Comparison of Non-HDL Cholesterol and Waist Circumference vs. Triglyceride Levels and Waist Circumference in Predicting Coronary Heart Disease Risk

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ABSTRACT

BACKGROUND: Dyslipidemia is recognized as a major cause of coronary heart disease (CHD). Emerged evidence suggests that the combination of triglycerides (TG) and waist circumference can be used to predict the risk of CHD. However, considering the known limitations of TG, non-high-density lipoprotein (non-HDL = Total cholesterol – HDL cholesterol) cholesterol and waist circumference model may be a better predictor of CHD.

PURPOSE: The Framingham Offspring Study data were used to determine if combined non-HDL cholesterol and waist circumference is equivalent to or better than TG and waist circumference (hypertriglyceridemic waist phenotype) in predicting risk of CHD.

METHODS: A total of 3,196 individuals from Framingham Offspring Study, aged ≥ 40 years old, who fasted overnight for ≥ 9 hours, and had no missing information on non-HDL cholesterol, TG levels, and waist circumference measurements, were included in the analysis. Receiver Operator Characteristic Curve (ROC) Area Under the Curve (AUC) was used to compare the predictive ability of non-HDL cholesterol and waist circumference and TG and waist circumference. Cox proportional-hazards models were used to examine the association between the joint distributions of non-HDL cholesterol, waist circumference, and non-fatal CHD; TG, waist circumference, and non-fatal CHD; and the joint distribution of non-HDL cholesterol and TG by waist circumference strata, after adjusting for age, gender, smoking, alcohol consumption, diabetes, and hypertension status.

RESULTS: The ROC AUC associated with non-HDL cholesterol and waist circumference and TG and waist circumference are 0.6428 (CI: 0.6183, 0.6673) and
0.6299 (CI: 0.6049, 0.6548) respectively. The difference in the ROC AUC is 1.29%. The p-value testing if the difference in the ROC AUCs between the two models is zero is 0.10. There was a strong positive association between non-HDL cholesterol and the risk for non-fatal CHD within each TG levels than that for TG levels within each level of non-HDL cholesterol, especially in individuals with high waist circumference status.

CONCLUSION: The results suggest that the model including non-HDL cholesterol and waist circumference may be superior at predicting CHD compared to the model including TG and waist circumference.
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LIST OF ABBREVIATIONS

Apo B – Apolipoprotein B
BMI – Body mass index
CHD – Coronary heart disease
CI – Confidence interval
CVD – Cardiovascular disease
HDL – High-density lipoprotein
HR – Hazard ratio
IDL – Intermediate-density lipoprotein
MEC – Mobile Examination Centre
NCEP ATP – National Cholesterol Education Program – Adult Treatment Panel
NHANES – National Health and Nutrition Examination Survey
NHLBI – National Heart Lung and Blood Institute
Non-HDL – Non-high-density lipoprotein
LDL – Low-density lipoprotein
ROC AUC – Receiver operator characteristic area under curve
TG – Triglycerides
VLDL – Very-low-density lipoprotein
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CHAPTER 1. INTRODUCTION

Coronary heart disease (CHD) is the single most common cause of death among middle-aged individuals around the world. In the U.S., it affects approximately 13 million people (Lofgren et al., 2004). The risk of mortality and morbidity resulting from cardiovascular disease is of great public health concern. Therefore, it is important to identify the individuals in the population that are at increased risk of CHD. Many risk factors are associated with the increased risk of CHD, however much attention has been focused on the elevated lipid profile and its atherogenic properties as a very powerful risk factor for the cardiovascular disease (CVD). Elevated triglyceride (TG) levels, TG-rich very low density lipoprotein (VLDL) cholesterol, low density lipoprotein (LDL) cholesterol, and low levels of high-density lipoprotein (HDL) cholesterol are characteristic risk factors of CHD. Research from various studies suggests that dyslipidemia is a major cause of CVD. It is also known that lowering the lipid levels can slow the progression and reduce the risk of cardiovascular events (Brown et al., 2000; Grundy, 2000).

Nevertheless, recent research suggests that factors other than the traditional lipoproteins and lipids may be better predictors of CHD risk. In this regard, concentrations of apolipoprotein B (apo B), insulin resistance, and high level of small dense LDL cholesterol, which has been named the metabolic triad, was proposed as an important predictor of CHD risk (St-Pierre et al., 2007). Metabolic triad is also highly prevalent among individuals with high accumulation of visceral adipose tissue (Lemieux et al., 2000). However, there are increased costs associated with the measurement of insulin, apo B, and small dense LDL cholesterol, thereby limiting its use in clinical
practice. To overcome this limitation, waist circumference was suggested as a substitute for the measurement of visceral adipose tissue accumulation and fasting TG concentrations have been used to predict LDL size (St-Pierre et al., 2002; Lemieux et al., 2000). Hypertriglyceridemic waist phenotype is defined as concurrently having plasma TG levels of $\geq 177$ mg/dL and waist circumference of $\geq 90$ cm in males (Lemieux et al., 2000), and TG levels of $\geq 150$ mg/dL and waist circumference of $\geq 88$ cm in females (LeMonte et al., 2003). However, the role of hypertriglyceridemia as an independent predictor for the risk of CHD is controversial. Various problems may arise as a result of using the hypertriglyceridemic waist phenotype to predict CHD risk. For example, it is difficult to determine if hypertriglyceridemia can independently predict the risk of CHD because of its association with abnormal lipid metabolism as well as other CHD risk factors (McBride, 2007; Ginsberg, 2002). Further, it is known that TG does not directly participate in the process of atherosclerosis but it exerts its effects indirectly through the accumulation of cholesterol-rich VLDL remnants in the extracellular matrix (Packard et al., 2004). In addition, elevated TG levels indirectly affect the formation of atherosclerotic plaque by causing structural abnormalities in LDL and HDL particles. It causes the formation of small dense LDL cholesterol and reduces the size and quantity of HDL cholesterol thereby reducing its protective effects (Packard et al., 2004). Therefore, a measurement of atherogenic lipoproteins, that include all the potential atherogenic lipoproteins such as LDL, IDL, VLDL and its remnants, and is a simple and an inexpensive measure, may be a better clinical tool.

Research indicates that elevated TG-rich lipoproteins such as VLDL may be an independent risk factor for CHD. As a result, the possible measure is the non-high density lipoprotein (non-HDL) cholesterol, which combines all atherogenic lipoproteins into a
single fraction, making the use of non-HDL cholesterol a simple and relatively inexpensive indicator of CHD risk. It is calculated by subtracting the HDL cholesterol from total cholesterol. Non-HDL cholesterol incorporates LDL, IDL, and VLDL cholesterol, and it reflects the cholesterol content of all apo B containing lipoproteins. Non-HDL cholesterol can be used as a predictor of CHD in individuals with and without high TG levels, type 2 diabetes, and metabolic syndrome (Hoenig, 2008; Liu et al., 2006; Packard et al., 2004; Lehto et al., 1997). There is less day-to-day variability in non-HDL cholesterol compared to TG concentrations and is unaffected by TG levels. Unlike TG, non-HDL cholesterol requires measurements of only total cholesterol and HDL cholesterol which can be measured reasonably accurately in the non-fasting state and it is readily derived from the routine lipoprotein profile. For these reasons non-HDL cholesterol may be a highly useful lipid measure for predicting the risk of CHD and evaluating response in treatment of hyperlipidemia.

Obesity is another risk factor for CHD. In 2002, worldwide there were more than one billion adults categorized as overweight (BMI ≥ 25.0 kg/m²) and 300 million as obese (BMI ≥ 30.0 kg/m²) (Haffner, 2006). Increased abdominal adiposity is clearly associated with increased risk for CVD and premature death (Smith et al., 2005). Various studies have indicated that high waist circumference is associated with increased rates of cardiovascular and metabolic risk factors and an increased risk of chronic diseases, including diabetes, hypertension, and CHD (Smith et al., 2005). Therefore, combining non-HDL and waist circumference may prove to be a better indicator of CHD risk compared to hypertriglyceridemic waist phenotype. Unfortunately, there is lack of information with respect to the combination of non-HDL cholesterol and waist
circumference and its association with risk of CHD compared to hypertriglyceridemic waist phenotype.

The purpose of this study is to compare using non-HDL cholesterol and waist circumference, versus using TG and waist circumference in predicting the risk of non-fatal CHD.
CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Research from experimental animals, observational studies, and clinical trials indicate that dyslipidemia and abdominal obesity are two major independent risk factors of CHD (Carr et al., 2004; Paccaud et al., 2000; Zhou et al., 2000). LDL cholesterol has been used for a long time in management of dyslipidemia (Gotto, 1993).

Although, hypertriglyceridemic waist phenotype has been proposed to predict the risk of CHD, several concerns regarding using TG in this method are raised. In particular, the requirement of overnight fasting and larger intra-individual variation may limit its application in clinical practice.

This chapter will review both lipid risk factors and non-lipid risk factors in the process of atherosclerosis, and explore the rational of using non-HDL cholesterol and waist circumference in detecting the risk of CHD.

2.2 Structure, Function, and Classification of Lipids and Lipoproteins

The components of lipids include fatty acids, TG, cholesterol, and lipoproteins. Lipids serve several important physiologic functions within the human body. Lipids are an important source of energy and are essential component of the cell structure. For cell membranes, cholesterol provides an essential stabilizing function and facilitates membrane transport. It is required for biosynthesis of hormones and is a precursor of adrenal and sex hormones. Cholesterol is also required for the synthesis of bile acids; bile acids are crucial for the absorption of dietary fat in the small intestine. TG offers an ideal
means of energy storage and production. Through lipolysis, the cardiac and skeletal muscle cells extract and convert the TG from circulating lipoproteins to fatty acids and glycerol. Fatty acids are a major energy source for muscle cells. In a number of cells, such as muscle and liver cells, fatty acids can also be converted to glucose through gluconeogenesis (Kingsbury et al., 2003).

Although lipids serve several important functions within the body, they are hydrophobic and cannot be transported directly in the blood. Therefore, they bind to specific proteins called apolipoproteins to form soluble lipid-protein complexes called lipoproteins (Jain et al., 2007). Plasma lipoproteins are composed of TG and cholesterol esters at the core making the core hydrophobic, and phospholipids and apolipoproteins form the outer polar layer (Kingsbury et al., 2003). Thus, the amphipathic properties of apolipoproteins make the lipids soluble in an aqueous environment and thereby act as an interface between plasma and the core components. It is in this form that the major lipids circulate in the plasma. Several lipoprotein complexes exist and they are identified based on their size, density, and lipid and apolipoprotein composition as seen in Figure 1. (Homma, 2006; Sawle et al., 2002). There are five main types of lipoproteins present in the plasma including chylomicrons, very low-density lipoprotein (VLDL) (pre-β-lipoproteins), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) (β-lipoprotein), and high density lipoprotein (HDL) (α-lipoprotein) cholesterol (Lewis, 1973). Generally, in clinical practice, LDL and IDL are combined into a single fraction termed LDL cholesterol. According to size, LDL and HDL are small particles, VLDLs are medium sized particles, and chylomicrons are large particles (Lewis, 1973). These lipoproteins contain TG, cholesterol esters, and phospholipids in different proportions.
LDL contains cholesterol as its major lipid, while VLDL and chylomicrons contain TG as their major lipid component (Lewis, 1973).

2.3 Basic Description of Lipids and Lipoproteins

2.3.1 LDL Cholesterol

LDL cholesterol makes up 60 to 70% of the total serum cholesterol. It contains a single apolipoprotein called apo B-100 (apo B). LDL is the major atherogenic lipoprotein and has been identified as the primary target of cholesterol lowering therapy by the National Cholesterol Education Program – Adult Treatment Panel III (NCEP ATP III, 2002).

2.3.2 HDL Cholesterol

HDL cholesterol normally makes up 20 to 30% of the total serum cholesterol. The major apolipoproteins of HDL cholesterol are apo A-I and apo A-II (NCEP ATP III, 2002). HDL cholesterol levels are inversely associated with CHD risk. Some evidence shows that HDL cholesterol protects against the development of atherosclerosis, although low levels of HDL cholesterol reflects the presence of other atherogenic factors.

2.3.3 VLDL Cholesterol

The VLDL cholesterol contains TG-rich lipoproteins, but contains only 10 to 15% of the total serum cholesterol. The major apolipoproteins of VLDL are apo B-100, apo C-I, apo C-II, apo C-III, and apo E (NCEP ATP III, 2002). VLDL are precursors of LDL cholesterol and are produced by the liver. VLDL remnants, a form of VLDL is known to be atherogenic. VLDL remnants are partially degraded VLDL and are rich in cholesterol ester.
2.3.4 Chylomicrons

Similar to VLDL, chylomicrons are also TG-rich lipoproteins. They are formed in the epithelium of the small intestine from dietary fat. They contain the same lipoproteins as VLDL except that apo B-48 is present instead of apo B-100 (NCEP ATP III, 2002). Partially degraded chylomicrons known as chylomicron remnants are also atherogenic.

Although LDL is the primary target for lipid lowering therapy, growing evidence indicates that VLDL plays important roles in the process of atherosclerosis.

2.4 Pathways of Lipid Transport

There are three pathways involved in the generation and transport of lipids. These include the exogenous pathway, the endogenous pathway, and the reverse cholesterol transport pathway. The exogenous pathway is the pathway of dietary lipid transport from intestine to the liver (Figure 2). After digestion and absorption of dietary fat, chylomicrons, composed of TG, cholesterol, and apolipoproteins, are formed in the epithelial cells of the intestines (Jain et al., 2007). In the bloodstream, chylomicrons collect lipids and transport dietary TG from the intestines to adipose tissues and muscles for storage. Dietary cholesterol is then transported to the liver. On the endothelial cells of the adipose tissue and muscles, the TGs undergo hydrolysis via the action of the enzyme lipoprotein lipase and fatty acids are released (Jain et al., 2007). Some of the components of the chylomicrons are repackaged into other lipoproteins, for example, some apolipoproteins are transferred to HDL, and the remaining chylomicron remnant particles are taken up by the liver via LDL receptors (Jain et al., 2007). This results in dietary TG storage in adipose tissue and muscles.
The endogenous pathway is the route for synthesizing and transporting lipoproteins from the liver to the target cells (Figure 2). TG and cholesterol from the liver are packaged and then released in the circulation as VLDL particles. VLDL is transported to adipose tissue and muscles where lipoprotein lipase hydrolyzes it and fatty acids and glycerol are released (Jain et al., 2007). The fatty acids are taken up by muscle cells and adipose tissue for production of energy and storage respectively. After the hydrolysis of VLDL, it becomes a VLDL remnant. Most of the VLDL remnants are taken up by the liver by means of LDL receptors and the remaining remnant particles become IDL (Jain et al., 2007). Some of the IDL particles are reabsorbed by the liver via the LDL receptors while the others circulate until they hydrolyzed in the liver by hepatic-triglyceride lipase to form LDL (Jain et al., 2007). At each hydrolysis step TG are lost to the adipose tissue and muscles resulting in LDL particles rich in cholesterol. This results in the transfer of TG from liver to target cells via LDL. LDL is the main carrier of circulating cholesterol in the body because it transports cholesterol from the plasma and delivers it to the peripheral tissues. LDL represents the final stage in the catabolism of VLDL. Most LDL particles are removed from the plasma through LDL receptors in the liver while the remaining LDL is removed by the scavenger pathways in the cells (Jain et al., 2007). As LDL is taken up by receptors, free cholesterol is released and accumulated within the cells. The uptake of LDL by the LDL receptors regulates the LDL concentrations in the blood via several mechanisms including decreasing the synthesis of hydroxyl-3-methylglutaryl coenzyme A reductase (which controls the rate of cholesterol synthesis), suppressing the synthesis of new LDL receptors in the cells, and activating acyl-coenzyme A cholesterol acyltransferase (which esterifies free cholesterol into cholesterol ester, storing cholesterol in the cell) (Jain et al., 2007).
Finally, the third pathway is the reverse cholesterol pathway. Reverse cholesterol transport is a pathway where cholesterol is transported from the tissues and returned to the liver to be excreted into the feces via bile. HDL is involved in the reverse cholesterol transport and the transfer of cholesteryl esters between lipoproteins. HDL is formed through a maturation process in which precursor particles secreted by the liver and the intestine go through a series of conversions to attract cholesterol from cell membranes and free cholesterol to the core of the HDL particle (Asztalos et al., 2003). As the cell dies and the cell membranes turnover, free cholesterol is released into the plasma. It is immediately absorbed into HDL particles, esterified with a long chain fatty acid by lecithin cholesterol acyl transferase, and transferred to VLDL or IDL by a cholesteryl ester transfer protein in plasma (Asztalos et al., 2003). Eventually, it is taken up by the liver as IDL or LDL, thus resulting in the recovery of cholesterol from cell membranes, which is then reincorporated into LDL or returned to the liver (Jain et al., 2007; Asztalos et al., 2003).

Abnormalities in these pathways may result in hyperlipoproteinemias. High TG and low HDL cholesterol levels are components of the metabolic syndrome which is discussed below in further detail.

2.5 LDL Cholesterol as the Primary Target of Therapy

LDL cholesterol is identified as the primary target for cholesterol lowering therapy in the NCEP ATP I (1988), ATP II (1994), and ATP III (2002). Evidence from animal, pathological, clinical, genetic, and different types of population studies has shown that LDL is the most abundant atherogenic lipoprotein. The role of LDL in causing CHD has been supported by controlled clinical trials. LDL cholesterol levels as low as 25 to 60 mg/dL is physiologically sufficient (Brown et al., 1986). Animal species that do not
develop atherosclerosis normally have LDL cholesterol concentrations below 80 mg/dL (NCEP ATP III, 2002). In contrast, high levels of LDL cholesterol are known to be atherogenic. For instance, the Framingham Heart Study (Wilson et al., 1998), and the Lipid Research Clinics trial (1984), found a direct association between LDL concentrations and the rate of CHD in males and females who were initially free of CHD. There is also a direct relationship between LDL cholesterol levels and recurring coronary events in individuals with pre-existing CHD (Rossouw et al., 1990; Pekkanen et al., 1990; Wong et al., 1991). Studies performed on different populations also show that those with higher levels of total or LDL cholesterol have higher rate of atherosclerosis and CHD than those who have lower levels (Keys et al., 1984a). People migrating from regions where cholesterol levels are low to regions with high cholesterol levels show an increase in their cholesterol levels as well as the risk for developing CHD (Kagan et al., 1974). Increased cholesterol levels in adolescents are also associated with development of CHD later in life (Stamler et al., 2000). In addition, elevated LDL causes CHD in individuals with genetic forms of hypercholesterolemia (Brown et al., 1986). In these individuals, atherosclerosis and CHD is known to occur even in the complete absence of other risk factors, thus providing strong evidence that LDL is a powerful atherogenic lipoprotein.

Even though high LDL cholesterol levels are associated with increased CHD risk and is considered as the primary target of therapy, estimation of LDL cholesterol has significant limitations, especially in individuals with high TG levels and those with diabetes mellitus. The limitations are due to the abnormal composition of TG-rich lipoproteins found in individuals with high TG levels, which are not considered in the Friedewald formula (Aguilar-Salinas et al., 2002). Non-HDL cholesterol may overcome
the limitations of LDL cholesterol. The cut-offs for LDL cholesterol levels are presented in Table 1.

### 2.6 Low HDL Cholesterol as an Independent Risk Factor for CHD

Epidemiological studies show low HDL cholesterol levels to be an independent risk factor for CHD even after controlling for other risk factors. In animal models, high levels of HDL levels have shown to have protective effect against atherosclerosis (Tangirala et al., 1999). Conversely, individuals with high HDL cholesterol levels have low CHD risk. Various studies show that the antioxidant and anti-inflammatory properties of HDL also inhibit atherosclerosis (Navab et al., 2000; van Lenten et al., 1995). Also, the Framingham Heart Study showed that high HDL cholesterol levels are associated with reduced risk for CHD. According to the NCEP ATP III report (2002), presence of high HDL cholesterol indicated a removal of one risk factor from the risk factor count used for setting treatment goals for LDL cholesterol. The cut-offs for HDL cholesterol levels are presented in Table 2.

### 2.7 Non-Lipid Risk Factors

A number of non-lipid risk factors are associated with increased CHD risk and must be considered in preventive efforts. Some of these risk factors are modifiable while others are non-modifiable and therefore their presence will require for more intensive lowering of LDL cholesterol.
2.7.1 Modifiable Risk Factors

The first goal for people with modifiable non-lipid risk factors is to alter them to reduce CHD risk. There are several modifiable risk factors that are associated with CHD risk.

2.7.1.1 Hypertension

The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (1997) defines hypertension as a systolic blood pressure ≥140 mmHg or diastolic blood pressure of ≥90 mmHg or current use of antihypertensive medication. Epidemiological studies have shown that high blood pressure is associated with increased risk of CHD (van den Hoogen et al., 2000; Franklin et al., 1999; Staessen et al., 1997). Further, blood pressure reduction in individuals with hypertension reduces the risk of CHD (NCEP ATP III, 2002). In individuals with elevated blood pressure, intensive LDL lowering therapy is required (NCEP ATP III, 2002).

2.7.1.2 Cigarette Smoking

Cigarette smoking is associated with increased risk of CHD (Pyorala et al., 1994). There is dose dependent relationship between smoking and CHD risk (NCEP ATP III, 2002). There is substantial reduction in CHD risk in those people who quit smoking. It is suggested that smoking reduces the risk of in CVD within months after quitting. NCEP ATP III (2002) recommends that prevention of smoking and smoking cessation should receive emphasis in order to reduce CHD risk.
2.7.1.3 Diabetes

Diabetes, defined as having fasting blood glucose of 126 mg/dL or greater (Gavin et al., 1998). Both type 1 and type 2 diabetes mellitus increases the risk of CHD. The NCEP ATP III (2002) has listed type 2 diabetes as a CHD risk equivalent, meaning persons with type 2 diabetes have a 10-year risk for major coronary events (myocardial infarction and CHD death) equivalent to the risk in CHD patients without diabetes. The mortality rates in diabetic individuals having CHD are higher than in non-diabetic individuals (Miettinen et al., 1998). The increase in CHD risk due to high blood glucose levels is independent of dyslipidemia and obesity status (NCEP ATP III, 2002). However, management of other risk factors is suggested to help reduce the incidence of CHD in individuals with diabetes. For instance, according to the UK Prospective Diabetes Study Group (1998) controlling high blood pressure in those with diabetes will help lower the risk of CHD. Therefore, presence of diabetes should lead to modification of treatment goals for LDL cholesterol.

2.7.1.4 Metabolic Syndrome

The metabolic syndrome is strongly associated with insulin resistance. Along with insulin resistance, the NCEP ATP III (2002) has considered high TG levels, low HDL cholesterol, abdominal obesity, and high blood pressure in their definition of metabolic syndrome. The criteria for clinical identification of metabolic syndrome are presented in Table 3. Individuals having three of the five risk factors are qualified as having metabolic syndrome (Cook et al., 2003). The clustering of these risk factors is associated with an increased risk for CVD compared to the risk associated with each condition considered separately. Metabolic syndrome and its consequences present a challenge to the healthcare
system, particularly due to the dramatic increase in the prevalence of obesity and type 2 diabetes mellitus. Insulin resistance and obesity are generally considered two important underlying risk factors for metabolic syndrome (Esmailzadeh et al., 2006; St-Onge et al., 2004). Insulin resistance may be caused by abdominal obesity and physical inactivity, and it is known to be associated with increased blood pressure (Copper-DeHoff et al., 2007).

2.7.1.5 Overweight/Obesity

Overweight is defined as having a BMI between 25.0 to 29.9 kg/m² and obesity is defined as a BMI of ≥30.0 kg/m² and (NCEP ATP III, 2002). Although some people, especially athletes, classified as overweight have increased muscle mass, most people have excess body fat. Overweight and obese people also have the presence of other CHD risk factors including high LDL cholesterol, low HDL cholesterol, high VLDL, high TG, type 2 diabetes, and hypertension. Overweight and obesity are associated with increase in the risk of CHD, stroke, and all-cause mortality (Calle et al., 1999; Manson et al., 1990). CHD risk is particularly higher in individuals who are abdominally obese, defined by a waist circumference greater than 102 cm in males and 88 cm in females. According to the NCEP ATP III report (2002), obesity is considered a direct target for clinical intervention instead of an indicator for modification of LDL treatment because the risk associated with overweight and obesity may be due to the presence of other major CHD risk factors.

2.7.1.6 Physical Inactivity

Physical inactivity is associated with increased risk for CHD. In contrast, physical activity is known to lower LDL cholesterol and TG levels, raise HDL cholesterol, improve insulin sensitivity, and lowers blood pressure in those with hypertension. Physical activity has been shown to reduce the risk of CHD. Therefore, physical inactivity
is considered to be one of the major modifiable risk factor for CHD (NCEP ATP III, 2002). One possible explanation for the increase in CHD risk may be that physical inactivity reduces energy expenditure and is associated with obesity and CHD risk factors (Grundy et al., 1999). Physical activity should be one of the important targets for lifestyle changes.

2.7.1.7 Atherogenic Diet

Diet high in saturated fatty acids and cholesterol increases the risk of CHD by raising LDL cholesterol levels. High sodium intake is also associated with hypertension. In contrast, individuals consuming diets high in fruits, vegetables, whole grains, and unsaturated fatty acids appear to have low risk of CHD due to the presence of antioxidants, folic acid, B-vitamins, omega-3 fatty acids, and other nutrients (Krauss et al., 2000).

2.7.2 Non-Modifiable Risk Factors

Several risk factors cannot be modified, their presence indicate the need for a more intensive LDL lowering therapy.

2.7.2.1 Age

The risk for CHD increases steeply with increasing age in both males and females. Compared to younger individuals, the risk for CHD is higher in older people for any given level of LDL cholesterol (Wilson et al., 1998). The main reason that the risk of CHD rises with age is that there is a continuous accumulation of atherosclerotic plaques with increasing age. Plaque ruptures produce acute coronary events such as unstable angina or myocardial infarction, or in cases where plaques grow large, coronary obstruction can
occur (NCEP ATP III, 2002). Development of atherosclerosis also reflects the presence of other CHD risk factors. Older people are exposed to the risk factors for a longer period of time compared to the younger people. The NCEP ATP III (2002) has suggested that age \( \geq 45 \) years in males and \( \geq 55 \) years in females is a positive risk factor for CHD.

2.7.2.2 Male Sex

At any given age males are at higher risk for CHD compared to females (Wilson et al., 1998). According to the NCEP ATP III (2002), risk in females is approximately 10 to 15 years behind that of males. The reasons for difference in CHD risk between males and females are not fully understood. One explanation may be that risk factors such as high LDL cholesterol, high blood pressure, and low HDL cholesterol are present at an earlier age in males than in females (NCEP ATP III, 2002). However, males and females have similar response to LDL lowering drug treatments (NCEP ATP III, 2002).

2.7.2.3 Family History of Premature CHD

CHD tends to cluster in families. The family history of premature CHD is positive if CHD or sudden death is reported in first degree male relatives younger than 55 years old and first degree female relatives younger than 65 years of age (NCEP ATP III, 2002). The first degree relative may be a parent, sibling, or offspring. Results from many prospective studies show that a family history of premature CHD is an independent risk factor even after adjusting for other risk factors. The risk of CHD increases with the number of first degree relatives affected and the age of onset of CHD (Pohjola-Sintonen et al., 1998). The siblings of the affected individual have the highest risk of CHD, may be due to common cultures, environments, exposures, and genetics.
2.8 Hypertriglyceridemic Waist Phenotype

Recent studies have indicated that the process of atherosclerosis is linked to components of the metabolic syndrome. The increase in the prevalence of obesity and type 2 diabetes is resulting in an increase in the incidence rates of CHD. There is increasing evidence that factors other than the traditional lipoprotein-lipid profile may further help in the identification of individuals at high risk for CHD. In this regard, The Quebec Cardiovascular Study found that hyperinsulinemia, increased levels of plasma apo B, and small dense LDL cholesterol are characteristics of insulin resistance and abdominal obesity and are known to predict increased risk of CHD (Senechal et al., 2005; St-Pierre et al., 2002; Lamarche et al., 1998). Hyperinsulinemia, increased levels of plasma apo B, and small dense LDL cholesterol are collectively known as the atherogenic metabolic triad. It is also known that the atherogenic metabolic triad is commonly found among abdominally obese individuals, especially those having increase amounts of visceral adipose tissue (St-Pierre et al., 2002; Despres et al., 2000). Obesity is also a risk factor for type 2 diabetes mellitus. Abdominal obesity is associated with changes in carbohydrate and lipid metabolism as well as with the inflammation resulting from atherosclerosis that contributes to increased CHD risk (St-Pierre et al., 2002). These studies indicate that atherogenic metabolic triad features and abdominal obesity measurements may predict the risk of CHD. However, there are increased costs associated with measurement of visceral adipose tissue accumulation and with the measurement of insulin, apo B, and LDL particle size thereby limiting its use in clinical practice. To overcome this limitation, waist circumference is used as a substitute for the measurement of visceral adipose tissue accumulation and fasting TG concentrations have been used to predict LDL size (St-Pierre et al., 2002; Lemieux et al., 2000). This is illustrated in Figure
3. Hypertriglyceridemic waist phenotype defined as concurrently having plasma TG levels of \( \geq 177 \) mg/dL and waist circumference of \( \geq 90 \) cm in males (Lemieux et al., 2000), and TG levels of \( \geq 150 \) mg/dL and waist circumference of \( \geq 88 \) cm in females (LeMonte et al., 2003) may be a simple predictor of atherogenic metabolic triad features and CHD risk.

The cut-offs for TG concentrations are shown in Table 4. According to the NCEP ATP III (2002), a normal TG level is defined as \(< 150\) mg/dL and hypertriglyceridemia is defined as TG concentrations of \( \geq 150 \) mg/dL. The role of hypertriglyceridemia as an independent predictor for the risk of CHD is controversial. Various studies have shown a strong association between hypertriglyceridemia and an increase in CHD risk (Bansal et al., 2007; Eberly et al., 2003; Abdel-Maksoud, 2002). It has repeatedly been found that many patients with CHD have elevated TG, small dense LDL cholesterol, and decreased levels of HDL cholesterol, which are characteristic of the atherogenic lipoprotein profile found in patients with metabolic syndrome and diabetes mellitus. A large meta-analysis of population-based prospective studies also indicated that hypertriglyceridemia is a predictor of CHD even after adjusting for other risk factors such as low HDL levels (Austin et al., 1998). Thus, there is strong evidence indicating that hypertriglyceridemia is a risk factor for CHD, but it is difficult to determine the strength of its contribution to CHD risk because it is known to be associated with abnormal lipid metabolism and other CHD risk factors such as obesity, insulin resistance, diabetes mellitus, hypertension, high LDL cholesterol, and low levels of HDL cholesterol (McBride, 2007; Ginsberg, 2002). Therefore one would conclude that individuals with hypertriglyceridemia are at increased risk for CHD, even when this increased risk cannot be explained by hypertriglyceridemia itself. Meta-analyses that support the independent association of elevated TG and CHD emphasizes the atherogenicity of TG-rich lipoproteins. The TG-rich lipoproteins include
the remnant particles of VLDL and IDL cholesterol. These remnant lipoproteins have many properties that are similar to the properties of LDL. In the bloodstream, most of the TG is carried by VLDL in the fasting state and chylomicrons in the fed state (Packard et al., 2004). Therefore, measurement of TG in the fasting state reflects VLDL concentrations. However, in conditions where TG levels are elevated to ≥500 mg/dL, chylomicrons are present in the circulation for a long period of time thereby contributing to the fasting plasma TG concentrations (Packard et al., 2004). Whether the chylomicrons themselves have atherogenic properties is still not known.

Further, it is also suggested that unlike cholesterol, TG itself is not deposited in the atherosclerotic plaques. Instead, the effect of TG on the formation of atherosclerotic plaques can be explained by the accumulation of cholesterol-rich VLDL remnants and the structural abnormalities in LDL and HDL particles caused by hypertriglyceridemia (Packard et al., 2004). At TG levels of approximately 200 to 500 mg/dL, most cholesterol occurring in VLDL cholesterol is contained in the small remnant VLDL particles that are then converted to IDL cholesterol (Bjorkegren et al., 2000). Generally, each remnant particle contains more cholesterol than an LDL particle (Packard et al., 2004). Recent studies have shown that elevated VLDL particles are atherogenic and are associated with increased CHD risk (Liu et al., 2006). Remnant lipoproteins cause the buildup of cholesteryl esters in macrophages in the arterial walls, promoting the formation of foam cells and forming the atherosclerotic lesions (Packard et al., 2004; Tanaka, 2004). Further, remnant particles have also been shown to promote platelet aggregation (Saniabadi et al., 1997), impair endothelial function (Kugiyama et al., 1998), promote adhesion of monocytes to endothelial cells (Kawakami et al., 2002), and promote proliferation of vascular smooth muscle cells (Kawakami et al., 2003). In experimental
animals, cholesterol-rich remnants have been known to cause atherosclerosis (Breslow, 1996). In clinical trials elevated levels of remnants were found to be strong predictors of coronary atherosclerosis or CHD (Karpe et al., 2001; Takeichi et al., 1999).

As mentioned earlier, the effects of TG on the process of atherosclerosis can also be explained by the abnormalities in the metabolism of HDL and LDL particles. Hypertriglyceridemia affects the metabolism of LDL and HDL particles by increasing the atherogenic properties of LDL and the reducing the cardioprotective effects of HDL. When TG levels are increased to >150 mg/dL, the large TG-rich VLDL levels are also elevated which enhances the action of cholesteryl ester transfer proteins (Packard et al., 2004). Therefore the cholesteryl ester transfer protein activity is regulated by TG concentrations. TG is transferred from VLDL into LDL and HDL, and cholesteryl esters are transferred from LDL and HDL into VLDL to promote remnant formation. As a result of lipid exchange, HDL is converted to TG-rich HDL and the normal size cholesteryl ester-containing LDL (LDL I and LDL II) are converted into TG-rich LDL particles (Packard et al., 2004). The TG-rich LDL can be hydrolyzed, via the action of hepatic lipase, into small, dense LDL. Small, dense LDL is a type of LDL considered to be more atherogenic than normal size LDL because it has decreased binding capacity to the LDL receptors, leading to an increase in the time spent in the plasma (Woods et al., 2006). It is also more readily oxidized than larger LDL particles and is more likely to be incorporated into the extracellular matrix during plaque formation and therefore is considered to be highly atherogenic (Packard et al, 2004).

Similarly, elevated plasma TG concentrations also affect HDL levels. The inverse association of HDL with TG is important since TG plays a crucial role in the regulation of HDL metabolism. TG plays a role in regulating the type, size, and quantity of HDL
particles. In the blood vessels, cholesteryl ester transfer proteins assist in the exchange of lipids and apolipoproteins between TG-rich lipoproteins and HDL cholesterol (Woods et al., 2006). Thus, elevated TG leads to the production of TG-rich HDL particles, which are hydrolysed by hepatic lipase. This hydrolysis results in the formation of small HDL particles which are more rapidly cleared from the blood than larger particles (Woods et al., 2006). Since they are more rapidly cleared from the blood, the concentrations of HDL are decreased and therefore its availability for transporting cholesterol from arterial walls back to the liver is also diminished (Yang et al., 2005). Normally, HDL promotes reverse cholesterol transfer, inhibits the formation of atherosclerotic plaques, and stabilizes lesions. However, when TG levels are elevated, the size and quantity of HDL particles are decreased thereby reducing these cardioprotective effects of the HDL cholesterol. The resulting lipid profile consisting of elevated TG, low HDL, and increase levels of small, dense LDL, is known as the atherogenic lipoprotein phenotype or the lipid triad, and is typically found in individuals with metabolic syndrome and type 2 diabetes, both of which are associated with increased CHD risk (Vinik, 2005).

In summary, even though it is suggested that hypertriglyceridemia is a risk factor for CHD, it is difficult to determine if it can independently predict the risk of CHD because of its association with abnormal lipid metabolism as well as other CHD risk factors. Further, it is clear that TG does not directly participate in the process of atherosclerosis but it exerts its effects indirectly through the accumulation of cholesterol-rich VLDL remnants in the extracellular matrix. Also, elevated TG levels cause the formation of small dense LDL cholesterol and reduce the size and quantity of HDL cholesterol thereby reducing the cardioprotective effects of HDL cholesterol.
2.9 Non-HDL Cholesterol

Although there are several assays available for identification and measurement of remnant lipoproteins including the ultracentrifugation, electrophoresis, and immunological techniques, they are not recommended for clinical practice (NCEP ATP III, 2002). Therefore, elevated VLDL cholesterol is used to indicate elevated atherogenic remnants in individuals with TG levels $\geq 200$ mg/dL. Since VLDL cholesterol is highly correlated with atherogenic remnant lipoproteins, it can be combined with LDL cholesterol to provide a better estimation of the risk of CHD in individuals with elevated TG concentrations. VLDL, IDL, and LDL cholesterol together are known as non-HDL cholesterol. Non-HDL cholesterol levels are available from the standard lipid profile and can be determined by subtracting HDL cholesterol from the total cholesterol. Non-HDL cholesterol provides information on the concentrations of all atherogenic lipoproteins including the LDL, VLDL, IDL, and lipoprotein (a). Non-HDL cholesterol is highly correlated with total apo B concentration which is the major apolipoprotein of all atherogenic lipoproteins and is a strong predictor of atherosclerosis and CHD (Lemieux et al., 2000; Larmarche et al., 1996).

In individuals with TG levels that are $<200$ mg/dL, elevation of both LDL and VLDL cholesterol are associated with increased risk of CHD (Liu et al., 2006). However, when TG levels are elevated to $\geq 200$ mg/dL, VLDL levels increase, and LDL concentrations are less well correlated with non-HDL concentrations. As a result, LDL concentrations alone cannot identify the risk associated with atherogenic lipoproteins and non-HDL cholesterol is considered to be a better marker for the total atherogenic lipoprotein levels compared to LDL cholesterol alone (Liu et al., 2006; Packard et al., 2004).
2.9.1 Predictive Value of Non-HDL Cholesterol

Various studies have indicated the ability of non-HDL cholesterol to predict cardiovascular risk. A recent study assessed the association of TG concentrations, LDL, HDL, and non-HDL cholesterol, and found that after adjusting for age, gender, race, cigarette smoking, hypertension, obesity, family history of premature CHD, and increasing quartiles of lipid levels, only the association of non-HDL cholesterol with coronary artery calcium scores remained statistically significant \((p = 0.002)\) (Orakzai et al., 2009). In a 24-year follow-up study done on Finnish males aged 30 to 61 years showed that non-HDL cholesterol was a significant predictor of CHD death (Keys et al., 1984b). Similar results were obtained in a 10 year follow-up of Italian males aged 45 to 65 years (Menotti et al., 1992). Another prospective study done on Finnish patients with type 2 diabetes aged 45 to 64 years showed that there was a twofold increase in the risk of CHD mortality or morbidity independent of other cardiovascular risk factors (Lehto et al., 1997). In the Lipid Research Clinics Program Follow-up Study, during the 19-year follow-up period, cardiovascular death occurred in 234 of 2,406 males and 113 of 2,056 females aged 40 to 64 years without cardiovascular disease at study entry (Cui et al., 2001). Compared to males with non-HDL cholesterol levels of < 160 mg/dL, males with 160 to 190 mg/dL, 190 to 220 mg/dL, and ≥ 220 mg/dL had increased risk of cardiovascular mortality by 14%, 43% and 114% respectively. Similarly, females with non-HDL levels of 160 to <190 mg/dL, 190 to 220 mg/dL, and ≥ 220 mg/dL had increased risk of cardiovascular mortality by 47%, 61% and 143% respectively. In this study, compared to total cholesterol and LDL cholesterol, non-HDL cholesterol was the strongest predictor of cardiovascular mortality in both genders. Another prospective study, the Bypass Angioplasty Revascularization Investigation trial showed that non-HDL
cholesterol level was a strong and independent predictor of outcomes among 1,514 elderly patients diagnosed with coronary artery disease (Bittner et al., 2002). Multivariate analyses with a large number of covariates showed that non-HDL cholesterol was the strongest predictor of myocardial infarction and the only predictor of angina with a 4.9% increase in risk for every 10 mg/dL increase in non-HDL concentrations. Triglyceride levels were only associated with a 1.6% increase in risk of myocardial infarction. Non-HDL cholesterol was independently associated with the risk of carotid atherosclerosis in individuals aged 65 years and older (Kawamoto et al., 2005) and in elderly patients with systolic hypertension (Frost et al., 1996). After adjusting for confounders, non-HDL cholesterol is also a strong predictor of CHD mortality in diabetic individuals (Liu et al., 2005; Schulze et al., 2004).

Overall, these research findings suggest that non-HDL cholesterol is a reliable marker for cardiovascular risk in individuals with and without disease. The theoretical considerations reviewed above, as well as available data on performance of non-HDL cholesterol in defining risk, suggest considerable utility of this measure in predicting the risk of CHD as well as a target in lipid-lowering therapy to reduce risk.

2.9.2 Applications of Non-HDL Cholesterol

Non-HDL cholesterol measurements can be used in clinical practice as a treatment target especially in patients with elevated TG levels since estimation of the LDL cholesterol is less accurate at high TG levels. The NCEP ATP III (2002) has already recommended using non-HDL cholesterol in patients with high TG, after the goals for LDL cholesterol are met. However, there are no specific goals identified for TG concentrations. An advantage of using non-HDL cholesterol in clinical practice is that it
does not limit treatment options in reducing atherogenic lipoproteins such as LDL and VLDL cholesterol (Packard et al., 2004). Focusing only on elevated TG levels might result in a treatment that specifically lowers TG concentrations but not the other atherogenic lipoproteins (Packard et al., 2004). In contrast, focusing on non-HDL cholesterol allows more treatment options. For example, statin therapy, which is recommended as the first-line therapy for most dyslipidemias, produces similar reductions in VLDL and LDL cholesterol, thereby also reducing atherogenic TG-rich remnants (Packard et al., 2004). Further, non-HDL cholesterol was chosen over plasma apo B as a secondary target of therapy in patients with elevated TG because of lack of standardized measures for apo B and the additional expense associated with this measurement (Packard et al., 2004). Another advantage of using non-HDL cholesterol is that it can be used to assess risk in individuals that are at increased risk of CHD due to dyslipidemia associated with the metabolic syndrome and diabetes (Orakzai et al., 2009). Further, non-HDL cholesterol has less day-to-day variability than TG and is not affected by increased TG levels (Wagner et al., 2005). Unlike TG, non-HDL cholesterol also does not require an overnight fast. Therefore, the use of non-HDL cholesterol may be a simple and relatively inexpensive indicator of CHD risk.

2.9.3 Summary

In summary, non-HDL cholesterol incorporates both LDL cholesterol and VLDL cholesterol, and it reflects the cholesterol content of all apo B containing lipoproteins. In individuals with elevated TG levels, non-HDL cholesterol is helpful in assessing the risk of CHD, since it includes the increased levels of atherogenic TG-rich remnant particles found in VLDL. When TG levels are elevated, VLDL levels increase and as a result, LDL
concentrations alone cannot define the risk associated with atherogenic lipoproteins and therefore, non-HDL cholesterol is considered to be a better marker for the total atherogenic lipoprotein levels compared to LDL cholesterol alone. Finally, overnight fast is not required for obtaining non-HDL cholesterol and it is readily derived from the routine lipoprotein profile. For these reasons non-HDL cholesterol may be a highly useful lipid measure for assessing risk and evaluating response in treatment of hyperlipidemia.

2.10 Waist Circumference and Coronary Heart Disease

Despite the continuing effort to emphasize that excessive weight raises the risk for CHD, the prevalence of overweight and obesity continues to increase. The National Health and Nutrition Examination Survey (NHANES) from 1960-1962, estimated the prevalence of obesity to be 13.4%. By 2000, the third NHANES estimated that 64.5% of U.S. adults were either overweight or obese, with the prevalence of obesity rising to 30.5% (Lofgren et al., 2004). Research shows that persons who are overweight or obese have a higher risk of developing insulin resistance, the metabolic syndrome, diabetes, hypertension, and CHD (Ross et al., 2000; Mokdad et al., 1999). Weight gain in adulthood is also associated with high rates of cardiovascular and metabolic risk factors. In 1998, The National Heart, Lung, and Blood Institute recommended guidelines that used BMI and waist circumference for screening overweight and obese adults (Lofgren et al., 2004). Waist circumference is used as a measure of abdominal fat, specifically visceral fat (Lofgren et al., 2004). Since abdominal fat predicts a higher risk for CHD and type 2 diabetes, waist circumference provides more information on risk assessment than BMI alone (Lofgren et al., 2004). Research has shown relationships between cardiovascular risk factors and metabolic risk factors and the development and progression of CVD. For
example, Rexrode et al. (2001) conducted a prospective cohort study, with a nine year follow-up period on U.S. males. Out of the total sample of 22,071, CHD event occurred in 552 individuals. In multivariate analysis, after adjusting for age, smoking, physical activity, parental history of myocardial infarction, alcohol intake, and multivitamin and aspirin use, males in the highest waist circumference quintile (≥ 103.6 cm) had a 60% greater risk for CHD compared to males in the lowest quintile (<88.4 cm). Another study conducted by Balkau and colleagues (2007) recruited 168,000 primary care patients from 63 countries across five continents. It was found that, in comparison to the lowest waist circumference group (< 94 cm in males and 80 cm in females), the age and region adjusted CVD odds ratios were 1.28 for males in the 94–102 cm group and 1.90 in the > 102 cm waist circumference group; for females, the odds ratios were 1.31 for 80–88 cm group and 1.97 for > 88 cm waist circumference group. The corresponding odds ratios for diabetes were higher, 1.60 and 2.65 in males, and 1.78 and 3.94 in females.

Another prospective cohort study was done among U.S. female registered nurses (Rexrode et al., 1999). During the eight year follow-up period, 320 CHD events occurred. In this study, after adjusting for BMI and other cardiovascular risk factors, a waist circumference of ≥96.5 cm was associated with a 144% increase in CHD risk compared with females of waist circumference <71.1 cm. Further, a study done on Finnish males aged 42-60 years with a follow-up period of 10.6 years concluded that abdominal obesity combined with smoking increased the risk of coronary events by 5.5 times, and abdominal obesity combined with poor cardiorespiratory fitness increased the risk of coronary events by approximately five times (Lakka et al., 2002).
These studies show that obesity, particularly central obesity, is an independent risk factor for the development of CVD. Therefore, combining non-HDL cholesterol with waist circumference may be a good predictor of CHD risk.

2.11 Pathogenesis of Coronary Heart Disease

CHD is caused by the narrowing of the coronary artery that supplies nutrients and oxygen to the heart. The main reason for the narrowing of the coronary artery is artherosclerosis. Atherosclerosis is a chronic disease affected by both genetic, behavioural, and environment factors. The independent risk factors for CHD as identified by the NCEP ATP include age, gender, hypertension, smoking, diabetes, family history of premature CHD, elevated plasma levels of LDL cholesterol ($\geq 160$ mg/dL), and low levels of HDL cholesterol (<40 mg/dL). Hyperlipidemia is one of the major causes of atherosclerosis and associated conditions such as CHD, ischemic cerebrovascular disease, and peripheral vascular disease (Jain et al., 2007). Atherosclerosis is the result of degenerative changes occurring in the intima layer of medium and large sized arteries (Jain et al., 2007). This degenerative change includes accumulation of lipids, carbohydrate molecules, blood and its components, and cellular waste products, along with the formation of fibrous tissues and calcium deposits in the intima of blood vessels (Jain et al., 2007). These deposits or plaques gradually decrease the lumen of the artery, reduce its elasticity, and the plaque may rupture leading to the occlusion of blood vessels.

The development of atherosclerosis is subdivided into various stages from the normal, healthy coronary artery to the final stage leading to its complete blockage resulting in myocardial infarction. The cross-sectional and the longitudinal view of the
coronary artery in different stages of the atherosclerosis process are illustrated in Figure 4.
and described below.

Before the onset of atherosclerosis, the coronary artery is smooth and free of any accumulation and deposits as seen in stage I in Figure 4. However, with increasing age, atherosclerotic lesions start to develop. Atherosclerotic lesions develop as a result of inflammation due to release of various cytokines, proliferation of smooth muscle cells, synthesis of connective tissue matrix, and accumulation of macrophages and lipid in the arterial wall (Crowther, 2005). The early stage in the development of the atherosclerotic plaque is the result of the changes in functions of the endothelium. Once the arterial wall is injured, the body’s defence mechanisms respond. Macrophages invade the injured area and stick to the endothelial wall. Atherosclerosis may also be initiated when endothelial cells increase the expression of adhesion molecules such as vascular cell adhesion molecule-1 in response to turbulent blood flow in the presence of abnormal lipid levels (Crowther, 2005). Stage II in Figure 4. show small amounts of lipid deposition and formation of fatty streaks but at this stage they do not produce any obstruction or symptoms. This is the beginning of the artherosclerosis process.

Lipids are important for the formation and development of the plaque. Three major atherogenic lipoproteins are deposited from the blood into the walls of the arteries. They include LDL, especially small dense LDL, remnant lipoproteins, and lipoprotein (a). These atherogenic lipoproteins, once deposited in the intima layer, are subjected to chemical modifications such as oxidation, thereby leading to a series of biological reactions (Longenbaker, 2005). Once the LDL cholesterol is deposited within the artery, there are three possible outcomes. The LDL may move back into the bloodstream, it may become oxidized through action of free radicals and activity of leukocytes, or it may be
taken up by monocyte or macrophages and converted to foam cells (Crowther, 2005). If the outcome is the latter, then macrophages bind the oxidized LDL particles to the receptors known as scavenger receptors. Macrophages filled with LDL are called foam cells. The uptake of oxidized LDL particles though the scavenger receptors reduces the mobility of the macrophages thereby promoting the accumulation of foam cells in the intima (inner layer of the artery) (Crowther, 2005). The foam cells secrete various cytokines and inflammatory mediators thereby promoting further recruitment and proliferation of smooth muscle cells, further LDL oxidation, recruitment of additional foam cells, and increasing the endothelial dysfunction (Crowther, 2005). A collection of foam cells creates a fatty streak. Enlargement of the atherosclerotic plaque is characterized by the accumulation of foam cells over time. As illustrated in stage III, the increased build-up of fatty layers in the coronary artery starts to interfere with the blood flow through the artery.

Over time, slowly growing plaques gradually accumulate lipid within foam cells. These foam cells along with proliferation of smooth muscle cells, components of the intracellular matrix, and calcium ions produce the definitive fibrous plaque (Longenbaker, 2005). In general, such plaques tend to have firm endothelial layers that are not prone to sudden disruption. This is seen in stage IV. However, in some cases, plaques within the inner lining of the coronary artery may develop a slight crack or rupture, which stimulates the production of the blood clots (Jain et al., 2007). The clots also get into the crack and cause further obstruction of the artery, as seen in stage V. The supply of the blood flow to the heart muscle is substantially reduced and the patient begins to have severe and prolonged chest pain that occurs at rest. This is known as unstable angina (Jain et al., 2007). In some cases the clot does not fully close the channel of the artery and sufficient
blood flow is maintained in the heart muscle, and a heart attack may not develop, provided appropriate and prompt treatment is effected. However, the clot may continue to grow as large as to completely block blood flow through an artery, causing the tissue supplied by the artery to die. This is shown in stage VI.

2.12 Summary of the Literature Review

In summary, even though it is suggested that hypertriglyceridemia is a risk factor for CHD, it is difficult to determine if it can independently predict the risk of CHD because of its association with abnormal lipid metabolism as well as other CHD risk factors. Further, it is suggested that unlike cholesterol, TG itself is not deposited in the atherosclerotic plaques. Instead, the effect of TG on the formation of atherosclerotic plaques can be explained by the accumulation of cholesterol-rich VLDL remnants and the structural abnormalities in LDL and HDL particles caused by hypertriglyceridemia. High levels of TG cause increase in small dense LDL cholesterol and decrease in the size and quantity of HDL cholesterol, thereby reducing cardioprotective effects of HDL cholesterol.

In contrast, non-HDL cholesterol has no such limitations. It includes all potential atherogenic lipoproteins, including LDL, IDL, and VLDL cholesterol. Non-HDL cholesterol is highly correlated with total apo B concentration which is the major apolipoprotein of all atherogenic lipoproteins. It can be used as a predictor of CHD in individuals with high TG levels, type 2 diabetes, and metabolic syndrome. There is less day-to-day variability in non-HDL cholesterol compared to TG concentrations and is unaffected by TG levels. Unlike TG, non-HDL cholesterol requires measurements of only total cholesterol and HDL cholesterol which can be measured reasonably accurately in the
non-fasting state. Finally, by combing all atherogenic lipoproteins into a single fraction, the use of non-HDL cholesterol may be a simple and relatively inexpensive indicator of CHD risk.

Overall, research findings suggest that non-HDL cholesterol is a reliable marker for cardiovascular risk in individuals with and without elevated TG levels. Studies have shown that central obesity measured in waist circumference, is an independent risk factor for the development of CHD. The theoretical considerations reviewed above, as well as results from other research studies suggest the use of non-HDL cholesterol and waist circumference in estimating the risk of CHD.
CHAPTER 3. STUDY OBJECTIVE

3.1 Purpose

Despite some indications from research conducted that non-HDL cholesterol is a better predictor of CHD risk than TG, there is lack of conclusive research in this area. Research is needed to determine whether, and to what degree, non-HDL cholesterol is a better predictor of CHD risk. As identified in the literature, there is lack of information with respect to the combination of non-HDL and waist circumference and its effect on the risk of CHD. The purpose of this study will be to examine the relationship between non-HDL cholesterol, waist circumference and the risk of CHD, and between hypertriglyceridemic waist phenotype and the risk of CHD.

3.2 Research Question

This study plans to address the following research question:

Is the combination of non-HDL cholesterol and waist circumference the same as or better than hypertriglyceridemic waist phenotype in predicting the risk of CHD?
CHAPTER 4. METHODOLOGY

The Framingham Offspring Study dataset was used to compare if using non-HDL cholesterol and waist circumference is same as or better than using hypertriglyceridemic waist phenotype in predicting the risk of CHD. In addition, the associations between non-HDL cholesterol, waist circumference and non-fatal CHD and between TG, waist circumference and non-fatal CHD were examined. This section describes the variables included in the analyses and the statistical procedures used to analyze the Framingham Offspring study data.

4.1 Study Sample

The public accessible dataset of the Framingham Offspring Study was obtained from the National Heart Lung and Blood Institute (NHLBI) after submitting the approved letter from the Research Ethics Board, Brock University for this secondary data analysis (Appendix III). The original Framingham Cohort Study began in 1948 under the U.S. Public Health Service. Participants were sampled from Framingham, Massachusetts. The Framingham Study is a longitudinal investigation of various risk factors influencing the development of CVD in males and females. Although the Framingham cohort is primarily white, the importance of the major CVD risk factors identified in this group have been shown in other studies to apply almost universally among racial and ethnic groups, even though the patterns of distribution may vary from group to group (NHLBI, 2007).

The Framingham Offspring Study began in 1971. A new cohort consisting of the offspring of the original participants and each offspring’s spouse were invited to participate in the Framingham Offspring Study. The Framingham Offspring Study was conducted to study the incidence and prevalence, trends, and family patterns of CVD and
its risk factors. The first examination took place between 1971 and 1975 which consisted of 5,124 males and females, ages 13 to 62 years. However, waist circumference measurements were not collected until the fourth examination so data from the fourth examination was used as the baseline. The fourth examination took place between 1987 and 1990. At the fourth examination, the participants underwent a routine medical history, a physical examination that included blood pressure measurement and anthropometry, and blood sampling after an overnight fast. Participants had completed three examinations with intervals of four years and have been followed for morbidity and mortality over that time period. After the fourth examination, the participants were followed up for an average of 11 years.

The fourth examination had 4,989 participants and after excluding those aged <40 years old there were 3,460 individuals. Further, excluding missing information on total cholesterol, HDL cholesterol, TG, waist circumference, and fasting less than nine hours, 3,196 individuals were eligible for the study, of which 1,572 were males and 1,624 were females. The drop in the study sample due to missing information was around 7.6%. Sample selection procedure is summarized in Figure 5.

### 4.2 Measurement of Study Variables

#### 4.2.1 Follow-up and Outcome Events

For a participant with a CHD event, the follow-up period was defined as the time interval from the fourth examination to the date the CHD event occurred. For a participant without CHD event, the follow-up time will be defined as the time interval from the fourth examination to the date that the individual was last known alive. The
amount of follow-up time was expressed in person-years for Cox proportional hazards models.

All the participants were monitored for CHD occurrence through periodic examinations. An endpoint adjudication committee consisting of three cardiologists reviewed hospitalization and physician office visit records for all suspected CHD events (Ingelsson et al., 2007). The diagnosis of incident CHD included MI recognized with diagnostic ECG, MI recognized with transaminase and history, MI recognized with autopsy evidence, silent and non-silent MI, angina pectoris, or acute coronary insufficiency. Events were considered fatal if the participant died before or during the hospitalization for the event, otherwise they were considered non-fatal.

4.2.2 Waist Circumference

Waist circumference was measured at the umbilicus while the participant was standing, with the tape measure parallel to the floor. In order to convert inches to centimetres, the waist circumference values were multiplied by 2.54.

4.2.3 Lipid measurements

Lipids and lipoproteins were determined on plasma samples collected after an overnight fast of ≥9 hours. Serum TG was measured using the semi-automated Lederer Kessler method (Kessler et al., 1965). Fasting venous blood samples were collected in tubes containing 0.1% EDTA. Total cholesterol levels were determined by the Abell-Kendell method (Abell et al., 1952). Managanese-heparin precipitation method was used to measure HDL cholesterol. Non-HDL cholesterol was calculated by subtracting the HDL cholesterol from the total cholesterol. LDL cholesterol was calculated from
measured values of total cholesterol, TG, and HDL cholesterol according to the Friedewald formula: 

\[ \text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \left( \frac{\text{TO}}{5} \right) \]

\( \left( \frac{\text{TO}}{5} \right) \) is an estimate of VLDL-cholesterol. The calculation is valid for \( \text{TG} \leq 400 \text{ mg/dL} \). LDL-cholesterol was reported only for participants fasting for at least nine hours. All lipid values are expressed in mg/dL.

Hypertriglyceridemic waist phenotype was defined as concurrently having plasma TG levels of \( \geq 177 \text{ mg/dL} \) and waist circumference of \( \geq 90 \text{ cm} \) in males (Lemieux et al. 2000), and plasma TG levels of \( \geq 150 \text{ mg/dL} \) and waist circumference of \( \geq 88 \text{ cm} \) in females (LeMonte et al., 2003). The criteria for non-HDL cholesterol and waist circumference and TG and waist circumference are listed in Table 5.

4.2.4 Covariates

a) Sex was coded as males=1 and females=0. In the analysis, females was used as the reference group.

b) Age at baseline was recorded in years and used as a continuous variable.

c) Smoking status was determined by asking the participants if they smoked cigarettes regularly in the past year and it was coded as yes=1 and no=0.

d) Alcohol consumption was recoded as the total alcohol consumption in ounces per week and used as a continuous variable.

e) Diabetes mellitus was defined as having a fasting glucose level of \( \geq 126 \text{ mg/dL} \). In the analysis, diabetes status was coded as yes=1 and no=0.

f) Systolic and diastolic blood pressure measurements (mmHg) were obtained by taking the average of two readings recorded by the physician at baseline examination. Hypertension status was determined if the participant had systolic
blood pressure of $\geq 140$ mmHg or diastolic blood pressure of $\geq 90$ mmHg or if the patient was receiving treatment for high blood pressure. Hypertension status was coded as yes=1 and no=0.

4.3 The receiver operating characteristic (ROC) curves

ROC curves provide a measure of classification or prediction accuracy and are used to compare the discrimination ability of various diagnostic tests (Goddard et al., 1990; Zweig et al., 1993). The ROC curve is a plot of sensitivity against one minus the specificity. Sensitivity is the proportion of true positives which are correctly identified as such by the diagnostic test. Specificity is the proportion of true negatives which are correctly identified by the test as being negative. One minus specificity, also known as false positive proportion is the proportion of true negatives that are falsely identified as positive by the diagnostic test. Sensitivity is plotted on the y-axis and one minus specificity is plotted on the x-axis. The area under the ROC curve (ROC AUC) is large for a model with high predictive accuracy. Conversely, ROC curve rises slowly and has a smaller area under the curve for models with low predictive accuracy (Metz, 1978). An ROC AUC of 0.5 represents chance prediction. If the two ROC areas under the curves are the same then the two tests provide equal detectability of the disease.

4.4 Statistical analyses

Descriptive statistics including means and proportions, stratified by gender were performed to get an overview of the sample at baseline. Age and gender adjusted means for the key study variables within the joint categories of non-HDL cholesterol and TG by waist circumference strata are reported. CHD event rates were calculated by dividing the number of CHD events by the person-years of follow-up.
Non-parametric ROC analyses were used to calculate the ROC AUC (Cleves, 2002). Logistic regression was used to generate the ROC curves for the predictors non-HDL cholesterol and waist circumference, and TG and waist circumference. The ROC AUC were compared to see if using non-HDL cholesterol and waist circumference is better than using TG and waist circumference in predicting the risk of CHD.

Cox-proportional hazards models were used to examine the association between non-HDL cholesterol, waist circumference and CHD, and TG, waist circumference and CHD. On the basis of the NCEP ATP III (2002) guidelines, the therapeutic goal for dyslipidemia management in subjects without CHD is a LDL cholesterol level <130 mg/dL. For non-HDL cholesterol, the cut-points are 30 mg/dL higher than for LDL cholesterol to account for the VLDL cholesterol fraction. Therefore, the cut-off for non-HDL cholesterol is ≥160 mg/dL. To examine the joint distributions of non-HDL cholesterol and waist circumference, four groups were created:

1. Non-HDL ≥160 mg/dL and waist circumference ≥ 90 cm (males) or ≥ 88 cm (females)
2. Non-HDL ≥160 mg/dL and waist circumference < 90 cm (males) or < 88 cm (females)
3. Non-HDL <160 mg/dL and waist circumference ≥ 90 cm (males) or ≥ 88 cm (females)
4. Non-HDL <160 mg/dL and waist circumference < 90 cm (males) or < 88 cm (females)

Group 4 was used as the reference group. Four Cox-proportional hazards models were created in order to examine the association of the joint distributions of non-HDL cholesterol and waist circumference with non-fatal CHD. The first model adjusted for age and gender, the second model adjusted for smoking status and alcohol consumption, the
third model adjusted for diabetes and hypertension status, and the fourth model adjusted for all the confounders.

Similarly, for the joint distributions of TG and waist circumference the following four groups were created:

1. TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females) and waist circumference ≥ 90 cm (males) or ≥ 88 cm (females)
2. TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females) and waist circumference < 90 cm (males) or < 88 cm (females)
3. TG < 177 mg/dL (males) or < 150 mg/dL (females) and waist circumference ≥ 90 cm (males) or ≥ 88 cm (females)
4. TG < 177 mg/dL (males) or < 150 mg/dL (females) and waist circumference < 90 cm (males) or < 88 cm (females)

Group 4 was used as the reference group. The four Cox-proportional hazards models were adjusted for in the same manner as described for non-HDL cholesterol and waist circumference.

For the joint distributions of non-HDL cholesterol and TG, eight groups were created on the basis of lipid levels and strata of waist circumference:

(I) Waist Circumference – males ≥ 90 cm, females ≥ 88 cm
   1. Non-HDL ≥ 160 mg/dL and TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females)
   2. Non-HDL ≥ 160 mg/dL and TG < 177 mg/dL (males) or < 150 mg/dL (females)
   3. Non-HDL < 160 mg/dL and TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females)
   4. Non-HDL < 160 mg/dL and TG < 177 mg/dL (males) or < 150 mg/dL (females)

(II) Waist Circumference – males < 90 cm, females < 88 cm
   5. Non-HDL ≥ 160 mg/dL and TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females)
   6. Non-HDL ≥ 160 mg/dL and TG < 177 mg/dL (males) or < 150 mg/dL (females)
   7. Non-HDL < 160 mg/dL and TG ≥ 177 mg/dL (males) or ≥ 150 mg/dL (females)
(8) Non-HDL <160 mg/dL and TG < 177 mg/dL (males) or < 150 mg/dL (females)

Group 8 was used as the reference group to which a hazard ratio of 1.0 for CHD was assigned for comparison purposes. The four Cox-proportional hazards models were adjusted for in the same manner as described for non-HDL cholesterol and waist circumference.

The proportionality assumption for the Cox-proportional hazards model was tested by creating an interaction term between the independent variable and the follow-up time and tested its p-value in SAS. If the p-value of the proportionality term was greater than 0.05 then the assumption was considered to be met. All statistical analyses were performed using SAS 9.1 (SAS Institute Inc. Cary, NC), except the ROC curves and significance testing of ROC AUC were prepared using STATA 10.1 (StataCorp, College Station, Texas).
CHAPTER 5. STUDY RESULTS

5.1 Characteristics of the Study Sample

Baseline characteristics of the Framingham offspring study stratified by gender are shown in Table 6. Among the 3,196 participants, 1,572 (49.2%) were males and 1,624 (50.8%) were females. The mean age at baseline was 53.9 years for males and 53.3 years for females. Compared to females, males were more likely to be diabetic (males vs. females: 6.3 vs. 3.6%; p-value: <0.0001), hypertensive (males vs. females: 44.1 vs. 34.5%; p-value: <0.0001), and consume more alcohol (males vs. females: 4.0 vs. 1.8 ounce/wk; p-value: <0.0001), while there was no significant difference in proportions of cigarette smokers (males vs. females: 23.6 vs. 25.3%; p-value: 0.2524). Means for main study variables, stratified by gender are reported in Table 7. Males had higher levels of non-HDL cholesterol, TG, LDL cholesterol, systolic blood pressure, and waist circumference, while females had higher levels of total and HDL cholesterol.

5.2 Coronary Heart Disease Incidence

Following the fourth examination, the average follow-up time was approximately 9.6 years in males and 10.1 years in females. There were 11 CHD deaths in males and four in females during the approximately 11 years of follow-up. A total of 416 non-fatal CHD events (304 in males and 112 in females) occurred during the follow-up period. All analyses were run on non-fatal CHD events (except for ROC curves which were run on both fatal and non-fatal CHD) due to the small number of fatal cases. However, when analyses were repeated using both fatal and non-fatal cases there was no significant change in the results obtained. The CHD event rate was 161.6 per 10,000 person-years (246.8 in males and 78.0 in females). The nonfatal CHD event rate was 155.9 per 10,000
person-years. Univariate analysis results presented in Table 8 indicate that although the hazard ratios were not significant, the association between lipids and non-fatal CHD showed similar trends in both males and females. Since the results for males and females were similar, males and females were grouped together for all subsequent analyses.

5.3 Adjusted Means for Key Study Variables within the Non-HDL Cholesterol and TG Groups Stratified by Waist Circumference Status

Age and gender adjusted mean values for key study variables within the non-HDL cholesterol and TG risk categories by waist circumference status are presented in Table 9. As expected, individuals with high non-HDL cholesterol and high TG levels had elevated total cholesterol, non-HDL cholesterol, TG concentrations, LDL cholesterol, waist circumference, systolic blood pressure, and low HDL cholesterol regardless of their waist circumference status. Despite having low TG, individuals with elevated non-HDL cholesterol still had elevated LDL cholesterol in both high and normal waist circumference groups. The adjusted means for all the risk factors were higher (lower for HDL cholesterol) in the four risk categories of elevated waist circumference group compared to the four risk categories of normal waist circumference.

5.4 ROC curves

ROC curves were generated and compared using logistic regression and are presented for both non-HDL cholesterol and waist circumference, and TG and waist circumference in Figure 6. The ROC AUC associated with non-HDL cholesterol and waist circumference and TG and waist circumference are 0.6428 (95% CI: 0.6183, 0.6673) and 0.6299 (0.6049, 0.6548) respectively. The difference in the ROC area under
the curves is 1.29%. The p-value testing if the difference in ROC AUCs between the two models is zero is 0.10.

5.5 Kaplan-Meier Curves

The Kaplan-Meier curve in Figure 7 shows survival rate of the four non-HDL cholesterol and waist circumference risk groups. The high level non-HDL cholesterol and high waist circumference group has the lowest survival rate, followed by the group having high level non-HDL cholesterol and low waist circumference. The group with low level non-HDL cholesterol and low waist circumference has the highest survival rate. The Kaplan-Meier curve in Figure 8 shows survival rate of the four TG and waist circumference risk groups. The high level TG and high waist circumference group has the lowest survival rate, followed by the group having low TG and high waist circumference.

5.6 Cox Proportional Hazards Models

5.6.1 Joint Distribution of Non-HDL Cholesterol and Waist Circumference Models

For the joint distribution of non-HDL cholesterol and waist circumference, four groups were created as described in the statistical analyses section for the Framingham Offspring Study in Chapter 4. Cox proportional hazards models were used for the multivariate adjustment. In this analysis, those who concurrently had non-HDL $\geq 160$ mg/dL and waist circumference $\geq 90$ cm in males or non-HDL $\geq 160$ mg/dL and waist circumference $\geq 88$ cm in females were considered as high risk group and the reference group is those who have a non-HDL $< 160$ and waist circumference $< 90$ cm (males) or $< 88$ cm (females). Multivariate adjusted HRs for the four models are shown in Table 10. In comparison to the reference group, those who were in the high risk group had a 4.3 higher risk of a non-fatal CHD (95% CI: 1.5, 11.9) after adjustment for age and gender in
the first model, a 6.9 higher risk (95% CI: 2.5, 19.0) after adjustment for the number of cigarettes smoked and alcohol consumption in the second model, a 5.5 (95% CI: 2.0, 15.0) higher risk after adjustment for diabetes and hypertension in the third model. In the fourth model, after adjustment for all covariates used in the previous three models, those who were in the high risk group still have a 3.3 (95% CI: 1.2, 9.1) times higher risk of a non-fatal CHD in comparison to the reference group. Age, gender, diabetes and hypertension status were significant predictors while cigarette smoking and alcohol consumption remained insignificant in the final model.

5.6.2 Joint Distribution of Triglyceride and Waist Circumference Models

Using a similar strategy, for the joint distribution of TG and waist circumference, four groups were created as described in the statistical analyses section for the Framingham Offspring Study in Chapter 4. In this analysis, the highest risk group was defined as those who had TG ≥177 mg/dL and waist circumference ≥90 cm in males and TG ≥155 mg/dL and waist circumference ≥88 cm in females, and the reference group was having TG <177 mg/dL and waist circumference <90 cm in males and TG <155 mg/dL and waist circumference <88 cm in females. Multivariate adjusted HRs for the four models are shown in Table 11. In comparing to the reference group, after adjusting for age and gender in the first model, the HR of a non-fatal CHD for those who were in the high risk group is 2.5 fold higher (95% CI: 1.2, 5.2). After adjustment for regular cigarette smoking and alcohol consumption in the second model, there was a 3.5 (95% CI: 1.7, 7.2) times higher risk of a non-fatal CHD in the high risk group. After adjustment for the diabetes and hypertension status in the third model, those who were in the high risk group had 2.5 (95% CI: 1.2, 5.2) times higher risk of a non-fatal CHD. After adjusting for all the
covariates used in the previous three models, the risk of a non-fatal CHD in those who were in the high risk group is 1.8 (95% CI: 0.8, 3.7) times higher in comparison to the reference group. Again, age, gender, diabetes and hypertension status were found to be significantly associated with non-fatal CHD risk.

5.6.3 Joint Distribution of Non-HDL Cholesterol and TG by Waist Circumference Levels

To examine the impact of the joint distribution of non-HDL, TG and WC on the nonfatal CHD risk, a total of eight groups were created as described in the statistical analyses section for the Framingham Offspring Study in Chapter 4. The HRs are adjusted for age, gender, smoking, alcohol consumption, diabetes and hypertension status and are shown in Figure 9. (Table 12 includes the HRs and the 95% CIs). The results indicate that elevated waist circumference was a significant risk factor for non-fatal CHD regardless of the lipid profile. However, in individuals with high waist circumference (≥90 cm in males or ≥88 cm in females), there was an increased risk of non-fatal CHD associated with a high level of non-HDL cholesterol (≥160 mg/dL) even when TG levels were normal. Further, there was no difference in the risk of non-fatal CHD associated with a high level of TG (≥177 mg/dL in males or ≥150 mg/dL in females) within the two levels of non-HDL cholesterol. In comparison to the reference group (waist circumference <90 cm (males) or <88 cm (females), TG <177 mg/dL (males) or <150 mg/dL (females), and non-HDL <160 mg/dL), individuals who had high waist circumference, high TG, and high non-HDL cholesterol had a 2.5 (95% CI: 1.4, 4.6) times higher risk of non-fatal CHD event. Individuals having high waist circumference and high TG, but normal levels of non-HDL cholesterol, also had a 2.5 (95% CI: 1.1, 5.5) times higher risk of a non-fatal CHD event. While those who had high waist circumference and low TG, but high non-
HDL, had a 2.8 (95% CI: 1.6, 5.0) times higher risk in comparison to the reference group. However, those having normal levels of TG and non-HDL but high waist circumference still had a 1.8 (95% CI: 1.0, 3.4) times higher risk of a non-fatal CHD event.

Among individuals who did not have high waist circumference, in comparison to the reference group, all other groups had approximately 2 to 3 times higher risk of non-fatal CHD although the risk in the group with high TG but low non-HDL was not significant.

The results from this analysis also confirmed that traditional risk factors such as age, gender, diabetes and hypertension all increase the risk of developing CHD. With every one year increase in age, the risk of non-fatal CHD increased by 1.0 (95% CI: 1.0, 1.1). Males had a 2.3 (95% CI: 1.6, 3.4) higher risk of non-fatal CHD in comparing to females. Individuals with diabetes had a 2.1 (95% CI: 1.3, 3.4) times higher risk of developing non-fatal CHD in comparing to non-diabetics, while individuals with hypertension had 1.5 (95% CI: 1.1, 2.0) times higher risk of developing a non-fatal CHD in comparing to those without hypertension.
CHAPTER 6. DISCUSSION

6.1 Comparison between the Predictive Value of Non-HDL Cholesterol, Waist Circumference, and TG, Waist Circumference on the Risk of CHD

Using the Framingham Offspring Study data, this study compared the predictive ability of non-HDL cholesterol and waist circumference, with that of TG and waist circumference on risk of developing non-fatal CHD. The ROC area under the curve associated with non-HDL cholesterol and waist circumference was 0.6428 (95% CI: 0.6183, 0.6673) and for TG and waist circumference was 0.6299 (0.6049, 0.6548). The difference in the ROC area under the curves was 1.29%. The p-value comparing the difference between the predictive ability of two models was 0.10. The results suggest that the model including non-HDL cholesterol and waist circumference may be superior at predicting CHD compared to the model including TG and waist circumference. Failure to achieve significance at the alpha error level of 0.05 may be due to low study power and the loss of study power because this cohort survival data were analyzed using logistic regression instead of the more sensitive Cox-proportional hazards regression analysis. Logistic regression analysis was used as opposed to Cox regression analysis because it is easier to generate the ROC curves and perform the statistical test comparing the ROC AUC (i.e. predictive ability). The ROC curves were generated for unadjusted models and therefore other predictor variables for CHD were excluded for two reasons. Since both models were prepared in the same population, there are no differences in the additional risk factors between the two models, so no adjustments for covariates were required to control for confounding. In addition, modeling TG and waist circumference and non-HDL cholesterol and waist circumference alone provided an absolute measure of the predictive
ability of the two factors under study. If adjusted for additional factors, their importance would be hidden.

The results from the joint distribution of non-HDL cholesterol and TG concentrations confirm that elevated waist circumference increases the risk for non-fatal CHD event. Non-HDL cholesterol and waist circumference was highly associated with the risk of non-fatal CHD in both groups of TG levels. The results also confirm that traditional risk factors including age, male gender, diabetes, and hypertension status are associated with increased risk of non-fatal CHD. Further, non-HDL cholesterol is strongly associated with non-fatal CHD risk, in individuals with both high and low waist circumference levels.

Several previous studies have examined the predictive role of non-HDL cholesterol level in risk assessment of CHD. A study conducted by Orakzai et al. (2009), analyzed the association of non-HDL cholesterol, LDL cholesterol, HDL cholesterol, and TG concentrations with coronary artery calcium, a marker of atherosclerosis. After adjusting for age, gender, race, cigarette smoking, hypertension, obesity, family history of premature CHD, and increasing quartiles of lipid levels, only the association of non-HDL cholesterol with coronary artery calcium scores remained statistically significant (p = 0.002). On the other hand, no such associations existed with LDL cholesterol, HDL cholesterol, and TG concentrations (Orakzai et al., 2009). In a study conducted on the Framingham cohort who were initially free from CHD, VLDL cholesterol predicted CHD risk after adjustment for LDL cholesterol (Liu et al 2006). Further, within each LDL cholesterol category, non-HDL cholesterol levels were predictive of CHD events but within each non-HDL cholesterol category, LDL cholesterol was not predictive of CHD events (Liu et al 2006). As expected, both LDL cholesterol and non-HDL cholesterol
predicted CHD events in patients with TG concentrations <200 mg/dL, however, LDL cholesterol was not effective in predicting CHD risk in patients with TG ≥200 mg/dL while non-HDL cholesterol remained predictive (Liu et al 2006). Hence, non-HDL cholesterol is a better predictor of CHD events and can be utilized to identify individuals with high risk of CHD regardless of their TG levels. Non-HDL cholesterol reflects the risks of both elevated TG levels and LDL cholesterol. The Lipid Research Clinics Program Follow-up study investigated the relationship between CHD and various lipoproteins over a 19 year follow-up period. It was found non-HDL cholesterol was a strong predictor of CHD death in both males and females, where a 30 mg/dL increase in non-HDL cholesterol levels results in a 19% increase in CHD death among males and 15% among females (Cui et al., 2001). Numerous studies have also shown that non-HDL cholesterol is a strong predictor of CHD deaths among individuals with diabetes (Liu et al., 2005; Lu et al., 2003) as well as among non-diabetics (Al-Daghri et al., 2007).

Although numerous studies have demonstrated the level of non-HDL cholesterol as an important predictor of the risk for CHD, based on an extensive review of the literature, this is the first study to have evaluated and compared the predictive value of the joint distribution of non-HDL cholesterol and waist circumference, and TG and waist circumference among individuals with CHD.

Non-HDL cholesterol measurement has several potential advantages for clinical practice. In clinical lipoprotein analysis, the level of LDL cholesterol is usually estimated using the Friedewald’s formula, based on the measurements of total and HDL cholesterol as well as TG concentrations, which are used to estimate the value of VLDL cholesterol. However, the estimate of this formula becomes progressively less accurate as TG levels increase, and the formula is no longer considered accurate enough for use when TG levels
reach 400 mg/dL. Although the Friedwald formula includes IDL cholesterol and lipoprotein (a) in the LDL cholesterol level measurement, they do not include remnant cholesterol particles. Since non-HDL cholesterol includes all the potentially atherogenic lipoproteins including the LDL cholesterol, IDL cholesterol, and VLDL cholesterol remnants, theoretically, it should be a better indicator of risk for non-fatal CHD than LDL cholesterol (Orakzai et al., 2009; Liu et al., 2006). In particular, the inclusion of VLDL remnants in the non-HDL cholesterol level measurements would enhance the assessment of CHD risk. Hence, measurements of non-HDL cholesterol levels are not limited by assumptions about the relationship between VLDL cholesterol and TG concentrations. In some individuals, especially in patients with diabetes, the relationship between VLDL cholesterol and TG levels may be altered, resulting in falsely low LDL cholesterol levels as calculated by the Friedwald formula, especially if TG levels are elevated to >400 mg/dL (Lu et al., 2003). In this setting, non-HDL cholesterol can be used to indicate the risk for CHD. Non-HDL cholesterol level may also be useful in identifying individuals who have the atherogenic lipoprotein phenotype found in patients with metabolic syndrome and diabetes mellitus and characterized by elevated TG-rich lipoproteins including the VLDL cholesterol and IDL cholesterol, and decreased levels of HDL cholesterol (Orakzai et al., 2009). It is estimated that approximately 20% of the American population have this phenotype (Orakzai et al., 2009). Further, studies that support the independent association of elevated TG levels and CHD emphasize the atherogenicity of VLDL cholesterol. Unlike cholesterol, TG itself is not deposited in the atherosclerotic plaques. Instead, the effect of TG on the formation of atherosclerotic plaques can be explained by the accumulation of TG-rich VLDL remnants and the structural abnormalities in LDL and HDL particles caused by high TG levels (Packard et al., 2004).
A recent study conducted by Liu et al (2006), has suggested that elevated VLDL cholesterol is an independent predictor of CHD risk regardless of the TG levels. VLDL cholesterol are atherogenic because they can be taken up by macrophages, resulting in increased formation of foam cells and speeding up the process of atherosclerosis (Lu et al., 2003). Elevated VLDL cholesterol levels are also associated with increased release of prothrombotic and procoagulant factors (Lu et al., 2003). Thus, the NCEP ATP III (2002) recommended the use of non-HDL cholesterol as a secondary target of lipid lowering, after achieving adequate control of LDL cholesterol and if TG levels are elevated to ≥200 mg/dL. Another benefit of using non-HDL cholesterol is that it can be calculated in the non-fasting state by simply subtracting the levels of HDL cholesterol from the total cholesterol and it is also known to have less day to day variability compared to TG levels (Wagner et al., 2005). Thus, simultaneous measurement and interpretation of non-HDL cholesterol and waist circumference may be a simple screening tool for identifying individuals with non-fatal CHD.

Abdominal obesity measured via waist circumference is also recognized as a major risk factor for CHD. The risk associated with increased waist circumference is due to the presence of visceral adipose tissue, which promotes insulin resistance, dyslipidaemia, and hypertension. A meta-analysis of the prospective cohort studies and randomized clinical trials showed that elevated waist circumference levels have strong significant association with the risk of CHD events and deaths (Koning et al., 2007) independent of BMI and conventional CHD risk factors (Canoy, 2008). Therefore, simple measures of abdominal obesity such as waist circumference should also be used along with non-HDL cholesterol to identify individuals with high CHD risk.
6.2 Strengths and Limitations

6.2.1 Strengths

The strengths of the study are 1) The Framingham Offspring Study is a prospective cohort study, originally designed to examine the risk factors of CHD. 2) The baseline information as well as the end point (non-fatal CHD) was well documented and the follow-up records are reliable. 3) Waist circumference measurements were used as an indicator of obesity instead of BMI. 4) The study was able to control for age, gender, cigarette smoking, alcohol consumption, diabetes and hypertension.

6.2.2 Limitations

Results should be interpreted with caution. The possible limitations include: 1) Since the obtained Framingham Offspring Study dataset was a limited version for public access, some variables were blocked in order to maintain confidentiality. 2) The Framingham Offspring Study dataset. Since the Framingham Offspring Study only recruited Caucasians, the findings may not be generalizable to the other ethnic groups. For instance, it is known that African Americans generally have lower levels of LDL cholesterol in comparison to Caucasians (Cohen et al., 2005) and yet they seem to have higher rates of cardiovascular mortality across all age groups (Mensah et al., 2005). 3) Adding more sample size or increasing the follow-up period would enhance the study power. 4) Significance testing of Cox proportional hazards models using C-statistic difference was not corrected out (needs boot strapping). 5) Calibration was not evaluated. 6) Diabetes status was determined by using fasting blood glucose concentration of ≥126 mg/dL. Doctor’s diagnosis of diabetes was not considered because no data was found in the Framingham Offspring Study dataset. 7) Other factors such as family history, physical
activity, diet, and socioeconomic status were not controlled for in the analyses since no data were available.

6.3 Conclusion

This is the first study to compare the predictive ability of non-HDL cholesterol, waist circumference and TG, waist circumference in predicting the risk of CHD. The results suggest that non-HDL cholesterol and waist circumference may be superior to TG and waist circumference in predicting the risk of CHD. The NCEP ATP III (2002) has recommended LDL cholesterol as the primary target of therapy. However, many individuals are at increased risk of CHD due to high concentrations of atherogenic lipoproteins not reflected in LDL cholesterol measurement, especially patients with dyslipidemia associated with the metabolic syndrome and diabetes. Use of non-HDL cholesterol as target for therapy in individuals with mild to moderate TG elevation will serve to improve treatment. Non-HDL cholesterol could be a simpler alternative and may give a more appropriate estimation of the risk of CHD. The use of non-HDL cholesterol and waist circumference as a primary target for cholesterol lowering therapy deserves further investigation. Further, substitution of non-HDL cholesterol and waist circumference for hypertriglyceridemic waist phenotype may help in screening individuals with high CHD risk so that measures can be taken to prevent the occurrence of CHD.

Future research should validate the findings of this study in a larger and a diverse population by using a more sensitive method for comparing the predictive ability of the two models. Comparison should also be made between non-HDL cholesterol, waist
circumference and other markers of atherogenic lipoproteins such as apo B, ratio of total to HDL cholesterol, and HDL cholesterol.
REFERENCES


## APPENDIX I

Table 1: NCEP ATP III Classification of LDL and Non-HDL Cholesterol

<table>
<thead>
<tr>
<th>Classification</th>
<th>LDL Cholesterol Level (mg/dL)</th>
<th>Non-HDL Cholesterol Level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>&lt;100</td>
<td>&lt;130</td>
</tr>
<tr>
<td>Near Optimal</td>
<td>100-129</td>
<td>130-159</td>
</tr>
<tr>
<td>Borderline High</td>
<td>130-159</td>
<td>160-189</td>
</tr>
<tr>
<td>High</td>
<td>160-189</td>
<td>190-119</td>
</tr>
<tr>
<td>Very High</td>
<td>≥190</td>
<td>≥220</td>
</tr>
</tbody>
</table>

(NCEP ATP III, 2002) – National Cholesterol Education Program Adult Treatment Panel III
<table>
<thead>
<tr>
<th>Classification</th>
<th>HDL Cholesterol Level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low HDL Cholesterol</td>
<td>&lt;40</td>
</tr>
<tr>
<td>High HDL Cholesterol</td>
<td>≥60</td>
</tr>
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</table>

(NCEP ATP III, 2002) – National Cholesterol Education Program Adult Treatment Panel III
# Table 3: Clinical Identification of Metabolic Syndrome

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining Level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abdominal Obesity (cm)</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&gt;102</td>
</tr>
<tr>
<td>Females</td>
<td>&gt;88</td>
</tr>
<tr>
<td><strong>High Triglyceride Level (mg/dL)</strong></td>
<td>≥150</td>
</tr>
<tr>
<td><strong>Low HDL Cholesterol Level (mg/dL)</strong></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Females</td>
<td>&lt;50</td>
</tr>
<tr>
<td><strong>Blood Pressure (mmHg)</strong></td>
<td>≥130/85</td>
</tr>
<tr>
<td><strong>Fasting Glucose (mg/dL)</strong></td>
<td>≥100</td>
</tr>
</tbody>
</table>

(NCEP ATP III, 2002) – National Cholesterol Education Program Adult Treatment Panel III
<table>
<thead>
<tr>
<th>Classification</th>
<th>Triglyceride Level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Borderline high</td>
<td>150-199</td>
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<tr>
<td>High</td>
<td>200-499</td>
</tr>
<tr>
<td>Very high</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

(NCEP ATP III, 2002) – National Cholesterol Education Program Adult Treatment Panel III
Table 5: Non-HDL, Waist Circumference and High Triglyceride, Waist Circumference Criteria

<table>
<thead>
<tr>
<th>Gender</th>
<th>Status</th>
<th>Non-HDL Cholesterol, Waist Circumference</th>
<th>Triglyceride, Waist Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>High</td>
<td>$\geq 160 \text{ mg/dL}$, $\geq 90 \text{ cm}$</td>
<td>$\geq 177 \text{ mg/dL}$, $\geq 90 \text{ cm}$</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>$&lt; 160 \text{ mg/dL}$, $&lt; 90 \text{ cm}$</td>
<td>$&lt; 177 \text{ mg/dL}$, $&lt; 90 \text{ cm}$</td>
</tr>
<tr>
<td>Females</td>
<td>High</td>
<td>$\geq 160 \text{ mg/dL}$, $\geq 88 \text{ cm}$</td>
<td>$\geq 150 \text{ mg/dL}$, $\geq 88 \text{ cm}$</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>$&lt; 160 \text{ mg/dL}$, $&lt; 88 \text{ cm}$</td>
<td>$&lt; 150 \text{ mg/dL}$, $&lt; 88 \text{ cm}$</td>
</tr>
</tbody>
</table>

Non-HDL Cholesterol, Waist Circumference Criteria: NCEP ATP III, 2002
Triglyceride, Waist Circumference Criteria: Lemieux et al. 2000; LeMonte et al., 2003
Table 6: Characteristics of Participants by Gender at the 4th Examination (1987-1990), Framingham Offspring Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>1,572</td>
<td>1,624</td>
<td></td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>53.9 (8.7)</td>
<td>53.3 (8.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Marital Status (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>5.0</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>85.3</td>
<td>76.6</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>2.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>6.5</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Separated</td>
<td>1.3</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetes (%)</strong></td>
<td>6.3</td>
<td>3.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>44.1</td>
<td>34.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Cigarette Smoking (%)</strong></td>
<td>23.6</td>
<td>25.3</td>
<td>0.2524</td>
</tr>
<tr>
<td><strong>Total Alcohol Consumption (ounce/wk)</strong></td>
<td>4.0 (5.4)</td>
<td>1.8 (2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Follow-up time (yrs)</strong></td>
<td>9.6 (2.8)</td>
<td>10.1 (2.1)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 7: Key Study Variables of Participants by Gender at the 4th Examination (1987-1990), Framingham Offspring Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1,572</td>
<td>1,624</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>53.9 (8.7)</td>
<td>53.3 (8.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>207.1 (36.9)</td>
<td>211.0 (40.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>43.0 (11.6)</td>
<td>55.5 (15.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-HDL Cholesterol (mg/dL)</td>
<td>164.1 (38.3)</td>
<td>155.5 (43.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>143.3 (103.3)</td>
<td>117.2 (105.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>136.7 (33.6)</td>
<td>132.4 (37.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>98.3 (10.3)</td>
<td>82.8 (13.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>130.4 (17.5)</td>
<td>127.1 (19.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHD Events (n)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fatal (n)</td>
<td>304</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Fatal (n)</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CHD Event Rate (per 10,000 person yrs)</td>
<td>246.8</td>
<td>78.0</td>
<td></td>
</tr>
</tbody>
</table>

*During follow-up
Table 8: Hazard Ratio* of Non-fatal Coronary Heart Disease Associated with Lipids and Waist Circumference, Framingham Offspring Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>1.3 (0.9, 1.8)</td>
<td>1.4 (0.9, 2.3)</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>0.7 (0.4, 1.1)</td>
<td>0.9 (0.5, 1.5)</td>
</tr>
<tr>
<td>Non-HDL Cholesterol (mg/dL)</td>
<td>1.3 (0.9, 1.9)</td>
<td>1.4 (0.9, 2.2)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>1.1 (0.9, 1.5)</td>
<td>1.1 (0.8, 1.5)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>1.0 (0.7, 1.7)</td>
<td>1.4 (0.8, 2.4)</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>1.3 (0.9, 1.8)</td>
<td>1.4 (0.8, 2.3)</td>
</tr>
</tbody>
</table>

* Adjusted for age, smoking, alcohol, diabetes, and hypertension
Table 9: Adjusted means* for the Key Study Variables within the Non-HDL Cholesterol and Triglyceride Groups Stratified by Waist Circumference Status, Framingham Offspring Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>nHDL≥160</th>
<th>nHDL≥160</th>
<th>nHDL&lt;160</th>
<th>nHDL&lt;160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG≥177 or 150 (mg/dL)</td>
<td>TG&lt;177 or 150 (mg/dL)</td>
<td>TG≥177 or 150 (mg/dL)</td>
<td>TG&lt;177 or 150 (mg/dL)</td>
</tr>
<tr>
<td>WC ≥ 90 or ≥ 88 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>416</td>
<td>594</td>
<td>135</td>
<td>622</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>54.5</td>
<td>54.3</td>
<td>55.5</td>
<td>53.9</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>242.4</td>
<td>233.9</td>
<td>178.6</td>
<td>180.9</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>38.7</td>
<td>47.8</td>
<td>37.9</td>
<td>49.5</td>
</tr>
<tr>
<td>Non-HDL Cholesterol (mg/dL)</td>
<td>203.7</td>
<td>186.2</td>
<td>140.7</td>
<td>131.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>282.6</td>
<td>112.2</td>
<td>232.5</td>
<td>90.4</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>154.0</td>
<td>163.6</td>
<td>97.2</td>
<td>113.2</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>101.2</td>
<td>98.2</td>
<td>102.5</td>
<td>99.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>136.0</td>
<td>130.7</td>
<td>135.6</td>
<td>131.2</td>
</tr>
<tr>
<td>CHD Events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fatal (n)</td>
<td>83</td>
<td>113</td>
<td>26</td>
<td>90</td>
</tr>
<tr>
<td>Fatal (n)</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Event Rate for CHD (per 10,000 person yrs)</td>
<td>275.0</td>
<td>245.7</td>
<td>257.8</td>
<td>188.0</td>
</tr>
</tbody>
</table>

| WC < 90 or < 88 cm          |          |          |          |          |
| n                          | 126      | 399      | 43       | 861      |
| Age (yrs)                  | 56.3     | 55.1     | 53.9     | 51.0     |
| Total Cholesterol (mg/dL)  | 248.8    | 238.6    | 184.9    | 182.6    |
| HDL Cholesterol (mg/dL)    | 40.7     | 51.7     | 46.7     | 57.6     |
| Non-HDL Cholesterol (mg/dL)| 208.1    | 186.8    | 138.3    | 125.0    |
| Triglycerides (mg/dL)      | 231.7    | 101.0    | 196.2    | 76.5     |
| LDL Cholesterol (mg/dL)    | 164.0    | 166.8    | 99.2     | 109.8    |
| Waist Circumference (cm)   | 83.2     | 80.1     | 82.2     | 77.8     |
| Systolic BP (mmHg)         | 130.3    | 126.5    | 127.5    | 121.8    |
| CHD Events                 |          |          |          |          |
| Non-fatal (n)              | 20       | 43       | 4        | 37       |
| Fatal (n)                  | 0        | 2        | 0        | 1        |
| Event Rate for CHD (per 10,000 person yrs) | 209.7 | 131.3 | 94.9 | 48.0 |

* Adjusted means for age and gender
Waist circumference (WC) – Males: 90 cm; Females: 88 cm
Triglyceride (TG) – Males: 177 mg/dL; Females: 150 mg/dL
<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
</tr>
<tr>
<td>nHDL ≥ 160 mg/dL &amp; Waist ≥ 90 or 88 cm (n = 1010)</td>
<td>4.3</td>
<td>(1.5, 11.9)</td>
<td>6.9</td>
<td>(2.5, 19.0)</td>
<td>5.5</td>
</tr>
<tr>
<td>nHDL ≥ 160 mg/dL &amp; Waist &lt; 90 or 88 cm (n = 525)</td>
<td>2.2</td>
<td>(0.7, 7.5)</td>
<td>2.6</td>
<td>(0.8, 8.8)</td>
<td>2.5</td>
</tr>
<tr>
<td>nHDL &lt; 160 mg/dL &amp; Waist ≥ 90 or 88 cm (n = 757)</td>
<td>1.5</td>
<td>(0.5, 4.8)</td>
<td>2.4</td>
<td>(0.8, 7.5)</td>
<td>2.0</td>
</tr>
<tr>
<td>nHDL &lt; 160 mg/dL &amp; Waist &lt; 90 or 88 cm (n = 904)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>1.0</td>
<td>(1.0, 1.1)</td>
<td>1.0</td>
<td>(1.0, 1.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.8</td>
<td>(2.0, 3.8)</td>
<td>2.2</td>
<td>(1.5, 3.1)</td>
<td>2.3</td>
</tr>
<tr>
<td>Female</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Regular Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.1</td>
<td>(0.8, 1.5)</td>
<td>1.1</td>
<td>(0.8, 1.5)</td>
<td>1.3</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Alcohol consumption (ounce/wk)</td>
<td>1.0</td>
<td>(1.0, 1.0)</td>
<td>1.0</td>
<td>(1.0, 1.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.4</td>
<td>(2.2, 5.3)</td>
<td>2.2</td>
<td>(1.4, 3.4)</td>
<td>2.1</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Hypertension</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.2</td>
<td>(1.6, 2.9)</td>
<td>1.6</td>
<td>(1.2, 2.2)</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
</tbody>
</table>
Table 11: Hazard Ratios in Predicting the Risk of Non-fatal Coronary Heart Disease Associated with the Joint Distribution of High Triglycerides and Waist Circumference, Framingham Offspring Study

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
<td>95% CI</td>
<td>HR</td>
</tr>
<tr>
<td><strong>TG≥ 177 or 150 mg/dL &amp; WC≥ 90 or 88 cm (n = 551)</strong></td>
<td>2.5</td>
<td>(1.2,5.2)</td>
<td>3.5</td>
<td>(1.7,7.2)</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>TG≥ 177 or 150 mg/dL &amp; WC&lt; 90 or 88 cm (n = 169)</strong></td>
<td>1.9</td>
<td>(1.0,3.7)</td>
<td>1.9</td>
<td>(1.0,3.8)</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>TG&lt; 177 or 150 mg/dL &amp; WC≥ 90 or 88 cm (n = 1216)</strong></td>
<td>2.0</td>
<td>(1.0,4.1)</td>
<td>3.1</td>
<td>(1.6,6.3)</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>TG&lt; 177 or 150 mg/dL &amp; WC&lt; 90 or 88 cm (n = 1260)</strong></td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>1.0</td>
<td>(1.0,1.1)</td>
<td>1.0</td>
<td>(1.0,1.1)</td>
<td>1.0</td>
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<tr>
<td>Gender</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.8</td>
<td>(2.0,3.8)</td>
<td>2.3</td>
<td>(1.6,3.2)</td>
<td>2.4</td>
</tr>
<tr>
<td>Female</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Regular Smoking</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.1</td>
<td>(0.8,1.5)</td>
<td>1.1</td>
<td>(0.8,1.5)</td>
<td>1.3</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
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<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(ounce/wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.4</td>
<td>(2.2,5.3)</td>
<td></td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
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<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.2</td>
<td>(1.6,2.9)</td>
<td>1.7</td>
<td>(1.2,2.2)</td>
<td>1.5</td>
</tr>
<tr>
<td>No</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
</tbody>
</table>
Table 12: Hazard Ratio* (95% CI) Results for Figure 8, Framingham Offspring Study

<table>
<thead>
<tr>
<th>Non-HDL Cholesterol (mg/dL)</th>
<th>WC &lt; 90 or 88 cm</th>
<th>WC ≥ 90 or 88 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG (mg/dL)</td>
<td>TG (mg/dL)</td>
</tr>
<tr>
<td>N</td>
<td>&lt;177 or 150</td>
<td>≥177 or 150</td>
</tr>
<tr>
<td>N</td>
<td>≥160</td>
<td>&lt;177 or 150</td>
</tr>
<tr>
<td></td>
<td>399</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>2.2 (1.6, 4.2)</td>
<td>3.1 (1.4, 7.1)</td>
</tr>
<tr>
<td></td>
<td>861</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.3 (0.7, 7.9)</td>
</tr>
</tbody>
</table>

Note: * Adjusted for age, gender, smoking, alcohol consumption, diabetes, and hypertension
APPENDIX II

TG/Cholesterol ratio, size and density of different lipoproteins

- Triglyceride
- Cholesterol

HDL
5% TG
95% Ch

LDL
10% TG
50% Ch

IDL
50% TG
50% Ch

VLDL
80% TG
20% Ch

Chylomicron
95% TG
5% Ch

Importance of waist girth and fasting triglyceride levels as screening tools

**ATHEROGENIC TRIAD OF NEW METABOLIC RISK FACTORS**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Apo B</th>
<th>Small, dense LDL</th>
</tr>
</thead>
</table>

**SCREENING TOOLS**

- Waist circumference: $> 90$ cm
- Triglycerides: $\geq 2.0$ mmol/L

Stage I  Normal coronary artery  
Stage II Beginning of atheroma
Stage III Beginning of encroachment of coronary artery  
Stage IV Blockages hardening into Plaques
Stage V Crack or rupture in coronary artery  
Stage VI Closing off the channel of artery

Total N at fourth examination = 4,989

Excluding age < 40 years: N = 3,460

Excluding missing total cholesterol: N = 3,380

Excluding missing HDL cholesterol: N = 3,363

Excluding missing TG and fasting < 9 hours: N = 3,212

Excluding missing waist circumference: N = 3,196

Figure 5: Sample Selection Procedure for the Framingham Offspring Study
Figure 6: Receiver Operating Characteristic Curves for Non-HDL Cholesterol and Waist Circumference, and Triglyceride and Waist Circumference, Framingham Offspring Study
Figure 7: Kaplan-Meier Curves for Non-HDL Cholesterol and Waist Circumference Groups, Framingham Offspring Study

Note: nhdl_wc = 1: non-HDL cholesterol ≥ 160 mg/dL and WC ≥ 90 or 88 cm
nhdl_wc = 2: non-HDL cholesterol ≥ 160 mg/dL and WC < 90 or 88 cm
nhdl_wc = 3: non-HDL cholesterol < 160 mg/dL and WC ≥ 90 or 88 cm
nhdl_wc = 4: non-HDL cholesterol < 160 mg/dL and WC < 90 or 88 cm
Figure 8: Kaplan-Meier Curves for Triglyceride and Waist Circumference Groups, Framingham Offspring Study

Note: tg_wc = 1: tg ≥ 177 or 150 mg/dL and WC ≥ 90 or 88 cm
tg_wc = 2: tg ≥ 177 or 150 mg/dL and WC < 90 or 88 cm
tg_wc = 3: tg < 177 or 150 mg/dL and WC ≥ 90 or 88 cm
tg_wc = 4: tg < 177 or 150 mg/dL and WC < 90 or 88 cm
Figure 9: Risk of Non-fatal Coronary Heart Disease by Non-HDL Cholesterol, Triglyceride, and Waist Circumference Status, Framingham Offspring Study

Note: Adjusted for age, gender, smoking, alcohol, diabetes, and hypertension
APPENDIX III

DATE: June 3, 2008

FROM: Michelle McGinn, Chair
Research Ethics Board (REB)

TO: Jain Liu, Community Health Science
Divya Joshi

FILE: 07-315 - LIU/JOSHI

TITLE: Non-HDL, Waist Circumference vs. Triglyceride, Waist Circumference in Detecting Risk of Cardiovascular Disease

The Brock University Research Ethics Board has reviewed the above research proposal.

DECISION: Secondary use of data accepted; however, please ensure that as the PI named on the data agreement, you have students sign confidentiality agreements. The confidentiality agreement form can be found on our website: https://www.brocku.ca/researchservices/forms/ethics_safety/Humans/GeneralStatementofConfidentiality.pdf

This project has received ethics clearance for the period of June 3, 2008 to August 01, 2008 subject to full REB ratification at the Research Ethics Board's next scheduled meeting. The clearance period may be extended upon request. The study may now proceed.

Please note that the Research Ethics Board (REB) requires that you adhere to the protocol as last reviewed and cleared by the REB. The Board must provide clearance for any modifications before they can be implemented. If you wish to modify your research project, please refer to http://www.brocku.ca/researchservices/forms to complete the appropriate form Request for Clearance of a Revision or Modification to an Ongoing Application.

Adverse or unexpected events must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants and the continuation of the protocol.

The Tri-Council Policy Statement requires that ongoing research be monitored. A Final Report is required for all projects, with the exception of undergraduate projects, upon completion of the project. Researchers with projects lasting more than one year are required to submit a Continuing Review Report annually. The Office of Research Services will contact you when this form Continuing Review/Final Report is required.
Please quote your REB file number on all future correspondence.

MM/kw

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