1.a. Full Title: The Association of Overweight and Obesity with Incident Coronary Heart Disease is Attenuated by Adjustment for Markers of Inflammation and Endothelial Dysfunction

b. Abbreviated Title (Length 26 characters): Obesity, Inflammation, Endothelial Dysfunction and Incident CHD

2. Writing Group (list individual with lead responsibility first):

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3. Timeline: All sample analyses have been completed by the Chemistry Laboratory, Hemostasis Laboratory or Lipid Laboratory. Statistical analysis will begin October 2002 and manuscript will be completed by January 2003.

4. Rationale:

Extensive epidemiologic evidence has shown that obesity is a major risk factor for cardiovascular disease, including coronary heart disease (CHD) (Hubert, 1983). Abdominal adiposity in general (Rexrode, 1998), and visceral adiposity in particular may confer a particularly high risk, and is more accurately assessed by waist-hip ratio (WHR) than body mass index (BMI). However, the independent causal role of obesity in the pathogenesis of CHD remains unclear due to its strong associations with other established cardiovascular risk factors such as hypertension (Havlik, 1983) and diabetes (Carey, 1997), which often are preceded by the overweight state. In fact, these cardiovascular risk factors often cluster together as the “metabolic syndrome” (consisting of hypertension, impaired glucose tolerance, truncal obesity, elevated triglycerides, decreased HDL cholesterol, and hyperinsulinemia due to insulin resistance). A prospective study of obesity in the ARIC population demonstrated an approximately 2-fold increased relative
risk of CHD (unadjusted for diabetes or hypertension) among women in the highest quartile of BMI, with a somewhat weaker relationship among men, and stronger associations seen with highest quartile of WHR (Folsom, 1998). A recent Framingham study found a statistically significant 50% increased risk of incident CHD among obese participants, after adjustment for hypertension, diabetes, hypercholesterolemia and smoking (Wilson, 2002).

An increasing amount of evidence indicates that atherosclerosis is an inflammatory disease which is initiated by the process of endothelial dysfunction (Ross, 1999). However, coronary events such as myocardial infarction are mediated by acute thrombosis of a coronary artery, a process involving platelets and the hemostatic system including fibrinogen. The processes of hemostasis and inflammation are closely interrelated. A number of studies have shown that various circulating markers of inflammation and hemostasis are associated with an increased risk of CHD, though this association is often attenuated by adjustment for standard cardiovascular risk factors. Serum levels of the inflammatory cytokine interleukin-6 (IL-6) have been found to predict incident CHD (Ridker, 2000), and tumor necrosis factor-alpha (TNF-alpha) levels are predictive of recurrent coronary events among persons with previous MI (Ridker, 2000). TNF-alpha is one of the major regulators of IL-6, which in turn stimulates the liver to produce acute-phase proteins such as CRP and fibrinogen. Increased levels of CRP (Ridker, 1997) and fibrinogen (Folsom, 1997) are also associated with increased risk of CHD. Fibrinogen may increase risk of CHD through its important roles in thrombosis, blood viscosity, or other effects (Meade, 1986); or be a general marker of enhanced inflammation. TNF-alpha also causes an increase in vWf, which is synthesized by endothelial cells and is a general indicator of endothelial dysfunction (Blann, 1993). vWf plays an important role in platelet adhesion and binds to and stabilizes factor VIII, an important cofactor in hemostasis and thrombosis (Ruggeri, 1992). Elevated WBC count, a general marker of increased inflammation, also is associated with increased cardiovascular risk (Lee, 2001), and may play an etiologic role through increased viscosity or direct endothelial damage (Ernst, 1987). Serum levels of albumin are consistently decreased in inflammatory states due to decreased hepatic synthesis under the control of IL-6, and low levels have been consistently shown to be associated with incident CHD (Danesh, 2000). This may be a non-specific association, but low levels of albumin may be due to endothelial dysfunction with increased vascular permeability (Fleck, 1985). Since albumin also has anti-oxidant properties and buffers free fatty acids, decreased levels may be directly pathogenic (McCarty, 1999). Among previously conducted prospective studies of the ARIC population, WBC, fibrinogen, vWf, and Factor VIII activity (Folsom, 1997), as well as low albumin (Nelson, 2000), have all been found to be associated with increased risk of incident CHD.

Many studies have found that obese persons have increased levels of inflammatory cytokines (IL-6 and TNF-alpha) as well as CRP (Yudkin, 1999). Studies have also shown a strong association of obesity with vWf and Factor VIII (Conlan, 1993), fibrinogen (Folsom, 1992), WBC (Schmidt, 1999) and low albumin (Nelson, 2000). Elevated fasting insulin levels are strongly associated with obesity, and although it is unclear whether its association is independent of other related risk factors, insulin has also been found to be predictive of incident CHD in many studies, including an analysis involving the ARIC population (Folsom, 1997) in which the association was found in
women only. Results from one study suggested that as much as 30% of the body’s IL-6 may arise from adipose tissue (Mohamed-Ali, 1997), and TNF-alpha has also been shown to be produced by adipose cells (McCarty, 1999). Both TNF-alpha and IL-6 increase lipolysis (Corry, 2001), leading to increased levels of free fatty acids. Free fatty acids elevated in obesity also increase oxidant stress in endothelial cells and enhance vascular inflammation (Yudkin, 2000). Furthermore, IL-6 enhances platelet activation, enhancing the pro-thrombotic state (Woods, 2000) and enhances hemostasis through increasing factor VIII and vWF levels (Kerr, 2001), providing a powerful connection between inflammation and hemostasis.

If inflammation, endothelial dysfunction and abnormal hemostasis mediate the relationship between obesity and atherosclerosis, then adjustment for these circulating markers may attenuate the increased CHD risk found among such patients. This possibility has important implications for novel pharmacologic and non-pharmacologic therapies designed to reduce the burden of CHD, and might also provide a target for sequential measurements to monitor response to anti-inflammatory therapies.

References:


5. **Main Hypotheses/Study Questions:**

In an update of previously performed ARIC analyses, it will be confirmed using updated follow-up data that:

1. Overweight and obesity are associated with an increased risk of CHD events.
2. Overweight and obesity are associated with increased markers of inflammation (WBC, fibrinogen, low albumin), and endothelial dysfunction (von Willebrand factor, factor VIIIc). Overweight and obesity are also associated with hypertension, diabetes, elevated triglycerides, decreased HDL, and fasting insulin.
3. Increased markers of inflammation and endothelial dysfunction are associated with incident CHD. Hypertension, diabetes, elevated triglycerides, decreased HDL and fasting insulin are also associated with incident CHD.

**Primary Hypothesis:** The association of overweight and obesity with incident CHD is attenuated by adjustment for markers of inflammation and endothelial dysfunction.
Secondary Hypotheses: If there is sufficient power, further models will be used to investigate whether this residual association of overweight and obesity with CHD is further reduced by adjustment for hypertension, diabetes, elevated triglycerides, low HDL and insulin separately or in combination. These models will also assess whether addition of these covariates reduces the relative contribution of markers of inflammation and endothelial dysfunction to the association of overweight and obesity with CHD.

6. Data (variables, time window, source, inclusions/exclusions):

Exposure variables: [fixed (Visit 1) and time-dependent for all participants] Height (cm), weight (kg), BMI (kg/m^2), waist circumference (cm), hip circumference (cm), and WHR.

Obesity and overweight will be defined by categories of BMI (body mass index, calculated from height and weight, in kg/m^2), as defined by the National Institutes of Health/World Health Organization (report, 1998):

- Normal weight, BMI 18.5 to 24.9 kg/m^2
- Overweight, BMI 25.0 to 29.9 kg/m^2
- Obesity Class I, BMI 30.0 to 34.9 kg/m^2
- Obesity Class II, BMI 35.0 to 39.9 kg/m^2
- Obesity Class III, BMI 40.0 kg/m^2 and greater

BMI and WHR will also be examined as predictor variables in quartiles, as well as continuous variables.

Outcome variables: Incident CHD case status (cardiac death, non-fatal MI, silent MI, cardiac procedure) and time to first CHD diagnosis will be obtained.

Covariates of interest: (fixed and time-dependent covariates) Systolic and diastolic blood pressure (mm Hg), hypertension (defined as BP>=140/90 mm Hg or use of anti-hypertensive medication), fasting glucose (mg/dL), diabetes (defined as fasting glucose>=126 mg/dL, non-fasting glucose >=200 mg/dL, use of anti-hyperglycemic medication, or physician history of diabetes), HDL cholesterol and triglycerides (mg/dL), fasting insulin (pmol/L).

Visit 1 only: Inflammatory markers - WBC, fibrinogen, albumin,
Endothelial dysfunction markers - von Willebrand factor, factor VIIIc

Nuisance variables: (fixed and time-dependent covariates) Smoking (current use, ever use, or none), Total Cholesterol and LDL cholesterol (mg/dL), physical activity (Visit 1 only)

Age, race, gender, center
Medication use: (time-dependent covariates) Aspirin, Statin, ACE-inhibitor or Angiotensin-receptor blocker (ARB), HRT, NSAID or corticosteroid use

Exclusion criteria include individuals with prevalent CHD or stroke.

**Analysis:**

Distribution of serum levels of markers of inflammation and hemostasis will be compared among participants within categories of BMI and WHR, and between cases and controls.

Cox proportional hazards regression will be used to compare the relative risk of CHD among participants stratified by BMI and WHR.

Multivariate Cox regression analyses will be performed adjusting for covariates of interest and nuisance variables:

- Model A: BMI or WHR, age, sex, race, center
- Model B: Model A plus remaining nuisance variables
- Model C: Model B plus markers of inflammation and endothelial dysfunction

Additional possible models:
- Model B plus combinations of hypertension, diabetes, elevated triglycerides, low HDL and insulin
- Model C plus combinations of hypertension, diabetes, elevated triglycerides, low HDL and insulin

Given the greatly increased prevalence of obesity among women in general and African-American women in particular, analyses by race and sex, and tests for interaction will be performed.

Comparison of BMI and WHR with regard to associations with incident CHD and traditional as well as inflammatory and endothelial dysfunction markers, hypertension, diabetes, elevated triglycerides, low HDL and insulin will also be performed.

Future considerations will include:
1. Use of additional inflammatory markers measured in case-control analyses
2. Analysis of attenuation of CHD risk associated with diabetes or metabolic syndrome by adjustment for inflammatory markers