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Atherosclerosis is highly associated with increased serum inflammatory markers. Coronary artery calcium (CAC) burden has allowed researchers to have a non-invasive proxy measure of atherosclerosis. We hypothesized that interleukin-6 (IL-6), after controlling for CV risk factors, would be associated with CAC scores, and this association will be modified by race/ethnicity. 344 subjects were recruited. IL-6 concentrations were measured, and computed tomography was used to calculate CAC scores. After accounting for age, gender, race, smoking, hypertension, diabetes, and cholesterol, a one-unit increase in IL-6 concentration is associated with 1.03 greater odds of an abnormal calcium score (OR: 1.03, 95% CI: 0.98, 1.07). Race/ethnicity did not modify this association. IL-6 did not prove to be a simple clinical marker of CAC.

INTERLEUKIN-6 AND ITS RELATIONSHIP TO

CORONARY ARTERY CALCIUM BURDEN-

NORTH TEXAS HEALTHY HEART STUDY

Nashila AbdulRahim, B.S.

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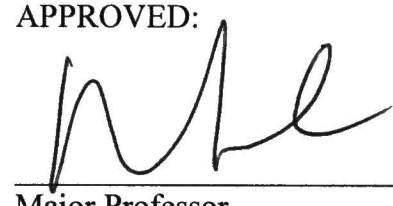

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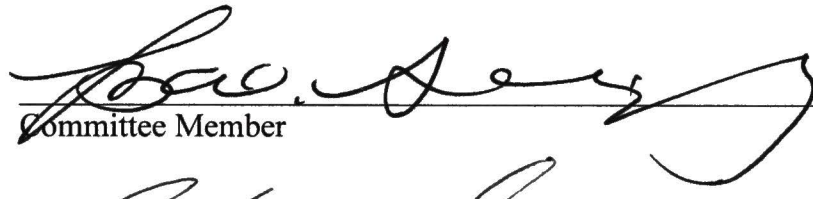

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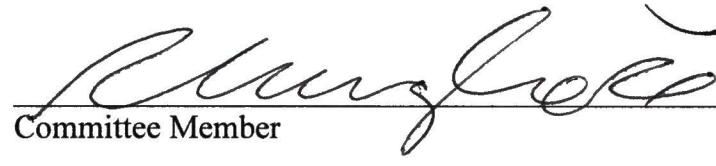

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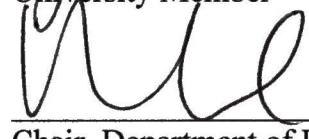

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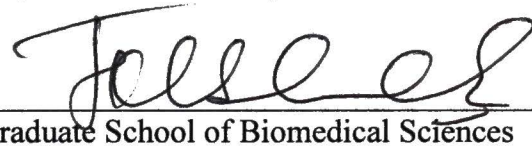

Dean, Graduate School of Biomedical Sciences

INTERLEUKIN-6 AND ITS RELATIONSHIP TO CORONARY ARTERY CALCIUM BURDEN – NORTH TEXAS HEALTHY HEART STUDY

THESIS

Presented to the Graduate Council of the

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For the Degree of

MASTER OF SCIENCE

By

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INTRODUCTION

Background

Significance of the Problem

Cardiovascular education programs are developed yearly to help millions of Americans since cardiovascular disease is the number one killer in the United States. Currently, 70 million Americans suffer from cardiovascular disease, and it is responsible for one death every 35 seconds[1]. Unfortunately, fifty percent of those who die from an acute cardiovascular event have no warning signs, symptoms, or traditional risk factors. Hence, it is crucial to identify more advanced screening tests that can identify those at risk of cardiovascular disease so serious and debilitating complications can be prevented[1]. Coronary heart disease is the primary cause of disability in the U.S. workforce resulting in billions of dollars in healthcare costs[1]. It is essential to pay particular attention to African Americans and Hispanics. The rising prevalence of hypertension, obesity, and other known risk factors in these minority groups has led to high morbidity and mortality rates[1, 2]. To aid in the identification and development of new cardiovascular risk factors, the understanding of the pathophysiology of atherosclerosis is warranted.

Atherosclerosis is considered an inflammatory disease and not merely a cholesterol-laden process. Endothelial dysfunction and the progression of atherosclerosis has been found to be associated with increased concentrations of serum inflammatory

markers[3]. Thus, measuring inflammation markers, such as interleukin-6 (IL-6) may potentially identify individuals at high risk for atherosclerosis. Fortunately, new non-invasive diagnostic measures of sub-clinical atherosclerosis, such as coronary artery calcium burden by multi-detector computed tomography, make such research possible[4-8]. Therefore, understanding the relationship between serum inflammatory markers and coronary artery calcium can eventually help clinicians better diagnose and target cardiovascular treatment.

Impact of Cardiovascular Disease

Cardiovascular disease is the number one cause of death in the United States for men and women (Figure 1) and all racial/ethnic groups[1, 2]. Although cardiovascular disease primarily affects those over the age of 65, the number of affected individuals aged 15-34 is rising steadily[1]. Seven million people worldwide each year die of coronary artery disease, a subset of cardiovascular disease[2]. All manifestations of cardiovascular disease, including hypertension and stroke, plague more than sixty million Americans[9]. Hospital discharge data on heart failure have shown an increase over the last two decades[9]. Close to 35% of deaths from heart failure occur in those less than 75 years old[9]. Morbidity secondary to cardiovascular disease has a significant economic impact[9]. In fact, it accounts for up to $403 billion of healthcare costs and lost productivity due to death and disability[1]. The problem is compounded by an aging American population making cardiovascular disease a rising public health concern.

Risk Factors for Cardiovascular Disease

Evidence from prospective research has led to discoveries of causal relationships between modifiable and unmodifiable risk factors and heart disease. Family history, older age, and male gender, although unmodifiable, should be taken into account during a clinical assessment of a patient[10]. Modifiable risk factors include high blood pressure, physical inactivity, obesity, diabetes, dyslipidemia, and cigarette smoking[9, 10]. In addition to this traditional list of risk factors, many studies have also shown hyperhomocysteinemia, C-reactive protein (CRP), and lipoprotein (a) to be independent risk factors for cardiovascular disease[9]. The strength of associations between each of these risk factors and stroke, peripheral arterial disease, and coronary artery disease differ significantly. For example, smoking, age, low high-density lipoprotein (HDL) cholesterol, and diabetes are more strongly associated to coronary artery disease than other risk factors, such as hypertriglyceridemia, male gender, hypertension, and hyperlipidemia[9]. (Table 1). Relative to other risk factors, hypertension and advanced age are more strongly associated with stroke. Similarly, when compared to other risk factors, hypertension and cigarette smoking are the two most strongly associated risk factors of peripheral arterial disease[9].

Epidemiologic studies have shown that higher blood pressure, especially systolic blood pressures in older populations, is directly related to worse cardiovascular outcomes[11]. Treating even mild hypertension leads to beneficial health outcomes[9, 11]. Vasan et al. found that even high normal blood pressure (130-139 mm Hg systolic, 80-89 mm Hg diastolic, or both) is associated with an increased risk of cardiovascular disease

after ten years[12]. The use of ramipril, an angiotensin-converting enzyme (ACE) inhibitor, has been shown to decrease fatal and nonfatal complications in those with established cardiovascular disease by lowering blood pressure an average of 20 mm Hg systolic or 10 mm Hg diastolic[9, 11, 13]. Those of advanced age, male gender, or with isolated systolic hypertension benefit the most in terms of cardiovascular mortality with control of hypertension. These risk factors are the strongest in terms of their association with acute cardiovascular events. Control of blood pressure to a normal range could prevent a significant number of cardiovascular events, specifically one-third fewer acute events in men and one-half fewer acute events in women. Even control to a high normal range leads to reduction of cardiovascular events[14].

Physical inactivity is another modifiable risk factor that has been shown to be associated with cardiovascular disease. It also increases blood pressure, triglyceride levels, and decreases HDL cholesterol levels[9]. Aerobic exercise leads to an improvement in insulin resistance and fibrinolysis and reduces obesity, another modifiable cardiovascular risk factor[9].

The incidence of obesity, especially in the United States, has grown over the past decade and is directly linked to cardiovascular disease by exacerbating hypertension, hypertriglyceridemia, low HDL cholesterol levels, insulin resistance, and ultimately diabetes mellitus[9, 15]. The risk for cardiovascular disease is two times greater in men and four times greater in women with type II diabetes compared to non-diabetics. This risk is higher in type I diabetics with renal manifestations such as proteinuria[9]. With weight loss and physical activity, there is an improvement in insulin resistance, a reduction in blood

pressure, an increase in size and decrease in density of low-density lipoprotein (LDL) particles, and an increase in HDL cholesterol[9, 15], all leading to further reduction in cardiovascular risk.

Dyslipidemia is a known modifiable risk factor for heart disease. The term "dyslipidemia" encompasses all lipid abnormalities, including low LDL, high total serum cholesterol levels, low HDL, and high triglyceride concentrations. Hypertriglyceridemia is associated with individuals with low HDL concentrations and can be independently related to cardiovascular disease in the diabetic population[9]. Although LDL cholesterol concentrations are frequently normal in diabetics, the particles are smaller and denser than in non-diabetics, thus more prone to atherogenesis[9]. The Adult Treatment Panel III (ATP III) considers diabetics in the high-risk category and recommends lowering their LDL to less than 100 mg/dL. A LDL concentration of less than 70 mg/dL is considered a reasonable "therapeutic goal option" based on available clinical evidence[16, 17]. Risk of cardiovascular disease is higher (10 year, 10-20% increase in cardiovascular events) in moderately high-risk patients with serum HDL concentrations less than 40 mg/dL or with LDL concentrations greater than 130 mg/dL. Patients in this group include those with two or more established risk factors, such as advanced age, male gender, cigarette smoking, hypertension, diabetes mellitus, and any form of dyslipidemia[16, 17]. Treatment options include lipid-lowering therapy plus life-style modification such as a diet low in sodium and fat and high in vegetables and fruits. The treatment goal for LDL cholesterol in the moderately high risk patient category is to achieve serum concentrations of less than 130 mg/dL, with less than 100 mg/dL as "a therapeutic option." The treatment goal for HDL

cholesterol is greater than 40 mg/dL[16, 17]. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have become a popular treatment option for lowering lipids. Besides having anti-inflammatory properties, statins reduce atherosclerotic plaque formation in blood vessels[9, 17]. The extent of their effects on inflammation has been shown in trials of both primary and secondary prevention of cardiovascular disease[18]. Evidence from trials such as Cholesterol and Recurrent Events (CARE) and Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) have shown that there is a tremendous benefit achieved, in terms of slowing the progression of cardiovascular disease[18, 19]. The CARE trial demonstrated that using statins lowered cardiovascular events even in individuals who have coronary artery disease but average LDL cholesterol concentrations. Those with pretreatment concentrations of total cholesterol in the high normal range also benefited in terms of decreased number of cardiovascular coronary events[19].

Finally, tobacco abuse triples to quadruples the rate of cardiovascular events. The incidence of heart disease, upon quitting, falls nearly to the same level as that of non-smokers in two years[9, 20]. Reducing risk factors has shown to both diminish severity of asymptomatic cardiovascular disease along with its clinical outcomes, such as myocardial infarction, strokes, and peripheral arterial disease. Quitting smoking dramatically improves function of the coronary arteries physiologically by lowering the inflammatory and plaque burden[16, 17, 21].

One focus of the ATP III is to use the Framingham 10-year absolute risk scale to recognize patients who need intensive treatment, especially those with greater than 20%

risk for future major cardiovascular events. Another focus is to identify patients with multiple risk factors who remain asymptomatic. Physicians can then intervene early through lifestyle modifications and medications in order to prolong life[16, 17, 21].

Atherosclerosis

Cardiovascular disease develops through two pathways: reduction or total occlusion of arterial lumens, as in atherosclerosis, and/or dilatation of arterial walls. Arterial walls are primarily made up of endothelial and smooth muscle cells, and their function and response to injury are the basis of cardiovascular disease outcomes including myocardial infarction and death[8].

A single layer of endothelial cells lines the entire cardiovascular system and is essential in maintaining the integrity of the blood vessels while acting as a semipermeable membrane for the transfer of nutrients and various solutes[8]. Inflammatory reactions associated with cardiovascular disease initiation and progression and growth of smooth muscle cells also occur at the endothelium lining. The endothelium's reaction in response to pathologic stimuli is referred to as endothelial activation[8]. The activators of endothelial cells include cytokines, bacterial products, hemodynamic forces, lipid products, advanced glycosylation end products, viruses, complement products, and decreased oxygen content or hypoxia. As a response to these activators, endothelial cells express cytokines and chemokines, adhesion molecules, growth factors, and vasoactive mediators[8, 20, 22]. The regulation of inflammation and immunity, an endothelial cell function, is done through production of cytokines, such as IL-6, and chemokines. The

initiation of atherosclerosis and other vascular lesions is, in part, due to endothelial dysfunction, allowing endothelial cells to attract inflammatory cells[8].

Atherosclerosis, medial calcific sclerosis, and arteriolosclerosis are three pathologic patterns of arteriosclerosis or "hardening of the arteries."[8] Atherosclerosis is the most significant and common process, characterized by lesions of the intima called atheromas or "fibrofatty plaques." These plaques obstruct lumens of blood vessels and weaken the media layer[8]. Fatty streaks appear early in life in the coronary arteries. Their development is not necessarily related to known risk factors of atherosclerosis. In other words, coronary arteries of healthy individuals without known traditional risk factors contain fatty streaks. Some evidence has supported the progression of fatty streaks to atheromas[8]. Not all fatty streaks become fibrofatty plaques. Many of these streaks occur in anatomical regions that are uncharacteristic of atheroma development later in life (Figure 2). In the past, two hypotheses for the initiation of atherosclerosis were proposed. One hypothesis focused on intimal cellular proliferation while the other centered on thrombus organization and growth[8]. Currently, "the response to injury" hypothesis integrates both of these ideas and incorporates established risk factors[8]. This theory suggests that there is a chronic inflammatory cascade in response to endothelial injury (Figure 3). The injury results in endothelial permeability and lipoprotein buildup, especially oxidized LDL (oxLDL), in the arterial vasculature. Furthermore, IL-6, a peripheral cytokine, activates leukocyte and endothelial cells in the presence of this inflammatory response, promotes assembly of CRP and other acute-phase reactants, and propagates the atherosclerotic process[23]. IL-6 is also responsible for the continual

expression of monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor (TNF) alpha[24]. IL-6 has many known functions in relation to the atherosclerotic process, including activation of leukocytes, promotion of acute phase response, upregulation of endothelial cell adhesion, and initiation of smooth muscle cell migration and proliferation. At time of endothelial injury, monocytes adhere and relocate into the intima, where they mature and differentiate into macrophages. With incorporation of oxLDL, macrophages then become foam cells. Macrophages secrete IL-1 and TNF alpha, leading to a greater accumulation of leukocytes. Other chemokines such as MCP-1 contribute to this process by translocating leukocytes into the developing plaque[8]. This cycle fosters and propagates a pro-inflammatory and a pro-thrombotic environment[23].

As described, inflammation is an integral process in the development of cardiovascular disease. Several studies have analyzed the relationship between inflammatory markers and various forms of atherosclerotic disease, such as peripheral arterial disease, acute myocardial infarctions, and valvular calcification[25-27]. Tzoulaki et al. examined IL-6 and CRP and their ability to predict peripheral arterial disease using the ankle-brachial index measure. They found IL-6 to be associated with peripheral atherosclerotic changes independent of traditional cardiovascular risk factors. IL-6 was a significant predictor of worsening ankle-brachial index measures at five and twelve-year follow-up[25]. Another study by Maier et al. reported that increased concentrations of IL-6 were found at ruptured plaque sites among patients experiencing acute myocardial infarctions. IL-6 can worsen myocardial damage and exacerbate heart failure secondary to acute infarctions[26]. Valvular disease, such as aortic stenosis, aortic sclerosis, and mitral

annular calcium, has also been linked to increased concentrations of IL-6, CRP, and MCP-1, suggesting an inflammatory process and its association with calcification[27]. Interestingly, it has been found that IL-6 is strongly and independently associated with clinical cardiovascular disease when compared to other cytokines and inflammatory markers, such as TNF-alpha and IL-2[28]. Hence, this project has focused on IL-6 and its role in sub-clinical atherosclerotic disease.

Is IL-6 the Answer? (Figure 4)

Several traditional risk factors previously discussed lie in the complex and interrelated pathway of inflammation and the progression to plaque formation. For example, tobacco use initiates vascular injury by causing endothelial dysfunction resulting in an inflammatory response[8, 20]. In fact, smoking is significantly associated with high levels of inflammatory markers, including CRP, fibrinogen, and white blood cell count. This explains how tobacco use elimination can decrease inflammation and overall cardiovascular risk[29]. Of note, CRP, fibrinogen, and WBC are all downstream inflammatory products of IL-6. The same principle applies to advanced glycosylated end-products (AGEs), found in higher concentrations in tissues of diabetics[22]. AGEs are the result of a chain of chemical reactions after an initial glycosylation reaction and are formed endogenously through normal metabolism and aging. During stressful hyperglycemic states, AGE levels can be increased beyond normal levels and, due to their function as endothelial cell activators, lead to clinical cardiovascular complications[22]. Lipid by-products, such as oxLDL, are endothelial cell activators resulting in endothelial dysfunction and inflammatory response[8]. IL-6 responds to the

chain reaction caused by these activators. IL-6 promotes the assembly of CRP and other acute phase reactants, which are key players behind inflammation and its path to cardiovascular disease. Consequently, it is proposed that IL-6 has a primary role in the complex intersecting inflammatory pathway in the development of cardiovascular disease.

Coronary Artery Calcium Burden

Ultrafast computed tomography (CT) scan or electron-beam CT, and multislice CT scanners non-invasively image coronary artery calcification associated with atherosclerotic plaques. These scanners allow fast noninvasive cardiac imaging in single breath hold. Coronary artery calcium has been repeatedly shown as a marker for sub-clinical atherosclerosis in epidemiologic studies. A significant association between coronary artery calcification and underlying atherosclerotic disease has been found at time of autopsy[30, 31]. Calcification is determined using a minimum radiographic threshold density of 130 Hounsefield units (HU) per pixel. Agaston is the most commonly-used coronary artery calcium scoring algorithm[31, 32] (Table 2). The score is derived from multiplying the calcification area in millimeters squared by the calcium density for each coronary segment. Adding the scores of each segment leads to a total coronary artery calcification score. Because the amount of calcification does not associate well with the level of the stenotic lesion, CT scanning is not a used for symptomatic cardiac disease[31]. Along with traditional risk factors, coronary artery calcium screening has shown to add additional information for predicting mortality in those who have asymptomatic cardiovascular disease[6, 31]. For this reason, it is an ideal diagnostic modality for a

population with traditional risk factors but undiagnosed cardiovascular disease. Latest research has shown that coronary artery calcium scoring can predict future cardiovascular events, especially among individuals with a Framingham 10-year risk of 10%-20%[4]. Event-free survival was found to be lower in individuals with a score of 100 or greater compared to those with a score of less than 100[30, 31]. Since coronary artery calcification is considered to be closely associated to atherosclerosis, it can be utilized as a sub-clinical measure and offer a good noninvasive diagnostic tool[5].

Race/Ethnicity as an Effect Modifier

Several hypotheses exist for African Americans having the highest overall cardiovascular mortality rate at younger ages and out-of hospital coronary death rate when compared to Caucasians and Hispanics[33]. They include a higher prevalence of cardiovascular risk factors, extended period of time before seeking medical care, psychosocial and socioeconomic factors, limited access to healthcare, and differential treatment of risk factors. The higher rate of cardiovascular disease still cannot be explained after accounting for these differences[34, 35]. Although the clinical continuum of cardiovascular disease in African Americans is the same as in Caucasians, African Americans present with higher rates of unstable angina and non-ST-elevation myocardial infarction (NSTEMI) than Caucasians. African Americans also carry an increased risk of sudden cardiac death[33]. Furthermore, African American women suffer from earlier cardiovascular events and hold the highest rate of mortality amongst all women[33, 35]. This suggests that there may be underlying factors associated with race/ethnicity that are

related to the development and progression of atherosclerosis and subsequent complications.

African Americans have fewer occluding lesions in their coronary arteries as evidenced by angiography compared to Caucasians[33, 36]. Budhoff et al. found lower levels of coronary artery calcium burden in Hispanics and African Americans when compared to Caucasians, hence the degree of calcification cannot explain the higher mortality rates suffered by African Americans[34]. This suggests that there may be an alternative process, such as plaque instability due to an inflammatory cascade that may be attributing to the disparate cardiovascular death rates.

The Problem and Hypothesis

Cardiovascular disease is a major health concern in the United States affecting millions of people. The Centers for Disease Control and Prevention, along with other organizations, continue to implement programs, such as the Prevention Research Center Network and State Cardiovascular Health Examination Surveys, to bring public health awareness to this leading cause of death in both men and women[1]. Certain racial and ethnic groups have been shown to be at increased risk for cardiovascular disease events. Hence, an earlier diagnosis of asymptomatic cardiovascular disease could result in a decrease in morbidity and mortality as a result of early intervention.

Initiation and progression of atherosclerosis is highly associated with increased concentrations of serum inflammatory markers[3]. Studies have investigated markers thought to be involved in the pathological processes of atherosclerosis such as IL-6, CRP,

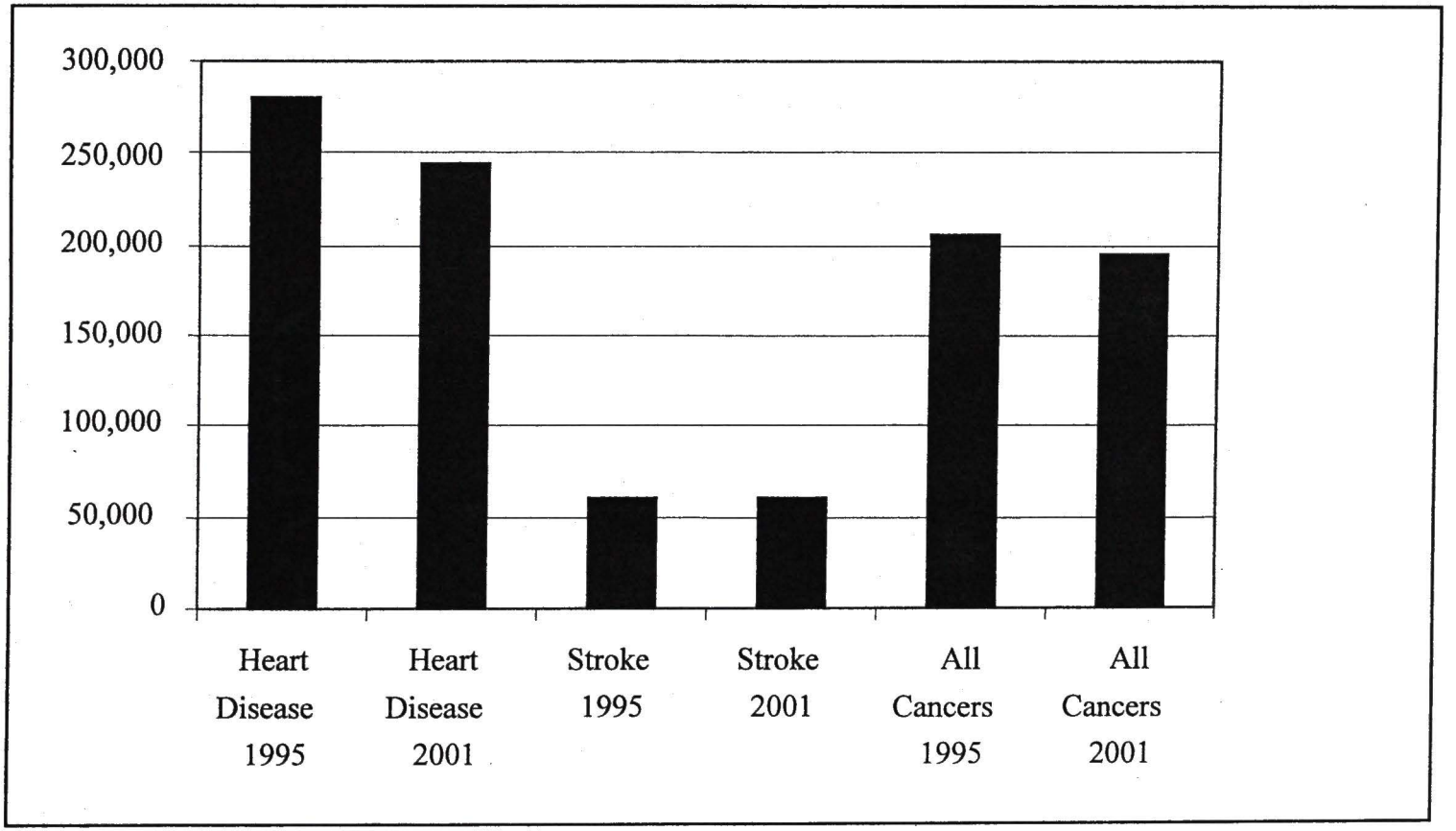
TNF-alpha, and MCP-1[6, 24-26, 28, 37, 38]. In the Fragmin and/or early Revascularization during InStability in Coronary artery disease (FRISC-II) trial, IL-6 concentrations greater than 5 ng/L predicted six and twelve-month mortality rates and identified individuals who would benefit the most from early invasive therapy[24, 38]. IL-6 has been found at the border of atherosclerotic plaques and can add to the plaque's instability by expressing matrix metalloproteinases (MMP-1) and TNF-alpha[24]. Acute coronary syndrome (ACS), which includes unstable angina, NSTEMI, and ST-elevation myocardial infarction (STEMI), has been associated with higher concentrations of IL-6 in the circulation[24]. Unfortunately, interventions may not be an option once ACS occurs because of its associated high mortality rate[39]. Therefore, using IL-6 serum concentration as a risk measure of atherosclerosis can allow early detection and intervention and subsequent survival.

Over the past several years, investigators have researched coronary artery calcium scanning using multi-detector or electron-beam CT and its role in an individual's cardiac risk profile[4, 7, 40]. Latest research has shown the value of coronary artery calcium scoring and its ability to predict future cardiovascular events, especially among individuals with a Framingham 10-year risk of 10%-20%[4]. The Framingham score determines the risk of developing a major cardiovascular event over the next ten years. Nonetheless, coronary artery calcium burden allows researchers to have a noninvasive proxy measure of atherosclerosis. No study has been found where IL-6, a unique and robust marker of inflammation, and coronary artery calcium burden has been specifically studied.

This study takes into account known cardiovascular risk factors based on personal history and laboratory data. It investigates an association between IL-6 as a marker of inflammation and coronary artery calcium burden, which is used as a proxy measure of atherosclerosis. Finally, the study also explores whether this relationship is modified by race and ethnicity. We hypothesize that IL-6, after controlling for traditional cardiovascular risk factors, is associated with coronary artery calcium scores, and that this association will be modified by race/ethnicity. The specific aims of the study are the following:

1. To assess whether IL-6 is associated with coronary calcium scores.
2. To assess whether the relationship between IL-6 and coronary calcium scores is modified by race and ethnicity.

Figure 1: Leading causes of death in the United States, 1995 and 2001*



*Adapted from National Center for Health Statistics, 2003[2]

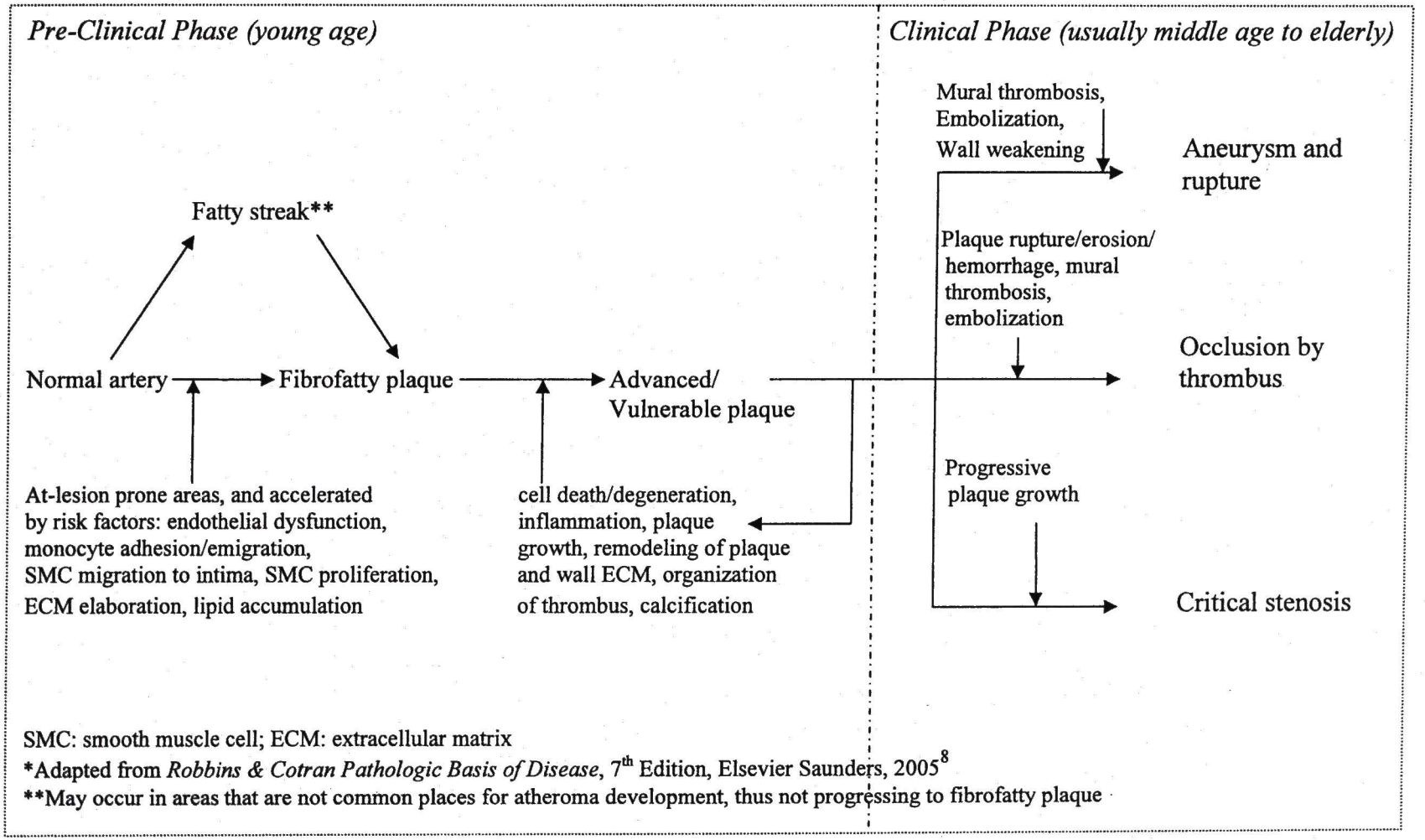
Table 1: Relative strength of established cardiovascular disease risk factors for coronary heart disease, stroke, and peripheral arterial disease*

	Coronary heart disease	Stroke	Peripheral arterial disease
Advanced age	+++	++++	+++++
Male gender	++	+	+
Cigarette smoking	+++	+	+++++
Hypertension	++	+++++	++
LDL cholesterol	++	+	+
Low HDL cholesterol	+++	+	+++
Triglycerides	++	+	+++
Diabetes mellitus	+++	+	+++++

LDL: low-density lipoprotein; HDL: high-density lipoprotein

*Adapted from *Cecil Textbook of Medicine*, 22nd Edition, Elsevier Saunders, 2004[9]

Figure 2: Phases of atherosclerosis buildup with its fundamental features and the major resulting complications in the coronary arteries*

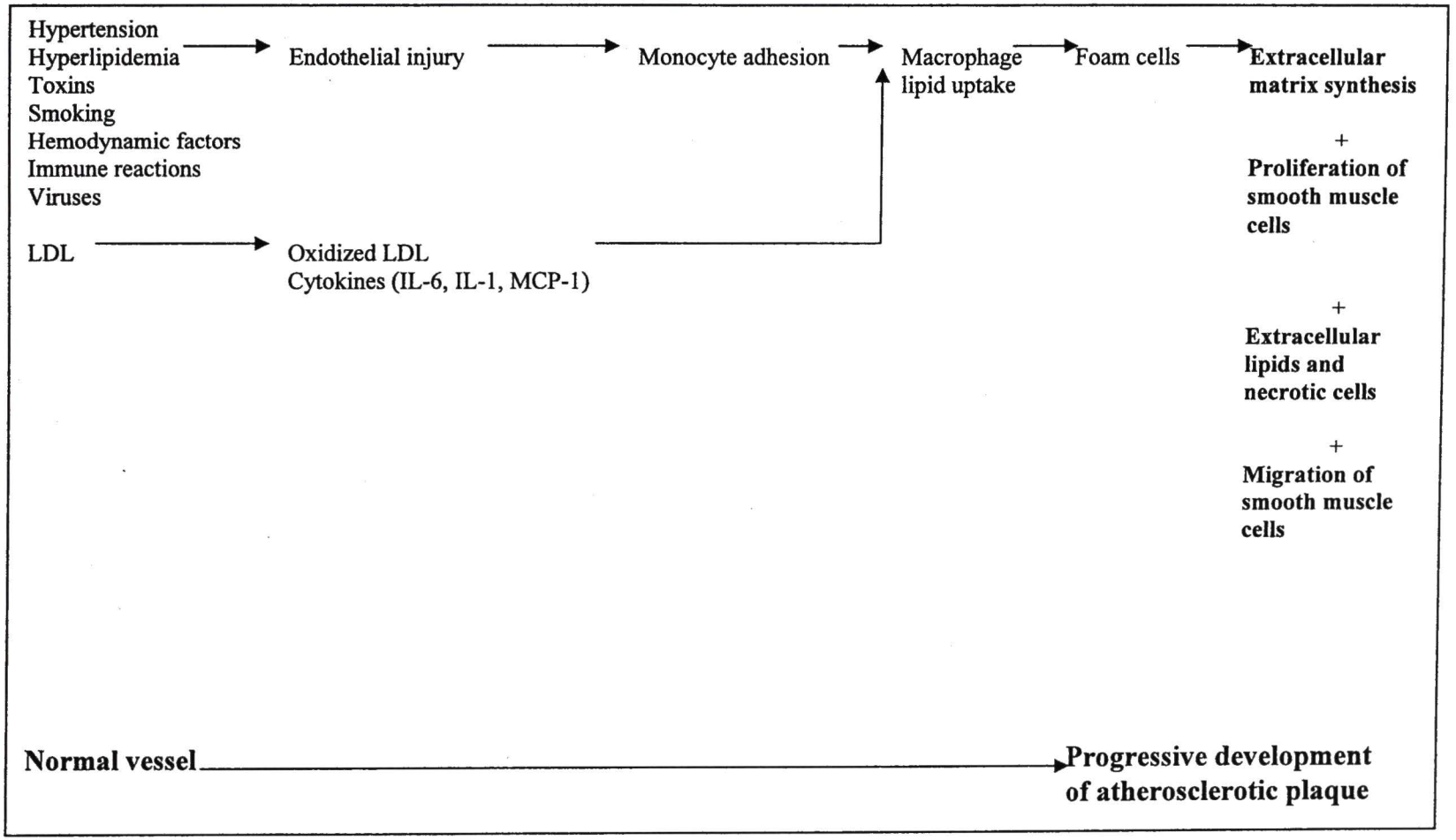


SMC: smooth muscle cell; ECM: extracellular matrix

*Adapted from *Robbins & Cotran Pathologic Basis of Disease*, 7th Edition, Elsevier Saunders, 2005[8]

**May occur in areas that are not common places for atheroma development, thus not progressing to fibrofatty plaque

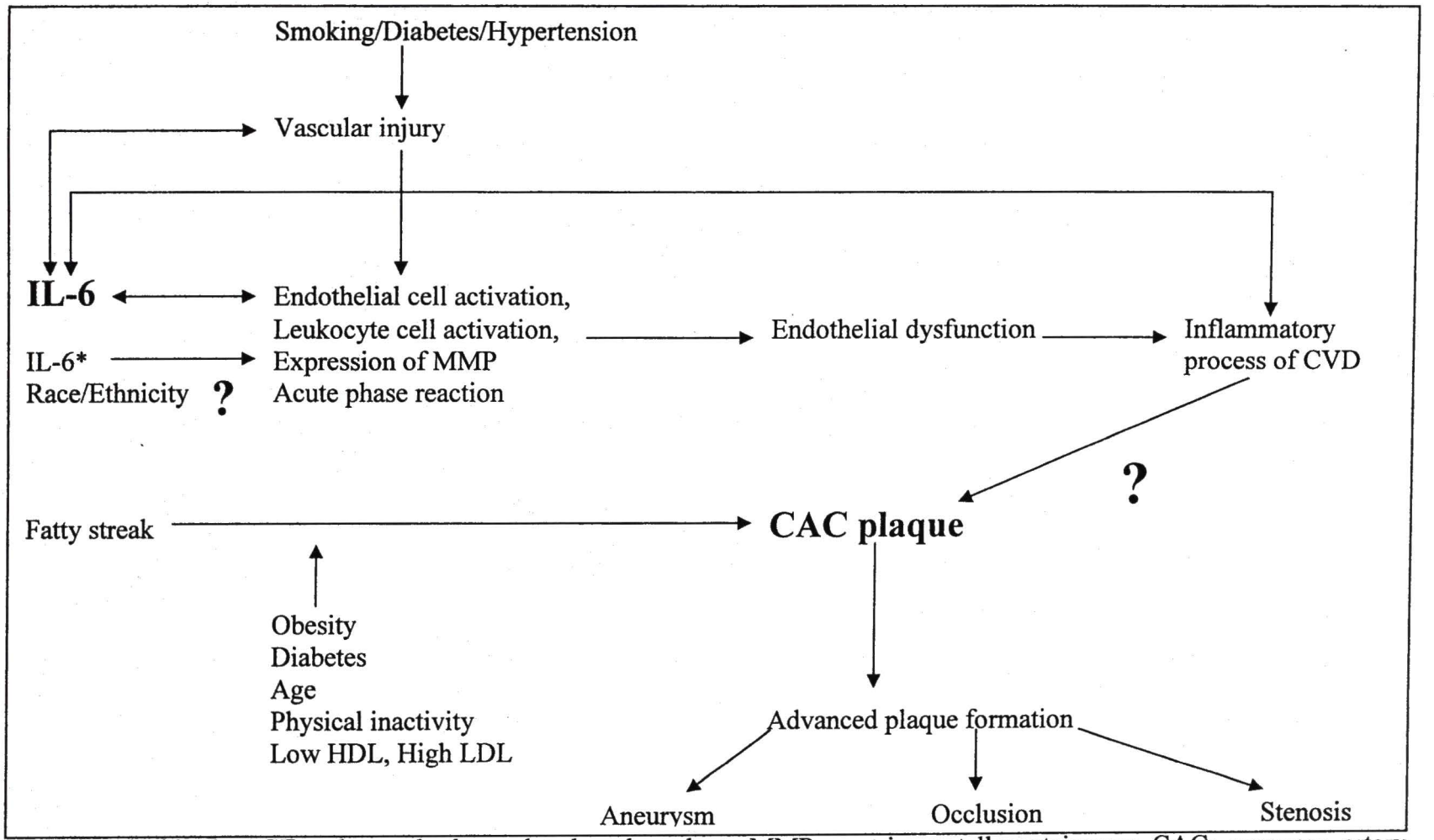
Figure 3: Sequence of events in atherosclerosis at a cellular level from endothelial injury to smooth muscle migration*



IL-6: interleukin-6; LDL: low-density lipoprotein; IL-1: interleukin-1; MCP-1: monocyte chemotactic protein-1

*Adapted from *Robbins & Cotran Pathologic Basis of Disease*, 7th Edition, Elsevier Saunders, 2005[8]

Figure 4: Proposed Association between Interleukin-6 and Coronary Artery Calcium



IL-6: interleukin-6; AGE: advanced glycosylated end-product; MMP: matrix metalloproteinases; CAC: coronary artery calcium; HDL: high density lipoprotein; LDL: low-density lipoprotein; CVD: cardiovascular disease; IL-6*Race/Ethnicity: IL-6 modified by race/ethnicity

Table 2: Calcium Densities and their associated Agaston score*

Calcium Density (Hounsfield units)	Agaston Score
0-129	0
130-200	1
201-300	2
301-400	3
401-higher	4

*Adapted from *Cecil Textbook of Medicine*, 22nd Edition, Elsevier Saunders, 2004[9]

CHAPTER II

RESEARCH DESIGN AND METHODOLOGY

Research Design (Figure 5)

A retrospective study was conducted using three hundred and seventy one subjects studied in 2006-2007 from eleven family medicine clinics of the North Texas Primary Care Practice-Based Research Network in Fort Worth, Texas, as part of the North Texas Healthy Heart Study. The convenient sample population consisted of 97 (28%) Caucasians, 124 (36%) Hispanics, and 123 (36%) African Americans. Subjects were of age 45 and older with no history of cardiovascular disease, kidney failure, or strokes. Twenty seven subjects were excluded due to either missing IL-6 measurements or calcium scores leaving a total of 344 for analysis. A urine pregnancy test was conducted on first visit in all females, and subjects with a positive test were excluded. All subjects were interviewed in either English or Spanish. They completed a medical history questionnaire asking personal information about themselves and their lifestyle. Ten milliliters of blood was taken to measure IL-6, fasting glucose, LDL cholesterol, HDL cholesterol, triglycerides, and total cholesterol concentrations. Finally, a non-contrast multi-slice CT scan of the coronary arteries was conducted at Radiology Associates Center for Diagnostic Imaging.

Informed Consent

All study procedures were approved by the University of North Texas Health Science Center Institutional Review Board. The study purpose, procedures, risks and discomforts, benefits, alternatives, and confidentiality were explained to each subject verbally and in an informed consent document. A research associate addressed any questions and concerns, and each subject signed the informed consent document. The person obtaining consent signed the document as well, recognizing the voluntary agreement of the subject to participate in the study. The subject received a copy of the document.

Measures (Table 3)

Each subject was assigned a unique identification number. Sociodemographic factors, such as gender, age, race/ethnicity, and level of education, were gathered. Race and ethnicity was categorized as Caucasian, African American, or Hispanic based on subject self-identification. All subjects completed a verbally-administered medical questionnaire available in English or Spanish. The questionnaire classified whether subjects had been diagnosed with high blood pressure, high cholesterol, diabetes, and/or heart disease. A list of current medications, if any, was collected, along with smoking status.

One hundred and seventy patients had blood pressure measured in the right arm while sitting; the blood pressure measurement was repeated in the left arm five minutes later with the patient in a sitting position. In order to quantify serum concentrations of

known cardiovascular risk factors, all subjects then had a venous blood sample taken after at least an eight hour fast. LDL, HDL, triglyceride, and total cholesterol concentrations were measured by a commercial laboratory (Quest Diagnostics®). IL-6 concentrations were also measured by a commercial laboratory (Quest Diagnostics®) using high sensitivity serum assays. A 16-slice multidetector CT scan was used to detect the presence of coronary calcium burden using standard techniques. The quantification of calcium was completed by a radiologist who was blinded to patient characteristics. The same radiologist read all of the scans.

Figure 5: Research design

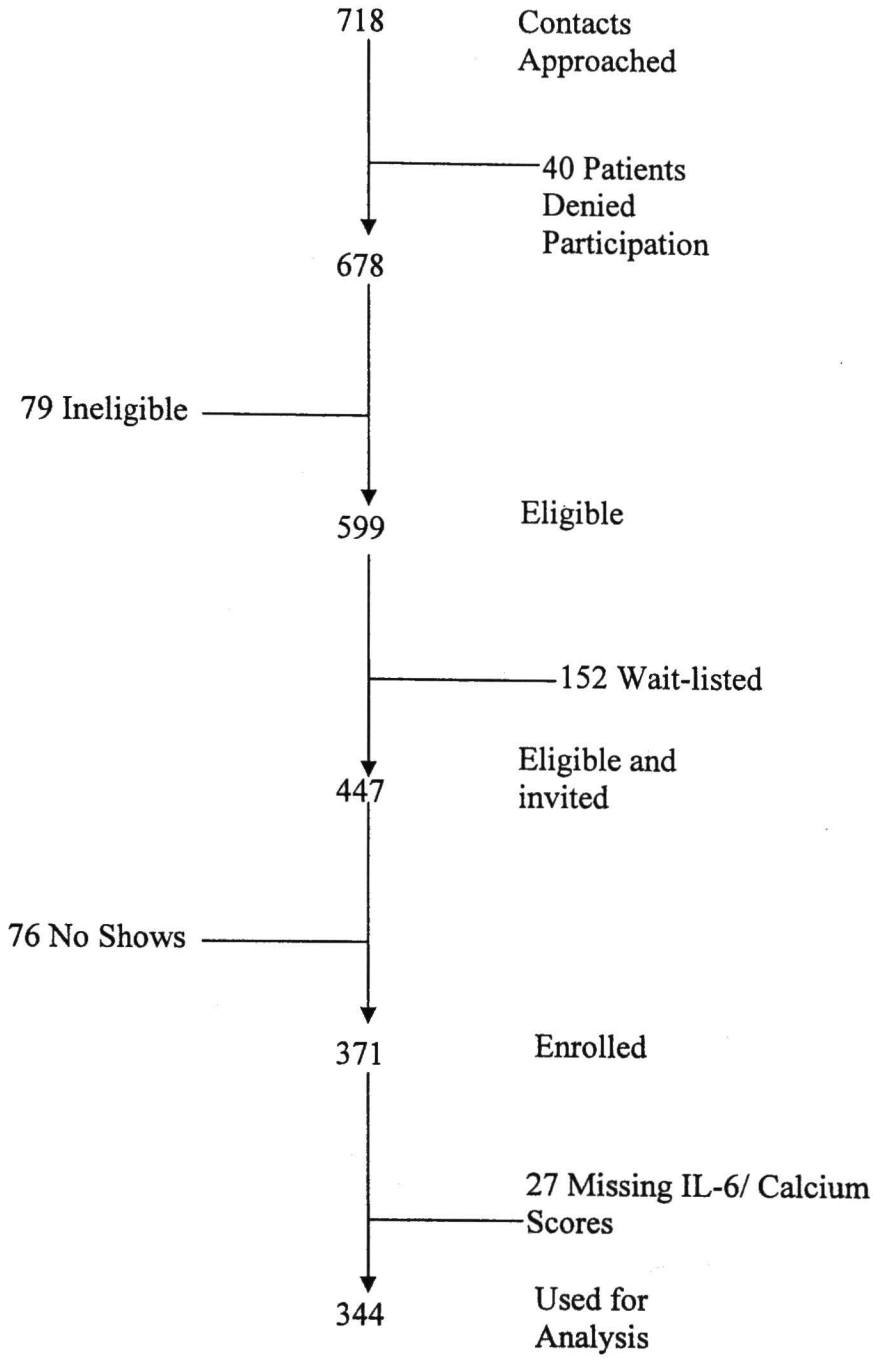


Table 3: List of variables and their types used in analyses

Variable Name	Options	Variable Type
IL6 (Interleukin-6)	(none) Normal range: 0.31-5 ng/L	Continuous
Calcium Score (Coronary Artery Calcium Scores)	1- Calcium score = 0 2- Calcium score: 1-10 3- Calcium score: 11-100 4- Calcium score: >100	Ordinal
Age	(none)	Continuous
Gender	Female Male	Dichotomous
Smoking Status	No smoking ever Smoker now or in the past	Dichotomous
Hypertension *If no Diabetes*: (systolic blood pressure > 140 OR diastolic blood pressure > 90 OR any hypertensive meds OR positive history of hypertension) *If Diabetes:* (systolic blood pressure > 130 OR diastolic blood pressure > 80 OR any hypertensive medication OR positive history of hypertension)	No Hypertension Hypertension	Dichotomous
Diabetes history of diabetes OR any glucose-lowering medication OR fasting glucose concentration >= 126 mg/dL	No Diabetes Diabetes	Dichotomous

Table 3: List of variables and their types used in analyses, continued

Cholesterol *If no Diabetes:* (history of hypercholesterolemia OR any cholesterol medication OR LDL > 160 OR HDL < 40 OR total cholesterol > 200 OR triglycerides > 150) *If Diabetes:* (history of hypercholesterolemia OR any cholesterol medication OR LDL > 100 OR HDL < 40 OR total cholesterol > 200 OR triglycerides > 150)	No Cholesterol Cholesterol	Dichotomous
Race/Ethnicity	Caucasians African Americans Hispanic	Nominal

CHAPTER III

ANALYSIS

Coronary artery calcium score was the dependent variable in all analyses. Because only 35.5% of the study population had calcium scores of greater than 0 and a few subjects had calcium scores of greater than one thousand, the dependent variable was largely skewed. Since population normality could not be assumed, linear regression was not performed. Several approaches were investigated in order to maximize the number of associations found. This included linear regression of transformed coronary artery calcium scores, such as natural log of calcium scores (excludes all subjects with 0 as their calcium score) and natural log of calcium score + 1 (ln (CAC+1)). Also, dichotomizing calcium scores was another option. These methods revealed fewer associations than ordinal regression. Ordinal regression was initially performed by categorizing calcium scores into five categories (Table 4). Category 4 and 5 were combined into one category because of their small sample sizes. Using ordinal regression or linear regression of transformed calcium scores to report scores would have been the most preferable in order to find the maximum number of associations as previously shown in other studies[41, 42]. The reason ordinal regression was chosen over natural log of calcium scores was because this methodology displayed the most clinically relevant data. The proportional odds assumption was met therefore ordinal regression could be used to explore the association between calcium scores and all cardiovascular disease predictors and IL-6. For each

predictor discussed, the odds of higher calcium score are the same across all categories. For instance, males in Category 1 are approximately two times more likely to have higher calcium scores than males in Category 2. Similarly, males in Category 2 are also approximately two times more likely to have higher calcium scores than males in Category 3. Reilly et al found that ln (CAC+1) and ordinal regression of categories of calcium scores uncovered the highest number of associations and more reliable results[41]. This held true for our study population as well.

Descriptive statistics, such as means, standard deviations, and percentages, were used to analyze all participant demographic and medical information, including age, gender, smoking status, hypertension, diabetes, hypercholesterolemia, and race/ethnicity. Analysis of variance (ANOVA) was used to test for differences amongst all categories of calcium scores. Univariate logistic regression was used to examine the relationship of IL-6 and traditional cardiovascular predictors to coronary artery calcium burden. Race/ethnicity was tested as an interaction term between IL-6 and coronary artery calcium scores in the fully-adjusted model. If race/ethnicity was significant as an interaction term, the results would have been stratified. Age, gender, smoking status, hypertension, diabetes, cholesterol, and race/ethnicity were added to a model to assess for confounding effects using ordinal regression. All analyses were performed with SPSS 15.0 at a 0.05 level of significance.

Diabetes is diagnosed in one of three following ways: plasma glucose concentration of 126 mg/dL or greater after an overnight fast, which should be confirmed with a repeat test; symptoms of diabetes and a random plasma glucose concentration of

200 mg/dL or greater; or oral glucose tolerance test that shows a plasma glucose concentration of 200 mg/dL or greater at two hours after a 75 gram glucose load[43]. As part of the study's protocol, the plasma glucose concentration after an overnight fast was performed once. The definition of diabetes was broadened to include subject self-identification as a diabetic. Also, if a subject took a glucose-lowering medication, he or she was also categorized as having diabetes (Table 3).

Although LDL cholesterol levels are frequently normal in diabetics, the particles are smaller and denser than in non-diabetics, thus more prone to atherogenesis[9]. The Adult Treatment Panel III (ATP III) considers diabetics in the high-risk category and confirms a reduction in their risk of developing cardiovascular complications with LDL lowering to less than 100 mg/dL[16, 17]. For this reason, 100 mg/dL was used for diagnosis of dyslipidemia in diabetics. For those in the low-risk category, the ATP III recommends LDL cholesterol lowering to 160 mg/dL[16, 17]. Thus, this level was used as a cutoff for diagnosis of dyslipidemia in non-diabetics (Table 3).

Hypertension is defined as systolic blood pressure of greater than 140 mm Hg or diastolic blood pressure of greater than 90 mm Hg in non-diabetics. The definition is based on the average of two or more readings taken at each of two or more visits after an initial screening[11, 44, 45]. In those with chronic kidney disease or diabetes, treatment of hypertension should be more aggressive with a goal blood pressure of less than 130/80 mm Hg[44, 46]. For this reason, the algorithm for diagnosis of hypertension in a diabetic reflected this current guideline. Subjects had two readings done five minutes apart; the average of the two readings was used in order to determine whether a subject was

categorized as hypertensive. The definition was broadened to include self-identification as a hypertensive. Also, taking a blood pressure lowering medication would automatically categorize the subject as having hypertension (Table 3).

Table 4: Categories of calcium scores with respective sample size before and after collapse of categories 4 & 5

Category	Agaston Scores	Sample Size
1	0	222
2	1-10	33
3	11-100	52
4	101-400	19
5	401+	18

Category	Agaston Scores	Sample Size
1	0	222
2	1-10	33
3	11-100	52
4	101+	37

CHAPTER IV

RESULTS

Overall, 122 individuals (35.5%) had a calcium score of greater than zero. Mean IL-6 concentrations were not significantly different amongst the categories. The mean age for those without any coronary artery calcium (53.76 years ± 7.2) was significantly lower than those in Category 2 (56.1 years ± 7.6), Category 3 (57.9 years ± 9.0), and Category 4 (62.1 years ± 7.6). A total of 218 females participated in the study, of which 154 (70.6%) were in Category 1, 15 (6.9%) in Category 2, 32 (14.7%)in Category 3, and 17 (7.8%) in Category 4. Among all males, 68 of the 126 (54.0%) were in Category 1, while 18 (14.3%) were in Category 2, and 20 (15.9%) were in Category 3 and 4 each. Among all smokers, 47 (13.7%) were current smokers or have smoked in the past. Of those that have never smoked, the majority (63.4%) were in Category 1, followed by Category 3, 4, and 2. 51.2% had hypertension, the majority of who were in Category 1 (58.0%). 19 (10.8%), 31 (17.6%), and 24 (13.6%) of those who had hypertension made up Category 2, 3, and 4 respectively. 120 (71.4%) subjects without hypertension were in Category 1, followed by 14 (8.3%) in Category 2, 21 (12.5%) in Category 3, and 13 (7.7%) in Category 4. 64 (18.6%) subjects were diabetics; exactly half of this group had evidence of calcium burden. The majority of non-diabetics (67.9%) had no calcium burden. 282 (82%) had dyslipidemia, of which 170 (60.3%) were in Category 1, 32 (11.3%) in Category 2, 47 (16.7%) in Category 3, and 33 (11.7%) in Category 4. 52

(83.9%) out of 62 subjects without dyslipidemia had no calcium burden. 97 (28.2%) were Caucasians, 123 (35.8%) were African Americans, and 124 (36%) were Hispanics. The majority of each of these racial/ethnic groups belonged to Category 1. Caucasians with calcium score of greater than 0 were mostly in Category 4 while African Americans with a calcium burden were mostly in Category 3. Hispanic subjects with a calcium burden were distributed equally into Category 2, 3, and 4.

Table 6 shows the results of the univariate logistic analyses. In the analyses, IL-6 was not associated with an increase in coronary artery calcium scores. However, age, gender, hypertension, diabetes, race/ethnicity, and dyslipidemia were significantly associated with an increase in calcium scores. With regards to age, a one year increase in age was associated with a 9% greater odds of having higher coronary artery calcification (OR: 1.09, 95% CI: 1.06, 1.12). Males were 1.97 times more likely to have higher calcification than females (OR: 1.97, 95% CI: 1.25, 3.08). African Americans and Hispanics were at 0.54 (OR: 0.54, 95% CI: 0.31, 0.92) and 0.40 greater odds (OR: 0.40, 95% CI: 0.23, 0.69), respectively, of having higher calcification than Caucasians. In other words, Caucasians were 1.85 and 2.5 times more likely to have a higher calcium burden over African Americans and Hispanics, respectively. Diabetics were at 2.20 greater odds of having higher coronary artery calcification than non-diabetics (OR: 2.20, 95% CI: 1.28, 3.76). Dyslipidemia was 2.31 times more associated with greater calcification than a normal lipid profile (OR: 2.31, 95% CI: 1.46, 3.67). Hypertension was 1.83 times more associated with calcium score greater than 0 than those without hypertension (OR: 1.83,

95% CI: 1.17, 2.86). Smoking status (OR: 0.86, 95% CI: 0.45, 1.63) was not shown as a significant predictor of coronary artery calcium.

Results of ordinal regression are presented in Table 7. In these analyses, IL-6 was not a significant predictor of coronary artery calcium scores after taking age, gender, race, smoking status, hypertension, diabetes, and dyslipidemia into account. Age, gender, race, diabetes, and dyslipidemia were significantly associated to high coronary artery calcium scores after taking other factors into account. Race/ethnicity was not a significant modifier thus the results were not stratified as such (p-value > 0.1). A one year increase in age was associated with 1.09 greater odds of having higher coronary artery calcification (OR: 1.09, 95% CI: 1.06, 1.13). Males were 2.41 times more likely associated with having higher calcification than females (OR: 2.41, 95% CI: 1.46, 3.97). African Americans and Hispanics were at 0.41 (OR: 0.41, 95% CI: 0.22, 0.74) and 0.33 greater odds (OR: 0.33, 95% CI: 0.18, 0.61), respectively, of having higher calcification than Caucasians. In other words, Caucasians were 2.44 and 3.03 times more likely associated with having higher calcium burden than African Americans and Hispanics, respectively. Diabetics were at 1.88 greater odds of having higher coronary artery calcification than non-diabetics (OR: 1.88, 95% CI: 1.01, 3.50). Dyslipidemia was 1.87 times more associated with higher calcification than a normal lipid profile (OR: 1.87, 95% CI: 1.11, 3.13). Hypertension (OR: 1.28, 95% CI: 0.76, 2.17) and smoking status (OR: 1.06, 95% CI: 0.51, 2.19) were not shown as significant predictors of coronary artery calcium scores.

Table 5: Demographics of the Study Population (N=344)

	Category 1 n (%)	Category 2 n (%)	Category 3 n (%)	Category 4 n (%)	p-value*
Total	222 (64.5)	33 (9.6)	52 (15.1)	37 (10.7)	
IL-6, Mean (SD**)	2.5 (1.9)	3.5 (7.5)	4.3 (10.8)	2.8 (2.4)	0.69
Age, Mean (SD**)	53.8 (7.2)	56.1 (7.6)	57.9 (9.0)	62.1 (7.6)	<0.001
Gender					0.01
Females	154 (70.6)	15 (6.9)	32 (14.7)	17 (7.8)	
Males	68 (54.0)	18 (14.3)	20 (15.9)	20 (15.9)	
Smoking Status					0.79
No Smoking Ever	180 (63.4)	28 (9.9)	45 (15.8)	31 (10.9)	
Smoker Now/ Past	32 (68.1)	4 (8.5)	5 (10.6)	6 (12.8)	
Hypertension					0.01
No Hypertension	120 (71.4)	14 (8.3)	21 (12.5)	13 (7.7)	
Hypertension	102 (58.0)	19 (10.8)	31 (17.6)	24 (13.6)	
Diabetes					<0.001

Table 5: Demographics of the Study Population (N=344), continued

	Category 1 n (%)	Category 2 n (%)	Category 3 n (%)	Category 4 n (%)	p-value*
No Diabetes	190 (67.9)	26 (9.3)	39 (13.9)	25 (8.9)	
Diabetes	32 (50)	7 (10.9)	13 (20.3)	12 (18.8)	
Cholesterol					0.01
No Cholesterol	52 (83.9)	1 (1.6)	5 (8.1)	4 (6.5)	
Cholesterol	170 (60.3)	32 (11.3)	47 (16.7)	33 (11.7)	
Race/Ethnicity					<0.001
Caucasian	51 (52.6)	10 (10.3)	17 (17.5)	19 (19.6)	
African American	80 (65.0)	13 (10.6)	24 (19.5)	6 (4.9)	
Hispanic	91 (73.4)	10 (8.1)	11 (8.9)	12 (9.7)	

*P-value corresponds to a two-tailed p-value for ANOVA; **SD, standard deviation; Category 1: Agaston score 0; Category 2: Agaston score 1-10; Category 3: Agaston score 11-100; Category 4: Agaston score >100

Table 6: Univariate Logistic Regression to Assess the Relationship of IL-6 and Traditional Cardiovascular Predictors to Coronary Artery Calcium Burden (N=344)

	OR*	95% CI**	p-value
IL-6	1.03	0.99- 1.07	0.21
Age	1.09	1.06-1.12	<0.001
Gender			
Females	…	…	…
Males	1.97	1.25-3.08	<0.001
Smoking Status			
No Smoking Ever	…	…	…
Smoker Now Past	0.86	0.45-1.63	0.64
Hypertension			
No Hypertension	…	…	…
Hypertension	1.83	1.17-2.86	0.01
Diabetes			

Table 6: Univariate Logistic Regression to Assess the Relationship of IL-6 and Traditional Cardiovascular Predictors to Coronary Artery Calcium Burden (N=344), continued

	OR*	95% CI**	p-value
No Diabetes	…	…	…
Diabetes	2.20	1.28-3.76	<0.001
Cholesterol			
No Cholesterol	…	…	…
Cholesterol	2.31	1.46-3.67	<0.001
Race/Ethnicity			
Caucasian	…	…	…
African American	0.54	0.31-0.92	<0.001
Hispanic	0.40	0.23-0.69	<0.001

*OR, Odds Ratio; **CI, Confidence Interval; …, Referent Categories

Table 7: Ordinal Regression Models to Assess the Relationship of IL-6 and Traditional Cardiovascular Predictors to Coronary Artery Calcium Burden (N=344)

	OR*	95% CI**	p-value
IL-6	1.03	0.98-1.07	0.25
Age	1.09	1.06-1.13	<0.001
Gender			
Females	…	…	…
Males	2.41	1.46-3.97	<0.001
Smoking Status			
No Smoking Ever	…	…	…
Smoker Now/ Past	1.06	0.51-2.19	0.89
Hypertension			
No Hypertension	…	…	…
Hypertension	1.28	0.76-2.17	0.35

Table 7: Ordinal Regression Models to Assess the Relationship of IL-6 and Traditional Cardiovascular Predictors to Coronary Artery Calcium Burden (N=344), continued

	OR*	95% CI**	p-value
Diabetes			
No Diabetes	…	…	…
Diabetes	1.88	1.01-3.50	0.05
Cholesterol			
No Cholesterol	…	…	…
Cholesterol	1.87	1.11-3.13	0.02
Race/Ethnicity			
Caucasian	…	…	…
African American	0.41	0.22-0.74	<0.001
Hispanic	0.33	0.18-0.61	<0.001

*OR, Odds Ratio; **CI, Confidence Interval; …, Referent Categories

CHAPTER V

DISCUSSION

The primary endpoint, IL-6, did not prove to be a significant predictor of coronary artery calcium scores either alone or after adjusting for age, gender, race, smoking status, dyslipidemia, hypertension, and diabetes. A possible explanation for this is that there is no relationship between IL-6 and coronary artery calcium burden. In addition, relative IL-6 concentration found at the periphery of atherosclerotic plaque may be higher than that found in the serum, i.e. its concentration in venous blood is considerably diluted[26]. Also, a large circadian variation exists in IL-6 concentrations[24, 26, 47, 48]. IL-6 is secreted in a biphasic circadian pattern with two nadirs at about 08:00 AM and 09:00 PM, and two peaks at about 07:00 PM and 05:00 AM[48]. One study found IL-6 concentrations to be significantly higher at 02:00 AM compared to 09:00 AM in subjects with acute myocardial infarctions and in healthy controls[47]. For this reason, this marker may be limited in its usefulness if only measured at one point during the day. Additionally, circulating cytokines, such as IL-6, generally have a shorter half-life[28] than their receptors, thus this may limit their usefulness in measuring chronic sub-clinical disease processes. Finally, only 35.5% of the study population had coronary artery calcification. Of these, only 37 subjects (10.8%) had moderate to high calcium scores (Agaston score greater than 100). Thus, not enough subjects with advanced atherosclerosis were assessed to find an association between IL-6 and coronary artery calcium scores.

For a one year increase in age over 45, the odds of higher calcium scores increase by 1.09. Endothelial changes that occur in the cardiovascular system with aging include increased deposition of protein in the sub-endothelial layer, buildup of lipid by-products, arterial wall modification, and alterations secondary to vasoactive stimuli. These changes to cardiovascular physiology and structure increase incidence of atherosclerosis[49]. Males were also approximately two times more likely to have a higher calcium score than females. This is secondary to androgens increasing lipoprotein lipid levels and lowering HDL with aging. An independent inverse association between testosterone concentration and atherosclerosis in men has been shown in multiple studies. Middle-aged men with testosterone deficiency and symptoms of andropause show increased carotid atherosclerosis[72]. Relative to men 55 years and older with the highest concentration of testosterone, men in the same age group but with the lowest concentration of testosterone were at a higher relative risk for the presence of severe aortic atherosclerosis according to the Rotterdam Study[73]. Females are also at an increased risk after menopause because of estrogen decline leading to LDL increase[50]. Associations of age and gender were consistent with findings of previous studies confirming these two as traditional risk factors of atherosclerosis[50-54]. Although traditional risk factors are very valuable in predicting the development of cardiovascular disease in women, many women without established risk factors for atherosclerosis suffer cardiovascular events[53, 54]. Our study affirms that coronary artery calcium scanning can be used to estimate the overall atherosclerotic plaque burden in women. It can also be used to diagnose its presence and

determine its extent. This paves the way for future studies to establish various therapeutic options regarding changes in or regression of calcium burden.

Smoking was not found to be a significant predictor of coronary artery calcium burden. This finding was not consistent with previous studies that have found cigarette smoking to be a significant independent predictor of calcium burden[52-54]. Since only 14.2% of the study population were smokers, our data may not have been sufficiently powered to find this difference. The low prevalence of smoking could contribute to the statistical difference between the populations of smokers and non-smokers. Hoff et al. screened approximately 31,000 asymptomatic individuals and used logistic regression analysis to compare odd ratios for established risk factors. Although they found associations for several established risk factors, such as age and gender, cigarette smoking and hypercholesterolemia were not associated with higher calcium scores[41, 42]. Several types of atherosclerotic lesions have been identified, such as thin cap fibroatheromas (vulnerable plaque) and fibroatheroma with or without the presence of dense calcium[55]. Thus, it is possible that smoking may not be associated with calcium-laden plaques. Further research may be needed to elucidate the pathophysiologic process in which smoking contributes to the development of atherosclerosis.

Race was found to be a significant independent predictor of coronary artery calcium. Caucasians were 2.5 and approximately three times more likely to have a higher calcium score than African-Americans and Hispanics, respectively. In the fully-adjusted model, Caucasians were 1.85 and 2.5 times more likely to have higher calcium scores than African-Americans and Hispanics respectively. Similarly, Caucasians with calcium

scores in Category 2 were 1.85 and 2.5 times more likely to have higher calcium scores than African-Americans and Hispanics in Category 3, respectively. This finding was consistent with previous studies, which have found lower calcium scores in African-Americans and Hispanics[34, 56]. White men have a greater incidence of plaque rupture than African American men, especially in their fourth and fifth decades[57]. Our study results suggest significant difference in the presence and severity of calcification according to ethnicity, independent of atherosclerotic risk factors. However, race did not modify the relationship between IL-6 and coronary artery calcium ($p > 0.9$), therefore, the results were not stratified. These results further provide evidence that there are alternate explanations for the increased number of cardiovascular related deaths in African Americans and Hispanics. Minorities in the U.S. have less access to health care, receive lower quality health care, and have higher rates of illnesses and premature deaths. Nearly 60% of Hispanics and 43% of African-Americans were uninsured in the earlier part of the decade compared to 24% of Caucasians[58]. Low income levels and social and cultural barriers further explain these health disparities. Income level, in particular, is strongly associated with the use of health services and health outcomes. The tendency of the economically disadvantaged to delay seeking treatment often until the advanced stages of disease points to the need for early prevention efforts[58].

Diabetes was a significant predictor of coronary artery calcium scores independent of other risk factors. Diabetics were approximately two times more likely to have coronary artery calcium present than non-diabetics. This was consistent with previous studies using electron-beam CT to evaluate calcium in the coronary arteries[59, 60].

The structural abnormalities of coronary vasculature in diabetics include endothelial dysfunction and reduced compliance. Also, AGEs interact with specific receptors that lead to overexpression of a range of cytokines. These processes lead to activation of hemodynamic pathways which are further exacerbated by lipid modulation, hyperglycemia, growth factor activation, systemic hypertension, and non-enzymatic glycosylation[61]. These activators of endothelial cell dysfunction contribute to fibrofatty plaque buildup, lead to more advanced and vulnerable atherosclerotic plaque formation, and subsequently increase risk for myocardial infarction and stroke[8, 61]. Patients with diabetes have a significant increase in the prevalence of high calcium scores (greater than or equal to 400) compared with the randomly selected and matched non-diabetic control groups. Scores in this range were reported to be highly predictive for abnormal stress tests and subsequent coronary events[59, 60]. Therefore, our results suggest a significant occurrence of calcium burden in asymptomatic diabetic subjects compared with non-diabetic control subjects. They also indicate that, if confirmed by prospective studies, multi-slice CT scans may be a useful approach for further evaluation of diabetic subjects for sub-clinical cardiovascular disease.

Hypertension has been found to be a predictor of coronary artery calcium in past studies[62]. Our results found those with hypertension to be 1.8 times more likely to have an increase in calcium burden when compared to non-hypertensive subjects. However, this association became non-significant in the fully-adjusted model. This implies another variable in the model, such as age, may be confounding the association between hypertension and calcium scores. Systolic blood pressure increases with age secondary to

endothelial dysfunction, activation of the renin-angiotensin system and, therefore, vascular remodeling. Elevated blood pressure, particularly systolic blood pressure, represents altered cardiovascular physiology and structure. Hypertension is associated with leukocyte adhesion, macrophage accumulation, smooth muscle cell migration and proliferation, and intimal thickening. It also facilitates atherosclerotic progression by causing endothelial injuries through oxidative stress, which result in further leukocyte recruitment into arterial wall[63]. Accumulation of lipids and formation of atherosclerotic plaque follow endothelial injury and lead to increases in large artery stiffness, which translates to an increase in systolic blood pressure[64, 65].

Dyslipidemia was found to be a significant predictor of coronary artery calcium scores. Subjects with dyslipidemia were approximately two times more likely to be associated with calcium burden than those without dyslipidemia. This association remains essentially unchanged in the presence of other predictors in the ordinal regression model. The increase in LDL uptake by endothelial cells and retention of LDL into the vessel wall leads to further progression of atherosclerotic plaques[66]. Along with deposited lipids, smooth muscle cells and their connective tissue products are also major contributors of plaque progression. Lipid deposition though occurs in lesion-prone areas of the coronary arteries prior to smooth muscle cell emigration and proliferation[66, 67]. Previous studies have found coronary artery calcium regression by lowering LDL cholesterol with statin therapy[68]. Coronary artery calcium is a well-accepted marker of atherosclerosis, with dyslipidemia as one of the main risk factors for atherosclerosis. Thus, it is reasonable to conceptualize that calcium burden may be halted or even

reversed by lowering of LDL cholesterol with statin therapy. The benefits of statins in the primary and secondary prevention of coronary artery disease appear to occur much sooner and are bigger than those expected from changes in lipid concentrations alone. This implies effects of statins beyond merely cholesterol lowering. Studies have suggested other benefits such as improvement of endothelial function, stabilization of atherosclerotic plaque, decrease of oxidative stress and inflammatory burden, and thrombogenic inhibition[69]. Lovastatin, for example, is effective in total plasma cholesterol and LDL cholesterol lowering enough to decrease morbidity and mortality by 30%, thus justifying the efficacy of statins for primary and secondary prevention of cardiovascular disease outcomes. Therapy with lovastatin for two years has shown to significantly improve coronary calcium scores and reduce the incidence of new lesions when compared with placebo[68, 70]. This strongly suggests that statin therapy can treat not only dyslipidemia, but slow progression and lower atherosclerotic burden in asymptomatic individuals with sub-clinical cardiovascular disease.

Our results are subject to a number of limitations. Since the patient population consists of only those from Fort Worth, Texas, it cannot be generalized to a more heterogeneous target population. The sample of patients obtained from all clinics is convenient and not random. This also affects our results' generalization to the target population. Because our subjects seek medical attention, traditional risk factors may be underrepresented in this sample than a more heterogeneous sample population which consists of subjects not seeking health care. Also, no causal relationships can be established between risk factors and atherosclerosis since the study was cross-sectional.

The plasma glucose concentration after an overnight fast was used to diagnose diabetes; this was performed only once, thus leading to an underestimation of the actual risk of a subject. In order to offset this, the definition of diabetes was broadened to include subject self-identification as a diabetic. If a subject took glucose-lowering medication, he or she was also categorized as having diabetes.

Although two blood pressure readings were obtained during the patient visit, two more readings are required at a subsequent visit for proper diagnosis. This could lead to an overestimation of hypertension in our study population. Furthermore, greater than two-thirds of the study population did not have any blood pressure measurements during the day of visit, thus leading to undiagnosed hypertension. Thus, the definition of hypertension was broadened to include self-identification as a hypertensive. Also, taking a blood pressure lowering medication would automatically categorize the subject as having hypertension.

The process of atherosclerosis is chronic and takes many years to develop, while our measure of inflammation, IL-6, was measured only one time. Future studies must have repeated measurements of IL-6 concentrations at its proposed peak secretion times. Also, blood samples measuring IL-6 concentrations should be taken at a site closer to the atherosclerotic lesion rather than in the serum since this inflammatory biomarker is found at the border of an atherosclerotic plaque[24, 26]. Furthermore, research has found that circulating IL-6 receptors may be present at more constant concentrations over time and generally have longer half-lives than the cytokine itself. The association between IL-6 receptor concentration and cardiovascular disease, especially during its early phases, has

been hypothesized[28, 71]. Thus, further investigation should be conducted to note whether IL-6 receptor concentration can uncover a possible association with calcium burden.

Several traditional risk factors were found to be significantly associated with coronary artery calcium burden. It remains to be determined whether calcium scores provide additional prognostic information over traditional, established risk factors in those with greater than 20% risk of developing cardiovascular events over ten years[4]. Future studies are needed to analyze and confirm these associations and the cost-effectiveness of using emerging cardiovascular markers and coronary artery calcium scores to predict cardiovascular risk. Regardless, understanding a possible relationship of inflammation and the development of atherosclerosis can potentially help researchers and clinicians to better diagnose and target treatment options for individuals.

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