown individually.
(Zoppini, Verlato et al. 2003)	Verona Diabetes Study	Control for all mediators at once	BMI	<ul style="list-style-type: none"> <li>• Duration of diabetes</li> <li>• Fasting glucose</li> <li>• Hypertension</li> </ul>	Mortality: <ul style="list-style-type: none"> <li>• All-cause</li> <li>• CVD</li> <li>• Malignancies</li> </ul>	The mediators attenuated what were mostly non-significant risks in those < 65 years. For those $\geq 65$ , BMI was non-significantly protective.

**Table 2.4. Comparison of beta coefficients for body mass index and waist circumference from same time (cross-sectional) and three-year lag (longitudinal) analyses**

	Body Mass Index			Waist Circumference		
	<i>B<sub>BMI</sub></i>	95% CI	Wald p-value*	<i>B<sub>WC</sub></i>	95% CI	Wald p-value*
<b>Systolic blood pressure</b>						
Same time	0.46	(0.41,0.50)		0.15	(0.13,0.16)	
Three-year lag	0.41	(0.36,0.47)	.1149	0.15	(0.13,0.16)	.9293
<b>Diastolic blood pressure</b>						
Same time	0.22	(0.19,0.25)		0.07	(0.06,0.08)	
Three-year lag	0.12	(0.09,0.15)	<.0001	0.04	(0.03,0.05)	<.0001
<b>Glucose</b>						
Same time	0.47	(0.44,0.50)		0.15	(0.14,0.17)	
Three-year lag	0.43	(0.40,0.47)	.0208	0.16	(0.14,0.17)	.8271
<b>Total cholesterol</b>						
Same time	0.43	(0.31,0.54)		0.19	(0.15,0.23)	
Three-year lag	-0.11	(-0.22,0.01)	<.0001	-0.01	(-0.05,0.03)	<.0001
<b>HDL cholesterol</b>						
Same time	-0.90	(-0.94,-0.85)		-0.29	(-0.31,-0.28)	
Three-year lag	-0.77	(-0.82,-0.73)	<.0001	-0.28	(-0.30,-0.27)	.2259
<b>LDL cholesterol</b>						
Same time	0.56	(0.45,0.67)		0.21	(0.17,0.25)	
Three-year lag	0.16	(0.05,0.27)	<.0001	0.08	(0.04,0.11)	<.0001
<b>Triglycerides</b>						
Same time	3.93	(3.69,4.18)		1.43	(1.35,1.52)	
Three-year lag	2.90	(2.64,3.16)	<.0001	1.11	(1.02,1.21)	<.0001

\*Testing whether the longitudinal estimate differs from the cross-sectional estimate

### **III. METHODS**

#### **A. The ARIC Study data**

The ARIC study is a prospective, multi-site investigation of atherosclerosis and cardiovascular disease (ARIC Investigators 1989). Baseline data were collected from 1987-1989 in 15,792 African American and White men and women, 45-64 years of age. Races other than African American or White (n=48) and African Americans in Minneapolis and Washington County (n= 55) were excluded per standard ARIC protocol because their numbers are too small to allow ethnic and field-center specific analyses, leaving 15,689 participants. These participants were from four communities in the United States: Forsyth County, NC (12% African American; 88% White); Jackson, MS (100% African American); the northwestern suburbs of Minneapolis, MN (100% White); and Washington County, MD (100% White). A range of physiologic and behavioral risk factors were measured. Participants were measured in a maximum of four clinic visits at approximately three year intervals (Table 3.1). The ARIC study uses a combination of cohort and community surveillance for the ascertainment and validation of cardiovascular events and vital status. Currently less than 1% of the ARIC cohort is lost to follow-up. Data were available through December 31<sup>st</sup>, 2005 for this analysis.

#### **1. Variables**

##### **a. Height and weight**

Height was measured in visits 1, 3 and 4. Weight was measured at all 4 visits. Height was measured to the nearest centimeter using a metal rule attached to a wall and a standard

triangular head board. Weight was measured to the nearest pound using a beam balance with subjects in a scrub suit and no shoes.

b. CHD

Incident CHD is defined as cardiac death, non-fatal MI, silent MI or cardiovascular surgery or coronary angioplasty. To identify events, interviewers contacted participants' homes annually to obtain information on all hospitalizations and deaths. ARIC staff also surveyed discharge lists from local hospitals and local obituaries and conducted an annual review of vital statistics tapes. Death certificates were obtained for all deaths. Trained abstractors obtained hospital charts and recorded the presenting signs and symptoms. To detect incident CHD, abstracted information included chest pain, cardiac enzymes, and related clinical information (White, Folsom et al. 1996). Up to three 12-lead electrocardiograms were photocopied and sent to the University of Minnesota where they were visually coded (Prineas, Harland et al. 1982). Out of hospital deaths were investigated using interviews with the next of kin (in approximately 81% of cases) and questionnaires completed by the patients' physicians (in approximately 91% of cases). Two members of the ARIC Morbidity and Mortality Classification Committee reviewed all potential clinical CHD events, and the chair of the Committee adjudicated differences between the two reviewers. Unrecognized MI was defined by a major Q wave or a minor Q wave with ischemic ST-T changes in the first and subsequent ARIC examinations or by computerized NOVACODE (Rautaharju, Warren et al. 1981) criteria, confirmed by side-by-side ECG comparison.

Collection of data on events in the ARIC cohort is on-going. Closure of incident CHD files lags real time by at least one year. We had 16-19 years of follow-up available for this analysis (from entry in 1987-89 through December, 2005). Both occurrence and date of

event were available. For all hazard analyses, time to event was defined as time from the subject's baseline visit to the event date or to the censored date (12/31/05).

c. Physiologic risk factors

Systolic and diastolic blood pressures were measured three times after a 5-minute rest using a random zero sphygmomanometer on the right arm of the seated participant. The last two measures were averaged and recorded. Study participants were asked to fast for 12 hours prior to their clinic visit and fasting blood samples were sent to the ARIC Central laboratories. Total plasma cholesterol (Siedel, Hagele et al. 1983) and triglycerides (Nagele, Hagele et al. 1984) were determined by enzymatic methods. HDL cholesterol was measured after dextran-magnesium precipitation (Warnick, Benderson et al. 1982). Serum glucose levels were measured by a hexokinase/ glucose-6-phosphate dehydrogenase method and insulin by radioimmunoassay (<sup>125</sup>Insulin Kit; Cambridge Medical Diagnostics, Inc., Billerica, MA). Insulin resistance was calculated using the Homeostatic Model Assessment equation (plasma glucose (mmol/l) times serum insulin (mU/l) divided by 22.5) (Bonora, Targher et al. 2000). We considered using the spreadsheet macro which employs the algorithm that the equation is supposed to approximate (Wallace, Levy et al. 2004). However, at high values of glucose and insulin, we found the macro to be highly unstable and the resulting values for insulin resistance were less correlated with our other variables than were the values calculated using the equation.

d. Medication use

Medication records were collected at each clinic visit. Participants were reminded to bring all medications used in the previous two weeks. Names of the medications were transcribed and coded by the ARIC medication coding system, developed by a pharmacist at

UNC. The ARIC medication codes were then mapped to Medi-Span Therapeutic Classification (MTC) codes and American Hospital formulary Service Classification Compilation (AHFSCC) codes. Subjects were classified as taking medications for blood pressure or lipids if they self-reported taking medications in general in the last two weeks, and the medications they brought included an appropriately classified medication. Use of glucose-lowering medications is based on self-report of taking medications for diabetes or high blood sugar in the past 2 weeks.

e. Covariates

Age (date of birth), race/ethnicity, and gender were self-reported. Additional covariates were assessed by interviewer-administered questionnaires. We categorized education as less than a high school education, high school graduate, or at least some college. Physical activity was assessed with the Baecke leisure time physical activity questionnaire (Baecke, Burema et al. 1982) and categorized in tertiles. Self-reported cigarette smoking status and alcoholic beverage consumption were categorized as current, former, or never. Participants were asked about a number of possible sources of health insurance, categorized for this analysis as insured or uninsured. Participants were also asked if either parent had a history of myocardial infarction, which we categorized as yes if either parent did and no if no parent did. Family history of high blood pressure and of diabetes were coded in the same way. A race-field center variable was created because all field centers did not have both race groups.

**2. *Quality control***

ARIC field centers used a computer-assisted data collection system in which staff directly recorded the information collected from interviews and examinations. Rigorous

quality control procedures were developed and implemented for all parts of the examination to ensure that data were collected uniformly at each center and over time. The Collaborative Studies Coordinating Center at UNC-Chapel Hill served as the study coordinating center. Several papers evaluating quality control in the ARIC study have been published (Chambless, McMahon et al. 1992; Chambless, McMahon et al. 1992; Chambless, McMahon et al. 1993; Ma, Folsom et al. 1995; Sorlie, Cooper et al. 1996; Stevens, Metcalf et al. 1996; Coady, Sorlie et al. 2001; Schroeder, Whitsel et al. 2004).

## **B. Analytic methods**

Methods used in individual papers are summarized in those chapters. Here I present additional detail about methods used and considered.

### **1. Mediation**

The regression-based approach to assessing mediation seen most often in the epidemiologic literature simply runs two regressions – one without a proposed mediator and one with the mediator, e.g.:

Model 1: Outcome = Main exposure

Model 2: Outcome = Main exposure + mediator

If the mediator is significant in model 2, mediation is assumed to occur. If the main exposure is no longer significant in model 2, then the mediator is considered to fully mediate the relationship between the main exposure and the outcome. If the main exposure is still significant, mediation is assumed to be partial.

In 1986, Baron and Kenny proposed an alternative modeling approach, using three regression equations (Figure 3.1):



Model 1 (Step 1): Outcome = Main exposure

Model 2 (Step 2): Mediator = Main exposure

Model 3 (Steps 3 and 4): Outcome = Main exposure + mediator

With this approach, the product of  $a \times b$  equals the indirect effect, and is equivalent to  $c-c'$ . The advantage of this approach is that a standard error can be calculated for  $a \times b$ , and the significance of the indirect effect can be tested. More recently, methods for testing indirect effects in small samples and with non-linear models have been developed, including bootstrapping (Shrout and Bolger 2002; MacKinnon, Fairchild et al. 2007).

## **2. *Structural equation modeling***

SEM uses variance and covariance matrices to test relationships among all specified paths in a model at once (e.g., Figure 3.2). It returns values (standardized and unstandardized) for all paths as well as measures of overall model fit (variance in the outcome explained by the model). Path coefficients represent change in the dependent variable associated with a 1-unit change in the independent variable (the same as in regression).

SEM has several advantages over standard regression. It allows the researcher to specify paths among the variables in the model and to leave out paths where a relationship is hypothesized not to exist. It does not assume that independent variables are measured without error, but instead can explicitly model that error. We had hoped to take advantage of this feature, as the ARIC study has reliability data on the exposure and mediators of interest for this study, but were not able to do so (see “Additional methodologies considered”, below). SEM permits comparison of model fit and significance of the indirect effect across race-gender groups. The use of a time-to-event outcome in SEM is a recent technique, and as

such has not been implemented often, especially using continuous survival data. However, a few papers on the methods exist (Larsen 2004; Larsen 2005; Asparouhov 2006), and there are at least two examples of usage in health literature (Moustaki and Steele 2005; Christ, Lee et al. 2008).

a. SEM Software

All mediation analyses used Mplus statistical software. Mplus is a software package that can be used for a variety of analyses, including linear and logistic regression, multi-level modeling, survival analysis, factor analysis and structural equation modeling.

**3. *The delta method***

The delta method was used in Mplus to calculate standard errors for indirect effects. The delta method is a technique for calculating the variance around some function of an estimate by estimating a linear approximation of the function and calculating the variance of that function (Xu 2005). As long as the function satisfies certain requirements, such as being differentiable, the delta method can be used (Greene 2003). It is appropriate for linear and non-linear combinations of parameters.

The mixed model analysis of stacked data presented in the secondary aim used an unstructured correlation matrix selected based on fit indices for several possible final models with both SBP and DBP as the outcome.

**4. *Additional methodologies considered***

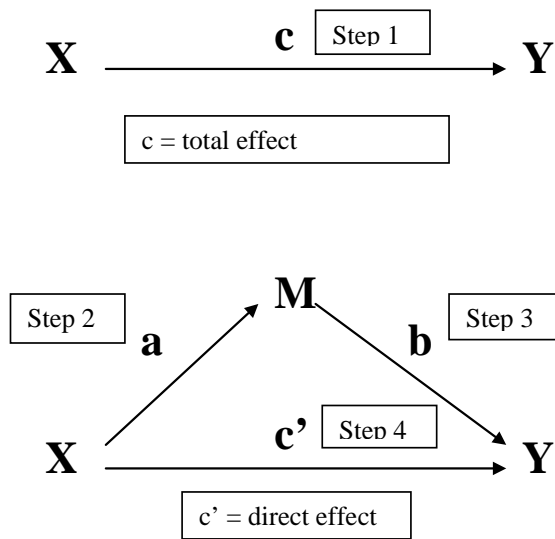
SEM does not assume that independent variables are measured without error, but instead can explicitly model that error. Where measurement error has been estimated, as for several variables in the ARIC study, those values can be entered. Measurement errors for relevant variables in ARIC are shown in Table 3.2. We had hoped to incorporate these errors

into the model as shown in Figure 3.2 (example for lipids), but the models did not converge with error terms incorporated, so we were unable to take advantage of that aspect of SEM. Fortunately, reliability for the main variables of interest is high.

**Table 3.1. Number of ARIC participants by race-gender and clinic visit**

	African American women	White women	African American men	White men	Totals
Visit 1 (1987-1989)	2,609	6,050	1,602	5,428	15,689
Visit 2 (1990-1992)	2,225	5,675	1,303	5,054	14,257
Visit 3 (1993-1995)	1,883	5,249	1,072	4,603	12,807
Visit 4 (1996-1998)	1,686	4,792	940	4,169	11,587
Completed all	1,520	4,657	824	4,070	11,061

**Figure 3.1: Baron and Kenny Mediation**

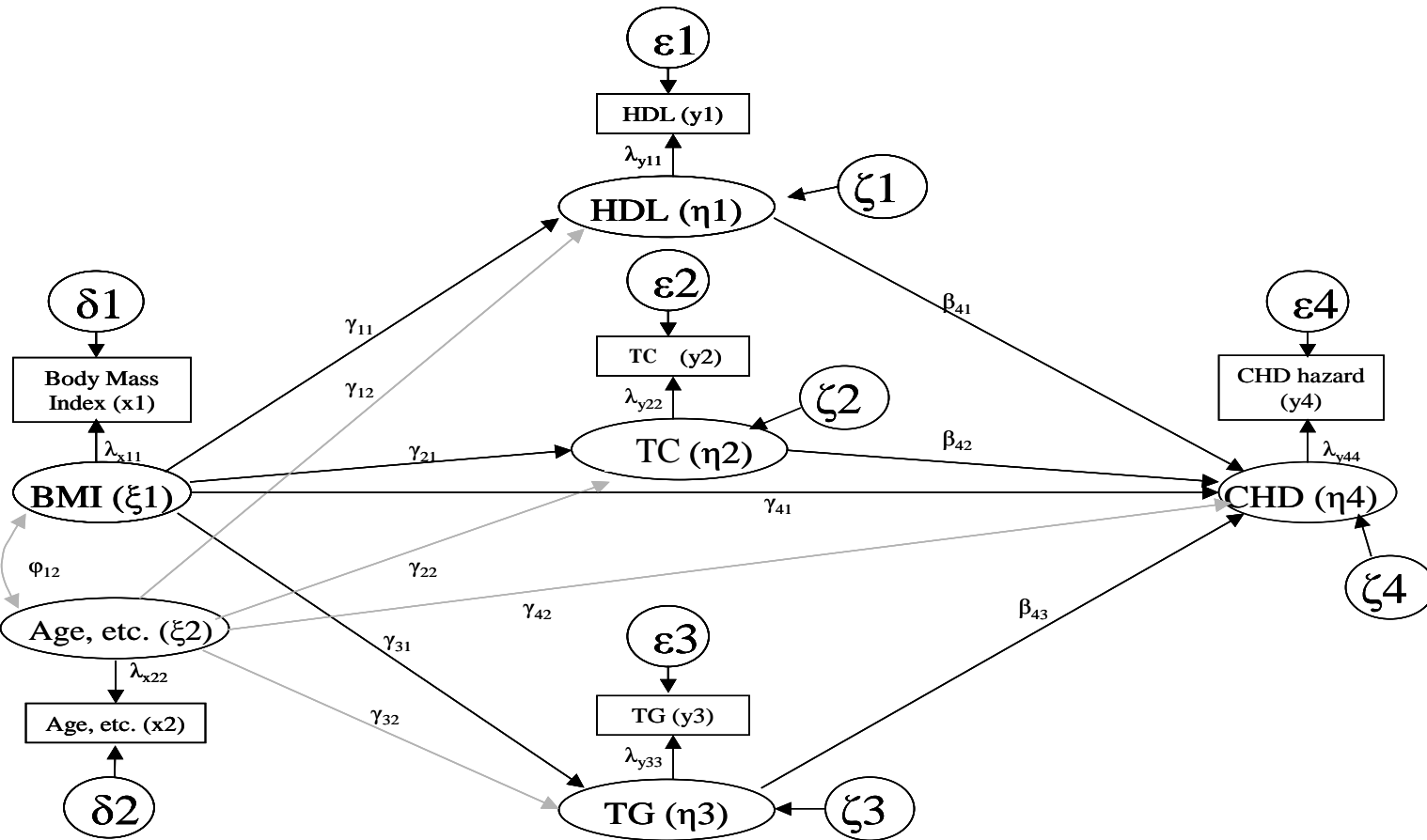


**Table 3.2. Reliability coefficients (R) for key variables**

<b>BMI</b> (Klipstein- Grobusch, Georg et al. 1997)	<b>TC</b> (Chambless , McMahon et al. 1992)	<b>HDL</b> (Chambless , McMahon et al. 1992)	<b>TG</b> (Chambless , McMahon et al. 1992)	<b>Glucose</b> (Eckfeldt, Chambless et al. 1994)	<b>SBP</b> (Weatherley , Chambless et al. 2006)
.99	.94	.94	.85	.84	.74

\*An R of 1.0 reflects perfect reliability

Figure 3.2: Structural equation model with variables modeled as single-indicator latent variables



η=latent endogenous variable; ζ=latent error term; y=observed endogenous variable; ε= observed error term; ξ=latent exogenous variable; x=observed exogenous variable; δ=disturbance term; φ=covariance of exogenous variables; λ=factor loading; γ=path coefficient for association of an endogenous variable with an exogenous variable; β=path coefficient for association of an endogenous variable with another endogenous variable

#### **IV. MEDIATION OF EFFECTS OF BODY MASS INDEX ON CORONARY HEART DISEASE BY ESTABLISHED PHYSIOLOGIC RISK FACTORS IN A BI-ETHNIC COHORT: THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY**

##### **A. Abstract**

We used structural equation models to determine whether established physiologic risk factors mediated the association between body mass index (BMI) and coronary heart disease (CHD) and whether mediation differed by race or gender. The Atherosclerosis Risk in Communities (ARIC) Study is a cohort of African American and White adults aged 45-64 years at baseline (1987-1989), followed through 2005. Mediators examined were total and HDL cholesterol (TC, HDL), triglycerides, systolic and diastolic blood pressure (SBP, DBP) and insulin resistance. Participants with prevalent CHD, who had not fasted, or were missing variables were excluded, leaving a final sample of 13,721. The indirect (mediated) effect of BMI on CHD through each mediator was calculated as the predicted hazard ratio associated with the change in the risk factor predicted by 3 specific contrasts of BMI: 25 vs. 20 kg/m<sup>2</sup>, 30 vs. 25 kg/m<sup>2</sup> and 35 vs. 30 kg/m<sup>2</sup>. The effect of BMI on CHD was curved, with a steeper slope between a BMI of 20 and 25 kg/m<sup>2</sup> than between 30 and 35 kg/m<sup>2</sup>. In the model without mediators, hazard ratios (HRs) and 95% confidence intervals for the effect of BMI on CHD were 1.39 (1.26,1.54) for the 25 vs. 20 kg/m<sup>2</sup> contrast and 1.20 (1.15,1.26) for 35 vs. 30 kg/m<sup>2</sup>. In models that included all 6 hypothesized mediators, BMI was not directly associated with CHD. Mediated effects of BMI on CHD tended to be strongest through HDL cholesterol (HR: 1.24 (95% CI: 1.15,1.34) for the 25 vs. 20 kg/m<sup>2</sup> and 1.10 (1.06,1.14) for the 35 vs. 30 kg/m<sup>2</sup> contrast) and through insulin resistance (1.09 (1.03, 1.16) and 1.11 (1.08,



1.13) for the respective contrasts). Mediation was more moderate through total cholesterol (1.06 (1.04,1.07) and 1.00 (1.00,1.01), respectively) and SBP (1.06 (1.04, 1.08) and 1.05 (1.04, 1.06), respectively), and was null through DBP and triglycerides. We found no differences in mediation by race or gender. The effect of BMI on CHD was completely mediated in this population by total and HDL cholesterol, SBP and insulin resistance. The relative importance of these mediators varied by level of BMI.

## **B. Background**

Obesity is associated with increased risk of coronary heart disease (CHD) (Folsom, Stevens et al. 1998; Kannel, Wilson et al. 2002; Mann, Lee et al. 2006), which is the leading cause of death in the United States (AHA 2007). This association is thought to occur at least partly via effects on blood pressure, lipids and glucose (Ledoux, Lambert et al. 1997; Zhu, Heshka et al. 2004), risk factors for CHD (Folsom, Szklo et al. 1997; NCEP 2001; Lloyd-Jones 2007). Few studies have attempted to quantify the mediation of the obesity-CHD relationship by these established physiologic risk factors. Epidemiologic analyses of mediation typically do not calculate indirect (mediation) effects through individual risk factors, but instead assess whether the direct effect of obesity is reduced after potential mediators are added to a model, often in one block (Rosengren, Wedel et al. 1999; Batty, Shipley et al. 2006; Kim, Meade et al. 2006). This approach is limited in that indirect effects through individual risk factors are not calculated or compared (MacKinnon, Lockwood et al. 2002).

Path analysis in a structural equations model (SEM) permits the calculation of individual mediation paths in single- and multiple-mediator models, and allows comparisons across mediators. We are not aware of any analyses of obesity and CHD that have used this

methodology, although it is commonly used in the social sciences to quantify and compare indirect effects. Further, we know of no studies that have addressed racial differences in the mediation of obesity's effects on CHD using any methodology. Racial differences have been found in the prevalences of obesity (Li, Ford et al. 2007; Ogden 2007), CHD risk factors (Winkleby, Kraemer et al. 1998; Sundquist, Winkleby et al. 2001) and CHD outcomes (Clark, Ferdinand et al. 2001; Jones, Chambless et al. 2002; American Heart Association 2008), as well as in the associations among these variables (Despres, Couillard et al. 2000; Harris, Stevens et al. 2000; Jones, Chambless et al. 2002; Carnethon, Lynch et al. 2006; Shai, Jiang et al. 2006; Abell, Egan et al. 2008), so differences in mediation might be expected.

The objective of this analysis was to quantify the direct and indirect effects of body mass index (BMI) on CHD through established physiologic risk factors and to compare the relative strength of the mediators. The risk factors examined were total and high-density lipoprotein (HDL) cholesterol, triglycerides, systolic and diastolic blood pressure, and insulin resistance. We also compared mediation across race (African American and White) and gender groups.

## **C. Methods**

### **1. *Study population***

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective investigation of atherosclerosis and cardiovascular disease in a cohort of 15,792 African American and White adults aged 45-64 years at baseline (1987-1989). Participants were from four communities in the United States: Forsyth County, NC, Jackson, MS, Minneapolis, MN, and Washington County, MD. Participants were examined at four study visits spaced approximately 3 years apart. Details of the study design have been described previously

(ARIC Investigators 1989). Races other than African American or White (n=48) and African Americans in Minneapolis and Washington County (n= 55) were excluded per standard ARIC protocol because their numbers are too small to allow ethnic and field-center specific analyses. This study was approved by the Institutional Review Board (IRB) at each field center, and this analysis was approved by the University of North Carolina Public Health-Nursing IRB on research involving human subjects.

## **2. *Measurements***

Body weight was measured at all clinic visits in a scrub suit to the nearest pound by use of a beam balance scale. Standing height (without shoes) was measured to the nearest centimeter using a metal rule and a standard triangular headboard. BMI was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressures were measured three times after a 5-minute rest using a random zero sphygmomanometer on the right arm of the seated participant. The last two measures were averaged and recorded. Participants were asked to fast for 12 hours prior to clinic visit. Serum glucose levels were measured by a hexokinase/ glucose-6-phosphate dehydrogenase method and insulin by radioimmunoassay (<sup>125</sup>I Insulin Kit; Cambridge Medical Diagnostics, Inc., Billerica, MA). Insulin resistance was calculated using the Homeostatic Model Assessment equation (plasma glucose (mmol/l) times serum insulin (mU/l) divided by 22.5) (Bonora, Targher et al. 2000), and was log-transformed for analysis. Total plasma cholesterol (Siedel, Hagele et al. 1983) and triglycerides (Nagele, Hagele et al. 1984) were determined by enzymatic methods. Triglycerides were also log-transformed for analysis. HDL cholesterol was measured after dextran-magnesium precipitation (Warnick, Benderson et al. 1982).

Coronary heart disease is defined as: cardiac death, non-fatal myocardial infarction (MI), silent MI detected by echocardiogram (ECG), or cardiovascular surgery or coronary angioplasty (White, Folsom et al. 1996). Follow-up of CHD events was available through December 31<sup>st</sup>, 2005. For all hazard analyses, time to event was defined as time from the subject's baseline visit to the event date or censored date. Prevalent CHD at baseline was defined for exclusion in these analyses as a reported history of a physician-diagnosed MI, MI detected by ECG, or cardiovascular surgery or coronary angioplasty. Age (date of birth), race/ethnicity, and gender were self-reported. Additional covariates were assessed by interviewer-administered questionnaires. We categorized education as less than a high school education, high school graduate, or at least some college. Physical activity was assessed with the Baecke leisure time physical activity questionnaire (Baecke, Burema et al. 1982) and categorized in tertiles. Self-reported cigarette smoking status and alcoholic beverage consumption were categorized as current, former, or never. Participants were asked about a number of possible sources of health insurance, categorized for this analysis as insured or uninsured. Participants were also asked if either parent had a history of myocardial infarction, which we categorized as yes if either parent did and no if no parent did. Family history of high blood pressure and of diabetes were coded in the same way. A race-field center variable was created because all field centers did not have both race groups.

### **3. *Statistical analyses***

We excluded persons with prevalent or missing CHD at baseline (n=1,102) and participants who had not fasted at least 8 hours (n=515), as well as participants missing BMI (n=22), systolic or diastolic blood pressure (n=4), lipids (n=188), glucose or insulin (n=9) or covariates (n=128). The final sample size was 13,721, comprising 5,635 White women,

2,147 African American women, 4,650 White men and 1,289 African American men. Mean follow-up time was 15.2 years.

We used structural equation models in conjunction with Cox proportional hazards regression in Mplus 5.0 to assess the indirect effect of baseline BMI on time to CHD through each of the physiologic risk factors, as well as the direct effect of BMI on CHD. BMI and all of the risk factors were analyzed as continuous variables. Risk factors were examined as mediators in separate models and in a combined model with all risk factors together.

The conceptual model for the mediation analysis is illustrated in Figure 4.1. Structural equation models incorporate several regression equations simultaneously to obtain path coefficients for each relationship. Linear regression was used to examine the relationship between BMI and physiologic risk factors (path A), and Cox proportional hazards regression was used to examine the relationships between risk factors and CHD (path B) and between BMI and CHD (path C', the direct effect). The total effect (path C, not shown) is the association of BMI with CHD in a proportional hazards model with no mediators in the model.

We examined quadratic terms and interactions for BMI and mediator variables. We kept quadratic terms that resulted in nonlinear estimates. Final equations for all paths are shown in Table 4.1. A quadratic relationship was found for the association of BMI with each risk factor and with CHD, so the BMI-risk factor (path A) associations and the total and direct effects of BMI (paths C and C') were predicted and reported for 3 specific contrasts of BMI: 25 vs. 20, 30 vs. 25 and 35 vs. 30 kg/m<sup>2</sup>. Quadratic associations of risk factors with CHD were observed for total cholesterol, triglycerides, diastolic blood pressure and insulin resistance. We therefore calculated hazard ratios for 2 specific contrasts of each risk factor:

the mean plus one standard deviation vs. the mean, and the mean vs. the mean minus one standard deviation. Interactions with BMI were observed for associations of HDL-cholesterol, triglycerides and diastolic blood pressure with CHD in individual-mediator models, but did not affect estimates in the combined mediator model and so were dropped from all models in favor of parsimony and simplicity of presentation of results.

The indirect (mediated) effect is the change in the outcome variable associated with the change in the mediator estimated for a given change in the exposure variable. In models without quadratic terms, the indirect effect is calculated by multiplying the path coefficients from the BMI-risk factor (path A) and the risk factor-CHD (path B) (Bollen 1989), where A is the change in the mediator for each 1-unit increment in exposure, and B is the change in the log hazard ratio (HR) of the outcome per 1-unit increment in the mediator. Because of the non-linear associations in our models, we calculated indirect effects by predicting the value of each risk factor for a BMI of 20, 25, 30 and 35 kg/m<sup>2</sup>, using mean values of covariates, and then estimating the log CHD hazard ratios associated with the contrast between the predicted risk factor values. We report the indirect (mediated) hazard ratio, which was the exponentiated change in the log CHD hazard ratio for each of these 5-unit increments in BMI. Standard errors for the indirect hazard ratios were calculated using the delta method.

All mediation models were adjusted *a priori* for age (centered), gender, race, field center, smoking, alcohol use, physical activity, education and insurance status. An age-squared term was also included in all of the models because age effects were curved in some of them. Family history of myocardial infarction, diabetes or hypertension was also included in portions of the model with relevant outcomes. Total calories and sodium intake were also

considered as covariates, but were not significant predictors of the outcome variables and did not change the main effects. Analyses were stratified by race-gender groups, and differences in A paths, B paths and indirect effects across groups were assessed using a Wald test.

#### **D. Results**

Sample characteristics at baseline are shown in Table 4.2 by BMI categories. Obese individuals were disproportionately African American women. Obese individuals had higher mean levels of all of the risk factors. They were less educated and less likely to have health insurance, less likely to be current drinkers or smokers and more likely to be former drinkers or smokers. Obese participants were less likely to be in the highest and more likely to be in the lowest tertile of leisure activity and were more likely to have a family history of diabetes or hypertension. Overweight and obese persons had a higher unadjusted incidence of CHD events than leaner individuals.

Table 4.3 shows the predicted effects of BMI on physiologic risk factors (A paths) for the 3 BMI contrasts. BMI was a strong predictor of all of the risk factors examined. The associations became weaker at higher BMI levels for all of the risk factors, especially total cholesterol.

Hazard ratios for the effect of standard deviation contrasts of physiologic risk factors on CHD are shown in Table 4.4 for single-mediator models and for the model with all mediators together. Each risk factor was significantly associated with CHD in single-mediator models. In the multiple-mediator model, triglycerides no longer predicted CHD. Diastolic blood pressure was null for the contrast above the mean, but weakly protective below. The association with insulin resistance was somewhat reduced. There was little difference between the single-mediator and multiple-mediator models for other risk factors.

Total and direct effects of BMI on CHD (C and C' paths) are shown in Table 4.5. BMI was associated with CHD in the model without mediators (C-path HRs for 5-unit contrasts of BMI range from 1.20 to 1.39). The addition of single mediators generally attenuated, but did not eliminate the effect of BMI on CHD (C' path). Total cholesterol had little effect on the BMI estimate, whereas insulin resistance substantially attenuated the direct effect of BMI. BMI was no longer a direct predictor of CHD in the model with all mediators together.

Table 4.6 and Figure 4.2 show the indirect (mediated) effects of the 3 contrasts of BMI on CHD through each mediator. When mediators were entered individually, all of the confidence intervals excluded the null except for total cholesterol and diastolic blood pressure for the 35 vs. 30 kg/m<sup>2</sup> contrast. Mediation tended to be weaker at higher BMI levels for all risk factors except systolic blood pressure. In the multiple-mediator model, indirect effects of BMI through total and HDL cholesterol, systolic blood pressure and insulin resistance were observed. However, mediation by insulin resistance no longer weakened with increasing BMI, as it had in the single-mediator model.

The relative strength of the mediation varied with the level of BMI. HDL was the strongest mediator for the 25 vs. 20 kg/m<sup>2</sup> contrast, with total cholesterol, systolic blood pressure and insulin resistance acting as lesser mediators. Whereas for the 35 vs. 30 kg/m<sup>2</sup> contrast, HDL cholesterol and insulin resistance acted as similar and relatively strong mediators of BMI, with less mediation through systolic blood pressure and none through total cholesterol.

We found few differences by race-gender groups in effects of risk factors on CHD (B paths) or in mediation (indirect effects), and there was no clear pattern in the few differences



that were seen. Given the large number of paths tested and the modest  $p$ -values for the few differences found, we did not report stratified results. We did observe some differences in associations of BMI with risk factors (A paths). Compared to Whites, African American women had weaker associations of BMI with systolic and diastolic blood pressures, especially at lower BMIs. African American women also had weaker associations of BMI with triglycerides than White men or women or African American men. Associations of BMI with insulin resistance were stronger in men than in women, and were weakest in African American women, especially at higher BMIs. None of the differences observed in the A paths persisted in the indirect effects.

## **E. Discussion**

Consistent with other longitudinal studies (Jousilahti, Tuomilehto et al. 1996; Bogers, Bemelmans et al. 2007), BMI predicted CHD in this analysis. The association was curvilinear, with a steeper gradient at lower BMIs and a flatter slope at higher BMIs, though the association remained positive through the BMI range shown here. A similar shape has been seen in some studies with similar baseline age and outcome definition (Rexrode, Buring et al. 2001; Yarnell, Patterson et al. 2001), though others have seen a linear (Manson, Colditz et al. 1990) or J-shaped association (Tunstall-Pedoe, Woodward et al. 1997). It is difficult to compare many studies to the results observed here because BMI is commonly categorized, so that a flattening of the slope above the highest threshold may be obscured.

The association of BMI with CHD has been assessed in other analyses using the ARIC data. A 1998 paper examined the association of BMI quartiles with fatal and non-fatal coronary heart disease, excluding cardiac procedures (Folsom, Stevens et al. 1998). A roughly J-shaped association was observed in men, and a more linear association was

observed in women. The 11 years of additional follow-up time and use of continuous BMI in the present analysis could account for differences observed. The exclusion of cardiac procedures does not appear to account for the differences based on the results of a sensitivity analysis, discussed below. Also using ARIC data, Chambless *et al.* found no association with BMI when it was added to a baseline model based on the Framingham risk score that already included smoking, systolic blood pressure, total cholesterol, HDL cholesterol and diabetes status (Chambless, Folsom *et al.* 2003). This agrees with our observation that there was no direct effect of BMI on CHD when all of the mediators were included in the model, indicating that all of the association of BMI with CHD can be accounted for by the physiologic risk factors we examined.

The association between BMI and CHD observed here was mediated by total and HDL cholesterol, systolic blood pressure and insulin resistance. A number of other cohort analyses have examined mediation of the relationship between CHD and weight-related variables by established physiologic risk factors using more traditional methods. The majority found that the assessed mediators attenuated but did not eliminate the relationship between overweight and CHD (most examined fatal CHD only) (Manson, Colditz *et al.* 1990; Jousilahti, Tuomilehto *et al.* 1996; Rosengren, Wedel *et al.* 1999; Song and Sung 2001; Kannel, Wilson *et al.* 2002; Dyer, Stamler *et al.* 2004; Hu, Tuomilehto *et al.* 2005), including a meta-analysis of 21 cohort studies (Bogers, Bemelmans *et al.* 2007) that found a relative risk for a 5-unit change in BMI of 1.27 (95% CI: 1.21, 1.33) without and 1.15 (95% CI: 1.11, 1.19) with BP and cholesterol in the model. A minority of studies found that the mediators eliminated the association with CHD (Jousilahti, Tuomilehto *et al.* 1996; Huang, Rodreiguez *et al.* 1997; Tanne, Medalie *et al.* 2005), in that the confidence interval for the obesity

variable included the null, but the absolute reduction in the effect was often similar to the other studies. Rarely, the hypothesized mediators had no attenuating effect (Kim, Meade et al. 2006). Since mediators were generally added as a group or successively, it is difficult to conclude from these studies which mediators were more important.

The few studies that tested mediators individually were inconsistent. Kim et al.(Kim, Meade et al. 2006) assessed BMI as a predictor of fatal CHD in the Northwick Park Heart Study. The addition of cholesterol strengthened rather than attenuated the relative risk of BMI, but the further addition of SBP attenuated the effect. Jousilahti et al.(Jousilahti, Tuomilehto et al. 1996) examined the relationship between BMI and CHD mortality in Finnish men and women aged 30-59. Although total cholesterol was significant in a model with BMI, the addition of cholesterol did not attenuate the relationship between BMI and CHD mortality. The addition of blood pressure did attenuate (and inconsistently reduced to null, depending on gender and age group) the relative risk for BMI. Tanne et al (Tanne, Medalie et al. 2005) assessed hazard ratios for CHD in male Israeli civil servants. The addition of SBP to a model with BMI and age attenuated the HR for the effect of BMI on CHD, whereas the further addition of diabetes, smoking and socioeconomic status produced little change. None of the studies we found compared mediation in African Americans versus Whites.

Mediation in the present analysis occurred through the same physiologic risk factors identified in several risk scores developed in other cohorts, such as the Framingham Risk Score (D'Agostino, Vasan et al. 2008), SCORE (Conroy, Pyorala et al. 2003), ASSIGN (Woodward, Brindle et al. 2007) and QRISK (Hippisley-Cox, Coupland et al. 2007), all of which emphasize total and HDL cholesterol, SBP and diabetes in addition to non-physiologic

risk factors such as smoking. The Framingham risk score has been validated in the ARIC data (D'Agostino, Grundy et al. 2001). The ARIC cohort has also been used to develop the Personal HEART score (Mainous, Koopman et al. 2007) based on self-reported rather than measured risk factors (e.g., self-reported history of high cholesterol). This score compared favorably with Framingham and SCORE. The Personal HEART score included BMI in the risk prediction for women, but most scores based on measured physiologic risk factors do not explicitly include BMI because it was no longer statistically significant once other risk factors were entered into the model (Woodward, Brindle et al. 2007; D'Agostino, Vasan et al. 2008), a finding also seen here. Nevertheless, the indirect effects reported here indicate that BMI is an important driver of the risk factors included in these scores.

To our knowledge, this study is the first to show that the relative strength of the indirect (mediated) effects varied with the level of BMI. Mediation through lipids weakened as BMI rose, whereas effects through insulin resistance and blood pressure remained similar. At lower BMI levels, most of the effect of BMI on CHD was mediated by HDL cholesterol. This suggests that the increase in CHD risk associated with higher BMI among normal weight individuals may be primarily due to HDL cholesterol, which drops sharply with increasing BMI below the threshold of obesity ( $\text{BMI} = 30 \text{ kg/m}^2$ ) and then changes very little above the threshold (Table 4.1). It also underscores the gradient of risk that exists within the normal weight category, a phenomenon that has received strong attention in Asians, but is less appreciated in Caucasians and African Americans (Stevens 2003). HDL cholesterol is known to be an important risk factor for CHD, and is included in most CHD risk scores, but its observed impact may be diminished in cohorts dominated by overweight subjects due to the increased relative effects of systolic blood pressure and insulin resistance at higher BMIs.

We chose not to control for use of antihypertensive, anti-hyperlipidemic or diabetic medications. Exclusion, stratification and adjustment for medication use have all been shown to bias results (Tobin, Sheehan et al. 2005) because medication use in observational data is so profoundly confounded by indication that the medication use variable acts as a marker for the risk factor itself. As a result, statistical adjustment for medication use will usually bias estimates for the effect of BMI on risk factors towards the null (Schisterman, Cole et al. 2009), which it does in these (data not shown) and other data (Tobin, Sheehan et al. 2005). Imputation is considered to be an appropriate solution for handling medication when the risk factor is the outcome variable, and various methods have been proposed (Cook 1997). However, for mediation, imputation would not have been not optimal because we were not only interested in the treated risk factors as outcomes (A paths), but also as exposures for CHD (B paths). In the latter case, we wished to estimate effects for the actual experienced (treated) value of the risk factor, and we did not wish to assign different values of the risk factor for path A and path B.

We conducted several sensitivity analyses to confirm our decision: 1) imputing treated values of the risk factors, 2) adjusting for medication use, and 3) excluding participants taking antihypertensive, antihyperlipidemic or diabetes medications (final N=9,399). Hazard ratios for indirect effects changed very little for all analyses, differing by no more than 0.02 from original estimates for all mediators except insulin resistance in the adjusted and exclusion analyses. We also repeated the analysis excluding cardiac procedures because of concerns about bias due to race, gender and other sociodemographic differences in use of these procedures (Lee, Folsom et al. 2001). We found only very slight differences in the overall population and minor differences by race and sex that did not change our

conclusions or our decision to present overall rather than race and/or gender-specific results. Our analysis adjusted for insurance and education, as well as race and gender, which may explain why our results changed little despite known differences in use of procedures.

We found few differences by race or gender in the relationships examined here. Effects of BMI on risk factors (A paths) initially appeared to be quite different in African American women compared to the other three race-gender groups, but addition of the quadratic BMI term reduced and/or eliminated the differences. Racial differences have been observed in the association of BMI with risk factors (Colin Bell, Adair et al. 2002) and of both BMI (Abell, Egan et al. 2008) and physiologic risk factors (Carnethon, Lynch et al. 2006; Shai, Jiang et al. 2006) with CHD in other large cohorts. However, observed racial differences in multifactorial conditions like obesity and heart disease, or in associations among such conditions, are particularly subject to unmeasured confounding (Kaufman 2008). While it is important to describe differences observed, care should be taken with etiologic inference.

The indirect HRs reported here were not large even though they are reported per 5 units of BMI, which represent about 30 lbs for a 5'6" person, a clinically large increment. Five BMI units is approximately the standard deviation in this population and is also the difference between commonly used cutpoints for overweight (BMI = 25 kg/m<sup>2</sup>) and obese (BMI = 30 kg/m<sup>2</sup>). This increment is comparable to gains seen in U.S. adults over 15-30 years (Stevens, Tyroler et al. 1998; Gordon-Larsen, Hou et al. 2009). Given that two thirds of the US population is overweight (Ogden, Carroll et al. 2006), the 5-10% increased hazards observed through most of the mediators for this size increment represent a large number of individuals at increased risk for CHD through each mediator.

This study had several strengths, including carefully measured CHD endpoints over a long follow-up period, high reliability of BMI (Klipstein-Grobusch, Georg et al. 1997) and physiologic risk factor measures (Chambless, McMahon et al. 1992) (Eckfeldt, Chambless et al. 1994) (Weatherley, Chambless et al. 2006), and a large bi-ethnic cohort, permitting comparisons by racial group. The use of path analysis in structural equations models allowed us to quantify and compare individual indirect effects, and the use of a Cox proportional hazards model in SEM is relatively novel (MacKinnon, Lockwood et al. 2002; Christ, Lee et al. 2008).

There are inherent limitations in drawing causal inferences from observational data, and these limitations may be exacerbated in an analysis in which multiple estimated parameters are used to calculate an additional parameter, as we have done for the indirect effects. A further limitation was the inability to fully account for medication use. We reported the results of sensitivity analyses, but no known method for addressing medication use could have adequately addressed all the issues in an observational study design.

The association of BMI on CHD was curvilinear in this population, with a stronger association at the lower BMI range and a flattening of the slope as BMI increased. The effect of BMI was entirely mediated by total and HDL cholesterol, SBP and insulin resistance. These indirect effects did not differ by race or gender, but were observed to vary by level of BMI. At lower BMIs, the indirect effect through HDL was substantially larger than for other mediators, but at higher BMIs, which are associated with more CHD events, HDL and insulin resistance were similarly important. Excess body mass, through its effect on various risk factors, is an important contributor to CHD risk. In leaner individuals, the effect of body

mass may occur primarily through HDL-cholesterol levels. This study underscores the importance of weight control for reducing CHD risk, even among lean individuals.



**Figure 4.1: Mediation analysis paths**

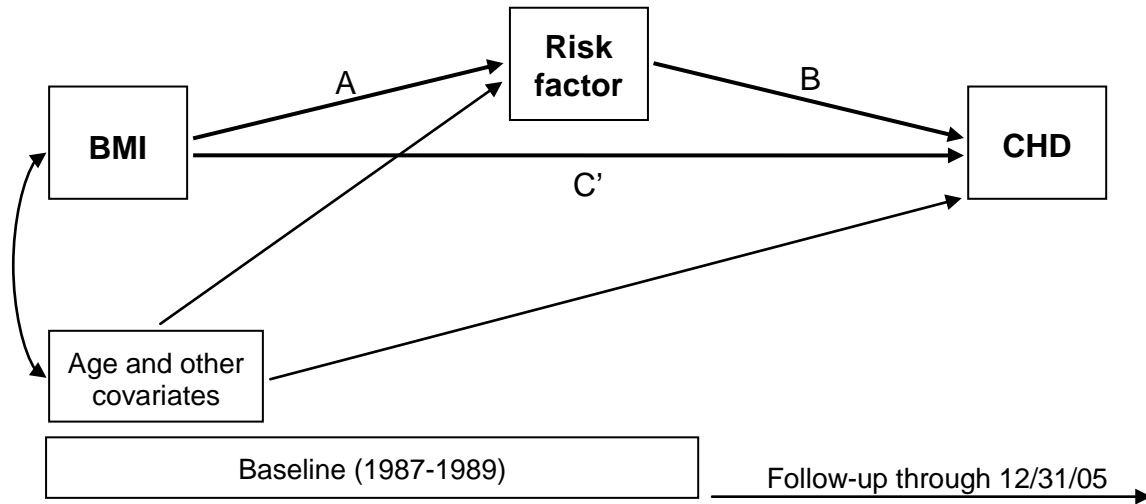


Figure 4.1: Conceptual model for the mediation analysis. BMI, physiologic risk factors and covariates were measured at baseline. Linear regression was used to examine the relationship between BMI and risk factor variables (path A), and Cox proportional hazards regression was used to examine the relationships between risk factors and CHD (path B) and between BMI and CHD (path C', the direct effect). Path C (the total effect, not shown) is the path from BMI to CHD in a model with no mediator.

**Table 4.1. Equations used in each of the structural equation models**

Model	Equations for A paths	Equations for C path (without mediator) and B and C' paths (with mediators)
No mediator	N/A	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + C )$
<u>Models with mediators</u>		
Total cholesterol	$\text{TC} = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{TC}_i + \beta_4 \text{TC}_i^2 + C )$
HDL cholesterol	$\text{HDL} = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{HDL}_i + C )$
Triglycerides	$\text{Ln}(\text{TG}) = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{TG}_i + \beta_4 \text{TG}_i^2 + C )$
Systolic Blood Pressure	$\text{SBP} = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{SBP}_i + C )$
Diastolic Blood Pressure	$\text{DBP} = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{DBP}_i + \beta_4 \text{DBP}_i^2 + C )$
Insulin resistance	$\text{Ln}(\text{IR}) = \alpha + \beta_1 \text{BMI} + \beta_2 \text{BMI}^2 + C$	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{IR}_i + \beta_4 \text{IR}_i^2 + C )$
All mediators in one model	A paths are all of the above.	$h_i(t) = \lambda_0(t) \exp ( \beta_1 \text{BMI}_i + \beta_2 \text{BMI}_i^2 + \beta_3 \text{TC}_i + \beta_4 \text{TC}_i^2 + \beta_5 \text{HDL}_i + \beta_6 \text{TG}_i + \beta_7 \text{TG}_i^2 + \beta_8 \text{SBP}_i + \beta_9 \text{DBP}_i + \beta_{10} \text{DBP}_i^2 + \beta_{11} \text{IR}_i + \beta_{12} \text{IR}_i^2 + C )$

C = covariates.

**Table 4.2. Means (SD) and prevalences of selected sample characteristics**

Variable	Body Mass Index (kg/m <sup>2</sup> )				
	<20 (n=445)	20-<25 (n=4,212)	25-<30 (n=5,404)	30-<35 (n=2,480)	≥35 (n=1,180)
Age (years)	54 (6)	54 (6)	54 (6)	54 (6)	53 (6)
Total cholesterol (mg/dL)	204 (40)	211 (40)	217 (42)	219 (44)	213 (40)
HDL cholesterol (mg/dL)	67 (21)	58 (18)	50 (16)	47 (14)	48 (14)
Triglycerides (mg/dL)	93 (43)	108 (61)	135 (88)	152 (106)	145 (99)
Systolic blood pressure (mmHg)	115 (19)	116 (18)	121 (18)	125 (18)	130 (19)
Diastolic blood pressure (mmHg)	69 (11)	71 (11)	74 (11)	76 (11)	78 (11)
Insulin resistance (mU*mmol/L <sup>2</sup> )	1.4 (2)	2.0 (7)	3.4 (8)	5.5 (11)	7.7 (14)
<u>Race-gender group (%)</u>					
White women	68	55	32	34	39
African American women	10	8	14	23	39
White men	11	29	44	33	16
African American men	10	8	10	11	7
<u>Education (%)</u>					
< High school	20	17	22	28	33
Finished high school	44	43	40	40	41
Some college	36	40	38	32	26
<u>Alcohol use (%)</u>					
Current drinkers	63	63	59	50	37
Former drinkers	17	16	18	20	23
Never drinkers	20	21	23	31	40
<u>Smoking status (%)</u>					
Current smokers	47	31	24	21	15
Former smokers	19	28	35	33	31
Never smokers	33	40	41	46	54
<u>Leisure time physical activity (%)</u>					
Highest tertile	25	33	31	23	15
Middle tertile	41	43	43	46	44
Lowest tertile	34	24	26	31	41
Health insurance (% with)	90	94	92	89	83
Family history of diabetes (%)	18	21	24	28	33
Family history of hypertension (%)	53	51	53	56	59
Family history of MI (%)	38	40	39	38	40
CHD events (n (%))	40 (9%)	438 (10%)	833 (15%)	424 (17%)	180 (15%)
Follow-up time (person-years)	6,522	65,084	81,522	36,941	17,865

**Table 4.3. Predicted differences in risk factors for 5-unit contrasts of BMI, the Atherosclerosis Risk in Communities Study**

	<i>BMI contrast</i>					
	<i>25 vs. 20 kg/m<sup>2</sup></i>		<i>30 vs. 25 kg/m<sup>2</sup></i>		<i>35 vs. 30 kg/m<sup>2</sup></i>	
	<b>Estimate</b>	<b>95% CI</b>	<b>Estimate</b>	<b>95% CI</b>	<b>Estimate</b>	<b>95% CI</b>
Total cholesterol (mg/dL)	<b>7.23</b>	5.90, 8.55	<b>3.69</b>	2.90, 4.49	0.16	-0.55, 0.88
HDL cholesterol (mg/dL)	<b>-8.88</b>	-9.44, -8.32	<b>-6.35</b>	-6.67, -6.03	<b>-3.82</b>	-4.08, -3.55
Log triglycerides	<b>0.25</b>	0.23, 0.27	<b>0.18</b>	0.17, 0.19	<b>0.10</b>	0.10, 0.11
Systolic blood pressure (mmHg)	<b>3.87</b>	3.32, 4.42	<b>3.52</b>	3.18, 3.85	<b>3.16</b>	2.85, 3.47
Diastolic blood pressure (mmHg)	<b>2.04</b>	1.71, 2.36	<b>1.75</b>	1.55, 1.94	<b>1.45</b>	1.27, 1.64
Log insulin resistance	<b>0.57</b>	0.54, 0.59	<b>0.46</b>	0.45, 0.47	<b>0.36</b>	0.34, 0.37

**Table 4.4. Predicted CHD hazard ratios (HRs) for standard deviation contrasts of risk factors, the Atherosclerosis Risk in Communities Study**

	<i>-1SD to Mean</i>		<i>Mean to +1SD</i>	
	<b>HR</b>	<b>95% CI</b>	<b>HR</b>	<b>95% CI</b>
<b><u>Mediators entered singly</u></b>				
Total Cholesterol (mean (SD) = 215 (42) mg/dL)	<b>1.34</b>	1.25, 1.44	<b>1.29</b>	1.23, 1.35
HDL cholesterol (SD = 17 mg/dL))	<b>0.66</b>	0.62, 0.71		
Log triglycerides (mean (SD) = 4.7 (0.5))	<b>1.43</b>	1.32, 1.55	<b>1.33</b>	1.27, 1.40
Systolic blood pressure (SD = 19 mmHg)	<b>1.32</b>	1.25, 1.38		
Diastolic blood pressure (mean (SD) = 74 (11) mmHg)	<b>1.10</b>	1.02, 1.18	<b>1.17</b>	1.12, 1.23
Log insulin resistance (mean (SD) = 0.86 (0.83))	<b>1.34</b>	1.23, 1.46	<b>1.41</b>	1.33, 1.49
<b><u>All mediators entered in one model</u></b>				
Total Cholesterol (mean (SD) = 215 (42) mg/dL)	<b>1.40</b>	1.30, 1.52	<b>1.31</b>	1.24, 1.38
HDL cholesterol (SD = 17 mg/dL))	<b>0.67</b>	0.61, 0.72		
Log triglycerides (mean (SD) = 4.7 (0.5))	1.00	0.90, 1.10	0.97	0.91, 1.04
Systolic blood pressure (SD = 19 mmHg)	<b>1.35</b>	1.27, 1.45		
Diastolic blood pressure (mean (SD) = 74 (11) mmHg)	<b>0.89</b>	0.82, 0.96	0.94	0.88, 1.01
Log insulin resistance (mean (SD) = 0.86 (0.83))	<b>1.14</b>	1.05, 1.24	<b>1.26</b>	1.18, 1.34

HRs are shown for standard deviation contrasts. For risk factors that had a quadratic relationship, two different contrasts are shown: from -1SD to the mean and from the mean to +1SD.

**Table 4.5. Predicted direct (unmediated) CHD hazard ratios (HRs) for 5-unit contrasts of BMI in mediation models, the Atherosclerosis Risk in Communities Study**

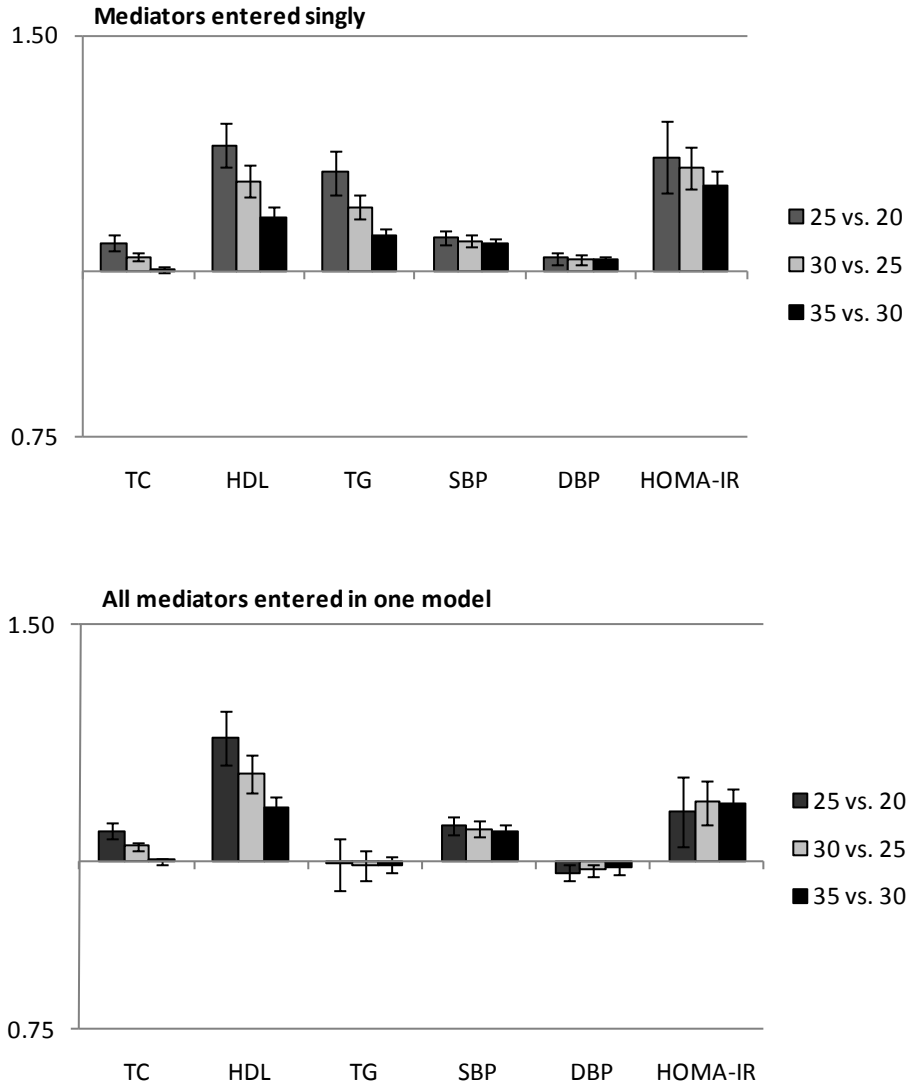
	<i>BMI contrast</i>					
	<i>25 vs. 20 kg/m<sup>2</sup></i>		<i>30 vs. 25 kg/m<sup>2</sup></i>		<i>35 vs. 30 kg/m<sup>2</sup></i>	
	<b>HR</b>	<b>95% CI</b>	<b>HR</b>	<b>95% CI</b>	<b>HR</b>	<b>95% CI</b>
No mediator	<b>1.39</b>	1.26, 1.54	<b>1.29</b>	1.22, 1.37	<b>1.20</b>	1.15, 1.26
All mediators together	0.94	0.85, 1.06	0.96	0.89, 1.03	0.98	0.92, 1.03
<u>Mediators entered singly:</u>						
Total cholesterol (mg/dL)	<b>1.34</b>	1.21, 1.48	<b>1.27</b>	1.20, 1.35	<b>1.21</b>	1.15, 1.26
HDL cholesterol (mg/dL)	<b>1.17</b>	1.05, 1.29	<b>1.14</b>	1.07, 1.22	<b>1.12</b>	1.07, 1.17
Log triglycerides	<b>1.19</b>	1.08, 1.32	<b>1.16</b>	1.09, 1.24	<b>1.13</b>	1.08, 1.19
Systolic blood pressure (mmHg)	<b>1.32</b>	1.19, 1.46	<b>1.23</b>	1.16, 1.31	<b>1.15</b>	1.09, 1.20
Diastolic blood pressure (mmHg)	<b>1.37</b>	1.23, 1.51	<b>1.27</b>	1.19, 1.35	<b>1.18</b>	1.12, 1.24
Log insulin resistance	1.10	0.99, 1.23	1.07	0.99, 1.14	1.03	0.98, 1.09

**Table 4.6. Predicted indirect (mediated) CHD hazard ratios (HRs)\* for 5-unit contrasts of BMI through established risk factors, the Atherosclerosis Risk in Communities Study**

	<i>BMI contrast</i>					
	<i>25 vs. 20 kg/m<sup>2</sup></i>		<i>30 vs. 25 kg/m<sup>2</sup></i>		<i>35 vs. 30 kg/m<sup>2</sup></i>	
	<b>HR</b>	<b>95% CI</b>	<b>HR</b>	<b>95% CI</b>	<b>HR</b>	<b>95% CI</b>
<b><u>Mediators entered singly</u></b>						
Total cholesterol (mg/dL)	<b>1.05</b>	1.03, 1.06	<b>1.02</b>	1.02, 1.03	1.00	1.00, 1.01
HDL cholesterol (mg/dL)	<b>1.24</b>	1.19, 1.29	<b>1.17</b>	1.14, 1.20	<b>1.10</b>	1.08, 1.12
Log triglycerides (log mg/dL)	<b>1.18</b>	1.14, 1.23	<b>1.12</b>	1.09, 1.14	<b>1.06</b>	1.05, 1.07
Systolic blood pressure (mmHg)	<b>1.06</b>	1.04, 1.07	<b>1.05</b>	1.04, 1.06	<b>1.05</b>	1.04, 1.06
Diastolic blood pressure (mmHg)	<b>1.02</b>	1.01, 1.03	<b>1.02</b>	1.01, 1.03	1.02	1.01, 1.03
Log insulin resistance (log mU*mmol/L <sup>2</sup> )	<b>1.22</b>	1.14, 1.29	<b>1.19</b>	1.15, 1.24	<b>1.16</b>	1.13, 1.19
<b><u>All mediators entered in one model</u></b>						
Total cholesterol (mg/dL)	<b>1.05</b>	1.04, 1.07	<b>1.03</b>	1.02, 1.03	1.00	1.00, 1.01
HDL cholesterol (mg/dL)	<b>1.24</b>	1.18, 1.29	<b>1.16</b>	1.13, 1.20	<b>1.10</b>	1.07, 1.12
Log triglycerides (log mg/dL)	1.00	0.95, 1.04	0.99	0.97, 1.02	0.99	0.98, 1.01
Systolic blood pressure (mmHg)	<b>1.06</b>	1.05, 1.08	<b>1.06</b>	1.04, 1.07	<b>1.05</b>	1.04, 1.06
Diastolic blood pressure (mmHg)	<b>0.98</b>	0.97, 0.99	<b>0.99</b>	0.98, 1.00	<b>0.99</b>	0.98, 1.00
Log insulin resistance (log mU*mmol/L <sup>2</sup> )	<b>1.09</b>	1.03, 1.16	<b>1.11</b>	1.07, 1.15	<b>1.11</b>	1.08, 1.14

\*HRs are predicted at mean values of covariates

**Figure 4.2. Predicted indirect (mediated) CHD hazard ratios (HRs)\* for 5-unit contrasts of BMI through established risk factors, the Atherosclerosis Risk in Communities Study**



\*HRs are predicted at mean values of covariates



## **V. IMPACT OF ANTIHYPERTENSIVE MEDICATION USE ON ASSOCIATIONS OF BODY MASS INDEX WITH BLOOD PRESSURE AND CORONARY HEART DISEASE IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY**

### **A. Abstract**

Antihypertensive medication use is highly prevalent, so it is important to understand the impact of medication use on observed associations of body mass index (BMI) with blood pressure (BP) and coronary heart disease (CHD) in the population. The objective of this study was to use of a variety of analytic strategies to aid in interpretation of observed associations. Data were from the Atherosclerosis Risk in Communities Study, a cohort of 15,792 White and African American adults aged 45-64 in 1987-89, followed through 2005. We used linear regression and Cox proportional hazards regression to compare untreated to treated participants. Models were adjusted for age, age<sup>2</sup>, gender, race, field center, physical activity, insurance, education, smoking, alcohol and family history of hypertension and myocardial infarction. In cross-sectional and mediation analyses, associations of BMI with BP and CHD were markedly attenuated in the treated compared to the untreated population. When the untreated population was limited to hypertensives, however, associations of BMI with BP were lower than in treated participants. New users of medication had similar associations between BMI and both SBP and DBP before and after initiating medication use; these associations were similar in magnitude to those seen in the treated participants. Associations of BMI with BP and CHD at the population level may be markedly reduced in treated compared to untreated individuals, but this may not be driven by effects of

medication use. Analysts should carefully consider the handling of medication status in analyses of observational data.

## **B. Background**

Thirty percent of U.S. adults 20 years and over had high blood pressure (systolic blood pressure (SBP)  $\geq 140$  mmHg, diastolic blood pressure (DBP)  $\geq 90$  mmHg or on blood pressure medication) in 1999-2002; another 28% of U.S. adults were pre-hypertensive (SBP 120-139 mmHg or DBP 80-89 mmHg) (Centers for Disease Control 2007). High blood pressure is associated with excess body mass (Ledoux, Lambert et al. 1997; Mokdad, Ford et al. 2003; Zhu, Heshka et al. 2004) and predicts coronary heart disease (CHD) (Pi-Sunyer 1993; Folsom, Stevens et al. 1998; Kim, Meade et al. 2006; Mann, Lee et al. 2006), the largest single cause of death in the United States (AHA 2007). Analyses of predictors and effects of high blood pressure (BP) must account for use of antihypertensive medications. Hypertension medication use has risen markedly in the last decade, especially among overweight and obese individuals (Hajjar, Kotchen et al. 2006; Ong, Cheung et al. 2007).

Associations among body mass index (BMI), blood pressure, CHD and antihypertensive medication use in the community are complex. The Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC) recommends that physicians add and/or change medications as needed to achieve a goal blood pressure of  $<140/90$  mmHg (Chobanian, Bakris et al. 2003). Initial BP is likely to be higher in obese persons, but the goal is lower ( $<130/80$  mmHg) for patients with diabetes or chronic kidney disease, both of which are associated with obesity (Chobanian, Bakris et al. 2003), so goals for change in blood pressure may be substantially higher in obese persons. Further, some medications may be contraindicated and/or less effective in overweight or

obese persons (Pi-Sunyer 2007). Given these complexities, it may be useful to examine and describe how medication use is associated with relationships among BMI, blood pressure and CHD at the community level.

Ideally, we would like to know not only how medication use is associated with these relationships, but if the medication itself is the cause of those differences. However, causal inferences about medication use from cohort data require caution because of confounding by indication. Medication use may be associated with increased rather than reduced blood pressure in a community sample because antihypertensive medications are indicated for high blood pressure, and even with treatment the average blood pressure of treated persons may be higher than that of the rest of the population. A medication use variable may then function as an indicator of hypertension rather than reflecting effects of medication on blood pressure. Observed associations of medication use with other variables in the community would therefore be at least partly confounded (Tobin, Sheehan et al. 2005). For this reason, analyses of medication use in cohort data are few. However, given the high prevalence of antihypertensive use (18% of all U.S. adults (Cutler, Sorlie et al. 2008) and 45% of those aged 60 and over (Ostchega, Dillon et al. 2007) (author's calculations from tables in cited references)), it is important to understand the impact of medication use on observed associations in the community. A careful examination of observed associations could have substantive and methodological value, and use of a variety of analytic strategies can aid in interpretation of observed associations. We are not aware of any study that has published such data.

The objective of this research was to investigate the impact of antihypertensive medication use in cohort data on the observed relationships among BMI, blood pressure and

coronary heart disease. We used several methodologies to examine differences by antihypertensive medication status in associations between BMI and blood pressure. We also examined differences by medication use status in mediation by blood pressure of the relationship between BMI and CHD events.

## **C. Methods**

### **1. Study population**

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective investigation of atherosclerosis and cardiovascular disease in a cohort of 15,792 African American and White adults aged 45-64 at baseline (1987-1989). Participants were from four communities in the United States: Forsyth County, NC, Jackson, MS, Minneapolis, MN, and Washington County, MD. Participants were examined at four study visits spaced approximately 3 years apart. Details of the study design have been described previously (ARIC Investigators 1989). Races other than African American or White (n=48) and African Americans in Minneapolis and Washington County (n=55) were excluded per standard ARIC protocol because their numbers are too small to allow ethnic and field-center specific analyses. The study was approved by the Institutional Review Board (IRB) at each field center, and this analysis was approved by the University of North Carolina Public Health-Nursing IRB on research involving human subjects.

### **2. Measurements**

Body weight was measured at all clinic visits in a scrub suit to the nearest pound by use of a beam balance scale. Height (without shoes) was measured to the nearest centimeter using a metal rule and a standard triangular headboard. BMI was calculated as weight in kilograms divided by height at baseline in meters squared. Systolic and diastolic blood

pressures were measured three times after a 5-minute rest using a random zero sphygmomanometer on the right arm of the seated participant. The last two measures were averaged and recorded.

Coronary heart disease is defined as: cardiac death, non-fatal myocardial infarction (MI), silent MI detected by echocardiogram, or cardiovascular surgery or coronary angioplasty (White, Folsom et al. 1996). Follow-up on CHD events was available through December 31st, 2005. For hazard analyses, time to event is defined as time from the subject's baseline visit to the event date or to the censored date. Subjects were classified as taking antihypertensive medications if they self-reported taking antihypertensive medications in the last two weeks or if the medications they brought to their clinic visit included an appropriately classified medication (alpha blocker, beta blocker, calcium channel blocker, ace inhibitor, angiotensin II receptor blocker, centrally acting adrenolytic, vasodilator or diuretic).

Age (date of birth), race/ethnicity, and gender were self-reported. Additional covariates were assessed by interviewer-administered questionnaires. We categorized education as less than a high school education, high school graduate, or at least some college. Physical activity was assessed with the Baecke leisure time physical activity questionnaire (Baecke, Burema et al. 1982) and categorized in tertiles. A 66-item, semi-quantitative food frequency questionnaire was used to assess diet (Willett, Sampson et al. 1985). We examined total energy and sodium, but these variables were dropped because they had no impact on any of the associations examined. Self-reported cigarette smoking status and alcoholic beverage consumption were categorized as current, former, or never. Participants were asked about a number of possible sources of health insurance, categorized for this

analysis as insured or uninsured. Participants were also asked if either parent had a history of myocardial infarction and of hypertension, each of which was categorized as yes or no. A race-field center variable was created because not all field centers had both race groups.

### **3. *Datasets and exclusions***

For the baseline data, we excluded participants missing BMI (n=40), SBP or DBP (n=9), antihypertensive medication status (n=8) or covariates (n=157). The final baseline dataset contained 15,475 participants.

For the analysis that included data from all study examinations, we created a “stacked” dataset such that each participant had between 1 and 4 repeated observations. We excluded observations missing BMI (obs=172), SBP or DBP (obs=17), antihypertensive medication status (obs=8) or covariates (obs=963). The final dataset contained 15,475 participants with 53,180 observations.

For the new user data, we constructed a dataset comprising individuals who reported taking antihypertensive medication at examination 2, 3 or 4 and were not taking medication at any of their prior visits (n=2,773). We excluded individuals missing blood pressure (n=2), BMI (n=11) or covariates (n=41) at either visit, for a total sample of 2,719.

### **4. *Statistical analyses***

#### **a. BMI - blood pressure analyses**

We examined differences by antihypertensive medication status in the association of BMI with systolic and diastolic blood pressure four different ways using baseline and stacked datasets. We first compared the treated population to the entire untreated population, which included untreated hypertensives and normotensives, and then stratified on blood pressure level (stacked dataset only) in order to compare treated persons to untreated hypertensives.

We also examined the association between BMI and blood pressure in the new users pre- and post-treatment.

Linear regression (PROC REG in SAS 9.1, SAS Institute, Cary, NC) was used with the baseline and new user data to evaluate the association of BMI with systolic and diastolic blood pressure in untreated compared to treated participants. For the stacked data, we used linear regression in SAS PROC MIXED. We used fixed effects with repeated measures and specified an unstructured correlation matrix, which provided the best model fit. We repeated these analyses dividing the untreated participants into untreated normotensives (SBP<140 mmHg and DBP<90 mmHg) and untreated hypertensives (SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg). We divided the treated participants in the same way for comparison.

For all cross-sectional analyses, BMI and BP variables were continuous. Higher order terms were considered for BMI and for age, and a BMI-age interaction was also assessed. Differences by medication status were assessed using the Wald test. All cross-sectional analyses were adjusted for age, age-squared, gender, race-field center, family history of hypertension, smoking, alcohol use, physical activity, education and insurance status. The stacked and new user analyses were also adjusted for calendar year.

b. Mediation analyses

We examined total, indirect (mediated by blood pressure) and direct (not mediated by blood pressure) effects of BMI on CHD by antihypertensive medication status. Systolic and diastolic blood pressures were examined as mediators in separate and combined models.

For the mediation analysis, exclusions were the same as in the baseline data with the additional exclusion of persons with prevalent (n=756) or missing (n=310) CHD at baseline. Final sample sizes were 10,302 untreated and 4,107 treated participants. There were 1,246

(12.1%) incident CHD cases among the untreated participants and 787 (19.2%) among the treated participants.

We used structural equation models in Mplus 5.0 to assess effects of baseline BMI on time to CHD through baseline blood pressure variables. Structural equation models incorporate several regression equations simultaneously to obtain path coefficients for each relationship. The conceptual model for the mediation analysis is illustrated in Figure 5.1. Linear regression was used to examine the relationship between continuous BMI and blood pressure variables (path A), and Cox proportional hazards regression was used to examine the relationships between blood pressure and CHD (path B) and between BMI and CHD (path C', the direct effect). Path C (the total effect, not shown) is the path from BMI to CHD in a model with no mediator.

The indirect (mediated) effect of BMI on CHD through a given blood pressure measure was calculated by multiplying the path coefficients from paths A and B, where A is the change in the blood pressure measure for a 5-unit increment of BMI, and B is the change in the log hazard ratio (HR) of CHD for a 1-unit increment of blood pressure. The indirect effect is therefore the change in the log CHD hazard ratio for a 5-unit increment of BMI, acting through that blood pressure measure. Exponentiation of this parameter yields an indirect hazard ratio for the effect of BMI on CHD through that measure. The standard error for the indirect effect was calculated using the delta method. All mediation models were stratified by baseline antihypertensive medication status and were adjusted for the same variables as the cross-sectional analysis plus family history of myocardial infarction in portions of the model with CHD as the outcome. Differences by medication status were assessed using a Wald test.



## **D. Results**

### **1. BMI - blood pressure analysis**

Sample characteristics at baseline and for the new user analysis are shown in Table 5.1 by antihypertensive medication treatment status. The stacked data (not shown) were similar to the baseline data in patterns of demographic and behavioral characteristics by treatment group. In the baseline data, treated participants had higher mean BMI than untreated participants. They were also older and more likely to be African American, female, without health insurance and to report low levels of physical activity. They were less likely to report being current smokers or drinkers. The new users reported some changes in behaviors between visits, in particular reductions in the prevalences of current smoking and drinking. There were small decreases in physical activity and in the percent uninsured. Table 5.2 shows the levels of SBP and DBP in the untreated compared to the treated observations for each of the three analysis datasets. Systolic blood pressure was 10 mmHg higher in the treated than in the untreated observations for both the baseline and stacked data, and diastolic was 3-5 mmHg higher in the treated observations. In contrast, there was a 6 mmHg drop in blood pressure from the pre-treatment visit to the post-treatment visit in the new users.

In the models not stratified by treatment status, the antihypertensive medication status - BMI interaction was highly significant ( $p < 0.0001$ ) for both blood pressure measures in the baseline and stacked data. The cross-sectional associations between BMI and SBP and DBP (Table 5.3) were much stronger in untreated than in treated participants for the baseline and stacked data. At baseline, for every 1-unit increment in BMI, SBP increased by 0.71 mmHg (95% CI: 0.65, 0.78) in the untreated compared to a substantially lower increase (0.31 mmHg

(95% CI: 0.21, 0.41)) in subjects taking antihypertensive medication. Similar patterns were observed for DBP (0.35 mmHg (95% CI: 0.31, 0.38) in the untreated vs. 0.09 mmHg (95% CI: 0.03, 0.14) in the treated). Associations in the stacked data were somewhat stronger than in the baseline data, but differences by treatment status followed the same pattern in both datasets. In contrast to the results from the baseline and stacked data, the associations between BMI and both SBP and DBP were not different in the new users at their post-treatment visit compared to their pre-treatment visit (approximately three years earlier). The magnitude of the BMI-BP associations observed in the new users at both visits was similar to what was seen in the treated participants in the other two analyses.

Table 5.4 shows the results from the stacked analyses stratified by blood pressure levels. There were many more normotensive than hypertensive observations. In both high and low blood pressure observations, the association was reduced in the treated compared to the untreated. The associations between BMI and both systolic and diastolic blood pressure among the observations with high blood pressure were markedly reduced compared to the associations seen in those with lower blood pressure. However, we did not see a curved relationship when we assessed the quadratic association of BMI with BP in the unstratified models.

## **2. *Mediation analysis results***

All effects of BMI (total, indirect and direct, as well as A paths) were substantially stronger in untreated than in treated participants. The adjusted HR for the effect of a 5-unit increment of BMI on CHD with no mediators in the model (the total effect) was 1.23 (95% CI: 1.16, 1.31) in untreated participants and a significantly lower 1.09 (95% CI: 1.03, 1.15) in treated participants ( $p=0.003$ , Figure 5.2a).

Figures 5.2b - 5.2d show beta coefficients for the effect of a 5-unit increment of BMI on SBP and DBP (the A paths) and HRs for the effects of 10-unit increments of SBP and DBP on CHD (B paths). While the effects of BMI on systolic and diastolic blood pressure (A paths) were substantially larger in untreated than in treated participants, in contrast, the B path effects of blood pressure on CHD were almost identical in the treated and untreated participants.

The direct effect of BMI on CHD was attenuated in mediation models (C' paths, Figures 5.2b-5.2d) compared to the model without mediators (C path, Figure 5.2a), but BMI remained a significant direct predictor of CHD for both untreated and treated participants. The direct effect of BMI was not attenuated by addition of DBP to the model containing SBP (comparing Figure 5.2d to 5.2b) in either untreated or treated participants.

Table 5.5 shows the indirect (A times B) effects of BMI on CHD through blood pressure. All confidence intervals excluded the null except for the effect through treated DBP with SBP in the model. Indirect effects were greater through systolic than through diastolic blood pressure and in untreated than in treated participants. The smaller indirect effects in treated participants were almost entirely due to the much smaller A coefficients. In the combined model, the indirect effect through SBP was increased when DBP was added to the model in the untreated participants, while the indirect effect through DBP became weakly protective.

## **E. Discussion**

Physicians are advised to adjust patient medications until goal levels of blood pressure are reached (Chobanian, Bakris et al. 2003). Therefore, in a community sample, we expected to see reduced associations of BMI with blood pressure and with CHD mediated by

blood pressure in treated individuals compared to untreated individuals, which is indeed what was observed. In the baseline and stacked data analyses, associations between BMI and blood pressure were substantially smaller in participants using antihypertensive medication compared to participants not taking such medication. We expected that the new user analysis we conducted would confirm these findings, but we observed no difference in the associations of BMI with systolic or diastolic blood pressure between the participants pre- and post-treatment.

The associations observed in the new user data were comparable in magnitude to those observed in the treated individuals in the other two analyses and greater than those observed in the untreated hypertensives. These observations suggested that the differences we observed in the baseline and stacked data might have been due to factors other than effects of medication use on blood pressure. Differences between the populations, rather than effects of treatment, might have led to the observed differences. The two populations were different on many baseline characteristics (Table 5.1), including blood pressure. We adjusted for these characteristics in the pooled analyses, but there were undoubtedly some unmeasured differences.

Observed differences could also have been the result of truncation of the data associated with subdividing the populations. A plot of SBP on BMI at baseline illustrates this problem (Figure 5.3). The lowest blood pressures occur only in individuals with low BMI, whereas high blood pressure occurs across the range of BMI. It is readily apparent from the figure that subdividing these individuals into high and low blood pressure will result in very different observed effects of BMI on SBP in the two groups, as we have indeed seen (Table 5.4). However, this apparent interaction is not indicative of a curved relationship

between BMI and blood pressure in either treated or untreated participants. Lower observed associations of blood pressure with BMI in the treated and new user groups may be driven by the elimination of some individuals with very low blood pressure from the dataset rather than by reductions in blood pressure caused by use of medication.

We conducted an additional analysis of the association of BMI with change in BP in new users compared to individuals who never reported treatment and to those who remained on antihypertensive medication across consecutive study examinations. Both SBP and DBP decreased in the new users (-5.1 mmHg and -4.7 mmHg, respectively). SBP increased slightly among participants whose medication status did not change (3.7 mmHg for those who were taking antihypertensive medication at both visits and 2.4 mmHg for those who were not), but DBP did not change or decreased (0.08 mmHg and -1.3 mmHg, respectively). Associations of mean BMI with change in blood pressure across consecutive visits were very small (less than 0.5 mmHg for a 5-unit increment of BMI) regardless of continuity or change in antihypertensive medication status. Obesity can diminish physiologic response to medication but may also affect choice of medication and dosage (Pi-Sunyer 2007). These effects might cancel each other out, or might be too weak to cause any association of change in blood pressure with BMI.

We are not aware of any other studies examining effects of BMI on blood pressure by medication status. Randomized trials of antihypertensive medication do not often examine how treatment affects associations of blood pressure with other risk factors such as BMI. Other studies with blood pressure outcomes typically dichotomize blood pressure into hypertensive (which includes people on medication) and not hypertensive, by-passing the need to address how medication use affects blood pressure. Previous work in the ARIC

cohort examined effects of weight loss on blood pressure in treated participants compared to untreated. Weight loss in treated participants was associated with slightly smaller decreases in blood pressure and less remission of hypertension than in untreated participants, but, as in this analysis, the authors could not account for reductions in medication number or dosage which may have occurred (Juhaeri, Stevens et al. 2003).

Consistent with other longitudinal studies (Jousilahti, Tuomilehto et al. 1996; Bogers, Bemelmans et al. 2007), BMI predicted CHD in the ARIC study. The association was mediated, in part, by blood pressure, particularly systolic pressure. Overall, the association between BMI and CHD was attenuated in treated individuals. This was true for the direct path (not through BP), as well as for the indirect (BP mediated) path. Other papers addressing mediation of the effect of BMI on CHD by blood pressure have found similar mediation effects, but have not addressed the possible impact of medication use on their analyses (Jousilahti, Tuomilehto et al. 1996; Tanne, Medalie et al. 2005; Kim, Meade et al. 2006). A meta-analysis of 21 cohort studies (Bogers, Bemelmans et al. 2007) found a relative risk of CHD for a 5-unit change in BMI of 1.29 (95% CI: 1.22, 1.35). Comparable HRs from the present analysis tended to be slightly smaller. In the meta-analysis the HR for BMI was attenuated to 1.16 (95% CI: 1.11, 1.21) with both blood pressure and cholesterol in the model. We conducted a supplementary analysis (not shown) in which the addition of total cholesterol, high-density lipoprotein cholesterol and insulin resistance (from the Homeostasis Model Assessment equation) to the model eliminated the direct effect of BMI, but SBP and DBP paths changed very little.

Associations between blood pressure and CHD in this study were not different in the treated and untreated groups. A few studies have examined differences by treatment status in

the effects of blood pressure on CHD (the “B” paths in this analysis). In the Copenhagen II cohort, HRs increased with increasing blood pressure categories in untreated subjects, but were null in treated subjects (Jensen, Nyboe et al. 1991). These results differed from the present study, which found no difference. An analysis of the NHANES I Follow Up Study data (baseline age 33-87) found HRs for the effects of SBP and DBP on CHD in treated participants very similar in magnitude to those presented here (Greenberg 2005). The HRs in untreated hypertensives (they excluded normotensives) were greater than what we found, but did not appear to be statistically different from their treated participants (they did not test this hypothesis formally, but confidence intervals were shown). In addition, a secondary analysis of randomized medication trial data in 4,632 adults  $\geq 60$  years with isolated systolic hypertension attempted to address differences in effects of BP on CHD by treatment status, but after 5 years there were no significant effects of blood pressure on CHD in the treatment or placebo groups (Vaccarino, Berger et al. 2001).

The direct effect of BMI on CHD (i.e., the effect not mediated by either SBP or DBP) was lower in treated participants. This implies that antihypertensive medication was associated with both BMI and CHD independently of blood pressure and other variables for which we adjusted, and may therefore be confounded. This agrees with our conclusion from the cross-sectional analyses that differences in treatment groups may be due to factors other than effects of medication use. In addition to differences in covariate values, there were also differences in overall CHD risk between treated and untreated participants, and there are likely to be differences in other metabolic CHD risk factors that could mediate the BMI-CHD relationship. Another possible complication is that some blood pressure medications also have effects on weight, lipids and glucose (Pi-Sunyer 2007). Diuretics can reduce (water)

weight, increase insulin resistance and, in high doses, contribute to dyslipidemia. Beta-blockers can cause weight gain, contribute to impaired glucose metabolism and can raise triglycerides and lower HDL-cholesterol. Alpha-blockers, on the other hand, can increase insulin sensitivity and lower triglycerides and LDL-cholesterol (Pi-Sunyer 2007). Further, participants treated with antihypertensive medications were more likely to be taking glucose and lipid-lowering medications in our data. Effects of all of these medications on risk factors for CHD may serve to dilute the association between obesity and CHD in treated individuals. Future work in this area should carefully consider the types of antihypertensive medications used.

There has been much discussion in the literature concerning the correct way to measure the effect of obesity on overall mortality and to estimate the number of lives lost due to obesity (Durazo-Arvizu, McGee et al. 1998; Allison, Fontaine et al. 1999; Flegal, Graubard et al. 2005). It is generally agreed that mediators (e.g. blood pressure) should not be included in models as covariates when estimating the risk associated with elevated BMI on an outcome such as CHD or mortality. There is less agreement concerning the possibility that pharmaceutical treatments for hypertension and cholesterol may impact results (Mark 2005; Flegal, Graubard et al. 2007; McTigue and Kuller 2008; Whitlock, Lewington et al. 2009) or help to explain the decrease in the association of obesity with mortality over time observed in some analyses (Ding 2005). This raises the question of whether the intent of the study is to estimate the risk of BMI in the population as it currently exists (with about 1/5 of US adults taking antihypertensive medications) or to estimate the theoretical, potential effect of elevated BMI were medications not used. The former estimate can be obtained given population-based data, whereas the latter effect is more difficult to estimate in a population



like that of the United States. If the association of BMI with blood pressure is markedly lower in those who use antihypertensive medications, statistical adjustment for medication use would likely yield an observed effect of BMI on outcomes associated with blood pressure (such as CHD and mortality) that is intermediate between the effect expected in the non-medicated and the attenuated effect seen among the medicated. Analyses of only non-medicated individuals could result in an underestimate of the effect that would be found were the entire sample not on medication, since a large portion of individuals prone to hypertension will have been removed from the sample. The proper handling of medication in these situations depends on whether observations of differences across medication status reflect effects of medication or are confounded by indication. Our results suggest that at least partial confounding is likely. Examination of young adults in whom medication use is rare could be helpful in sorting out these effects, but long follow-up periods would be needed to collect CHD or mortality events, and information on the initiation of medications over time collected.

This analysis had several limitations. The untreated participants in this analysis include both normotensive and hypertensive individuals. We examined associations in these subgroups separately, but untreated hypertensives are not directly comparable to treated participants. The former may be untreated for a variety of reasons and may have previously been treated. In short, this population is representative of the community, but is not properly controlled to assess causal effects of medication use. We had no information on medication dosage per se, and incomplete data on types of antihypertensive medications did not allow us to examine this variable. The age range of the cohort studied here allowed the observation of CHD events, but meant that obesity, blood pressure and medication status earlier in life were

not measured. The simultaneous inclusion of SBP and DBP in the combined model could have lead to bias in one or both of the estimates of BP effects on CHD (B paths) due to correlated errors. Though we cannot rule out such bias, the combined model estimates are consistent with known effects of pulse pressure (Vaccarino, Holford et al. 2000; Miura, Dyer et al. 2001; Lewington, Clarke et al. 2002).

Medication use was assessed as usage in the past two weeks, and while we excluded persons who were taking medication at prior visits, we cannot be sure that our “new” users have not taken medication at any point in the past. Also, the new users may have been on medication for only a few weeks or for 3 or more years. Medication doses may have been adjusted over time and such adjustments may have differed by weight status. Further, blood pressure values in our new user analysis may be particularly subject to regression to the mean from the first visit to the second because letters were sent to the physicians of participants with high observed blood pressure at each visit; individuals whose measured blood pressure was at the high end of their usual range at a particular visit may have been more likely to be on medication at the next visit than individuals with the same usual blood pressure but no spuriously high measurement at their prior ARIC visit. In sum, while the new user analysis adds to our understanding of the impact of medication use in this cohort, our finding that medication use does not change the association between BMI and blood pressure in this group is not strong evidence of a null effect.

Strengths of this study include the excellent data quality and multiple examinations in the ARIC cohort. The use of several different analytic methods to examine how medication use modified associations of BMI with blood pressure and CHD provided a broad picture of its potential impact on analyses of cohort data. We caution against interpretations of our

results as addressing causal effects of medication use. Antihypertensive medication use in the community is profoundly confounded by indication – i.e., only persons with high blood pressure are given antihypertensive medication, and thus medication use is highly associated with disease at the community level. This contrasts sharply with actual effects of medication use, which reduce disease in randomized controlled trials (Neaton, Grimm et al. 1993). We reported the associations of BMI with blood pressure and CHD by antihypertensive medication use status and used various strategies to try to understand how medication use might be affecting the associations in this cohort.

This study illustrates the impact of antihypertensive medication use on observed associations among BMI, BP and CHD in population-level data and does not necessarily imply that body mass is less important as a risk factor for disease in treated individuals. Indeed, excess body weight may have been an important factor in the manifestation of hypertension and the need for antihypertensive medication in many of our participants. Further, given that high blood pressure is more resistant to effects of treatment in obese individuals (Cushman, Ford et al. 2002; Pi-Sunyer 2007), an analysis of subjects randomized to treatment might expect to find a stronger association of body mass with BP in treated individuals rather than the weaker association seen here. An analysis of randomized trial data could address causality but may suffer from different limitations due to a narrower range of starting BP levels and potential lack of generalizability. We are not aware of any such analyses to date.

Future analyses in cohort data might also prove illuminating. When we compared the associations seen in the treated population and new users to the subset of the untreated population that had high blood pressures levels (those more likely to become new users), we

observed that the associations between BMI and BP in the untreated hypertensive individuals were even lower than the associations in the treated and new user populations. This analysis could be further refined with the use of propensity scores (predicting likelihood of treatment using a vector of covariates) to provide a better matched untreated group for comparison to the treated population.

Associations of BMI with blood pressure and CHD were attenuated in individuals treated with antihypertensive medications compared to individuals not taking such medications in this cohort, even for the effect on CHD independent of blood pressure. In contrast, analyses of the association between BMI and BP limited to the same individuals before and after beginning treatment showed no differences by treatment status, providing evidence that the attenuation observed in the overall cohort may have been attributable to factors other than causal effects of medication use. However, the intra-individual analysis also suffered from limitations. More research, possibly including secondary analyses of clinical trial data, could help to clarify the causal relationships. Meanwhile, analysts of observational data should consider the potential impact of the handling of antihypertensive medication treatment on analyses of body mass index and cardiovascular outcomes regardless of whether blood pressure is explicitly included in the analysis.

**Figure 5.1: Mediation analysis paths**

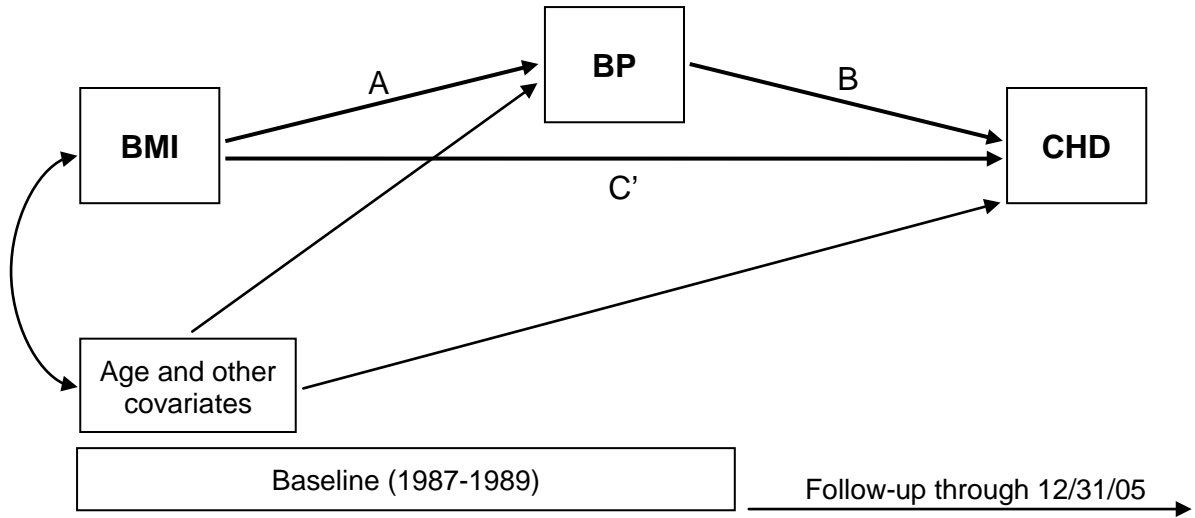


Figure 5.1 legend: Conceptual model for the mediation analysis. BMI, BP and covariates were measured at baseline. Linear regression was used to examine the relationship between BMI and blood pressure variables (path A), and Cox proportional hazards regression was used to examine the relationships between blood pressure and CHD (path B) and between BMI and CHD (path C'). Path C (not shown) is the path from BMI to CHD in a model with no mediator.

**Table 5.1. Means (SD) and frequency distributions of selected sample characteristics by antihypertensive treatment status in the Atherosclerosis Risk in Communities Study**

Variable	Baseline data		New user data	
	Untreated (N=10,733)	Treated (N=4,742)	Time 1 (pre-treatment)	Time 2 (post-treatment)
Body mass index (kg/m <sup>2</sup> , mean (SD))	27 (5)	30 (6)	29 (5)	29 (5)
Age (years, mean (SD))	54 (6)	55 (6)	57 (6)	60 (6)
Race (% African American)	22	38	26	26
Gender (% female)	53	60	50	50
Education (% < high school)	20	32	22	22
Health insurance (% without)	9	12	8	7
Alcohol use (% current drinkers)	60	47	55	50
Cigarette smoking (% current smokers)	27	24	22	17
Physical activity (% lowest tertile)	26	33	27	26
Family history of hypertension (% with)	49	63	54	54
Family history of myocardial infarction (% with)	38	42	–	–

**Table 5.2. Mean (SD) systolic and diastolic blood pressure by antihypertensive treatment status for three analytic datasets in the Atherosclerosis Risk in Communities Study**

	Baseline data		Stacked data		New user data	
	Untreated ( <i>N</i> =10,733)	Treated ( <i>N</i> =4,742)	Untreated ( <i>obs</i> =34,260)	Treated ( <i>obs</i> =18,920)	Pre-treatment ( <i>obs</i> =2,719)	Post-treatment ( <i>obs</i> =2,719)
Systolic blood pressure (mmHg)	118 (17)	128 (20)	120 (17)	130 (20)	133 (20)	127 (18)
Diastolic blood pressure (mmHg)	72 (11)	77 (12)	71 (10)	74 (11)	78 (12)	72 (11)

**Table 5.3. Increase in systolic and diastolic blood pressure for a 1-unit increment of body mass index in the Atherosclerosis Risk in Communities Study**

	Baseline data				Stacked data				New user data			
	Untreated*		Treated*		Untreated*		Treated*		Pre-treatment		Post-treatment	
	<i>(N=10,733)</i>		<i>(N=4,742)</i>		<i>(obs=34,260)</i>		<i>(obs=18,920)</i>		<i>(obs=2,719)</i>		<i>(obs=2,719)</i>	
	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI
Systolic blood pressure (mmHg)	0.71	0.65, 0.78	0.31	0.21, 0.41	0.91	0.86, 0.96	0.38	0.32, 0.45	0.37	0.22, 0.51	0.34	0.21, 0.47
Diastolic blood pressure (mmHg)	0.35	0.31, 0.38	0.09	0.03, 0.14	0.43	0.40, 0.46	0.14	0.10, 0.17	0.12	0.04, 0.21	0.11	0.04, 0.18

Adjusted for age, age-squared, gender, race-field center, family history of hypertension, smoking, alcohol use, physical activity, education and insurance status, as well as calendar year for the stacked and new user analyses.

\*P<0.001 for differences by antihypertension medication status (untreated vs. treated), assessed using the Wald test.



**Table 5.4. Increase in systolic and diastolic blood pressure for a 1-unit increment of body mass index in the Atherosclerosis Risk in Communities Study**

	Antihypertensive medication status							
	<u>Normotensive (SBP&lt;140 and DBP&lt;90)</u>				<u>Hypertensive (SBP≥140 or DBP≥90)</u>			
	Untreated (obs = 29,525)		Treated (obs = 13,352)		Untreated (obs = 4,735)		Treated (obs = 5,568)	
	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI	$\beta_{\text{BMI}}$	95% CI
Systolic Blood Pressure (mmHg)	0.67	0.63, 0.71	0.31	0.27, 0.35	0.11	0.04, 0.19	-0.03	-0.10, 0.05
Diastolic Blood Pressure (mmHg)	0.34	0.31, 0.37	0.11	0.08, 0.14	0.02	-0.04, 0.07	-0.07	-0.12, -0.01

\*Adjusted for age, age-squared, gender, race-field center, family history of hypertension, smoking, alcohol use, physical activity, education and insurance status.

\*\*Differences by antihypertension medication status (untreated vs. treated) were assessed using the Wald test.

**Figure 5.2: Mediation paths by antihypertensive treatment status**

Figure 5.2a. No mediation

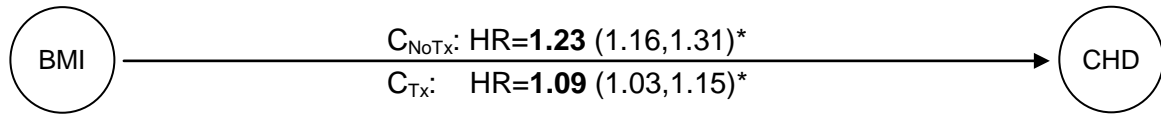


Figure 5.2b. Mediation by systolic blood pressure

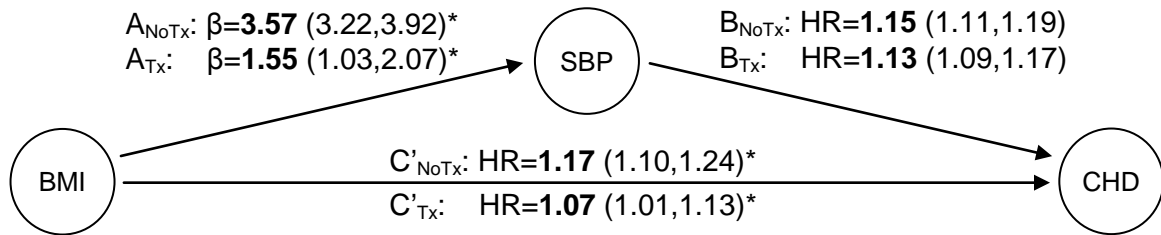


Figure 5.2c. Mediation by diastolic blood pressure

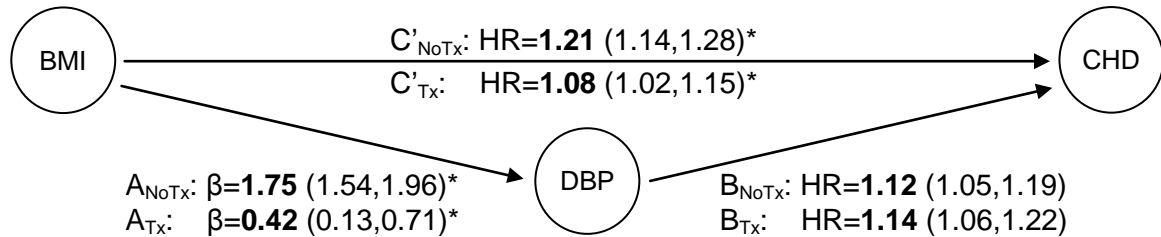


Figure 5.2d. Mediation by systolic and diastolic blood pressure

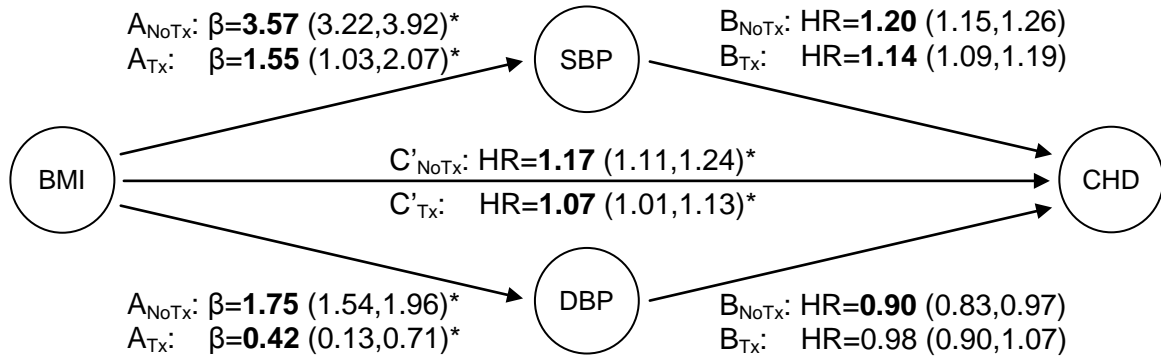


Figure 5.2a-d legend:

Beta coefficients (A paths) and hazard ratios (HRs) (exponentiated B and C' path coefficients) for structural equation models of the relationships among body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and coronary heart disease (CHD). BMI effects are for a 5-unit increment of BMI. BP effects are shown for a 10-unit increment of the BP measure. All models were adjusted for age, age-squared, gender, race-field center, family history of hypertension and myocardial infarction, smoking, alcohol use, physical activity, education and insurance status. Estimates in bold type are significantly different from zero.

\* Estimates are different between treatment groups (Wald test,  $\alpha=0.05$ ).

**Table 5.5. Indirect effects\* of body mass index on coronary heart disease through blood pressure, by antihypertensive treatment status in the Atherosclerosis Risk in Communities Study**

	Antihypertensive medication status				<i>P</i> -value**
	Untreated (N=10,302)		Treated (N=4,107)		
	Indirect effect	95% CI	Indirect effect	95% CI	
<u>Single-mediator models:</u>					
SBP alone (Fig. 2b)	<b>1.05</b>	1.04, 1.06	<b>1.02</b>	1.01, 1.03	<0.001
DBP alone (Fig. 2c)	<b>1.02</b>	1.01, 1.03	<b>1.01</b>	1.00, 1.01	0.022
<u>Combined model:</u>					
SBP (with DBP, Fig. 2d)	<b>1.07</b>	1.05, 1.09	<b>1.02</b>	1.01, 1.03	<0.001
DBP (with SBP, Fig. 2d)	<b>0.98</b>	0.97, 1.00	1.00	1.00, 1.00	0.017

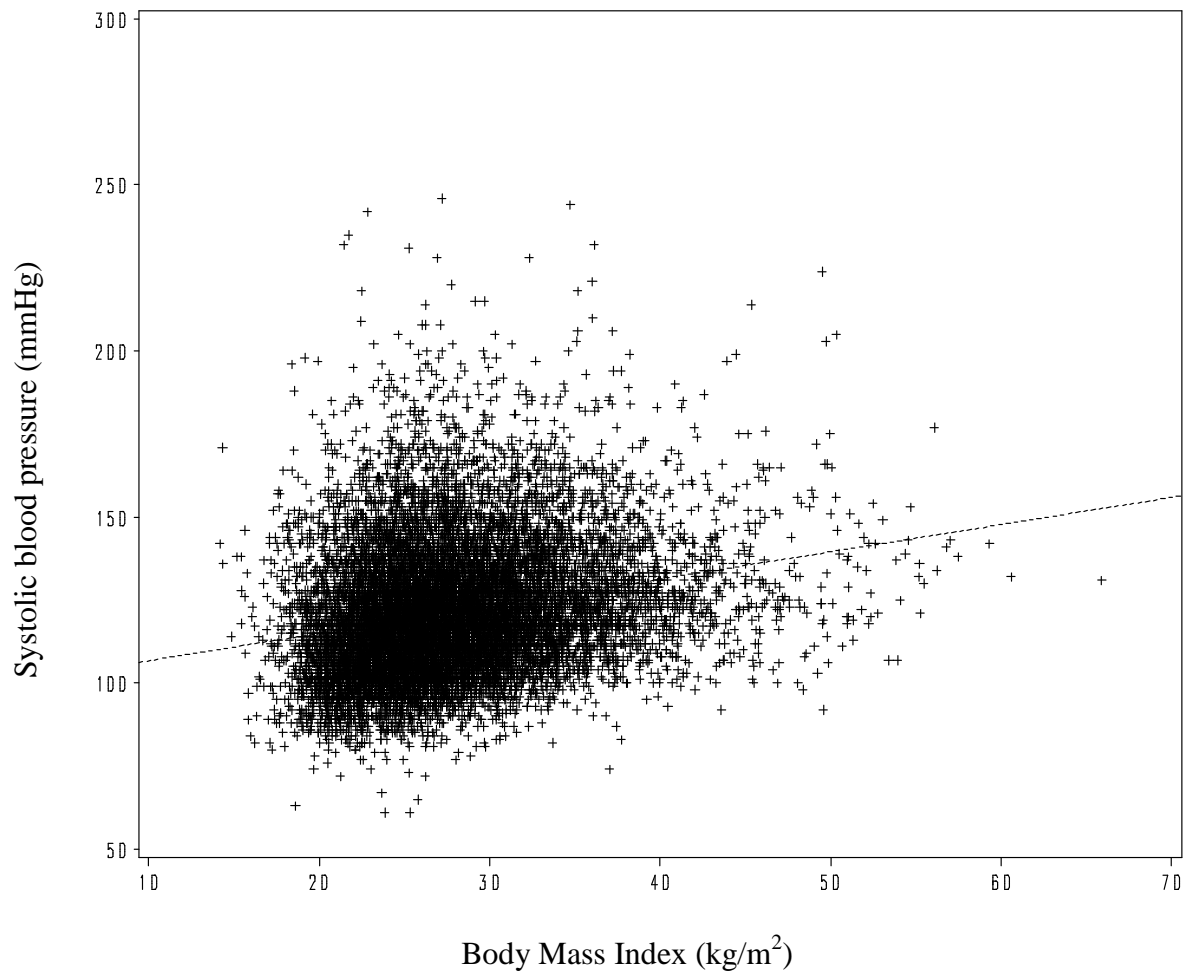
Indirect effects are calculated as the A path times the unexponentiated B path (log of the B paths shown in Figure 2). 95% CIs were calculated using the delta method.

\* All models adjusted for age, age-squared, gender, race-field center, family history of hypertension and myocardial infarction, smoking, alcohol use, physical activity, education and insurance status.

\*\*Differences by antihypertension medication status (untreated vs. treated) were assessed using the Wald test.

SBP=systolic blood pressure. DBP=diastolic blood pressure.

**Figure 5.3. Plot of systolic blood pressure on body mass index at baseline**



## **VI. SYNTHESIS**

### **A. Overview**

The primary aim of this dissertation was to examine mediation of the effects of obesity on CHD through traditional physiologic mediators in a bi-racial cohort of older adults. We observed complete mediation of BMI's effects on CHD through the mediators examined. I.e., there was no longer a direct effect of BMI on CHD when its indirect effects through the hypothesized mediators were all accounted for. This is consistent with much of the existing literature on mediation of the BMI-CHD relationship.

The most novel aspect of our primary aim lies in the comparison of individual mediation paths with all mediators examined together. These results were particularly interesting because of the curved nature of the relationships we found among BMI, the mediators and CHD. The total effect of BMI on CHD declined with increasing BMI, as did BMI's associations with all of the mediators, particularly the lipids. Effects of most mediators on CHD were also curved, but the direction of the curve varied. Effects of total cholesterol and triglycerides on CHD declined at higher levels, but effects of diastolic blood pressure and insulin resistance increased. Effects of HDL and systolic blood pressure were not curved at all.

As a result of the curves in the individual paths, most of the mediation effects were also curved, but to different degrees, which led to the interesting finding that the indirect (mediated) effect of BMI observed at lower BMIs was predominantly through HDL cholesterol, whereas at higher BMIs, indirect effects through HDL and insulin resistance

were of similar magnitude. Mediation by systolic blood pressure was moderate, but constant across levels of BMI. Total cholesterol was also a moderate mediator at lower BMIs and was not a mediator at all above a BMI of 30 kg/m<sup>2</sup>. Diastolic blood pressure and triglycerides were not mediators at any BMI after accounting for the other mediators.

We expected to find race and/or gender differences in the indirect effects because of the race and gender differences that have been found in the prevalences of many of these variables and in some of the associations among them. And indeed, when we did not model the curvature of the associations we examined, we did see differences, especially in effects of BMI on mediators, which tended to be lower in African American women than in the other race-sex groups. The differences disappeared when effects of BMI were allowed to vary with levels of BMI (by means of addition of a quadratic BMI term to the model), revealing that the differences initially observed were likely at least partially an artifact of the different distribution of BMI in African American women.

Our secondary aim was to determine how antihypertensive treatment might affect the various paths in our mediation analyses involving blood pressure. We were particularly concerned about effects of BMI on blood pressure because treatment has been shown to attenuate observed effects of predictor variables on continuous blood pressure (Tobin, Sheehan et al. 2005). Given the high prevalence of antihypertensive medication use in the ARIC cohort, the potential for bias was high.

We found that associations of BMI with blood pressure were attenuated in participants taking antihypertensive medication compared to participants who were not taking such medication. However, when the same comparison was made within individuals who transitioned from not taking antihypertensive medication to taking such medication, the

association of BMI with blood pressure in the treated observations was the same as when those individuals were not treated. While this “new user” analysis does not establish causality, it indicated that the differences observed between untreated and treated cohort participants may not have been due to effects of treatment.

## **B. Strengths**

The strengths of this dissertation include the large bi-ethnic cohort and the high quality of the ARIC data, the novel use of SEM with Cox proportional hazards analysis and the attention given to the proper handling of medication use in the analyses. The use of path analysis in a structural equation model allowed us to examine and compare mediation through individual risk factors, which adds substantially to the literature on effects of obesity on CHD.

## **C. Limitations**

Limitations of the study include the inability to completely account for medication use despite the attention devoted to understanding its impact; our analyses were limited to a description of how medication use influences associations of interest in cohort data and how those influences might be interpreted and addressed. Limitations of the primary aim include the inherent limitations in drawing causal inferences from observational data, which may be exacerbated in an analysis in which multiple estimated parameters are used to calculate an additional parameter, as we have done for the indirect effects.

## **D. Conclusion**

Overall, these results highlight the complexity of analyses of blood pressure in persons being treated with antihypertensive medication and underscore the importance of traditional physiologic risk factors, particularly HDL cholesterol and insulin resistance, as



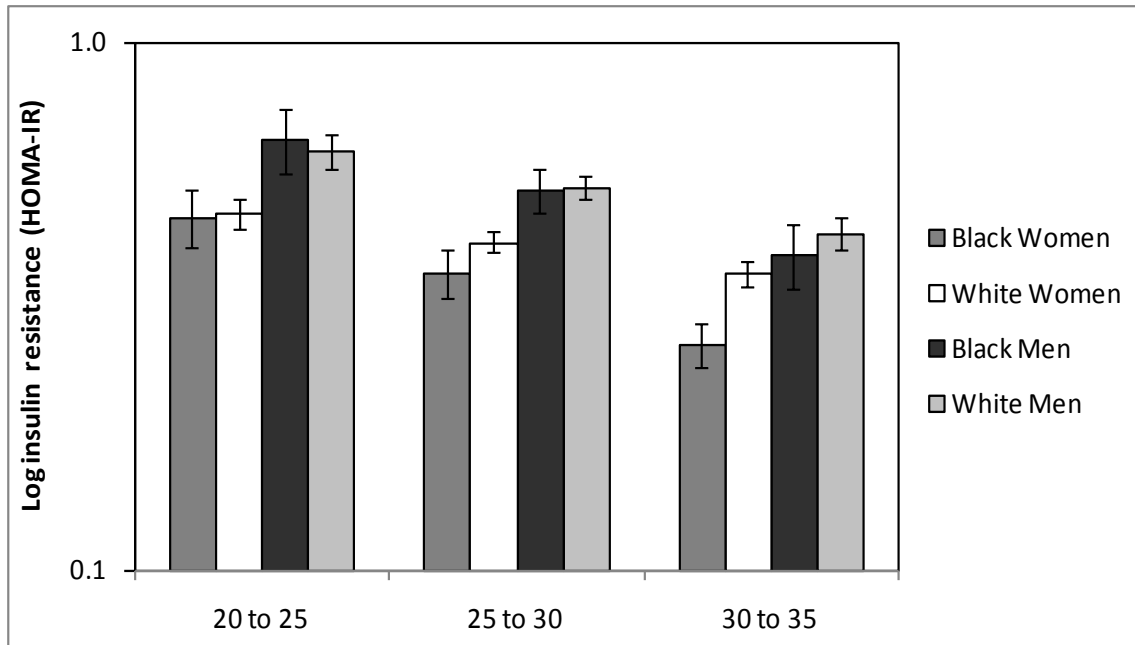
mediators of the effect of obesity on CHD. The variation we observed in mediation across levels of BMI has not been previously shown. Our work adds significantly to the literature on mediation through use of an improved methodological approach. Repetition of this approach in other large datasets with hard endpoints would add further to our understanding of how obesity affects morbidity and mortality.

## APPENDICES

### Appendix A: Race and gender results for Chapter 4 mediation analyses

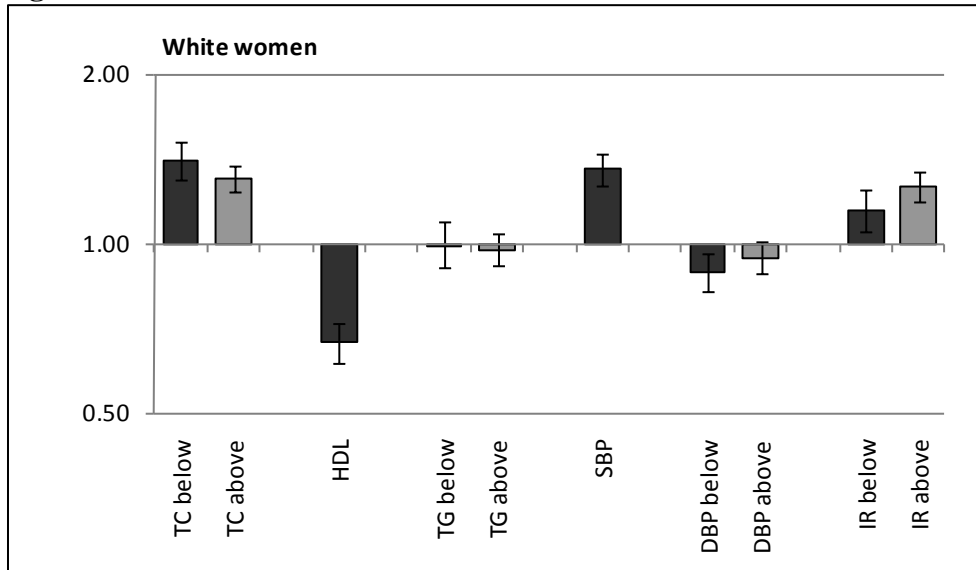
We did not observe race or gender differences in mediation of the BMI-CHD relationship (Chapter 4) or in any of the paths assessed except for the effect of BMI on insulin resistance, which varied by gender (A path, Figure A.1.). The effects of mediators on CHD (B paths, Figures A.2.a-d) and indirect effects of BMI on CHD through the indicated mediators (Figures A.3.a-d) are shown by race and gender below.

**Figure A.1. Increment in log insulin resistance associated with 5-unit contrasts of body mass index ( $\text{kg}/\text{m}^2$ ) by race and gender, the Atherosclerosis Risk in Communities Study**

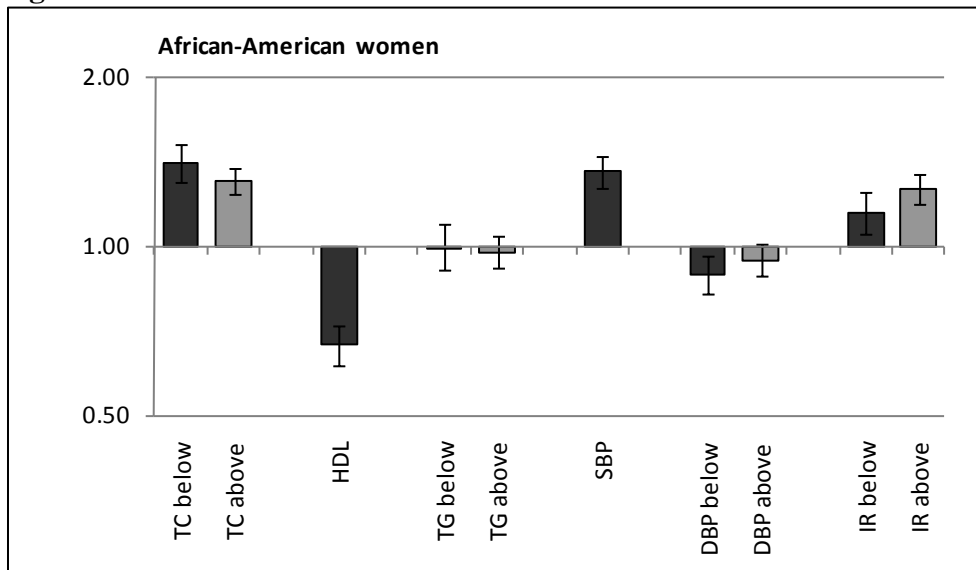


**Figure A.2. Predicted CHD hazard ratios (HRs) for standard deviation increments of risk factors by race and sex in the Atherosclerosis Risk in Communities Study**

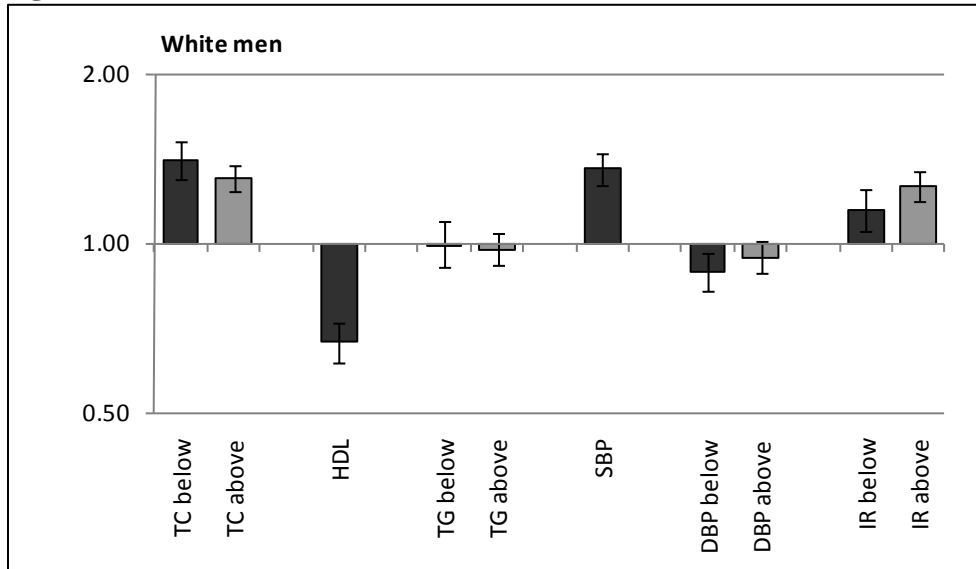
**Figure A.2.a.**



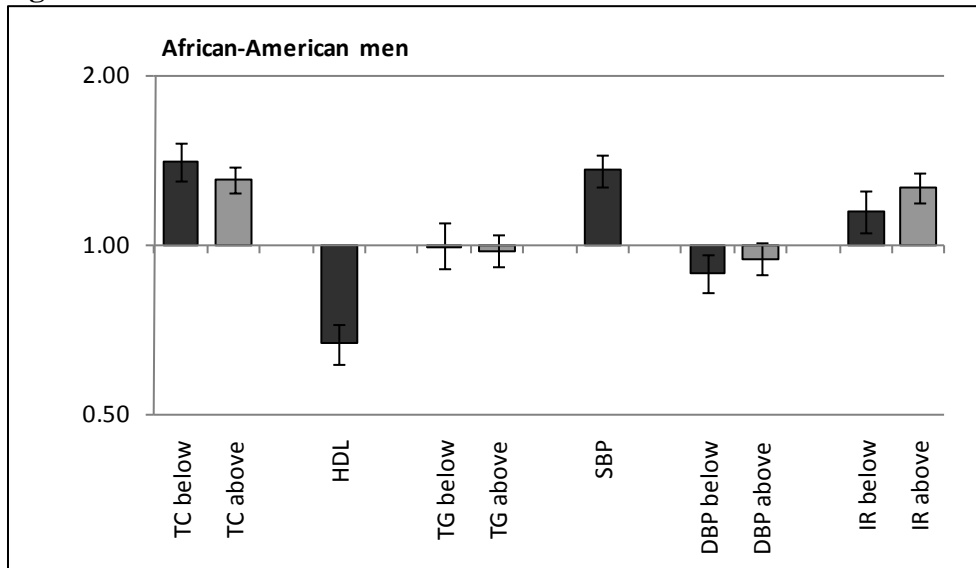
**Figure A.2.b.**



**Figure A.2.c.**

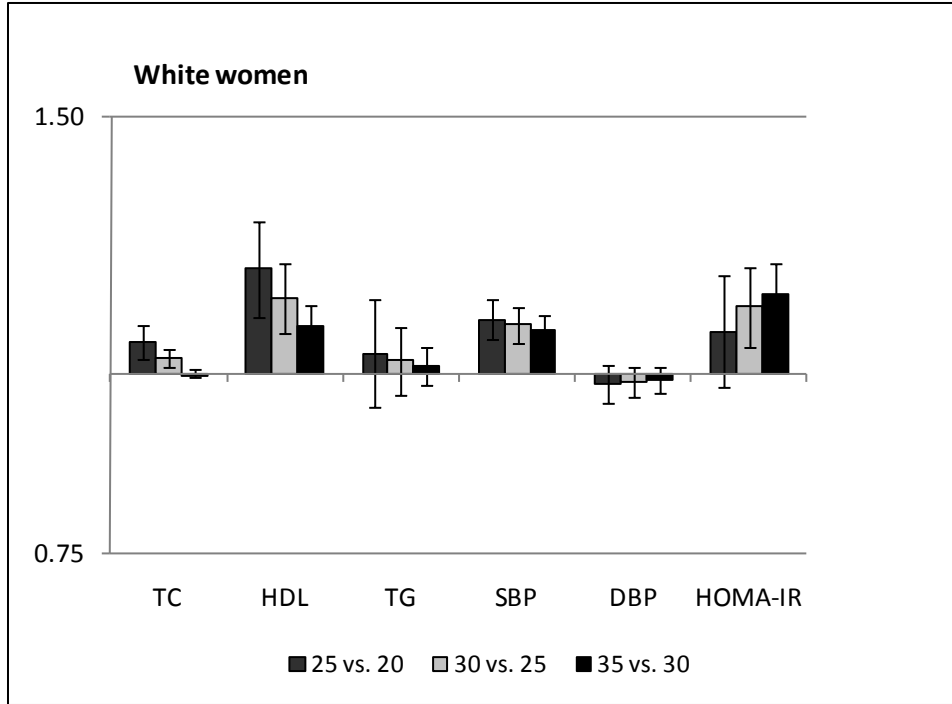


**Figure A.2.d.**

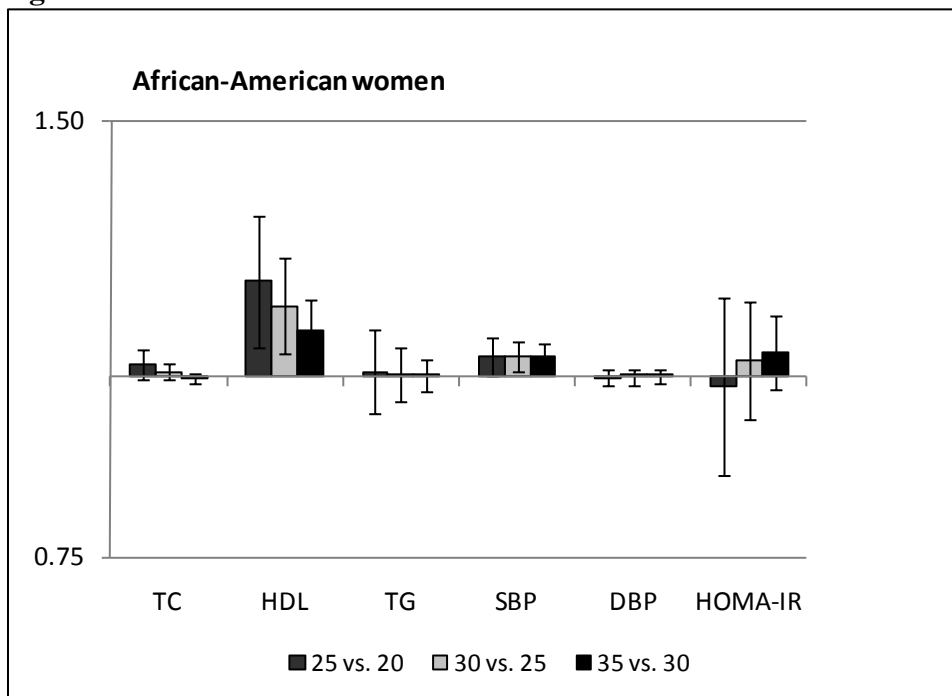


**Figure A.3. Predicted indirect (mediated) CHD hazard ratios (HRs) for 5-unit contrasts of body mass index (kg/m<sup>2</sup>) through established physiologic risk factors by race and sex in the Atherosclerosis Risk in Communities Study**

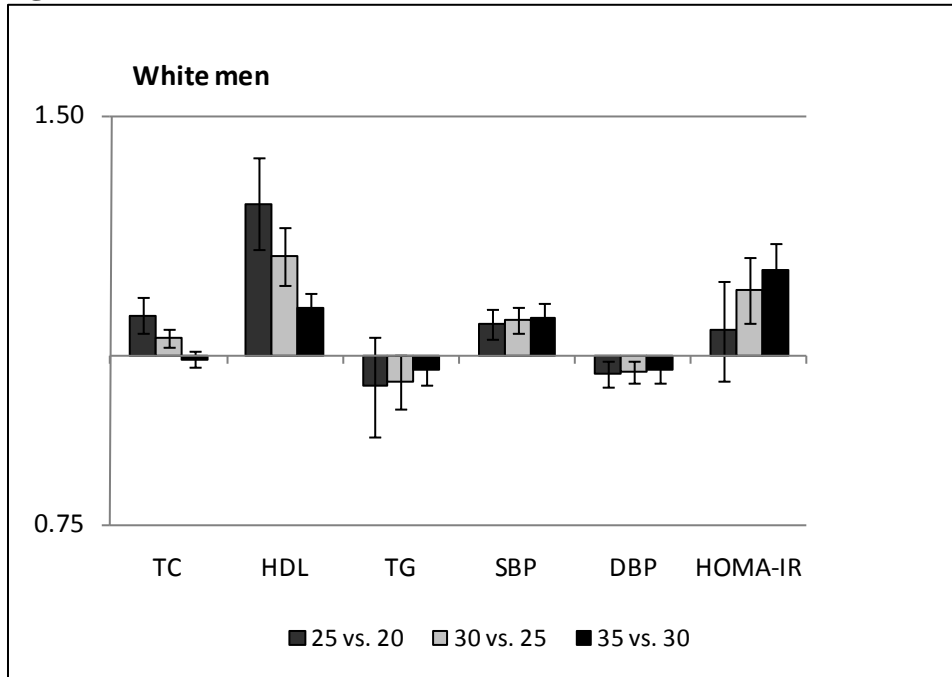
**Figure A.3.a.**



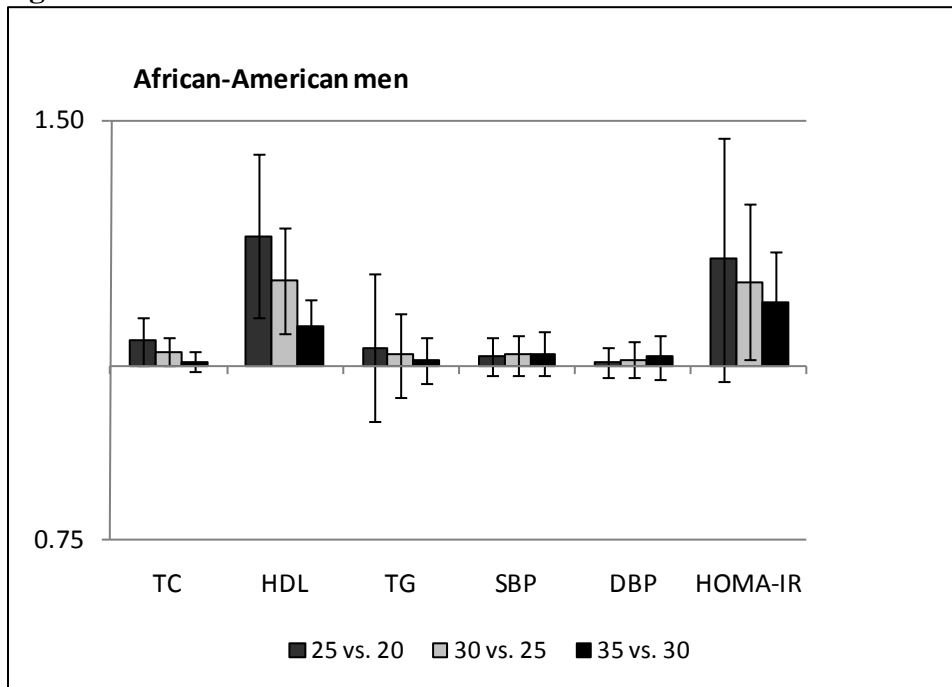
**Figure A.3.b.**



**Figure A.3.c.**



**Figure A.3.d.**



## **Appendix B: Alternative methods for handling antihypertensive medications in analyses of BMI, blood pressure and CHD**

The following tables include additional information on medication use in the ARIC cohort (Table B.1.) and the results of additional analyses of mediation under various scenarios for handling antihypertensive medication (Table B.2.). We conducted the main mediation analysis presented in Chapter 5 in untreated and treated participants separately (as in Chapter 5) and in the entire population with and without statistical adjustment for treatment and using imputed values of the risk factors for the treated participants. We used a simple imputation method, as our purpose was not to accurately estimate effects but to explore the impact of treatment methods. Participants being treated with antihypertensive medication were assigned a value for SBP or DBP 10mmHg higher than their observed value. Untreated persons retained their observed value. The sample sizes are slightly smaller than in Chapter 5 because participants taking lipid-lowering and diabetes medications were also excluded from the data presented here.

**Table B.1. Means (SD) and prevalences of selected baseline sample characteristics among participant taking and not taking medications to treat hypertension, dyslipidemia and/or diabetes, the Atherosclerosis Risk in Communities Study**

<b>Variable</b>	<b>Whole population (N=13,569)</b>	<b>Taking no meds (N=9,399)</b>	<b>Taking HT meds (N=3,796)</b>	<b>Taking lipid meds (N=356)</b>	<b>Taking DM meds (N=490)</b>
Body Mass Index (kg/m <sup>2</sup> )	28 (5)	27 (5)	29 (6)	28 (4)	31 (6)
Age (years)	54 (6)	53 (6)	55 (6)	56 (5)	56 (5)
SBP (mmHg)	121 (18)	118 (17)	128 (20)	122 (17)	130 (21)
DBP (mmHg)	74 (11)	72 (11)	77 (11)	72 (9)	74 (11)
Total cholesterol	215 (42)	212 (40)	219 (44)	235 (46)	220 (49)
HDL cholesterol	52 (17)	53 (17)	50 (16)	47 (17)	45 (15)
Triglycerides	129 (86)	120 (77)	148 (100)	185 (150)	184 (134)
Homa-IR	3.6 (8.8)	2.6 (2.6)	5.3 (12)	5.6 (19)	20 (40)
Education (% < HS)	22	19	30	22	41
Education (% HS)	42	42	40	44	38
Health insurance (% with)	91	92	88	95	83
Drinkers (% current)	57	61	48	53	29
Drinkers (% former)	18	16	21	23	32
Smokers (% current)	25	26	24	20	20
Smokers (% former)	32	32	31	38	30
Leisure activity (% highest tertile)	28	31	22	31	16
Leisure activity (% 2 <sup>nd</sup> tertile)	44	43	45	45	46
Family history of diabetes (%)	24	23	25	29	48
Family history of hypertension (%)	54	49	64	58	56
Family history of MI (%)	39	38	41	44	37
CHD events (N(%))	1,886 (14)	1,072 (11)	714 (19)	77 (22)	179 (37)

TC = Total Cholesterol HDL = HDL cholesterol lnTG = Log triglycerides SBP = Systolic blood pressure DBP = diastolic blood pressure lnIR = Log insulin resistance (HOMA-IR)



**Table B.2. Beta coefficients and HRs for mediation paths in a model of effects of BMI on CHD through blood pressure, under various scenarios for handling antihypertensive medication, the Atherosclerosis Risk in Communities Study**

Mediation Path (estimate)	Endogenous Variable	Exogenous Variable ( $\Delta$ )	Unadjusted for HTmeds (n=13,569)	Untreated participants only (n=9,773)	Treated participants only (n=3,796)	Adjusted for HTmeds variable (n=13,569)	Imputation of treated BP* (n=13,569)
A ( $\beta$ )	SBP	BMI (5 kg/m <sup>2</sup> )	3.31 (3.02, 3.60)	3.59 (3.24, 3.94)	1.66 (1.13, 2.20)	2.89 (2.60, 3.19)	4.08 (3.77, 4.39)
A ( $\beta$ )	DBP	BMI (5 kg/m <sup>2</sup> )	1.57 (1.40, 1.74)	1.80 (1.58, 2.01)	0.49 (0.20, 0.78)	1.32 (1.14, 1.49)	2.34 (2.15, 2.54)
B (HR)	CHD	SBP (10 mmHg)	1.18 (1.14, 1.22)	1.20 (1.14, 1.25)	1.13 (1.08, 1.19)	1.17 (1.13, 1.21)	1.17 (1.13, 1.21)
B (HR)	CHD	DBP (10 mmHg)	0.95 (0.89, 1.01)	0.91 (0.83, 0.99)	0.97 (0.88, 1.07)	0.94 (0.88, 1.00)	1.00 (0.94, 1.06)
Total effect (C path, HR)	CHD	BMI (5 kg/m <sup>2</sup> )	1.22 (1.17, 1.27)	1.22 (1.15, 1.29)	1.10 (1.04, 1.17)	1.17 (1.12, 1.22)	1.22 (1.17, 1.27)
Direct effect (C' path, HR)	CHD	BMI (5 kg/m <sup>2</sup> )	1.16 (1.11, 1.21)	1.16 (1.09, 1.23)	1.08 (1.02, 1.15)	1.13 (1.08, 1.18)	1.14 (1.09, 1.19)
Indirect effect through SBP (AxB, HR)	CHD	BMI (5 kg/m <sup>2</sup> )	1.06 (1.04, 1.07)	1.07 (1.06, 1.09)	1.02 (1.01, 1.03)	1.05 (1.03, 1.06)	1.07 (1.05, 1.08)
Indirect effect through DBP (AxB, HR)	CHD	BMI (5 kg/m <sup>2</sup> )	0.99 (0.98, 1.00)	0.98 (0.97, 1.00)	1.00 (0.99, 1.00)	0.99 (0.98, 1.00)	1.00 (0.99, 1.01)

\* 10mmHg was added to SBP and to DBP if the individual was taking antihypertensive medication.

SBP = systolic blood pressure; DBP = diastolic blood pressure; CHD = coronary heart disease; HR = hazard ratio; BMI = body mass index; HTmeds = antihypertensive medication.

## **Appendix C. Change in blood pressure associated with BMI**

Table C.1 below presents the change analyses mentioned in the discussion of Chapter 5. We used stacked data to examine associations of mean BMI with change in BP across consecutive study examinations at which a participant either 1) remained off antihypertensive medication, 2) remained on antihypertensive medication or 3) transitioned from not taking to taking antihypertensive medication.

**Table C.1.. Change in systolic and diastolic blood pressure between consecutive visits predicted by mean BMI for participants according to change in antihypertensive medication status in the Atherosclerosis Risk in Communities Study**

Medication transition from time 1 to time 2	Adjusted mean change in SBP (mmHg)	Change in Systolic Blood Pressure		Adjusted mean change in DBP (mmHg)	Change in Diastolic Blood Pressure	
		$\beta_{\text{BMI}}$	95% CI		$\beta_{\text{BMI}}$	95% CI
No, No	3.7	0.05	0.03, 0.08	0.08	-0.01	-0.03, 0.001
Yes, Yes	2.4	-0.03	-0.07, 0.01	-1.3	-0.03	-0.05, -0.004
No, Yes	-5.1	-0.01	-0.14, 0.11	-4.7	-0.01	-0.08, 0.06

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