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Endothelial Function in Familial Hyperlipidemia Children

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Endothelial Function in Familial Hyperlipidemia Children

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Children with familial hypercholesterolemia (FH) and familial combined hyperlipidemia (FCH) are at risk for advanced atherosclerosis; however, they are often undiagnosed and undertreated. To examine the association among atherogenic lipids, inflammation, and endothelial dysfunction in children FH and FCH, a descriptive cross-sectional study was conducted. Dependent variables included endothelial function, as measured by brachial flow-mediated dilation, and inflammation, as measured by hsCRP. Independent variables included lipid profile and non-lipid variables. Lipid profile was measured by total cholesterol, triglycerides, LDL-C, HDL-C and nonHDL-C. Non-lipid predictors were measured by gender, age, BMI, blood pressure, and fasting blood glucose. Person Correlation Coefficients were calculated to evaluate the association between dependent variables and independent variables. Multiple linear regressions were modeled to identify predictors for endothelial function and inflammation.

Among 64 children with familial hyperlipidemia, the multiple regression models identified age as a significant independent variable for decreased brachial FMD. Increased fasting blood glucose and lower nonHDL-C were identified as significant independent variables for increased hsCRP. Age was a significant predictor, contributing 7% variance in brachial flow-mediated dilation ($R^2 = 0.074$, $F = 4.978$, $p = 0.029$). Beta coefficient indicated higher age was associated with higher brachial flow-mediated dilation. Individually, fasting blood glucose accounted for 14% variance and nonHDL-C

accounted for 7% variance in hsCRP ($R^2 = 0.232$, $F = 9.205$, $p = 0.000$). Beta coefficients indicated lower fasting blood glucose and lower nonHDL-C were associated with higher LoghsCRP. Increased fasting blood sugar was associated with higher hsCRP and lower brachial flow-mediated dilation.

Although controversial to traditional findings, our data sheds a light on the complex impact of atherogenic lipids and inflammation on endothelial function in children. The important role of fasting sugar manifesting inflammation combined with the variability of total cholesterol, LDL-C and nonHDL-C in FH and FCH children aged 7 to 19 years old implicate a need for further investigation.

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CHAPTER ONE

Introduction

Heart disease was the fifth leading cause of death for American youth age 12-19 years in the turn of twentieth-first century (Minino, 2010). During 1999 to 2006, an estimate of 491 youth (0.2%) died of heart disease every year. Despite low mortality in youth, atherosclerotic cardiovascular diseases (CVDs) remain the leading causes of death in the American adult population since 1950's (Roger et al., 2011). In 2007, CVDs accounted for one death in every 39 seconds and 2,200 deaths every day in Americans aged less than 65 years. In the National Health and Nutrition Examination Survey (NHNES) 2005-2008, 14% of men and 9.7% of women aged 20-39 years, and 39% of men and 37% of women aged 40-59 years suffered from CVDs. Clearly, mortality and morbidity of CVDs increase with age and preventive measures must begin in young age.

Cumulative pathological evidence indicates that fatty streaks can develop in a fetus with a hypercholesterolemic mother (Napoli et al., 1999), and that advanced atherosclerotic lesions develop in young adults with multiple CVD risk factors (Berenson et al., 1998; McGill et al., 2000). Although hypercholesterolemia has been established as a risk factor for CVD (Kannel, Dawber, Kagan, Revotskie, & Stokes, 1961) the atherogenic mechanisms of lipid disorders remain inconclusive. As a result, recommendations set for screening and pharmacological treatment in children with lipid disorders are not unified. The inconsistent guidelines set by American Academic of Pediatric (Daniels, Greer, and the Committee on Nutrition, 2008) and The US Preventive Services Task Force (US Preventive Services Task Force, 2007) implicated the complexity in prevention and management of metabolic lipid disorders in youth. Such

inconsistency may hinder medical management of the 20% of American youth at risk for early development of atherosclerosis due to abnormal lipid levels (Centers for Disease Control and Prevention, 2010).

Recent confirmatory human genome-wide linkage studies indicated the importance of genetic control of coronary heart disease (Musunuru & Kathiresan, 2010) and its association with metabolic lipid disorders including low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and total cholesterol (Teslovich et al., 2010). Despite genetic predisposition, metabolic lipid disorders have also been described as established modifiable CVD risk factors in the Framingham Heart Study (Kannel et al., 1961). In the past 50 years, environmental factors such as diet and lifestyle have also been proven determinants for lipid phenotypic presentations (Gidding et al., 2009). Children born with predisposed lipid disorders are at high risk for progressive atherosclerosis development and premature coronary heart disease (Hopkins et al., 2003). Although current medical interventions can't alter genetic composition, many therapeutic treatments are available for modifying genetic expression and treating familial lipid disorders. Early detection and timely intervention offer the best opportunity to manage the lifetime risks of children with familial lipid disorders in developing premature CVD.

In 2008, both the American Academy of Pediatrics (AAP) and the British National Institute for Health and Clinical Excellence (NICE) presented different guidelines for diagnosing and treating for children with familial lipid disorders (Daniels et al., 2008; Wierzbicki, Humphries, & Minhas, 2008). Both sets of recommendations also included some controversial issues in the respective countries (De Ferranti, &

Ludwig, 2008; Mihas et al., 2009). In 2011, the American National Lipid Association Expert Panel (ANLAEP) on Familial Hypercholesterolemia (FH) published more refined recommendations (Daniels, Gidding, & De Ferranti, 2011). In the recommendations, the ANLAEP accepted three criteria for diagnosing FH: the Simon Broome Criteria (Scientific Steering Committee of the Simon Broome Register Group, 1991), Dutch Lipid clinic Network Criteria (Civeira, 2004) and the United States Make Early Diagnosis Prevent Early Deaths (MEDPED; Williams et al., 1993). The ANLAEP also suggested the use of non-high-density lipoprotein (non HDL-C) ≥ 145 mg/dl for screening and non HDL-C ≥ 190 mg/dl for diagnosing suspected FH in individuals younger than 20 years old (Daniels et al., 2011).

The goal of this research is to assess the development of atherosclerosis in children with familial lipid disorders and to evaluate ANLAEP 2011 recommendations. As a complex disease, atherosclerosis is described by three theories, endothelial dysfunction theory (Gimbrone, 1989), response-to-retention theory (Williams & Tabas, 1995) and response-to-inflammation theory (Ross, 1999). Endothelial dysfunction, as an early event of atherosclerosis, will be assessed by brachial artery flow-mediated dilation (FMD), a non-invasive ultrasound measure of endothelium-dependent vascular hyperemic reactivity. Response-to-retention (lipid retention) will be assessed by biochemical analysis of atherogenic lipid profile including LDL-C and non HDL-C levels, a parameter suggested by ANLAEP 2011 (Daniels et al, 2011). Response-to-inflammation will be assessed by biochemical analysis of the inflammatory biomarker high sensitive c reactive protein (hs-CRP). Using three measures will provide a comprehensive assessment of pre-clinical atherosclerosis in children with familial lipid

disorders. By identifying the associations among endothelial dysfunction, lipid retention and inflammation, newly found scientific evidences could improve our capacity to screen and treat children with familial lipid disorders.

Statement of the Problem

Familial hypercholesterolemia (FH) and familial combined hyperlipidemia (FCH) are two of the most commonly occurring genetic lipid disorders among children and adolescents (Daniels et al., 2011). Both familial lipid disorders are also under-diagnosed and under-treated in the pediatric population (Defesche, 2010). The prevalence of heterozygous FH is estimated to affect one in 300 to 500 people in most ethnic groups across the United States. The prevalence of homozygote is one in one million persons worldwide (Hopkins, Toth, Ballantyne, & Rader, 2011). The prevalence of FCH varies from 1% to 6% in the general population (Hopkins et al., 2003; Jarvik, Brunzell, & Motulsky, 2008). Among individuals with a family history of premature coronary heart disease (CHD), the prevalence for FCH can increase to 36-48% (Williams et al., 1990).

Issues on Diagnosing FH

In the 1930's, Norwegian physicians began to notice an association between hereditary hyperlipidemia and cardiovascular diseases in patients with chest pain and xanthomatosis (Muller, 1939). A decade later, American physicians began reporting comprehensive family pedigree studies (Wilkinson, Hand & Fliegelman, 1948). In the 1960's, familial hyperlipidemia was classified Type I-V hyperlipoproteinemia according to lipid presentations (phenotype) on patients with coronary heart disease (Fredrickson, Levy, & Lee, 1967). Familial hypercholesterolemia was defined as Type II

hyperlipoproteinemia (hyperbeta-lipoproteimemia) with characteristics of two to four times the levels of LDL-C. Early recognition of familial combined hyperlipidemia was characterized by increased total cholesterol and triglyceride (Goldstein, Schrott, Hazzard, Bierman, & Motulsky, 1973). Thanks to advancements in genetic and biochemical technologies, over the last two decades different diagnosing criteria based on national averages have been proposed. To better understand the debate over diagnosing and treating children with FH and FCH three criteria proposed by ANLAEP will be briefly discussed in the following paragraphs. Because homozygous FH and FCH are very rare, this dissertation will be focused on heterozygous carriers.

Dutch Lipid Clinic Network Criteria for Diagnosing FH.

The Dutch lipid clinic network diagnosis of FH is a scoring system used to help identify affected individuals among family members of index cases (De Sauvage Nolting, Buirma, Hutten, Kastelein, 2002). The diagnostic criteria include five categories: family history, clinical (personal) history, physical examination, laboratory analysis and DNA-analysis. Premature coronary heart disease is defined as men younger than 55 years and women younger than 60 years. LDL-C concentrations are estimated by the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972) calculation. The Friedewald formula using unit in mg/100 ml (mg/dl) is LDL-C = total cholesterol – HDL-C – TG/5, or using unit in mmol/l as DLD-C = TC – HDL-C – TG/2.2 (Descamps et al., 2011). Children younger than 18 years with LDL-C > 95th percentile are given two points. LDL-C levels of 4.0-4.9 mmol/l (155-189 mg/dl), 5.0-6.4 mmol/l (190-249 mg/dl), 6.5-8.4 mmol/l (250-329 mg/dl), >8.5 mmol/l (>330 mg/dl) are given 1, 3, 5, and 8 points respectively. The presence of functional mutation in LDLR earns eight points. Physical sign of tendon

xanthomata is assigned six points, and arcus cornealis below the age of 45 years is assigned 4 points. FH diagnosis is confirmed if an individual's score is greater than 8 points and verified by genetic testing of known LDL receptor mutations. Probable cases are those with a score of 6-8 points, and possible cases receive a score of 3-5 points.

Since 1994, Dutch lipid clinics have employed these criteria to identify index cases and used targeted screening (cascade screening) on relatives of the index cases based on family or personal history (Umans-Eckenhausen, Defesche, Sijibrands, Scheerder, Kastelein, 2001). Five years into the targeted screening program, 37 Dutch lipid clinics found 526 index cases, of which 62% were verified by genetic tests, 45% males and 20% females with history of CHD and a mean age of onset at 47 years (De Sauvage Nolting et al., 2003). Among these index cases, CVD risk factors including age, hypertension, diabetes, increased BMI (body mass index), triglycerides and low HDL-C were found as well.

In addition, based on more than 1,000 Dutch children's data, investigators found LDL-C>3.5 mmol/l (135 mg/dl), HDL-C<1.0 mmol/l (38.6 mg/dl) and lipoprotein (a)>300 mg/l to have diagnostic value in children with a known family history (Wiegman, et al., 2003). Despite a high participation rate, the ability of genetic testing to identify causal mutation was less than 50% in patients with clinical diagnosis.

British Simon Broome Register Group Definition of FH.

Inspired by Dr. J Slack (Slack, 1969), British Simon Broome Register Group began to enroll adult FH patients (older than 20 years) in 1980 (Scientific Steering Committee of the Simon Broome Register Group, 1991). The initial criteria were: total

cholesterol level above 7.5 mmol/l (290 mg/dl) or LDL-C calculated by Freidewald formula above 4.9 mmol/l (190 mg/dl); family history of CHD below age 60 years in parents and 50 years in grandparents; personal history of obesity, hypertension, diabetes, CVD; physical signs of corneal arcus, or xanthelasma. Among 526 defined FH patients, investigators reported 100 times increase in the relative risk of mortality from CHD in treated subjects aged 20-39 years.

Based on 650 men and 580 females with FH the committee proposed including tendon xanthomas in a patient or a parent, child, grandparent, sibling, uncle or aunt as a new condition (Scientific Steering Committee of the Simon Broome Register Group, 1999). The cutoff points for children under 16 were total cholesterol level above 6.7 mmol/l (260 mg/dl) and LDL-C level above 4.0 mmol/l (155 mg/dl). Updated criteria included genetic test of LDLR mutation or familial defective Apo B-100. A definite diagnosis of FH could be made by total cholesterol level, or LDL-C level plus tendon xanthomas or genetic testing (Marks, Thorogood, Neil, & Humphries, 2003). In 1999, the committee acknowledged that excessive mortality from CHD could be reduced by the use of statins.

In 2008, British National Institute for Health and Clinical Excellence (NICE) based on the Simon Broome criteria, published guidelines for screening, diagnosing and managing FH (Wierzbicki, Humphries, & Minhas, 2008; National Institute for Health and Clinical Excellence, 2008). The guidelines adopted a cascade testing with age- and gender-specific LDL-C cutoffs for diagnosing first-degree family members with FH (Starr et al., 2008). Calculated LDL-C cutoffs points were 3.11 mmol/l (120 mg/dl) for male age 0-14 years, 3.37 mmol/l (130 mg/dl) for female aged 0-14 years; 3.01 mmo/l

(116 mg/dl) for male aged 15-24 and 3.32 mmol/l (128 mg/dl) for female aged 15-24. Children older than 15 years are acknowledged to have lower LDL-C levels than younger children.

MEDPED Criteria for FH

The MEDPED Program supported by the Center for Disease Control (CDC) and led by Williams and colleagues, tracked medical pedigrees with FH in Utah (Williams et al., 1993). Based on their study of 207 people in five large FH pedigrees in Utah, Williams and colleagues proposed the MEDPED criteria in 1993 (Bild et al., 1993). The MEDPED criteria included cutoff points of total cholesterol > 270 mg/dl and LDL-C > 200 mg/dl and triglyceride < 100 mg/dl for individuals younger than 20 years old with unknown family histories. For individuals younger than 20 years of age with a known family history, the cutoff points were lower, which included total cholesterol > 220 mg/dl and LDL-C >155 mg/dl (Williams et al., 1993).

In 1985, the National Cholesterol Education Program (NCEP) NCEP was initiated by the National Heart, Lung, and Blood Institute (NHLBI). The purpose of NCEP was to reduce coronary heart disease morbidity and mortality through disseminating clinical evidence and promoting cooperative education among 20 participating organizations (Lenfant, 1986).

In 1992, the NCEP Expert Panel presented a set of criteria for children and adolescents (NCEP Expert Panel on Blood Cholesterol Levels in Children and Adolescents, 1992). Based on the Coronary Primary Prevention Trial, a series of epidemiologic, community-based studies hosted by Lipid Research Clinics (Tyrol, 1984),

the Expert Panel reported total cholesterol ≥ 200mg/dl and LDL-C ≥ 130mg/dl to be 95th percentile for children and adolescents aged 1-19 years. The Expert Panel also recommended selectively screening children and adolescents whose parents or grandparents had a history of CHD at age 55 years or younger. Lipid lowering medication might begin at age ten after an adequate diet therapy when the index case's LDL-C remained ≥ 190 mg/dl, for individuals with a family history of CHD and with LDL-C> 160 mg/dl.

In 2011, the American National Lipid Association updated its recommendations based on a panel of expert on FH (Daniels et al., 2011). Instead of targeted screening, the ANLAEP recommended universal screening at age 9 to 11 years used fasting total cholesterol and LDL-C levels or non-fasting non HDL-C levels. Screening could take place on children older than two with a known family history. A fasting lipid profile should be repeated in the case of non-fasting non HDL-C ≥ 145 mg/dl. Recommended cutoff points for individuals younger than 20 years were LDL-C ≥ 160 mg/dl or non HDL-C ≥ 190 mg/dl. Instead of using total cholesterol, the new recommendations used non HDL-C as an index for Apo-B-containing lipoprotein particles. Non HDL-C can be determined by subtracting HDL-C from total cholesterol. Scientists also acknowledged that phenotypic expressions of heterozygous FH are often influenced by environmental factors (Austin, Hutter, Zimmern, & Humphries, 2004). For individuals with a known family history, the gold standard for diagnosis is to confirm the presence of causal mutation in the low-density lipoprotein receptor gene (LDLR), or apolipoprotein B (Apo-B), or proprotein convertase subilisin/kexin type 9 (PCSK9) genes through genetic testing.

In summary, these criteria are different in the ages to define premature CHD, in cutoffs for abnormal total cholesterol and LDL-C for different age groups in defining FH index cases, and in the approaches for screening index cases. Although genetic testing is the gold standard for establishing diagnosis, the causal mutation detecting rate can be low and is not cost-effective. Additionally, there is an inconsistency in utilizing genetic tests to define index cases (Minhas, Humphries, Qureshi, & Neil, 2009). Currently, there are no universal recommendations and criteria in defining and treating children with familial hypercholesterolemia.

Issues on Diagnosing FCH

Current definitions for FCH are as controversial as those described by Fredrickson and colleagues five decades ago. In the 1960's, FCH was used to define familial lipid disorders including types IIa, IIb, IV or V based on the lipid particles presentation on the electrophoresis strips (Fredrickson, Levy, & Lees, 1967a). The characteristics included increased LDL-C alone (type IIa) or with hypertriglyceridemia (type IIb), or normal LDL-C with hypertriglyceridemia (type IV), or combined increased total cholesterol and increased triglyceride (type V). Six years later, an updated characterization of FCH was based on studies of 176 families using $\geq 95^{th}$ percentile population level for hypercholesterolemia and hypertriglyceridemia (Goldstein, Schrott, Hazzard, Bierman, & Motulsky, 1973). The diagnosis of FCH was made by finding a first-degree family member who had a different lipoprotein phenotype than the index case.

In the past decade, different FCH criteria have been proposed by American and European scientists. These criteria were based on different lipid analyzing technologies. Despite efforts to standardize lipid analyzing methods and assays, current plasma apoliprotein assays are found to have significant intra-individual, intra-familial and inter-racial variability (McNamara, Warnick, & Cooper, 2006). In 2002, the European Society for Clinical Investigation in Barcelona, the third workshop on FCH, proposed redefining FCH based on the Dutch studies (Sniderman et al., 2002). The workshop proposed a new term hyperTg hyperapoB for FCH. The proposed criteria included plasma triglyceride level > 2.0 mmol/l (77 mg/dl), and Apo-B > 125 mg/dl but excluded hypertriglyceridemia with normal total cholesterol level in the new definition.

Other popular criteria have also been suggested. The Italian Atherosclerosis and Dysmetabolic Disorders Study Group proposed including hypercholesterolemia (> 95% of population level) and/or hypertriglyceridemia (>95% of population level) in at least two members of the same family for diagnosis (Gaddi, Cicero, Odoo, Poli A, & Paoletti, 2007). A simplified monogram was proposed by Dutch scientists using cutoffs of triglycerides > 1.5 mmol/L (58 mg/dl) and Apo-B > 1200 mg/L for diagnosing FCH (Veerkamp, De Graaf, Hendriks, Demacker, & Stalenhoef, 2004).

Genetically, FCH is now believed to be a group of complex genetic lipid disorders (Suviolahti, Lilja, & Paiukanta, 2006). Genetic linkage and association studies have associated FCH with mutations in 1q21-q23, Apo-AV gene with Apo-AI/CIII/AIV cluster, 9p, 16q, 11q and gene encoding upstream transcription factor 1 (Gaddi et al., 2007). Specifically, Apo A-IV associated with low lipoprotein lipase function, chromosome 1 associated with VLDL over-production, gene cluster on chromosome 11

containing Apo-AI, Apo AVI, Apo-V, and Apo-CIII genes associated with triglyceride-rich remnants, VLDL and HDL, and Apo AV associated with hypertriglyceridemia (Wierzbicki, et al., 2008).

Issues with Blood Lipid Variations.

Cumulative studies since the 1980's have reported significant biological variability of lipid levels in children and adolescents (Freedman, Shear, Srinivasan, Webber, Berenson, 1985; Juhola et al., 2011; Porkka, Viikari, & Akerblom, 1994). In the Cardiovascular Risk in Young Finns Study, investigators found significant short-term and long term intra-individual variations among Caucasian children and adolescents aged 3-18 years. In nine years old boys, correlation coefficients of total cholesterol, LDL-C, HDL-C, triglycerides decreased from 0.9 to 0.77, 0.97 to 0.85, 0.87 to 0.63, and 0.71 to 0.49 respectively from two days to 7 day (Porkka, Viikari, & Akerblom, 1994). Male subjects aged from three to 18 years, had better correlation coefficients in lipid components than female subjects (Juhola et al., 2011). In females, correlation coefficients of total cholesterol, LDL-C, HDL-C, triglycerides varied from 0.39 to 0.57, 0.34 to 0.63, 0.41 to 0.58, 0.08 to 0.38 respectively in 3 years to 18 years. Among males, correlation coefficients of total cholesterol, LDL-C, HDL-C, triglycerides varied from 0.43 to 0.57, 0.48 to 0.61, 0.45 to 0.59, 0.13 to 0.36 respectively from three years to 18 years. In these predominately Caucasian children and adolescents, their triglycerides had most variability in both short-term and long-term follow-ups. However, in the racially mixed Bogalusa Heart Study, correlation coefficient for LDL-C was reported 0.8 in one year and 0.5 in 20 years (Freedman et al., 2010). The decrements of LDL-C were reported to be higher in > 190 mg/dl than < 70 mg/dl if taken within one year.

Lipid distribution in different genders and ethnic groups are reported with significant variations during puberty. In the Bogalusa Heart Study, the most dynamic changes of serum lipids and lipoproteins were reported in the first year of life and during sexual maturation (Berenson, Srinivasan, Cresanta, Foster, & Webber, 1981). Serum total cholesterol decreased gradually with increasing adolescent sexual maturation (between age 10 and 16 years for boys and 9-14 years for girls). Compared to white girls and black children, white boys had more decreases of HDL-C during puberty. Similar sexual maturation-related blood lipids changes were also reported by the Project HeartBeat study (Altwaijri et al., 2009). However, the ages of sexual maturation related lipid changes in the Project HeartBeat were from aged nine to 16 years in girls and from 10 to 17 years in boys (Dai et al., 2009).

In recent years, different recommendations for screening methods and treatment plans have been presented by different scientific groups in the United States and worldwide. The discrepancies can be attributed to recent discoveries of the complex genetic variants and new biochemical methodological variations. Original diagnostic criteria based on the phenotypic presentations of patients with coronary heart disease are now undergoing redefinition with the emergence of genetic information. In such a situation, continuous discordances in the scientific committees may hinder medical practitioners' care for children with familial lipid disorders.

Familial lipid disorders are treatable diseases, as demonstrated by the effective use of lipid-lowering drugs over the past thirty years (Vuprop et al., 2010). In children affected by familial lipid disorders, early detection with lifestyle interventions and timely medical treatment may prolong life without CVD. To enhance the capacity of identifying,

monitoring and managing children affected by familial lipid disorders, this research project proposes an integrative approach to assess three aspects of atherogenic processes, endothelial dysfunction, abnormal lipids levels and inflammation. By using validated measurements the relationships among these three processes can be identified. Through integrating physiological assessment of endothelial function and the biochemical assessment of abnormal lipid levels and inflammatory biomarkers, the investigator proposes testing atherosclerosis hypotheses based on endothelial dysfunction, lipid retention and inflammation. A retrospective, cross-sectional observational study will evaluate the relationship between endothelial dysfunction, inflammation and atherogenic lipid phenotype (Austin, King, Vranizan, & Krauss, 1990) in children and adolescents with FH or FCH. The use of comprehensive assessment can not only detect pre-clinical atherosclerosis for primary prevention but also monitor atherosclerosis progression for secondary prevention.

Purpose of the Study

A cross-sectional, descriptive study was designed to explore the relationship among three theories of atherosclerosis: endothelial dysfunction, response-to-inflammation and response-to-retention (lipid retention) in children with familial metabolic lipid disorders. Heterozygous FH and FCH children are born with pro-atherogenic genetic predisposition and at high risk for premature heart disease. They were hypothesized to exhibit decreased endothelial function, high levels of non HDL-C and increased inflammation. Improved understanding of the interplay among these three components may help to bring unified recommendations.

The primary aim is to investigate the correlation between atherogenic lipid profile and endothelial function, atherogenic profile and inflammation and endothelial dysfunction and inflammation in children with familial metabolic lipid disorders. The research was designated to address following questions:

Question 1. What are the levels of atherogenic lipid profile, calculated by non HDL-C, a parameter recommended by the ANLAEP 2011?

Question 2. What are levels of endothelial function as assessed by brachial flow-mediated dilation?

Question 3. What levels of inflammation are measured by hs-CRP?

Question 4. Is there an association between non HDL-C and brachial FMD?

Question 5. Is there an association between non-HDL and hs-CRP?

Question 6. Is there an association between brachial FMD and hs-CRP?

Significance

The significance of this study is to identify the correlations between lipid profile, endothelial dysfunction and inflammation. Increased and more effective detection, monitoring, and management of the progression of atherosclerosis in youth can be implemented by identifying critical components of atherogenic factors. Findings in this research project can provide more scientific evidence in forming unified recommendations in treating an international and multigenerational public health issue.

In addition, findings from this project may be generalized from children with primary metabolic lipid disorders (genetic) to children with secondary metabolic lipid disorders (without family history). Effective measures in screening and treatment will certainly benefit 20% of American teenagers who are known have abnormal lipid levels (Center for Disease Control and Prevention, 2011). To effectively address metabolic lipid disorders currently, early detection and timely intervention with theory based and clinically proven measures will result in long-lasting treatment and prevention.

CHAPTER TWO

Literature Review and Conceptual Framework

Because atherosclerosis is a complex disease none of current theory can completely describe this phenomenon. The conceptual framework of this study was built on a comprehensive approach to describe atherosclerosis with three theories: endothelial dysfunction, lipid retention and respond-to-inflammation. To illustrate this conceptual framework, each theory will be assessed by a validated measurement. Respectively, endothelial dysfunction was assessed by non-invasive ultrasound measurement of brachial artery flow-mediated dilation (FMD), lipid retention was assessed by biochemical analysis of atherogenic lipids including non HDL-C and LDL-C; and response-to- inflammation was assessed by biochemical analysis of the levels of inflammatory biomarker hs-CRP.

Children with familial hypercholesterolemia are born with an unfavorable genetic predisposition. If untreated, heterozygous carriers will have a 50% chance to develop CHD by age 50 if male or by age 60 if female (Slack, 1969). Although there is no long-term data available, statin therapy has shown short-term safety and efficacy in lowering LDL-C in children and adolescents with familial hypercholesterolemia (Arambepola, Farmer, Perera, & Neil, 2007; Avis et al., 2007). Affected children may prolong their lives if early detection, lifestyle intervention and medical treatment are implemented in time. To help unify recommendations, a theory based methodology was applied to explore the relationship among endothelial dysfunction, atherogenic lipids and response-to-inflammation and to evaluate recent recommendations set by the ANLAEP 2011.

The following literature review will provide theoretical backgrounds for endothelial dysfunction, lipid retention and response-to-inflammation and related mechanisms in the genesis of atherosclerosis (atherogenesis). Additionally, methodological issues on measuring lipids, endothelial function and inflammatory biomarker in children with familial metabolic lipid disorders will also be discussed.

Atherosclerosis as a Morphological Phenomenon

A paradigm shift from morphological development to cellular and molecular interactions becomes clear in a thematic review of the theories of atherogenesis. Repeated themes are identified among three theories of atherogenesis: "response-to-injury" (Ross, Glomset, & Harker, 1977), "response-to-retention" (Williams & Tabas, 1995), and "response-to-inflammation" (Ross, 1999). Scientific investigations have revealed a complex role of cholesterol metabolism and transport in the atherogenesis. Identified by their density and the complexes containing lipid and protein (lipoprotein), cholesterol particles have been widely studied in an effort to develop strategies to prevent and reverse atherosclerosis. Study of the vascular structure where cholesterol is transported has led to the discovery of the endothelium, and a cellular foundation for current theories of atherogenesis. The theory of endothelial dysfunction is hypothesized to link three theories in the development of atherosclerosis (Gimbrone, 1989).

Traditionally, the term *atherosclerosis* describes the morphological changes in arteries. In the 1830s, pathologist Lobstein appears to have been the first person to coin the term "arteriosclerosis" to describe hardening and thickening of the arterial wall (Duff, 1951). By the mid 1850s, two popular theories for the etiology of atherosclerosis

emerged. One was Rokitansky's atheroma theory and the other was Virchow's inflammatory theory (Duguid, 1949). Originally, Rokitansky believed that atherosclerosis started when an endogenous atheroma derived from blood fibrins began to deposit and thicken the arterial wall (Mayerl et al., 2006). Virchow opposed Rokitansky's view, believing that atherosclerosis was initiated by cellular inflammation (Methe & Weis, 2007). A thickened arterial wall was the result of an inflammatory process involving degeneration of intimal fatty tissue and proliferation of fibrous tissue. Debates between these two theories continued in the 20th century.

In the beginning of the century, Anichkov (Anitschkow in German literature) and his colleagues used Virchow's inflammatory theory to propose a lipid theory (Konstantinov, Mejevoi, & Anichkov, 2006; Steinberg, 2004). In the lipid theory, a causal association between high dietary levels of cholesterol and atherosclerosis was established and demonstrated by feeding cholesterol-enhanced diets to rabbits (Konstantinov et al., 2006). In the experimental rabbits, atherosclerotic lesions (fatty streaks) were induced. The suspicion was that due to a defect in the continuous movement of plasma lipid particles across the arterial wall from the lumen to the adventitial layer, some lipid particles interacted with the arterial walls, then precipitated, and then were ingested by lymphocytes or white blood cells, and eventually transformed into foam cells (Steinberg, 2004). These foam cells infiltrated the arterial wall under the influence of hemodynamic forces. The lesions grew in proportion to the duration of exposure and to the concentration of cholesterol in the blood. Although Anichkov was not able to demonstrate lipid theory in other animal models, the theory became an essential element of the modern response-to-injury theory (Finking & Hanke, 1997).

In the 1950's, theories about atherosclerosis were based on pathological and histological evidence. Duguid modified Rokitansky's view and proposed a thrombosis theory to explain thickening of the arterial wall (Duguid, 1949). In the thrombosis theory, atherosclerosis begins with a thrombus that occluded an artery. Under the influence of blood pressure, a channel might form in the thrombus, with the endothelium incorporating and transforming the thrombus into fibrous tissue of the arterial wall to thicken arterial wall.

Also in the 1950's, another group of scientists identified the stages of atherosclerotic progression by comparing postmortem pathological evidence with observations of the disease's progression in living human beings (Holman, McGill, Strong, & Geer, 1958). From pathological observations of cadavers acquired in different geographical regions, atherosclerosis was hypothesized to begin with fatty streaks in early life and to progress from fatty streaks to fibrous plaques and complicated lesions in 15 years (Holman et al., 1958). The fibrous plaques might revert to fatty streaks, or progress to become complicated lesions and thrombi, and eventually clinical manifestations of CHD (Strong & McGill, 1962). The progression of atherosclerosis could be reversed by controlling risk factors (Strong, McGill, Tejada, & Holman, 1958; Tejada, Gore, Strong, & McGill, 1958). Using data from the Pathological Determinants of Atherosclerosis in Youth study, investigators proposed to prevent CVD by controlling CVD risk factors (McGill, McMahan, & Gidding, 2008).

Current popular views of atherosclerosis have been based on Virchow's inflammation theory, Duguid's thrombosis theory, and Anitschkow's lipid theory. Ross extended Duguid's thinking and proposed a response-to-injury theory. In 1999, Ross

modified his theory to suggest a response-to-inflammation theory that has become the most widely accepted atherosclerosis theory. The response-to-inflammation theory was based on morphological evidence of inflammatory cells coexisting with lipids, smooth muscle cells, macrophages, and T lymphocytes in atherosclerotic plaque (Ross, 1999). Despite its popularity, some investigators argue that the response-to-inflammation theory cannot fully explain the success of statins-(lipid-lowering medications)-in reducing the prevalence of CVD (Brugts et al., 2009). Thus, the role of cholesterol in atherogenesis must be further explored.

Atherosclerosis and Cholesterol

The missing link between atherosclerosis and hyperlipidemia was made clear after the invention of the high-speed centrifuge (Moreton, 1947). By applying Stokes' law, scientists became able to separate plasma cholesterol and lipoproteins (proteins that carry lipid particles) according to their sizes and density. Two major cholesterol-carrying lipoproteins, high-density lipoprotein cholesterol (HDL-C) known as the "good" cholesterol and low-density lipoprotein cholesterol (LDL-C), "bad" cholesterol, were identified in the 1950's.

The discoveries of HDL-C, LDL-C and different kinds of apolipoproteins have revealed dynamic aspects of cholesterol metabolism and transport across the arterial wall (Field, Swell, Schools, & Treadwell, 1960). With these new discoveries, two popular views have emerged. One suggests that atherosclerosis is caused by lipid deposition in the arterial wall due to disordered lipid metabolism (Davison, 1951). The other view was

a modification of Virchow's inflammatory theory, which proposes that atherosclerosis is an inflammatory reaction caused by the formation of fatty streaks (Duguid, 1949).

Duguid's view of vascular thrombosis was based on continuous interaction between blood components and fatty streaks. On the basis of these two views, Duff (1951) further hypothesized that atherosclerosis is an arterial disease that begins with an intimal accumulation of cholesterol then extends to a medial atheroma with a necrotic center. By the 1960s, scientists agreed that atherosclerosis is an inflammatory and metabolic disease and not only the result of aging (Katz, 1962).

In the past two decades, the investigation of cholesterol metabolism and transport in relation to atherogenesis has advanced significantly. The investigation of a genetic disease called familial hypercholesterolemia led scientists to suspect an association between atherogenesis and cholesterol metabolism. The primary findings included overproduction of LDL and very low-density lipoproteins and a defect in the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a cholesterol metabolism enzyme, in cholesterol biosynthesis (Goldstein & Brown, 1973). In further studies of cholesterol metabolism, a LDL receptor that mediates endocytosis (substances taken into by a cell) was identified on the cell membrane (Brown, & Goldstein, 1979). Subsequently, the LDL receptor gene was isolated (Goldstein & Brown, 2009; Sudhof, Goldstein, Brown, & Russell, 1985). At about the same time, Japanese scientist Endo (2008) discovered statin drugs and found them very successful in lowering circulating levels of LDL. In a genome-wide study conducted in 2008, Sandhu et al. (2008) identified a locus for LDL regulation on chromosome 1p13.3 and suggested a causal role of LDL in the genesis of atherosclerosis.

Many families of apolipoproteins have been identified by monoclonal antibody assay and homologous assay (Albers, Brunzell, & Knopp, 1989; Chan & Watts, 2006). Structurally, these apolipoproteins (Apos) are protein particles similar to certain lipoproteins. Apolipoproteins including Apo A-1, Apo A-2, Apo B-48, Apo B-100, Apo C-1, Apo C-2, Apo C-3, and Apo E have been identified in the regulation of lipoproteins (Dedoussls, 2007). These apolipoprotein particles have been intensively investigated in genetically modified animal models for their role in atherogenesis. Apo A-1 (a main surface protein on HDL particles) and Apo B-100 (a main surface protein on LDL particles) are well-known biomarkers for CHD (Albers et al., 1989). Specifically, Apo A-1 is a biomarker associated with HDL while Apo B is a biomarker associated with very low-density lipoprotein, intermediate-density lipoprotein, and LDL (Chan & Watts, 2006).

Endothelium

Human endothelium is estimated to weigh between 110 grams (Pries, & Kuebler, 2006) and 1 kilogram (Sumpio, Riley, & Dardik, 2002). Despite its light weight, the endothelium is known for its complex function and heterogeneity (Aird, 2007). Endothelial cells adapt to rapid changes of the local blood flow environment indicating their ability to remodel vascular structure (Flaherty et al., 1972). This observation was supported by pathological findings of patchy distribution of fatty streaks and early atheroma at areas where turbulent flow was suspected (Caro, Fitz-Gerald, & Schroter, 1969; Karino et al., 1987). Recent evidence verifies the dynamic impact of blood flow on the morphological and physiological development of endothelial cells (Hove et al., 2003).

Endothelium is distributed throughout the body though blood vessels. Endothelial cells have different organelles and intercellular connections in relation to their location in the vascular beds (Fishman, 1982). This morphological diversity may relate to the endothelium's role in protecting end organs against hemodynamic forces, and controlling permeability for solute exchanges (Pries & Kuebler, 2006). Endothelial cells in different local environments can develop differently even when exposed to the same endothelial growth factors (Coultas, Chawengsaksophak, & Rossant, 2005). This demonstrates that endothelium is an organ capable of sensing, monitoring, commanding, modulating, and differentiating its functions in relation to local tissues.

Recent advancement in bioassays, have led to the identification of many labile endothelium-derived bioactive molecules (Vane, 2004). From studying isolated animal arteries, Furchgott and Zawadzki (1980) first discovered that endothelial cells had an obligatory role in relaxing arterial smooth muscles. Subsequently, endothelial cells were found to release prostacyclin, and nitric oxide which may dilate arteries (Palmer, Ferrige, & Moncada, 1987; Vanhoutte, 2009c). Nitric oxide can be released by endothelium and be broken down by interacting with reactive oxygen species (Gryglewski, Palmer, & Moncada, 1986; Moncada, Gryglewski, Bunting, & Vane, 1976). Nitric oxide stimulates guanylate cyclase to increase the synthesis of cyclic guanosine monophosphate (c-GMP), which then mediates vascular smooth muscle cells (VSMCs) relaxation. In addition, endothelial cells release endothelial-derived hyperpolarizing factors including hydrogen peroxide and epoxyeicosatrienoic acid to dilate resistance arteries (Parkington, Tare, & Coleman, 2008). Increased intracellular calcium probably hyperpolarize endothelial cells

via opening of calcium-activated potassium channels in the gap junction between endothelial cells and smooth muscle cells (Bellien, Thuillez, & Joannides, 2008).

To maintain vascular homeostasis, the endothelium also releases vasoconstrictors. Endothelin (Yanagisawa et al., 1988) and angiotensin II (Weiss, Sorescu, & Taylor, 2001) are two well known endothelium-derived vascular contracting factors that counteract vascular dilators. An over-expression of vascular constrictors or compromise of vascular dilators is believed to be the initial cause of the endothelial dysfunction (Vanhouttee, 2009b) that leads to atherogenesis. The identification and isolation of these bioactive molecules have led to the concept that the endothelium is an endocrine (secreting internally), exocrine (secreting outwardly), and paracrine (secreting locally) organ. Any imbalance in these secretory functions indicates endothelial dysfunction and is a sign of endothelial injury.

Endothelium and Atherosclerosis

Vascular endothelium has been intensively investigated being an important factor in atherogenesis because of its strategic position at the interface of the circulating blood and surrounding tissues. Recent animal observations showed caveolae on endothelial cell membranes to have numerous receptors for LDL, HDL, albumin, and interleukin 1 (IL-1), an immuno-stimulant. These caveolae receptors may provide a different pathway for molecular transport and play a significant role in endothelial transcytosis (molecules moving from blood vessel lumen to subendothelial space), endocytosis, and intracellular signal transduction and atherosclerosis (Frank & Lisanti, 2004; Frank, Pavlides, & Lisanti, 2009). Caveolae and their protein marker, caveolin-1, have exhibited a

proatherogenic role by facilitating transcytosis of LDL from the blood to the arterial intima that indicate their involvement in the formation of fatty streaks (Frank et al., 2009; Frank & Lisanti, 2004). However, caveolae in the VSMCs exhibit an anti-atherogenic role by inhibiting the cell migration, and signaling a pathway mediated by tumor necrosis factor α that retarded neointimal growth (Frank & Lisanti, 2004). From these observations, caveolae located in different vascular cells may be assumed to play opposing roles in the formation of atherosclerosis.

Endothelial cells have Weibel and Palade Bodies (WPBs) that store Willebrand factors (VWFs) and other bioactive factors (Metcalf, Nightingale, Zenner, Lui-Roberts, & Cutler, 2008; Wagner, Olmsted, & Marder, 1982). Endothelial cells release components of WPBs in response to inflammation, to maintain hemostasis, and to modulate vascular tonicity and angiogenesis (Metcalf et al., 2008). In endothelial cells, VWFs can be released spontaneously or in response to stimuli such as vascular injury and inflammation (Giblin, Hewlett, & Hannah, 2008). In an occluded artery, VWFs become active vascular ligands that trigger platelets rolling to the injury site. Their causative role in CHD has been noted in the Atherosclerosis Risk in Communities Study, in which elevated plasma levels of VWFs were found in patients with acute myocardial infarction (Folsom, Wu, Rosamond, Sharrett, & Chambless, 1997).

In addition to VWFs, some WPB components have been associated with inflammation. Specifically, E-selectin, an inflammatory adhesion molecule is released by endothelium (Woollard & Chin-Dusting, 2007). In addition, IL-8 (Wolff, Burns, Middleton, & Rot, 1998), eotaxin-3 (Oynebraten, Bakke, Brandtzaeg, Johansen, & Haraldsen, 2004), endothelin-1 (Ozaka, Doi, Kayashima, & Fujimoto, 1997), and

angiopoietin (Fiedler et al., 2004) are reportedly released by WPBs as inflammatory mediators. IL-8, in particular, rapidly attracts neutrophils to inflammatory sites (Oynebraten et al., 2004).

Other factors can also contribute to the initiation of atherosclerosis. For example, CD63 is a glycoprotein and scavenger receptor on platelets, monocytes, macrophages, endothelial cells, and smooth muscle cells (Vischer & Wagner, 1993). It is reported that CD63 can interact with oxidized LDL and help to trap macrophages in the endothelium (Collot-Teixeira, Martin, McDermott-Roe, Poston, & McGregor, 2007; Park, Febbraio, & Silverstein, 2009). Also, CD63 can interact with P-selectin and integrins (receptors on leukocytes) to attract leukocytes and enhance leukocyte adhesion (Harrison-Lavoie et al, 2006; Rondaij et al., 2007**)**. P-selectin can also be released by platelets and WPBs to induce leukocyte rolling, a key process in inflammation (Dole et al., 2005).

Endothelium and Vascular Tone

The endothelium regulates the exchange of molecules, maintains vascular tone, prevents inflammation, promotes fibrinolysis, and prevents coagulation. Endothelial injury (Ross et al., 1977) that leads to impaired endothelial permeability and oxidized LDL retention (Williams, & Tabas, 1995) has been associated with atherogenesis. An increased transcytosis and deregulated lipoprotein transport probably leads to retention of lipoproteins (Sima, Stancu, & Simionescu, 2009). Modification of trapped lipoproteins also triggers endothelial dysfunction and initiates atherogenesis.

To control vascular tone, the endothelium relies on locally produced vasodilators and vasoconstrictors for adequate tissue perfusion, buffer hemodynamic forces, and

systemic blood pressure maintenance. Three endothelium-mediated vasodilatation pathways have been proposed; they are mediated through nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors (Bryan, You, Golding, & Marrelli, 2005). In isolated animal arterial rings, a sudden increase in the flow rate of fluid was identified as an activating factor for an intact endothelium to release nitric oxide, a potent vasodilator (Rubanyi, Romero, & Vanhoutte, 1986). This original experiment demonstrated an endothelium-dependent mechanism in flow-mediated arterial dilatation. Prostacyclin is released by endothelial cyclooxygenase-2 (COX), has a very short biological half-life, and is another known potent vasodilator and anticoagulant (Arehart et al., 2008). Hydrogen peroxide and epoxyeicosatrienoic acids are endothelium-derived hyperpolarizing factors that have a persistent effect on dilating VSMC even when the effects of nitric oxide and prostacyclin are suppressed (Feletou & Vanhoutte, 1996). It is believed that endothelium-derived hyperpolarizing factors play an important role in the vasodilatation of resistance vessels (Parkington et al., 2008).

To counterbalance vasodilitation, the endothelium releases vasoconstrictors, such as endothelium-derived contracting factors (EDCFs), angiotensin II (Weiss et al., 2001), and endothelin (Yanagisawa et al., 1988). The EDCFs are named because their production is thought to be inhibited by nitric oxide and endothelium-derived hyperpolarizing factors (Vanhoutte & Tang, 2008). Recent evidence indicates that endothelium-derived vasoconstrictor prostanoids such as prostaglandin endoperoxides produced by cyclooxygenase are responsible for endothelial dysfunction in aging persons and those with essential hypertension (Vanhoutte, 2009a; Vanhoutte, 2009b; Vanhoutte & Tang, 2008).

Endothelins are known as the most potent and long-lasting endothelial vasoconstrictors (Barton & Yanagisawa, 2008). Endothelins exert vasoconstriction via a receptor-mediated mechanism. Angiotensin II is a peptide belonging to the renin-angiotensin system that can be released via multiple pathways such as vascular endothelial cells, cardiac muscles, and circulating plasma (Weiss et al., 2001). Recent evidence indicates that angiotensin II works with specific cell receptors to mediate the regulation of blood pressure (Schmieder, Hilgers, Schlaich, & Schmidt, 2007). In human hypertension, any increase in reactive oxygen species that leads to an over expression of angiotensin is associated with atherogenesis.

Nitric oxide and atherosclerosis. Although nitric oxide has been identified as an endothelium-derived relaxing factor for two decades, its biological pathway in the vascular system is still not well understood. Nitric oxide is known to have diverse roles in the cardiovascular system, including regulating vascular tone, neurotransmission, immune response, nutrition metabolism, and homeostasis (Jobgen, Jobgen, Li, Meininger, & Wu, 2007; Lowenstein, 2007). Nitric oxide is a free radical and signaling molecule with a biological half-life of a few seconds (Stamler, Singel, & Loscalzo, 1992).

In endothelial cells, endogenous nitric oxide can be derived from enzymatic and non-enzymatic pathways in the vascular system (Chen, Pittman, & Popel, 2008). The enzymatic production of nitric oxide is mediated by nitric oxide synthesis (NOS). The regulation of endothelial NOS is hypothesized through protein-protein interaction (enzymatic) and phosphorylation (non-enzymatic addition of phosphate) at different sites in endothelial cells (Fulton et al., 2008). In an enzymatic pathway, nitric oxide is

produced by degrading L-arginine to L-citrulline via endothelial NOS (Palmer et al., 1988), mediated by cofactors tetrahydro-L-biopterin and nicotinamide adenine dinucleotide phosphate (NADPH) (Forstermann & Munzel, 2006). In a non-enzymatic receptor-mediated pathway, nitric oxide production can be induced by agonists including acetylcholine, substance P, and bradykinin (Vanhoutte, 2009c). These neurotransmitters can induce an increase in intracellular calcium that enhances calcium binding to calmodulin (a calcium protein) and enhances production of nitric oxide (Heiss et al., 2006).

Nitric oxide can be broken down by the superoxide anion and preserved by superoxide dismutase and copper (Gryglewski et al., 1986). In humans, nitric oxide derived from endothelial cells is very unstable. It is rapidly oxidized to nitrite and nitrate and excreted in the urine (Helmke & Duncan, 2007; Jobgen et al., 2007). Nitrite and nitrate produce superoxides, that fuel the oxidative stress associated with atherosclerotic progression (Schulz, Jansen, Wenzel, Daiber, & Munzel, 2008). However, some clinical observations indicate that nitric oxide could be converted from nitrite during cardiac ischemia and infarction, exerting a vasodilatation effect (Gonzalez et al., 2008; Hendgen-Cotta et al., 2008). These findings suggest that nitrite may be a source of nitric oxide which can exert cardiac protection. This controversial role of nitrite in maintaining vascular health needs further validation (Grau et al., 2007).

Under normal physiological conditions, nitric oxide in low concentrations regulates blood pressure and maintains peripheral vascular tone (Vallance, Collier, Moncada, 1989) through direct activation of calcium-dependent potassium channels (Bolotina, Najibi, Palacino, Pagano, & Cohen, 1994). However, endothelial NOS may

coexist with the expression of inducible NOS in the atherosclerotic plaque. The excessive amounts of nitric oxide generated by endothelial and inducible NOS can interact with oxygen-derived radicals and produce the potent cytotoxic oxidant peroxynitrite (Herman & Moncada, 2005). Thus, the bioavailability of, not the concentration of, nitric oxide might play a key role in maintaining vascular health.

Four mechanisms have been proposed as causes of decreased bioavailability of nitric oxide, which can adversely affect vascular health and promote atherogenesis: decreased expression of NOS, uncoupling of endothelial NOS, enhanced breakdown of nitric oxide, and impaired transmission of signaling activities mediated by nitric oxide (Braam & Verhaar, 2007). Each of these mechanisms has become a specific target area for research and therapeutic intervention. Clinically, endothelium-derived nitric oxide's effect on arterial reactivity has been verified as the source of flow-mediated dilatation in the human brachial artery (Vallance et al., 1989).

Other endothelial vasodilators. In the past 10 years, the important role of endothelium-derived hyperpolarizing factors (EDHFs) in the regulation of vascular tone has been acknowledged. The factors are initially differentiated into endothelium-derived factors, neither nitric oxide nor prostanoids (Luksha et al., 2009; Moncada, 2006; Parkington et al., 2008). Currently, EDHFs are considered vasodilator substances with a different mechanism of endothelial control of vascular tone (Bryan et al., 2005). EDHFs appear to facilitate electrical coupling between endothelial cells and VSMCs through calcium-activated potassium channels (Triggle & Ding, 2002). This coupling presumably takes place in gap junctions connecting VSMCs and endothelial cells (Bellien et al., 2008; De Wit & Wolfle, 2007). In addition, EDHFs are associated with the control of

myogenic tone in both conduit arteries and resistance arteries (Bellien, et al., 2008). As such, EDHFs may play an important compensatory role in atherosclerotic arteries, where nitric oxide and prostacyclin vasodilitation actions are compromised.

Endothelial vasoconstrictors. The identification of endothelium-dependent vascular contracting factors has been controversial. Scientists have not yet reached consensus on which molecular compounds constitute endothelium-dependent vascular contracting factors. Some believe that vasoconstrictor prostanoids are endothelium-dependent vascular contracting factors produced by endothelial COX under certain pathological conditions (Hirao, et al., 2008; Vanhoutte & Tang, 2008). In animal models, an increase of calcium concentration in the endothelium (Tang et al., 2007) and increased levels of oxygen-derived free radicals (Gollasch, 2008; Shi, So, Man, & Vanhoutte, 2007) can stimulate COX to transform arachidonic acid into prostaglandin endoperoxides (Miller & Vanhoutte, 1985; Vanhoutte & Tang, 2008). Then, these endoperoxides can be converted into prostacyclin and thromboxane A_2. Under normal physiological conditions, prostacyclin (a vasodilator) is produced predominantly by the endothelial COX-2 and acts as an antagonist to thromboxane A_2, a vasoconstrictor predominantly produced by COX-1 in platelets (Vane, 2002). A counterbalance effect between prostacyclin and thromboxane A_2 (Cheng et al., 2002; Gryglewski, 2008) may result. As prostacyclin exerts its protective function by relaxing the vascular smooth muscle, thromboxane A_2 stimulates thromboxane-prostanoid receptors in the vascular smooth muscle to initiate vasoconstriction. A decrease in the bioactivity of nitric oxide can lead to an over expression of thromboxane A_2 which is believed to create a proinflammatory environment.

In the past 20 years, endothelins have been identified as one of the most potent and long-lasting vasoconstrictors (Haynes, & Webb, 1994; Yanagisawa et al., 1988). Endothelins are the focus of more than 20,000 scientific publications (Barton & Yanagisawa, 2008). Endothelins are not only potent vasoconstrictors but also cytokine-like peptides which play an important role in cellular inflammation (Barton & Yanagisawa, 2008). Endothelins work as autacoids and paracrine hormones to exert physiological function, including neurotransmission and the development of neural crest cells.

Human endothelins are categorized as three types of peptides each with 21 amino acids: endothelin-1, endothelin-2, and endothelin-3. In humans, endothelial cells can synthesize only endothelin-1. Endothelin-1 is the principal isoform found in both circulating human plasma (Lerman et al., 1990) and WPBs of vascular endothelial cells (Ozaka, et al., 1997). It is released continuously from endothelial cells by the stimulation of extracellular calcium (Yanagisawa et al., 1988) through endothelin-converting enzyme. Endothelin-1 works on the receptors on the endothelial cells and VSMCs to maintain endogenous vascular tone (Davenport & Maguire, 2006; Haynes & Webb, 1994). It is also released from WPBs upon external stimulation, including chemical and mechanical stimulations (Russell, Skepper, & Davenport, 1998).

Pathological over production of endothelin is apparently contributing factors to the genesis of atherosclerosis. In the atherosclerotic human coronary artery, an increase of endothelin can lead to an enhancement of VSMC proliferation and induction of extracellular matrix formation in both VSMCs and endothelial cells (Gossl & Lerman, 2006; Lerman et al., 1990, Lerman et al., 1991; Kinlay et al., 2001). Endothelin has been

postulated to activate nuclear factor kappa β, a key transcriptional factor in the inflammation cascade in human macrophages. This process will facilitate the development of atherosclerosis (Gossl & Lerman, 2006; Wilson, Simari, & Lerman, 2001). Experimental and clinical studies suggest a possible counterbalancing relationship between endothelin-1 and nitric oxide (Gossl & Lerman, 2006; Iglarz & Clozel, 2007). Eendothelin-1 may decrease production of nitric oxide by up regulating the expression of caveolin-1, a negative regulatory protein of endothelial NOS, which inhibits NOS activity (Ramzy et al., 2006). Or endothelin-1 may increase vascular production of reactive oxygen species, leading to an increase in degradation of nitric oxide and promotion of atherogenesis (Amiri et al., 2004). In summary, the atherogenic role of endothelin-1 may include abnormal vasoconstriction, the uncontrolled growth of VSMCs, and decreased bioavailability of nitric oxide.

Healthy endothelium maintains hemostasis by inhibiting cellular interaction with platelets, preventing coagulation, and controlling fibrinolysis (Arnout, Hoylaerts, & Lijnen, 2006; Becker, Heindl, Kupatt, & Zahler, 2000). Under physiological conditions, white blood cells, platelets, and red blood cells do not adhere to the endothelium or migrate into the local tissue. However, during endothelial injury, shear forces induced by disturbed blood flow introduce endothelial tissue factors (e.g., VWF) to blood causing those factors to become adhesion molecules that activate platelet aggregation (Arnout et al., 2006). The interaction between subendothelial factors, VWF, and platelets ultimately triggers coagulation cascade. Thrombin is generated and fibrinogen is converted into a fibrin network. The resulting blood clots then occlude the injured vasculature (Dahlback, 2000). After hemostasis is achieved, the fibrinolysis system is activated to undo the

coagulation cascade. That system converts plasminogen into the active plasmin through tissue-type or urokinase-type plasminogen activators. Then, plasmin digests the fibrin and removes the blood clot (Arnout, et al., 2006) to complete the healing process.

As previously discussed, endothelial control of platelets can be exemplified by the release of vasoactive factors such as nitric oxide. Through the c-GMP pathway, nitric oxide can inhibit platelet adhesion, activation, secretion, and aggregation (Radomski, Palmer, & Moncada, 1987; Rajendran & Chirkov, 2008). Endothelial anticoagulation properties can be demonstrated by releasing prostacyclin to counteract thromboxane A_2 (Gryglewski, 2008). To control fibrinolysis, endothelium-released plasminogen activator inhibitor might inhibit plasminogen activation (Fay, Garg, & Sunkar, 2007).

Theoretical Framework

Response to Injury Theory

The impact of blood flow on vascular endothelium has long been suspected in the genesis of atherosclerosis. Pathological evidence of disorganized endothelial nuclear patterns and unique patchy distribution of atherosclerotic lesions in the area of disturbed flow has led scientists to associate the formation of atherosclerosis with fluid shear stress (Caro et al., 1969; Flaherty et al., 1972; Glagov et al., 1988). Early morphological observations left scientists unsure about the impact of fluid shear stress they hypothesized that fatty streaks were located in areas of either high or low shear stress (Caro, 2009). In 1966, Fox and Hugh first hypothesized a boundary layer in the circulatory system where blood flow of very low momentum could allow platelets to interact with fibrin and

trapped lipid particles and produce fatty streaks. This boundary layer is now referred to as endothelium.

In the 1970s, Ross and colleagues proposed their response-to-injury theory, which modified Virchow's inflammation theory of atherogenesis (Ross et al., 1977). In the response-to-injury theory, high shear stress was hypothesized to be the cause of endothelial cell damage, cell loss, and detachment. Damaged endothelium would then expose the underlying collagen to platelets. Activated platelets would become adhesive, aggregate, and release factors that induce focal intimal proliferation of smooth muscle cells. This proliferation would be accompanied by formation of a connective tissue matrix from collagen, elastic fiber, and proteoglycans. Intracellular protein matrix would be formed and extracellular lipid would be deposited. Ross and colleagues hypothesized that atherosclerosis was the result of the endothelial cells' response to injury.

Scientists were also debating the causes of endothelial injury that induced atherogenesis. In animal models, atherosclerotic lesions in a healthy monkey induced by a mechanically removed endothelium resembled the atherosclerotic lesions in a monkey with hypercholesterolemia (Ross & Harker, 1976). However, in other animals the same results led to identification of shear stress on the arterial wall as a key source of injury that induces atherosclerosis (Caro, Fitz-Gerald, & Schroter, 1971). Scientists debating blood flow pulsation versus shear stress of the blood flow injured the endothelium and caused atherosclerotic lesions (Friedman, O'Brien, & Ehrlich, 1975). Then, Gimbrone and colleagues proposed an endothelial dysfunction theory which posited endothelial dysfunction as the cause of atherogenesis (Gimbrone, Nagel, & Topper, 1997).

Endothelial Dysfunction Theory

In 1989, Dr. Gimbrone first described the concept of endothelial dysfunction. He hypothesized that atherosclerosis was a mal-adaptation of the endothelium to blood components (Gimbrone, 1989). The endothelium performs multiple functions: containing blood; controlling vascular permeability; synthesizing and secreting hormones; monitoring, integrating, and transmitting blood-borne signals; regulating vascular tone and growth; responding to inflammation; and balancing haemostatic and thrombotic activities. Pathological derangement could take place even if anatomic integrity was preserved. A localized thrombotic event could take place whenever endothelial signal transduction is triggered by an imbalance of proinflammatory and anti-inflammatory or procoagulant and anticoagulant mechanisms. Consequently, atherosclerosis would be the manifestation (phenotype) of endothelial remodeling, the result of maladaptive interaction between genetic constitutions (genotype) and environmental risk factors (Gimbrone, 1989).

The concept of endothelial dysfunction as a key part of the pathogenesis of atherosclerosis has been widely accepted and tested clinically and experimentally over the past two decades. Based on the early observations of a transient increase in blood flow after an artery was occluded (reactive hyperemic response), scientists were able to quantify the correlation between the period of occlusion and the magnitude of augmentation of reactive hyperemia in isolated dog hearts and human forearms (Katz & Lindner, 1939). As a mechanotransducer, the endothelium is believed capable of sensing, transmitting, and responding to hemodynamic forces (Davies, 1995). Flow-mediated dilatation is a concept based on the hypothesis that vascular growth and adaptation are

mediated by biophysical, biochemical, and genetic interactions between endothelium and hemodynamic shear stresses.

Since the 1980s, scientists have begun using noninvasive ultrasound devices to document endothelium-dependent vasodilatation in humans and animals (Angus, Campbell, Cocks, & Manderson, 1983; Safar, Peronneau, Levenson, Toto-Moukouo, & Simon, 1981). Paradoxical vasoconstriction induced by intracoronary infusion of acetylcholine has been documented in human atherosclerotic coronary arteries by quantitative angiography (Ludmer et al., 1986). Further investigation revealed that both atherosclerotic and nonatherosclerotic coronary arteries were dilated in the presence of the endothelium-independent vasodilator nitroglycerin, but vasoconstriction in response to acetylcholine was found only in arteries with atherosclerotic lesions. Together, these reports indicate that endothelial dysfunction is responsible for compromised flow-mediated dilatation in atherosclerotic coronary arteries (Cox et al., 1989; Zeiher, Drexler, Wollshlager, & Just, 1991).

In recent years, endothelial dysfunction due to impaired bioactivity of endothelium-derived nitric oxide has been regarded as an early event in atherosclerosis (Thomas, Witting, & Drummond, 2008; Munzel, Sinning, Post, Warnholtz, & Schulz, 2008).

Response to Retention Theory

The response-to-retention theory addresses a different aspect of atherogenesis (Williams & Tabas, 1995), linking Anichkov's lipid hypothesis to atherosclerosis (Williams & Tabas, 1998). This theory suggests that- atherosclerosis begins with the

accumulation of lipoproteins among subendothelial extracellular proteoglycans (Williams, & Tabas, 1998). Normally, LDL-C and lipoproteins are thought to be transported between the blood and endothelium, often accumulating in arterial sites that susceptible to atherosclerosis (Williams & Tabas, 1995). Shear stress on the arterial wall contributes to the stimulation of intramural synthesis of subendothelial extracellular proteoglycans. In addition to proteoglycans, lipoprotein lipase and smooth muscle sphingomyelinase can interact with lipoproteins and promote the retention of lipid particles (Gustafsson et al., 2007).

Proteoglycan-LDL-C complexes have an increased affinity for the arterial wall and are prone to oxidation. Oxidized LDL-C acting as a pathogen induces the endothelium and VSMCs to activate monocyte migration (Cushing et al., 1990). Proatherosclerotic endothelial dysfunction can be activated both by the synergy of shear stress and oxidative lipoproteins (Camejo, Hurt-Camejo, Olsson, & Bondiers, 1993) and by interactions between proteoglycans and macrophages (Gustafsson & Boren, 2004; Nakashima, Wight, & Sueishi, 2008).

Data gathered from genetically modified animals and human clinical trials have provided additional support for the response-to-retention theory. In transgenic mice, atherosclerosis was induced and verified by direct observation of subendothelial (intimal) retention of lipoproteins (Gustafsson et al., 2007; Skalen et al., 2002). In the extracellular matrix of endothelial cells, proteoglycans containing negatively charged sulfate will interact with LDL-C. Modified LDL-C that contains Apo B has higher affinity and tends to be trapped in the arterial wall (Proctor, Vine, & Mamo, 2002). Recent discovery of novel bridging molecules such as lipoprotein lipase, sphingomyelinase, and

phospholipase A_2 at the binding sites of proteoglycans and Apolipoprotein B demonstrate that both the concentrations and interactions of these molecules were determinants of lipoprotein retention (Gustafsson & Boren, 2004; Tabas, Williams, & Boren, 2007).

Histological observations in humans performed by international researchers offer further support for the response-to-retention theory (Dalager, Paaske, Kristensen, Laurberg, & Falk, 2007; Nakashima, Fuji, Sumiyoshi, Wight, & Sueishi, 2007). The distinct distribution of atherosclerotic lesions in coronary arteries, carotid arteries, and femoral arteries, demonstrate the systematic nature of atherosclerosis (Dalager et al., 2007). A high prevalence of foam cell lesions and lipid core plaques was found in the coronary and carotid arteries of subjects who died of coronary artery disease. It is believed that the unique morphological expression of these foam cells and lipid cores is associated with a higher risk of death. Scientists associated the increased intima-media thickness in the carotid artery at the site of foam cell lesions with the impact of hemodynamic shear stress. Thickened intimal tissues are observed with fatty streaks co-localized with macrophages, smooth muscle cells, elastin, proteoglycans, and lipoproteins. This presentation indicates that extracellular accumulation of lipid and proteoglycans may have occurred before the infiltration of macrophages. Thus, scientists hypothesized that accumulations of lipid and proteoglycans are independent events that may trigger atherosclerosis (Nakashima et al., 2007; Nakashima et al., 2008). However, such claims do not explain why acute coronary syndrome and stroke occur in subjects with normal lipid profiles.

Response to Inflammation Theory

In 1999, Ross modified his response-to-injury hypothesis, suggesting a response-to-inflammation mechanism as the cause of atherosclerosis. The assumption is that low-grade atherosclerotic inflammatory lesions may be present throughout a person's lifetime. This type of lesion may consist of fatty streaks, macrophages, and T lymphocytes. Complex interactions between environmental and genetic risk factors may threaten endothelial function. Endothelial injury may then lead to endothelial dysfunction, with the endothelium becoming more permeable, proinflammatory, and procoagulant. The initiation of the inflammatory cascade stimulates migration and proliferation of VSMCs and aggregation of platelets. To compensate for a thickened arterial wall, the vascular tree remodels itself and becomes dilated. During continuous inflammation, cytokines, chemokines, and growth factors promote leukocyte migration to the affected sites enhancing migration and proliferation of VSMCs. This leads to the formation of advanced lesions with lipid and necrotic cores covered by fibrous caps. When arterial dilatation can no longer accommodate the increased wall thickening, these advanced complicated lesions protrude into the vessel lumen. The protrusion of these complicated lesions changes the local blood flow profile and further threatens the stability of the lesions. Any rupture of these complicated lesions will lead to acute coronary syndrome.

Over the past two decades, relying on new monoclonal antibody assays and genetically modified animal models, scientists have collected immunological evidence about atherogenesis (Nilsson & Hansson, 2008; Taleb, Tedgui, & Mallat, 2008; Van Den Elzen et al., 2008). The use of monoclonal antibodies to identify specific molecules on leukocytes, the cluster of differentiation (CD) protocol, has empowered scientists to

identify many subpopulations of the T lymphocyte family in human atherosclerotic plaque (Hansson & Jonasson, 2009).

In addition, the role of the major histocompatibility complex system in atherogenesis has been well documented. Of note, three classes of molecules in the major histocompatibility complex system, also called the human leukocyte antigen system, have been identified in human chromosome 6 (Muller & Young, 2001). Molecules in the human leukocyte antigen class I (A, B, C, D, E, F, and G) exist on all cells and serve to recruit cytotoxic T cells (endogenous antigen). Molecules in the human leukocyte antigen class II (DP, DR, DQ) exist on B cells and macrophages and serve to signal helper T cells to present exogenous antigens. Human leukocyte antigen class III molecules are proteins with immunological functions; examples include tumor necrosis factor and heat shock proteins. All three classes of human leukocyte antigen molecules have been implicated in atherogenesis.

The initiation of atherosclerosis can also be seen as an endothelial response to inflammation that is triggered by an accumulation of oxidized lipid particles. The progression of atherosclerosis is due to the vascular system's failure to balance between proinflammatory and anti-inflammatory processes. Risk factors such as obesity, hypertension, and diabetes trigger the resulting uncontrolled inflammation. Once triggered, the inflammatory cascade is characterized by leukocytes adhering to, rolling to, and migrating through the endothelial cells. These complex processes are facilitated by factors such as intercellular adhesion molecules, chemokine-triggered leukocyte integrins, and selectins (Bobryshev, 2006; Rao, Yang, Garcia-Cardena, & Luscinskas, 2007). These factors are released by endothelial cells (E-selectin), leukocytes (L-

selectin), platelets (P-selectin), T cells, and VSMCs, which have been identified in atherosclerotic plaque. Inflammation is therefore strongly indicated as an important contributing cause of atherogenesis.

The genesis and progression of atherosclerosis can theoretically be interpreted as the loss of balance between opposing mechanisms: proatherogenic versus anti-atherogenic, proinflammatory versus anti-inflammatory, pro-fibrinolysis versus anti-fibrinolysis, pro-coagulation versus anticoagulation, pro-oxidation versus anti-oxidation, and uncontrolled cell proliferation versus premature apoptosis of endothelial cells and arterial smooth muscle cells. To further illustrate the conceptual framework of this research project the in following discussion we describe the most recent evidence that supports the three theories and their application to children at risk for CVD.

Endothelial Function in Healthy Children

In 1992, Celermajer and colleagues presented a non-invasive ultrasound assessment of brachial artery hyperemic reactivity as an index of endothelial function (Celermajer et al., 1992). Based on the assumption that endothelial dysfunction is an early event of atherogenesis, children and adults at risk of atherosclerosis were found to have significant lower FMD. Ten children aged 8-16 years with familial hypercholesterolemia had a femoral artery FMD 0 ± 1% (mean ± standard deviation) with range 2 – 6%. At this age, in gender matched healthy children, the femoral FMD is 9 ± 1% with a range of 2-12%. The same method was employed to demonstrate a significantly lower brachial artery FMD in 20 adult cigarette smokers. This landmark experiment has demonstrated the utility of using non-invasive ultrasound assessment of

arterial hyperemic reactivity as an index for endothelial function in asymptomatic children and adults at risk for CVD.

To demonstrate the accuracy and reproducibility of brachial FMD, a standardized imaging protocol with lower arm occlusion was conducted (Sorensen et al., 1995). Forty healthy adults aged 22-51 were scanned with intervals between scans of 1-2 day, 1-2 weeks, and 2-4 months. Compared with the phantom scan, the mean artery diameter error was 0.04 millimeter and overall coefficient of variation for FMD was 1.8%. The FMD was calculated as [peak diameter – (mean baseline diameter at pre-hyperemic and pre-nitroglycerin)] / (mean baseline diameter at pre-hyperemic and pre-nitroglycerin) X 100%. The coefficient of variation was obtained by a non-traditional method, which was using nested 240 measurements to calculate standard deviation of the mean differences between scans divided by the overall mean flow mediated dilation and expressed as a percentage.

For the past two decades, non-invasive ultrasound assessment of arterial hyperemic reactivity has been used to test endothelium-dependent dilation in the femoral artery radial artery and brachial artery. Other, study protocol variations include distal (forearm) occlusion versus proximal (upper arm) occlusion, cuff inflation time for three to five minutes, reporting FMD using area under the dilation response curve or time curve between 30 and 180 seconds after cuff deflation. These have resulted inconsistent FMD even in healthy population. In order to identify a normal FMD for children, systematic review of published clinical trials with standardized study protocols (Corretti eta l., 2002) in healthy children will be presented. A table of brachial FMD assessed in healthy children in different ages is listed in the appendix (Table 69).

In the Avon Longitudinal Study of Parents and Children (ALSPAC), healthy 10 year olds had compromised endothelial function even with mild infection (Charakida et al., 2010). Children with acute infection had significantly lower brachial FMD (6.3 ± 2.7%), compared to the healthy control group (9.7 ± 2.5%) and 90% of children would improve their brachial FMD once they recover from the infection. The impairment of brachial FMD during infection was associated with innate immunity genetic predisposition (Charakida et al., 2010). Such significant correlation between genetic heritability and brachial FMD was demonstrated in monozygotic but not in dizygotic twins (Hopkins, & Stratton et al., 2010). The heritability of brachial FMD was estimated at 0.44 in monozygotic.

Vascular endothelial function assessed by brachial FMD demonstrated correlation with physical activity in children aged 5-10 years (Abbott, Harkness, & Davies, 2002), in 10-11 years (Hopkins et al., 2009) and 13 years (Pahkala et al., 2008). Increased intensity of habitual physical activity was associated with higher brachial FMD in all three age groups. Seasonal changes in physical activity were also correlated with the changes of brachial FMD in 10 years old children (Hopkins et al., 2011). Higher intensity physical activity in summer resulted in higher brachial FMD (10.0 ± 4.3%) versus lower brachial FMD (7.9 ± 3.9%) with lower intensity activity in autumn. However, there was a weak correlation between brachial FMD and body composition in children aged 9-10 years (Hopkins, & Green et al., 2010). There was also a weak correlation between low birth weight and lower brachial FMD in children 9-11 years old compared to normal birth weight children (Leeson et al., 1997).

In the Special Turku Coronary Risk Factor Intervention Project for children (STRIP), asymptomatic boys demonstrated higher brachial FMD (9.62 ± 3.53 %) among boys who had been in dietary intervention groups since infancy than boys (FMD 6.36 ± 3.85 %) who had not been in the interventional group (Raitakari et al., 2005). However, there was no significant difference of brachial FMD between girls with dietary intervention and girls without. The contributing factor for improved brachial FMD was lower total cholesterol. Also, there was no significant difference of total cholesterol between girls with and without dietary intervention. This result indicated a gender difference in children's response to dietary interventions.

In the same study, children exposed to tobacco smoke had decreased brachial FMD, which was confirmed by higher serum cotinine levels (Kallio et al., 2007). Despite a high serum cotinine level and a decreased brachial FMD, children exposed to tobacco smoking did not have an increased hs-CRP. Interestingly, hs-CRP demonstrated an inverse relationship with brachial FMD in healthy 11 years old children (Jarvisalo et al., 2002). Investigators suggested that an increased hs-CRP might attenuate endothelial function and promote atherogenesis. However, it was not evidenced in this study.

Endothelial Dysfunction in Children with Familial Hyperlipidemia

As discussed earlier, brachial FMD was found to be significantly decreased in children with familial hypercholesterolemia (Aggoun et al., 2000; Celermajer et al., 1992). As a first-line intervention, many randomized clinical trials have been conducted in the past two decades. Endothelial function was improved using antioxidant vitamin therapy (Mietus-Synder, & Malloy, 1998), vitamin C and E treatment (Engler et al.,

2003), and docosahexaenoic acid treatment (Engler et al., 2004). The use of plant stanols significantly decreased LDL-C but did not improve brachial FMD (Jakulj et al., 2006).

Two randomized clinical trials on statin therapy demonstrated strong lipid lowering effects with improved endothelial function (de Jongh et al., 2002; Ferreira et al., 2007). In a group of children and adolescents aged 9-18 with FH, simvastatin treatment for 28 weeks improved absolute brachial FMD 3.9 ± 4.3% and restored it to 15.6 ± 5.4 %, a level comparable to healthy control (de Jongh et al., 2002). The improvement of brachial FMD was inversely correlated to changes of total cholesterol ($r= -0.31$, $p< 0.05$) and LDL-C ($r= -0.31$, $p< 0.05$). Similar result was demonstrated in 18 Brazil children and adolescents with FH aged 6-18 years (Ferreira et al., 2007).

Despite such a significant effect on lowering lipids, some interventions fail to improve endothelial function. Studying the impact of inflammation on endothelial function in children with familial metabolic lipid disorders is also inconclusive. Although current guidelines recommend the use of statins beginning at eight years old for children with familial hyperlipidemia, no long-term clinical trial has proven its safety. To address these concerns, a comprehensive approach to monitor vascular function can be helpful.

Assumptions

Based on the afore mentioned atherosclerosis theory, the following assumptions were made. First, endothelial function as an early event of atherosclerosis might be present in children with familial hyperlipidemia. Secondly, hyperlipidemia was hypothesized a possible cause of inflammation and children with familial hyperlipidemia might have ongoing inflammation. Third, increased inflammation was assumed to be

associated with worsening endothelial function. Children with familial hyperlipidemia might have abnormal endothelial function. Fourth, children with FH and FCH had different characteristics of lipid abnormality. By applying ANLAEP's cutoffs, children with FH and FCH who have LDL ≥ 160 mg/dl or non HDL-C ≥ 190 mg/dl might have increased inflammation or decreased FMD (Daniels, Gidding, de Ferranti, 2011).

Following these assumptions, the primary investigator assumed that a high atherogenic lipid profile was associated with increased inflammation and decreased endothelial function. Endothelial function would be assessed by brachial FMD. Inflammation would be measured by hs-CRP. Brachial FMD of children with LDL- C ≥ 160 mg/dl would be compared with children with LDL-C < 160 mg/dl. Brachial FMD of children with non-HDL ≥ 190 mg/dl would be compared with non-HDL≥ 190 mg/dl. To assess the impact of atherogenic lipid profile on inflammation, hs-CRP of children with LDL-C ≥ 160 mg/dl would be compared with LDL-C< 160 mg/dl, or hs-CRP of children with non HDL-C ≥ 190 mg/dl would be compared with children with non HDL-C< 190 mg/dl. Consequently, children with LDL-C ≥ 160 mg/dl or non HDL-C ≥ 190 mg/dl were hypothesized to have worse brachial FMD and higher hs-CRP levels.

Research Questions

This study investigated the correlation between atherogenic lipid profile and endothelial function, atherogenic profile and inflammation in children with familial hyperlipidemia. The research questions are as following:

Question 1. What are the levels of atherogenic lipid profile, calculated by non HDL-C, a parameter recommended by the NCL 2011?

Question 2. What are levels of endothelial function assessed by brachial flow-mediated dilation?

Question 3. What levels of inflammation are measured by hs-CRP?

Question 4. Is there an association between non HDL-C and brachial FMD?

Question 5. Is there an association between non-HDL and hs-CRP?

Question 6. Is there an association between brachial FMD and hs-CRP?

Definition of Terms

Brachial FMD is defined as the percentage of peak post hyperemic arterial dilation diameter subtracted from the mean baseline diameter and divided by the mean baseline diameter, FMD % = (peak hyperemic diameter – mean baseline diameter) / mean baseline diameter x 100%. Peak FMD is defined as the largest post hyperemic arterial diameter measured at the 30^{th} , 45^{th} , 60^{th} , 90^{th} and 120^{th} seconds. The arterial diameter of three consecutive cardiac cycles at each time points will be measured and averaged. Mean baseline arterial diameter is defined by averaging three arterial diameter at " R" wave on the ECG. All arterial diameters will be expressed by millimeter (mm).

Body mass index is defined as weight in kilograms divided by height in meter squared, kg/m^2. Biochemical analyses of lipids and hs-CRP are expressed as milligram/deciliter, mg/dl.

CHAPTER 3 METHODOLOGY

Research Design

A retrospective, cross-sectional study was designed to explore the relationships among the atherogenic lipid profile, endothelial function and inflammation in children with familial hyperlipidemia. Specifically, a descriptive study was designed to investigate the association between nonHDL-C, brachial artery FMD and hsCRP in children with familial hyperlipidemia.

Sample and Settings

Participants were referred from the Lipid Clinic at University of California, San Francisco Medical Center (UCSF Medical Center) to participate in the Endothelial Assessment of Risk from Lipid in Youth (EARLY) trial (Engler et al., 2003; Engler et al., 2004; Engler et al., 2005). The EARLY trial was a randomized clinical trial to study the effects of the National Cholesterol Education Program Step II (NCEP-II) diet and antioxidants supplements. The interventions included vitamins C and E, and docosahexaenoic acid with the primary outcome of endothelial function. Children diagnosed with FH and FCH, aged 7-19 years were recruited. Children's clinical diagnoses were based on serial lipoprotein profiles of the children and their parents. Children were diagnosed with FH if they had LDL-C > 130 mg/dl, normal triglycerides, and had an affected parent. Children were diagnosed of FCH if they had LDL-C > 130 mg/dl or fasting triglycerides > 150 mg/dl or both, and if at least one parent had one of these three phenotypes. Subjects had not received any cholesterol-lowering medication.

Exclusion criteria included chronic diseases with or without secondary lipid disorders, current smoking, and pregnancy.

Human Subjects Assurance

Subjects were referred by and recruited from the Lipid Clinic at UCSF Medical Center. Members of the research team contacted all children and parents or guardians. Informed consent forms were signed by parent or guardian, and child assent was also obtained. The study was monitored and approved by the Committee on Human Research at University of California, San Francisco.

Parents accompanied their children participating in the EARLY trial throughout the study. Children's personal privacy was protected as carefully as possible. The ultrasound study was conducted in a single private room with minimal exposure to the public. Children were not left alone without supervision throughout the study. The study was stopped whenever the children requested. Each child was assigned a study identification number and no personal identification was used in the study. All the research materials related to study subjects were kept in a locked cabinet. There was no data sharing.

Criteria for Sample Selection

A total of 80 children were recruited for the EARLY trial. Among the participants, 65 completed a physical exam, a blood draw for the lipid profile, and an ultrasound assessment of brachial FMD. There were 33 FH carriers, 31 FCH carriers and one child with diagnostic code of obesity. Because children were referrals from Lipid

Clinic at UCSF Medical Center, no further genetic testing was done to verified their diagnostic code while entering EARLY trial.

Participating children were excluded in the final data analysis if data was missing on total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C, FMD, hsCRP, gender, age, BMI, height, weight, heart rate, systolic blood pressure (sys bp), diastolic blood pressure (dys bp) and fasting plasma glucose (glucose). Children with a diagnostic code other than FH and FCH were also excluded in the final analysis. Pre-analytical exclusion criteria also included unreadable ultrasound images and duplicating study identification code.

Data Collection Methods

Techniques. Investigators recorded a medical history, a physical examination, height, and weight. Participating children's height and weight were measured using standardized scales. Blood draws for lipid analysis and ultrasound assessment for endothelial-dependent arterial dilation were conducted in the Pediatric Clinical Research Center (PCRC) at UCSF Medical Center.

Experienced nurses at the PCRC drew blood. Blood samples were centrifuged and separated into aliquots for immediate analysis. Cholesterol and lipoprotein levels were determined using an enzymatic technique, and triglyceride levels were determined by a glycerokinase reaction (Kane et al., 1990). The high sensitivity c reactive protein (hsCRP) assay was a latex-enhanced immunoephelometric assay conducted on a BNI analyzer by Dade Bering, Newark, DE, USA (Rifai, Tracy, & Ridker, 1999). In this assay, serum CRP was introduced to CPR antibodies coated on polystyrene beads. The

CRP concentration was in proportion to light emitted by the CRP-antibodies complex, and measured by the nephelometer.

Instruments. Ultrasound assessment of brachial artery hyperemic reactivity was performed with a 15 MHz linear array vascular transducer and ultrasound system (Sequoia C256, Acuson, Mountain View, CA, USA). Brachial ultrasound images were recorded digitally for off-lint analyses using the Brachial Ultrasound Workstation (Medical Imaging Applications, Iowa City, IA, USA).

Ultrasound assessment of brachial artery endothelial function. Ultrasound assessment of endothelium-dependent hyperemic reactivity was performed in the morning after an overnight fast. Subjects were kept supine in a dimly lit, thermally controlled room. Three baseline blood pressure measurements were obtained by Dinamap on the left arm after ten minutes of acclimation.

Reliability and validity. Biochemical measurements included: total cholesterol (mg/dl), LDL-C (mg/dl), VLDL-C (mg/dl), HDL-C (mg/dl), triglycerides (mg/dl), hs-CRP (mg/dl). Total cholesterol and triglyceride levels were standardized against reference material supplied by the Standardization Program of the National Center for Disease Control (Kane et al., 1990). High sensitivity CRP assay coefficient variations were 6.4%, 3.7% and 2.9% for CRP concentration of 0.47 mg/dl, 10.5 mg/dl, 54.9mg/dl respectively (Rifai, et al., 1999).

Brachial artery assessment was performed by a few sonographers for different interventions during the trials. This researcher began ultrasound scanning and involved with the study since 2010. This researcher has undergone a rigorous training program and

has been certified with technical sufficiency since 2007. Within rater reproducibility was demonstrated by a reliability of Cronbach's Alpha 0.999 and an intraclass correlation 0.999 for baseline diameter and a reliability of Cronbach's Alpha 0.998 and an intraclass correlation 0.998 for post-hyperemic FMD on 10 subjects on two visits with one week apart.

Procedure

The imaging protocol was followed by standardized recommendations (Corretti et al., 2002). Three ECG leads were placed on each subject. After subject identification was entered into the ultrasound machine, a rapid inflation blood pressure cuff was placed around the widest part of the proximal right forearm at the widest part of forearm distal to antecubital fossa. Subject's right arm was comfortably positioned on an arm board, with the elbow positioned downward and the thumb pointing upward. Ultrasound preset elements included: 15 MHz linear-array transducer, not harmonic imaging, high dynamic range, standard depth of 4 cm.

The brachial artery was imaged about 2-10 cm proximal to the antecubital crease and positioned around 1.5 to 3 cm depth on the monitor. To optimize the image, time-gain compensation and overall gain setting were adjusted to identify the lumen and arterial wall interface. Landmarks were identified and marked on the monitor and recorded on the worksheet to ensure that the image segment of the brachial artery would be reproduced within and between studies. Anatomical approaches included distance between the transducer and antecubital fossa (cm), and angle of incidence to the plane of the bed using a protractor horizontally aligned to the bed plane were recorded.

Baseline Doppler flow velocity was acquired with sample position in the center of the vessel and aligned with flow with an incident angle of 60 degree, on a scale of 1 - 1.2 m/s. Then, turned to M-mode 2D to acquire non-gated baseline digital cine-loop and gated baseline cine-loop. The forearm blood pressure cuff was inflated to 200 mmHg for 5 minutes. Using the ultrasound system clock time, the cuff inflation time, cuff deflation time, reactive hyperemic 60-second (sec) time and reactive hyperemic 90 sec time were annotated. At the fourth minute of inflation, a gated cine-loop for pre-hyperemic 2D images was acquired. The blood pressure cuff was deflated at five minutes. Doppler images of post hyperemic reactivity at first five cardiac cycles were recorded on a 2.0 - 2.5 m/s scale. A continuous scan for 2D images of post hyperemic reactivity began at post cuff deflation 30^{th} sec, 45^{th} sec, 60^{th} sec, 90^{th} sec and ended at 120th sec. Post hyperemic recovery 2D images were acquired at 180^{th} sec. Digitalized images were stored for off-line analyses.

Data Analysis

Baseline Doppler flow velocity and post hyperemic peak Doppler flow velocity were measured using an ultrasound machine caliper. Digitalized 2D images were transferred to optical disks and analyzed using a Brachial Ultrasound Workstation (Medical Imaging Applications, Iowa City, IA, USA). Arterial diameters were taken at end-diastole incident gaited with the R wave. Mean baseline diameters were taken by averaging three cardiac cycles at baseline gated cine-loop. Reactive hyperemic reaction after cuff deflation was taken with an average of three cardiac cycles at 30^{th} sec, 45^{th} sec, 60^{th} sec, and 120^{th} sec. FMD was calculated by subtracting the post hyperemic diameter from the baseline diameter then dividing the result by baseline diameter as a percentage

of the baseline diameter (Celermajer et al., 1992). For example, FMD at 30th sec = (reactive hyperemic diameter at 30^{th} sec – mean baseline diameter) / mean baseline diameter X 100%. The highest FMD in the four time points was designated the peak FMD.

Statistical analysis was performed using SPSS Graduate Pack 19.0 for Windows. The primary outcome was the brachial FMD, expressed as mean ± standard deviation (mean ± sd) %. Continuous variables including lipids, age, height, weight, BMI, FMD, hsCRP, fasting blood glucose, heart rate, systolic blood pressure, diastolic blood pressure, were presented as mean ± sd. The hsCRP had a non-normal distribution therefore it was logarithm (Log10) transformed to meet the assumption of central tendency distribution.

Descriptive statistics was first conducted to analyze frequency distribution of diagnostic code and genders. Further frequency analysis was conducted to find mean, median, standard deviation, minimum and maximum for lipids including TC, TG, LDL-C, HDL-C, and non-lipids including age, BMI, systolic BP, diastolic BP, HR, and glucose. Non HDL-C was calculated by subtracting total cholesterol with HDL-C.

To measure central tendency and data dispersion, mean, standard deviation, median, minimum, and maximum were calculated for all the continuous variables including total cholesterol, triglycerides, LDL-C, HDL-C, non HDL-C, age, height, weight, BMI, systolic blood pressure, diastolic blood pressure, heart rates, glucose, FMD and hs-CRP. Frequency histograms for continuous variables including lipids, age, sys bp, dia bp, HR, glucose, FMD, hsCRP and log10 transformed hsCRP were drawn to demonstrate centralized distribution.

Bivariate correlation with Pearson Coefficients was used to analyze association between dependent variables, and independent variables (predictors). Continuous variables included two dependent variables, five lipid components, and six predictors. Two dependent variables were LoghsCRP and brachial FMD. Five lipid components were TC, TG, LDL-C, HDL-C and nonHDL-C. Six predictors including age, height, weight, BMI, systolic blood pressure, diastolic blood pressure, heart rate, and blood glucose. Specifically, bivariate Pearson Coefficients correlations were calculated between FMD and five lipids, FMD and six non-lipids predictors, LoghsCRP, and five lipids and LoghsCRP and six non-lipids predictors.

Predictors with Pearson Correlation Coefficients (r) reaching 0.05 significant level ($p < 0.05$) were chosen for linear regression analysis. By using the stepwise method, predictors were entered into linear regression model for their statistical significance.

Independent Sample T-Test was used to compare group means. Group means defined by diagnostic codes, genders, blood glucose cutoff point at mean, LDL-C at 200 mg/dl, LDL-C at 160 mg/dl, LDL-C at 130mg/dl, nHDL-C at 145 mg/dl, nHDL-C at 190 mg/dl, total cholesterol at 250 mg/dl were compared.

CHAPTER FOUR RESULTS

Preliminary Analysis

Defined by diagnostic code, there were 33 children with FH and 31 children with FCH (Table 1). Among them, were 30 males and 34 females (Table 2). The following descriptive analyses of two dependent variables include brachial FMD and hsCRP, and their associations with predictors including five lipid variables, and six non-lipid variables. All the continuous variables were expressed by mean and standard deviation (mean ± sd). A p-value < 0.05 was considered significant.

Descriptive Analysis

The study group's lipid analysis (Table 3) showed total cholesterol (TC) 256.6 ± 69.4 mg/dl, varied from 132 mg/dl to 468 mg/dl with a median at 251.5 mg/dl. Among subject children, 75% had TC ≥ 200 mg/dl (US Lipid Clinic criteria), or 64% had TC ≥ 220 mg/dl (MEDPED criteria) or 45% had TC ≥ 260 mg/dl (Simons criteria). Group lipids were higher than healthy children in the NHANES 2005-2008 survey (Table 69).

The study group's triglycerides (TG) were 125.3 ± 70.3 mg/dl, with a mean at 113.5 mg/dl and varied from 39 mg/dl to 410 mg/dl. Among subject children, 84% had TG ≥ 58 mg/dl (Dutch criteria) or 69% had TG ≥ 77 mg/dl. One 16 years old girl with FCH had TG 410 mg/dl.

The study group's LDL-C were 189.3 ± 71.5 mg/dl, with a median at 186.0 mg/dl, and varied from 72 mg/dl to 410 mg/dl. Among subject children, 75% had LDL-C > 130 mg/dl (US Lipid Clinic criteria), 73% had LDL-C ≥ 135 mg/dl (Dutch criteria),

67% had LDL-C ≥ 155 mg/dl (MEDPED and Simon criteria), and 63% children had LDL-C > 160 mg/dl (ANLAEP 2011's criteria).

The study group's HDL-C were 46.8 ± 11.1 mg/dl with median 45 mg/dl and varied from 22 mg/dl to 70 mg/dl. Among subject children, 22% had HDL-C < 39 mg/dl (Dutch criteria).

Group's nonHDL-C (nHDL-C) calculated 209.8 ± 68.3 mg/dl with median 209 mg/dl and varied from 85 mg/dl to 417 mg/dl. Among studied children, 59% had nonHDL-C ≥ 190 mg/dl (ANLAEP 2011's criteria).

Demographic descriptive analysis (Table 4) showed study children were 12.2 ± 2.8 years old and varied from 7 to 19 years old. Body mass index (BMI) for the study group was 22.0 ± 5.6 kg/m². Three children with FCH were obese: a 16 years old girl with BMI of 48.3 kg/m², a boy and a girl aged 11 years with BMI of 31 kg/m² and 33 kg/m² respectively. There were 22% children overweight with BMI ≥ 25 kg/m².

Vital signs analysis showed that the group's systolic blood pressure readings were 115 ± 11 mmHg and varied from 140 mm Hg to 83 mmHg. The study group's diastolic blood pressure readings were 59 ± 9 mmHg and varied from 86 mmHg to 37 mmHg. Their heart rates were 74 ± 15 beats and varied from 51 beats per minute to 115 beats per minute.

Fasting blood glucose analysis showed that the group's fasting glucose readings were 84.8 ± 9.1 mg/dl and varied from 62.0 mg/dl to 103.0 mg/dl and median fasting blood glucose was 85 mg/dl. Frequency analysis showed that 25% children had fasting

blood glucose < 80 mg/dl, 48% children fasting blood sugar ≤ 84 mg/dl, 51% children fasting blood sugar ≥ 85mg/dl and 27% children fasting blood sugar ≥ 90 mg/dl.

Frequency histogram analysis for data distribution showed all variables except hsCRP met central tendency assumption. In a frequency histogram, distribution of hsCRP was skewed to the left (Table 5). To meet central tendency assumption, it was log10 transformed. New variable Log10 hsCRP (LoghsCRP) showed a nearly normally distributed frequency histogram (Table 6). Further descriptive analysis (Table 7) showed hsCRP varied from 0.017 mg/dl to 4.27 mg/dl with mean and standard deviation 0.39 ± 0.73 mg/dl. Transformed LoghsCRP showed mean and standard deviation -0.85 ± 0.63 with median -0.92 and varied from -1.77 to 0.63.

Descriptive analysis of endothelium-dependent hyperemic reactivity, brachial FMD showed 5.27 ± 2.45 %, with median 4.88%. Frequency analysis showed the group's brachial FMD varying from 0.39% to 10.30 % and only 10 children had FMD ≤ 3%.

Pearson Correlation Analysis

Bivariate Pearson Correlation analyses showed inverse relationships between brachial FMD and hsCRP, brachial FMD and LoghsCRP, brachial FMD and nonHDL-C (Table 8). However, the associations were not statistically significant. Pearson Correlations Coefficients between brachial FMD and hsCRP, brachial FMD and LoghsCRP, brachial FMD and nonHDL-C were calculated and displayed in the correlation matrix (Table 8). Increased brachial FMD had no statistically significant association with decreased hsCRP ($r = -0.095$, $p = 0.458$). Increased brachial FMD had no statistically significant association with decreased LoghsCRP ($r = -0.147$, $p = 0.245$).

In addition, brachial FMD had no statistically significant association with nonHDL-C. Calculated Pearson Correlation Coefficient showed insignificant inverse relationship between brachial FMD and nonHDL-C ($r = -0.024$, $p = 0.853$). Decreased brachial FMD was associated with increased nonHDL-C.

In the bivariate correlation analysis (Table 8), both hsCRP and LoghsCRP had an inverse relationship with nonHDL-C. However, only correlations between LoghsCRP and nonHDL-C reached statistical significance. Calculated Pearson Correlation Coefficient showed an increase in hsCRP associated with a decrease in nonHDL-C ($r = -0.239$, $p = 0.058$); and an increase in LoghsCRP was weakly associated with a decrease in nonHDL-C ($r = -0.282$, $p = 0.024$). Despite being highly correlated with each other, hsCRP was not equal to LoghsCRP in their correlation with nonHDL-C in the statistical term.

In another bivariate correlation analysis (Table 9), brachial FMD was found to have no statistically significant association with all the traditional lipids including total cholesterol, triglycerides, LDL-C and HDL-C. Although not reaching statistical significance, lower brachial FMD was associated with higher total cholesterol ($r = -0.028$, $p = 0.825$), higher triglycerides ($r = -0.070$, $p = 0.585$), higher LDL-C ($r = -0.011$, $p = 0.931$) and higher HDL-C ($r = -0.031$, $p = 0.808$).

In additional bivariate correlation analysis (Table 10), LoghsCRP was found to have statistically significantly inverse relationships with total cholesterol ($r = -0.271$, $p = 0.03$), and with LDL-C ($r = -0.282$, $p = 0.024$). Higher LoghsCRP was weakly associated with lower levels of total cholesterol, and LDL-C. There was no statistically significant

relationship between LoghsCRP and triglycerides (r = -0.087, p = 0.492), and between LoghsCRP and HDL-C (r = 0.040, p = 0.752).

Among non-lipid independent variables, only age was found to have a statistically significant correlation with brachial FMD (Table 11). Increased age was weakly associated with higher brachial FMD (r = 0.273, p = 0.029). In addition to age, BMI and glucose were found to have statistically significant association with LoghsCRP (Table 12). Higher level LoghsCRP was associated with younger age (r = -0.259, p = 0.039), higher BMI (r = 0.258, p = 0.039) and higher fasting blood glucose (r = 0.375, p = 0.002).

In summary, brachial FMD as a dependent variable was found to have a positive correlation with only one predictor: age. As a dependent variable, LoghsCRP was found to have weakly positive correlations with three lipid components including total cholesterol, LDL-C, nonHDL-C and three non-lipid predictors including age, BMI and glucose. Increased LoghsCRP was weakly correlated with decreased age, and increased BMI. Increased LoghsCRP was moderately correlated with increased fasting blood glucose.

Linear Regression Analysis

In the simple linear regression analyses, predictors with statistically significant Pearson Correlation Coefficients were used. Using stepwise linear regression analysis, age BMI, glucose, total cholesterol, LDL-C and nonHDL-C were entered into the regression model. As a dependent variable, 7% variance in brachial FMD was explained by age (Table 13). The ANOVA was performed by using age (Table 14). A significant

regression equation was identified, F (1, 62) = 4.978, p = 0.029. Children's brachial %FMD was estimated equal to 2.379 + 0.237 (age) when age was measured in years (Table 15). Children's % FMD increased 0.237% for each increase of chronicle age.

Based on correlation analyses, predictors reaching statistical significance including age, BMI, glucose, TC, LDL-C and nonHDL-C were entered into regressing models. With multiple trials, a linear regression model with statistical significance was constructed to estimate the dependent variable, LoghsCRP. Using a stepwise linear regression method, glucose and nonHDL-C were identified as statistically significant predictors. In the linear regression model (Table 16), 14% variance in LoghsCRP was accounted by glucose ($R^2 = 0.14$, p = 0.002) and 9.2% variance in LoghsCRP was accounted by nonHDL-C ($R^2 = 0.232$, p = 0.000). A significant linear regression equation was identified (F(1, 62) = 10.116, p = 0.002) using glucose as a predictor and (F(2, 61) = 9.205, p =0.000) using glucose and nonHDL-C as predictors and displayed in a ANOVA table (Table 17). Estimated Linear Regression Coefficients were 0.027 for glucose and -0.003 for nonHDL-C with both variables entered into the model. Children's LoghsCRP was estimated to be equal to -2.549 + 0.027 (glucose) mg/dl + (-0.003) (nonHDL-C) mg/dl when glucose was measured in mg/dl and nonHDL-C was measured in mg/dl (Table 18). Children's LoghsCRP was predicted to increase 0.027 units with each mg/dl increase of glucose and decrease 0.003 units for each mg/dl decrease of nonHDL-C.

Independent Samples T - Test

Based on previous analyses, age was a statistically significant predictor for brachial FMD. To further explore the inference of age on lipids, fasting blood glucose,

BMI, brachial FMD and Log10 hsCRP, parametric inferential statistics were conducted. Using different cutoff ages, children were divided into younger and older groups. Group means of lipids, fasting blood glucose, BMI, brachial FMD and LoghsCRP were compared.

Comparisons of lipids. Based on the observation that mean total cholesterol peaks at 9 to 11 years of age (Daniels et al., 2008), children 9 years or older were compared with children younger than 9 years old (Table 19). There were no significant differences of means in total cholesterol, triglycerides, LDL-C and nonHDL-C between the older age group and younger age groups. Older children had higher total cholesterol (257.6 ± 69.7 mg/dl) than younger children (236.3 ± 71.6 mg/dl). The mean difference of total cholesterol between groups, 21.2 mg/dl was not statistically significant (95% CI:-61.3 to 103.7 mg/dl; t (62) = 0.514, p = 0.609, two-tailed). Older children had higher triglycerides (126.6 ± 71.1 mg/dl) than younger children (98.7 ± 54.5 mg/dl); t (62) = 0.669, p = 0.506 (two-tailed). The magnitude of the differences in the means (mean difference = 27.9 mg/dl, 95% CI: -55.6 to 111.4 mg/dl) was very small (eta squared = 0.007). Older children had higher LDL-C (190.5 ± 71.9 mg/dl) than younger children (165.0 ± 69.5 mg/dl); t (62) = 0. 6, p = 0.551 (two-tailed). The magnitude of the differences in the means (mean difference = 25mg/dl, 95% CI: -59.5 to 110.5 mg/dl) was very small (eta squared = 0.006). Older children had lower HDL-C (46.2 ± 11.1 mg/dl) than younger children (58.0 ± 5.7 mg/dl); t (62) = -1.821, p = 0.073 (two-tailed). The magnitude of the difference in the means (mean difference = -11.8 mg/dl, 95% CI: -24.7 to 1.1 mg/dl) was moderate (eta squared = 0.060). Old children had higher nonHDL-C (211.3 ± 68.3 mg/dl) than younger children (178.3 ± 75.4 mg/dl); t (62) = 0.814, p =

0.419 (two-tailed). The magnitude in the means (mean difference = 33.0 mg/dl, 95% CI: -48.0 to 114.0 mg/dl) was small (eta squared = 0.011).

Using age 10 as a cutoff point (Table 23), group means of total cholesterol, triglycerides, LDL-C, HDL-C and nonHDL-C of the older group were found to be lower. However, the differences in the means were not statistically significant. Children 10 years or older had lower total cholesterol (255.5 ± 69.4 mg/dl) than children younger than 10 years old (261.1 ± 72.2 mg/dl). The mean difference of total cholesterol between the two groups was -5.6 mg/dl (95% CI: -50.3 to 39.2 mg/dl; t (62) = -0.249, p = 0.805, two-tailed). Older children had lower triglycerides (125.6 ± 72.6 mg/dl) than younger children (123.8 ± 62.0 mg/dl); t (62) = 0.079, p = 0.938 (two-tailed). The magnitude of the differences in the means (mean difference = 1.8 mg/dl, 95% CI: -43.6 to 47.2 mg/dl) was very small (eta squared = 0.0001). Older children had lower LDL-C (188.4 ± 71.6 mg/dl) than younger children (193.2 ± 74.0 mg/dl); t (62) = -0.206, p = 0.837 (two-tailed). The magnitude of the differences in the means (mean difference =-4.8 mg/dl, 95% CI: -50.9 to 41.4 mg/dl) was very small (eta squared = 0.0007). Older children (45.8 ± 10.8 mg/dl) had lower HDL-C than younger children (50.9 ± 5.7 mg/dl); t (62) = -1.443, p = 0.154 (two-tailed). The magnitude of the differences in the means (mean difference = -5.1 mg/dl, 95% CI: -12.2 to 2.0 mg/dl) was small (eta squared = 0.03). Older children had lower nonHDL-C (209.7 ± 68.1 mg/dl) than it in younger children (210.1 ± 72.4 mg/dl); t (62) = -0.017, p = 0.987 (two-tailed). The magnitude of the differences in the means (mean difference = -0.4 mg/dl, 95% CI: -44.5 to 43.7 mg/dl) was very small (eta squared = 0.000).

Compared to the younger group (Table 27), children 11 years or older had lower total cholesterol, higher triglycerides, higher LDL-C, lower HDL-C and lower nonHDL-C. There was a significant difference of means in the HDL-C between children 11 years or older and those younger than 11 years old. Older children had higher total cholesterol (246.7 ± 69.6 mg/dl) than younger children (280.0 ± 64.7 mg/dl). The mean difference of total cholesterol between the two groups was -33.3 mg/dl (95% CI: -70.6 to 4.0 mg/dl); t (62) = -1.787, p = 0.079, two-tailed. Older children had lower triglycerides (128.2 ± 77.1 mg/dl) than younger children (118.3 ± 51.9 mg/dl); t (62) = 0.516, p = 0.608 (two-tailed). The magnitude of the differences in the means (mean difference = 10.0 mg/dl, 95% CI: -28.7 to 48.7 mg/dl) was very small (eta squared = 0.004). Older children had lower LDL-C (180.2 ± 72.3 mg/dl) than younger children (210.7 ± 66.6 mg/dl); t (62) = -1.577, p = 0.120 (two-tailed). The magnitude of the differences in the means (mean difference =-30.5 mg/dl, 95% CI: -69.1 to 8.2 mg/dl) was very small (eta squared = 0.04). There was a significant difference in HDL-C (Table 28) for older children (44.4 ± 10.5 mg/dl) and younger children (52.3 ± 10.9 mg/dl); t (62) = -2.687, p = 0.009 (two-tailed). The magnitude of the differences in the means (mean difference = -7.8 mg/dl, 95% CI: -13.6 to -2.0 mg/dl) was moderate (eta squared = 0.10). Older children had lower nonHDL-C (202.2 ± 68.9 mg/dl) than younger children (227.7 ± 65.2 mg/dl); t (62) = -1.371, p = 0.175 (two-tailed). The magnitude of the differences in the means (mean difference = -25.5 mg/dl, 95% CI: -62.6 to 11.7 mg/dl) was small (eta squared = 0.03).

Subsequent comparisons showed an inconsistent trend in the changes of lipid components between ages 12 to 16 (Table 29 –Table 33). The changes of differences in the means of total cholesterol were not statistically significant. Compared to the younger

group, children 12 years or older had lower total cholesterol (248.3 ± 76.7 mg/dl) than children younger than 12 years old (264.8 ± 61.3 mg/dl) with a mean difference -16.5 mg/dl (95% CI: -51.9 to 18.2 mg/dl; t (62) = -0.951, p = 0.345, two-tailed). Compared to the younger group, children 13 years or older had lower total cholesterol (242.7 ± 68.1 mg/dl) than children younger than 13 years old (267.4 ± 69.4 mg/dl) with a mean difference -24.7 mg/dl (95% CI: -59.3 to 10.0 mg/dl; t (62) = -1.423, p = 0.160, two-tailed). Compared to the younger group, children 14 years or older had higher total cholesterol (262.1 ± 71.2 mg/dl) than children younger than 14 years old (267.4 ± 69.4 mg/dl) with a mean difference 7.9 mg/dl (95% CI: -30.3 to 46.1 mg/dl; t (62) = 0.413, p = 0.681, two-tailed). Compared to younger group, children 15 years or older had lower total cholesterol (254.3 ± 80.3 mg/dl) than children younger than 15 years old (257.1 ± 67.5 mg/dl) with a mean difference -2.8 mg/dl (95% CI: -47.6 to 41.9 mg/dl; t (62) = -0.436, p = 0.511, two-tailed). Compared to younger group, children 16 years or older had lower total cholesterol (255.3 ± 88.2 mg/dl) than children younger than 16 years old (256.8 ± 66.8 mg/dl) with a mean difference -1.4 mg/dl (95% CI: -51.7 to 48.8 mg/dl; t (62) = -0.057, p = 0.306, two-tailed). Although the difference of means was not significant the differences in means went from negative to positive between age 13 and 14.

A similar change of means was also observed in LDL-C. However, the differences of means were not significant. Children 12 or older had lower LDL-C (180.2 ± 80.3 mg/dl) than children younger than 12 years old (198.4 ± 61.4 mg/dl). The mean difference between two groups was not statistically significant (-18.2 mg/dl; t (62) = -1.016, p = 0.314, tow tailed, 95% CI: -53.9 to 17.6 mg/dl). Children 13 years or older had lower LDL-C (173.9 ± 13.6 mg/dl) than children younger than 13 years old (201.3 ± 70.0

mg/dl). The mean difference between two groups was not statistically significant (-27.4 mg/dl; t (62) = -1.536, p = 0.130, 95% CI: -63.0 to 8.2 mg/dl). Children 14 years or older had higher LDL-C (193.5 ± 73.4 mg/dl) than children younger than 14 (187.5 ± 71.5 mg/dl). Although the mean difference was not statistically significant (5.9 mg/dl; t (62) = 0.301, p = 0.764, 95% CI: -33.5 to 45.3 mg/dl), it changed from negative to positive between 13 and 14 years. Children 15 years or older had lower LDL-C (180.3 ± 82.5 mg/dl) than children younger than 15 years old with a mean difference of -11.1mg/dl (t (62) = -0.483, p = 0.631, two tailed, 95% CI: -57.2 to 34.9 mg/dl). Children 16 years or older had lower LDL-C (178.7 ± 91.7 mg/dl) than children younger than 16 years old (191.0 ± 68.5 mg/dl) with a mean difference of -12.4 mg/dl (t (62) = -0.478, p = 0.634, tow tailed, 95% CI: -64.1 to 38.3 mg/dl).

Between age 9 and 16, HDL-C concentrations were always higher in the older age group. However, there was only a statistical significance in the difference of means for children 11 or older and children younger than 11 years old. Compared to the younger group, children 12 years or older had lower HDL-C (45.1 ± 10.4 mg/dl) than children younger than 12 years old (48.5 ± 11.7 mg/dl). The mean difference of HDL-C between these two groups was -3.4 mg/dl (95% CI: -9.0 to 2.1 mg/dl; t (62) = -1.227, p = 0.224, two-tailed). Compared to the younger group, children 13 years or older had lower HDL-C (44.7 ± 10.7 mg/dl) than children younger than 13 years old (48.4 ± 11.4 mg/dl). The mean difference of HDL-C between the two groups was -3.7 mg/dl (95% CI: -9.3 to 1.9 mg/dl; t (62) = -1.329, p = 0.189, two-tailed). Compared to the younger group, children 14 years or older had lower HDL-C (44.3 ± 9.4 mg/dl) than children younger than 14 years old (47.8 ±11.7 mg/dl). The mean difference of HDL-C between the two groups

was -3.5 mg/dl (95% CI: -29.6 to 2.6 mg/dl; t (62) = -1.145, p = 0.256, two-tailed). Compared to the younger group, children 15 years or older lower HDL-C (45.0 ± 11.1 mg/dl) than children younger than 15 years old (47.2 ± 11.2 mg/dl). The mean difference of HDL-C between the two groups was -2.2 mg/dl (95% CI: -9.3 to 5.0 mg/dl; t (62) = -0.606, p = 0.547, two-tailed). Compared to the younger group, children 16 years or older had lower HDL-C (41.7 ± 9.6 mg/dl) than children younger than 16 years old (47.6 ± 11.2 mg/dl). The mean difference of HDL-C between the two groups was -5.9 mg/dl (95% CI: -13.9 to 2.0 mg/dl; t (62) = -1.495, p = 0.140, two-tailed. In summary, older groups had consistently lower HDL-C.

The concentrations of triglycerides had been consistently higher in the older age group from age 12 to 16 years. In all age groups, there was a statistical significance of difference in means for children 16 years or older and children younger than 16 years old (Table 33). Children 12 years or older had higher HDL-C (125.6 ± 76.9 mg/dl) than children younger than 12 years old (125.0 ± 64.2 mg/dl) with a mean difference 0.6 mg/dl (t (62) = 0.035, p = 0.972, two-tailed; 95% CI: -34.8 to 36.0 mg/dl). Children 13 years or older had higher triglycerides (129.1 ± 80.6 mg/dl) than children younger than 13 years old (122.3 ± 62.1 mg/dl) with a mean difference of 6.9 mg/dl (t (62) = 0.385, p = 0.702, two-tailed; 95% CI: -28.8 to 42.5 mg/dl). Children 14 years or older had higher triglycerides (137.2 ± 84.3 mg/dl) than children younger than 14 years old (120.2 ±63.9 mg/dl) with a mean difference of 16.9 mg/dl (t (62) = 0.876, p = 0.384, two-tailed; 95% CI: -21.6 to 55.4 mg/dl). Children 15 years or older had higher triglycerides (146.3 ± 96.8 mg/dl) than children younger than 15 years old (120.4 ± 62.9 mg/dl) with a mean difference of 25.9 mg/dl (t (62) = 1.154, p = 0.253, two-tailed; 95% CI: -19.0 to 70.8

mg/dl). Children 16 years or older had higher triglyceride (169.4 ± 101.6 mg/dl) than children younger than 16 years old (118.1 ± 62.1 mg/dl). Although the sample size was small (9 children 16 or older and 55 children younger than 16) the mean difference between the two groups was 51.4 mg/dl; t (62) = 2.086, p = 0.041, tow tailed, 95% CI: 2.1 to 100.6 mg/dl (Table 34). The magnitude of the differences in the means was moderate (eta squared = 0.07).

In addition, nonHDL-C also had similar group means change in 13- and 14- year-old children. However, the differences of means were not statistically significant. Children 12 or older had lower nonHDL-C (203.2 ± 75.6 mg/dl) than children younger than 12 years old (216.3 ± 60.8 mg/dl) with a mean difference -13.1 mg/dl (t(62) = -0.762, p = 0.449, two tailed, 95% CI: -47.3 to 21.1 mg/dl). Children 13 years or older had lower nonHDL-C (198.0 ± 67.9 mg/dl) than children younger than 13 years old (218.9 ± 68.2 mg/dl) with a mean difference -20.9 mg/dl (t (62) = -1.221, p = 0.937, two tailed, 95% CI: -55.2 to 13.3 mg/dl). To the contrary, children 14 years or older had higher nonHDL-C (217.8 ± 67.9 mg/dl) than children younger than 14 years old (206.4 ± 70.0 mg/dl). Although the difference in means was not statistically significant (11.4 mg/dl; t (62) = 0.606, p = 0.547, tow tailed, 95% CI: -26.2 to 49.3 mg/dl) it change from negative to positive between 13 and 14 years old children. Children 15 years or older had very similar nonHDL-C (209.3 ± 75.8 mg/dl) to children younger than 15 years old (209.9 ± 67.3 mg/dl) with a mean difference -0.7 mg/dl (t (62) = -0.30, p = 0.976, two tailed, 95% CI: -44.8 to 43.4 m/dl). Children 16 years or older had higher nonHDL-C (213.7 ± 83.5 mg/dl) than children younger than 16 years old (209.1 ± 66.4 mg/dl) with a

mean difference 4.5 mg/dl (t (62) = 0.183, p = 0.856, two tailed, 95% CI: -45.0 to 54.0 mg/dl). Again, the differences in means changed from negative to positive.

Comparisons of brachial FMD and LoghsCRP. Group comparisons showed the older age group had consistently higher brachial FMD from age 9 to 16 (Table 21, Table 25, Table 35, Table 36, Table 37, Table 39, Table 41, Table 42). The differences of means were statistically significant in age 9 (Table 22), age 13 (Table 38) and age 14 (Table 40). Children 9 years or older (Table 21) had significantly higher brachial FMD (5.43 ± 2.38%) than children younger than 9 years old (2.00 ± 1.39%); t (62) = 2.461, p = 0.017 (two tailed). The magnitude of the differences in the means (mean difference 3.43%, 95% CI: 0.64 to 6.21%) was moderate (eta squared = 0.09). Children 10 or older had higher brachial FMD (5.39 ± 2.31%) than younger children (4.73 ± 3.03 %); t (62) = 0.848, p = 0.400 (two-tailed) with a difference in means of 0.67% (95% CI: -0.90 to 2.24%) was small (eta squared = 0.011). Children 11 or older had higher brachial FMD (5.49 ± 2.26%) than children older than 11 years old (4.75 ± 2.85%) with a difference in means 0.73% (t (62) = 1.094, p = 0.278, two tailed, 95% CI: -0.61 to 2.07%).

From age 12 to 16, children 13 years or older had higher brachial FMD (5.95 ± 2.53%) than children younger than 13 years old (4.74 ± 2.28%); t (62) = 2.018, p = 0.048 (two-tailed) (Table 38). The magnitude of the differences in the means (mean difference = 1.22, 95% CI: 0.01 to 2.42%) was moderate (eta squared = 0.06). Children 14 years or older had higher brachial FMD (6.25 ± 2.73%) than children younger than 14 years old (4.85 ± 2.23%); t (62) = 2.139, p = 0.036 (two-tailed) (Table 40). The magnitude of the differences in the means (mean difference = 1.39, 95% CI: 0.09 to 2.70%) was moderate (eta squared = 0.07).

Between ages 9 and 16, LoghsCRP was consistently lower in the older age groups (Table 21, Table 25, Table 35, Table 36, Table 37, Table 39, Table 41 & Table 42). There was a significant difference in LoghsCRP (Table 26) for older children 10 years or older (-0.943 ± 0.595) and children younger than 10 year old (-0.460 ± 0.624; t (62) = -2.513, p = 0.015 (two-tailed). The magnitude of the differences in the means (mean difference = -0.483, 95% CI: -0.867 to -0.099) was moderate (eta squared = 0.09). The difference in means between children 9 or older (-0.884 ± 0.618) and children younger than 9 years old was -0.673. The difference in means between children 11 or older (-0.944 ± 0.615) and children younger than 11 years old (-0.636 ± 0.608) was -0.308. The difference in means between children 12 or older (-0.988 ± 0.632) and children younger than 12 years old (-0.717 ± 0.597) was -0.271. The difference in means between children 13 or older (-0.954 ± 0.632) and children younger than 13 years old (-0.773 ± 0.615) was -0.181. The difference in means between children 14 or older (-1.027 ± 0.659) and children younger than 14 years old (-0.779 ± 0.602) was -0.249. The difference in means between children 15 or older (-1.099 ± 0.744) and children younger than 15 years old (-0.795 ± 0.588) was -0.303. The difference in means between children 16 or older (-0.924 ± 0.784) and children younger than 16 years old (-0.841 ± 0.603) was -0.084. The difference in means was smallest between children 16 or older and children younger than 16 years.

Comparisons of BMI and fasting blood glucose. Children in the older age groups had higher BMI (Table 20, Table 24, Table43, Table 44, Table 45, Table 46, Table 47, & Table 49). There was a significant difference in means of BMI for children 16 or older and children younger than16 years old (Table 50). The difference in the means of BMI between children 9 years or older (22.2 ± 5.6 kg/m²) and younger than 9 years old (18.5 ±

4.8 kg/m²) was 3.7 kg/m². The difference in the means of BMI in children 10 years or older (22.4 ± 5.8 kg/m²) and children younger than 10 (20.5 ± 4.6 kg/m²) was 1.9 kg/m. The difference in the means of BMI between children 11 years or older ((22.9 ± 5.6 kg/m²) and children younger than 11 years old (20.1 ± 4.1 kg/m²) was 2.7 kg/m². The difference in the means of BMI between children 12 years or older (23.1 ± 6.2 kg/m²) and children younger than 12 years old (21.0 ± 4.7 kg/m²) was 2.1 kg/m². The difference in the means of BMI between children 13 years or older (23.6 ± 6.3 kg/m²) and children younger than 13 years old (20.8 ± 4.6 kg/m²) was 2.8 kg/m². The difference in the means of BMI between children 14 years or older (24.1 ± 7.1 kg/m²) and children younger than 14 years old (21.2 ± 4.6 kg/m²) was 2.9 kg/m². The difference in the means of BMI between children 15 years or older (24.1 ± 8.2 kg/m²) and children younger than 15 years old (21.6 ± 4.8 kg/m²) was 2.6 kg/m². There was a significant difference in the means of BMI between children 16 years or older (225.5 ± 9.2 kg/m²) and children younger than 15 years old (21.5 ± 4.7 kg/m², $t(62) = 2.025$, $p = 0.047$, two tailed). The magnitude of the differences in the means (mean difference = 3.7 kg/m², 95% CI: 0.1 to 7.9 kg/m²) was moderate (eta = 0.06).

Fasting blood glucose was compared in the different age groups (Table 20, Table 24, Table43, Table 44, Table 45, Table 46, Table 47, & Table 49). There was a significant difference in the means for children 15 or older than children younger than 15 years old (Table 48). The difference in means of fasting blood glucose for children 9 years or older (84.9 ± 9.3 mg/dl) and children younger than 9 years old (83.3 ± 5.0 mg/dl) was 1.6 mg/dl (Table 20). The difference in means of fasting blood glucose for children 10 years or older (84.0 ± 9.14 mg/dl) and children younger than 10 years old (88.4 ± 8.3

mg/dl) was -4.4 mg/dl (Table 24). The difference in the means of fasting blood glucose for children 11 or older (84.0 ± 9.7 mg/dl) and children younger than 11 years old (86.7 ± 7.3 mg/dl) was -2.6 mg/dl (Table 43). Children 12 or older had lower fasting blood glucose (82.9 ± 9.3 mg/dl) than children younger than 12 years old (86.8 ± 8.7 mg/dl) with a difference in the means of -3.8 mg/dl (Table 44). Children 13 or older had lower fasting blood glucose (83.1 ± 9.7 mg/dl) than children younger than 13 years old (86.2 ± 8.5 mg/dl) with a difference in the means of-3.1 mg/dl (Table 45). Children 14 or older had lower fasting blood glucose (81.7 ± 11.0 mg/dl) than children younger than 14 years old (86.2 ± 7.9 mg/dl) with a difference in the means of-4.5 mg/dl (Table 46).

There was a significant difference in the means of fasting blood glucose for children 15 or older and (79.8 ± 9.6 mg/dl) and children younger than 15 years old (86.0 ± 8.7 mg/dl). The differences in the means between two group (Table 50) were significant (-6.1 mg/dl, 95% CI: -11.8 to 0.5 mg/dl, t (62) = -2.172, p = 0.034, two-tailed). The magnitude of the differences in the means was moderate (eta = 0.07). Compared to younger group, children 16 or older had lower fasting blood glucose (81.0 ± 9.3 mg/dl) than children younger than 16 years old (85.5 ± 9.0 mg/dl) with a statistically insignificant difference in the means (-4.5 mg/dl).

Furthermore, fasting blood sugar was explored for its impact on two dependent variables and lipids. Fasting blood glucose 84 mg/dl (group mean) was used as a cutoff point to divide children into two groups (Table 51). An independent samples t-test was used to compare means of brachial FMD, LoghsCRP and lipids between children with fasting blood sugar equal to and greater than 84 mg/dl and children with fasting blood sugar less than 84 mg/dl. There was a significant difference in brachial FMD for children

with fasting blood glucose lower than 84 mg/dl (6.27 ± 2.27%) and children with fasting blood sugar greater than 84mg/dl (4.67 ± 2.38%); t (62) = -2.648, p = 0.010 (two-tailed) (Table 52). The magnitude of the differences in the means (mean difference = -1.60, 95% CI: -2.81 to – 0.39) was moderate (eta squared = 0.10). Also, there was a significant difference in LoghsCRP for children with fasting blood sugar less than 84 mg/dl (-1.074 ± 0.591) and for children with fasting blood sugar greater than 84 mg/dl (-0.719 ± 0.614); t (62) = 2.268, p = 0.027 (two-tailed). The magnitude of the differences in the means (mean differences = 0.355, 95% CI: 0.042 to 0.667) was moderate (eta squared = 0.08).

Also, an independent samples t-test was used to compare children's lipids in children with fasting blood glucose greater than 84 mg/dl and children with fasting blood glucose less than 84 mg/dl (Table 53). There was no significant difference in total cholesterol for children with higher fasting blood sugar (260.4 ± 73.1mg/dl) and children with lower fasting blood sugar (250.3 ± 63.6mg/dl) with mean differences of 10.1 mg/dl. There was no significant difference in triglycerides for children with higher fasting blood sugar (130.8 ± 86.2mg/dl) and children with lower fasting blood sugar (121.6 ± 73.2mg/dl) with mean differences of -8.9 mg/dl. There was no significant difference in LDL-C for children with fasting blood sugar less than 84 mg/dl (192.1 ± 75.5 mg/dl) and children with fasting blood sugar greater than 84 mg/dl with mean differences of -7.4 mg/dl. There was no significant difference in HDL-C for children with fasting blood sugar less than 84 mg/dl (47.2 ± 12.1 mg/dl) and children with fasting blood sugar greater than 84 mg/dl (46.1 ± 9.5 mg/dl) with mean difference of 1.09 mg/dl. There was no significant difference in nonHDL-C for children with fasting blood sugar less than 84

mg/dl (213.2 ± 72.9 mg/dl) and children with fasting blood sugar greater than 84 mg/dl (204.2 ± 61.2 mg/dl) with mean differences of 9.0 mg/dl.

Comparisons of lipid criteria. To explore the inference of nonHDL-C, children were divided into two groups using cutoff points 145 mg/dl (ANLAEP 2011's screening criteria) and 190 mg/dl (ANLAEP 2011's diagnostic criteria). An independent samples t-test was used to compare group means of brachial FMD and LoghsCRP. An independent-sample t-test was conducted to compare brachial FMD for children with nonHDL-C equal to and greater than 145 and children with nonHDL-C less than 145 mg/dl (Table 54). There was no significant difference in brachial FMD for children with nonHDL-C greater than 145 mg/dl (5.17 ± 2.51%) and children with lesser nonHDL-C (5.67 ± 2.25%) with mean differences of -0.50%. There was no significant difference in LoghsCRP for children with greater nonHDL-C (-0.870 ± 0.617) and children with lesser nonHDL-C with mean differences of -0.089.

Using nonHDL-C 190 mg/dl as a cutoff point (Table 55), an independent samples t-test was used to compare group means of brachial FMD and LoghsCRP. Children with nonHDL-C equal and greater than 190 mg/dl and children with nonHDL-C less than 190 mg/dl had no significant difference in brachial FMD. Children with nonHDL-C greater than 190 mg/dl (5.18 ± 2.61%) had similar brachial FMD to children with lesser nonHDL-C (5.40 ± 2.23%) with mean differences of -0.22%. There was no significant difference in LoghsCRP for children with greater nonHDL-C (-0.910 ± 0.553) and children with lesser nonHDL-C (-0.768 ± 0.721) and the mean differences were -0.142.

Although higher nonHDL-C was associated with lower brachial FMD and lower LoghsCRP, the group means of brachial FMD and LoghsCRP were not statistically significantly different at either cutoff point. The levels of nonHDL-C had a very small effect on both brachial FMD and LoghsCRP in our study group.

To explore the inferences of LDL-C, children were divided into two groups by using cutoff points at 160 mg/dl (ANLAEP 2011's criteria), 155 mg/dl (MEDPED's & Simons' criteria), 135 mg/dl (Dutch's criteria) and 130 mg/dl (US Lipid Clinic's criteria).

An Independent samples t-test was used to compare brachial FMD in children with LDL-C equal to and greater than 160 and children with LDL-C lesser than 160 mg/dl (Table 56). There was no significant difference in brachial FMD for children with greater LDL-C (5.17 ± 2.60%) and children with lesser LDL-C (5.44 ± 2.22%); t (62) = -0.432, p = 0.667 (two-tailed). The magnitude of the differences in the means (mean difference = -0.28%, 95% CI: -1.55 to 1.00%) was very small (eta squared = 0.003). An independent samples t-test was used to compare LoghsCRP in children with LDL-C equal to and greater than 160 mg/dl and children with LDL-C lesser than 160 mg/dl (Table 56). There was no significant difference in LoghsCRP for children with greater LDL-C (-0.937 ± 0.593) and children with lesser LDL-C (-0.711 ± 0.664); t (62) = -1.413, p = 0.163 (two-tailed). The magnitude of the differences in the means (mean difference = -0.226, 95% CI: -0.547 to 0.094) was small (eta squared = 0.031).

An independent samples t-test was used to compare brachial FMD in children with LDL-C equal to and greater than 155 and children with LDL-C lesser than 155 mg/dl (Table 57). There was no significant difference in brachial FMD for children with

greater LDL-C (5.14 ± 2.55%) and children with lesser LDL-C (5.53 ± 2.27%); t (62) = -0.599, p = 0.551 (two-tailed). The magnitude of the differences in the means (mean difference = -0.39%, 95% CI: -1.70 to 0.92%) was very small (eta squared = 0.006). An independent samples t-test was used to compare LoghsCRP in children with LDL-C equal to and greater than 155 and children with LDL-C lesser than 155 mg/dl. There was no significant difference in LoghsCRP for children with greater LDL-C (-0.917 ± 0.587) and children with lesser LDL-C (-0.719 ± 0.692); t (62) = -1.195, p = 0.236 (two-tailed). The magnitude of the differences in the means (mean difference = -0.198, 95% CI: -0.530 to 0.133) was small effect (eta squared = 0.023).

An independent samples t-test was used to compare brachial FMD in children with LDL-C equal to and greater than 135 and children with LDL-C lesser than 135 mg/dl (Table 58). There was no significant difference in brachial FMD for children with greater LDL-C (5.21 ± 2.54%) and children with lesser LDL-C (5.44 ± 2.23%); t (62) = -0.315, p = 0.754 (two-tailed). The magnitude of the differences in the means (mean difference = -0.22%, 95% CI: -1.65 to 1.20%) was very small (eta squared = 0.002). An independent samples t-test was used to compare LoghsCRP in children with LDL-C equal to and greater than 135 and children with LDL-C lesser than 135 mg/dl (Table 58). There was no significant difference in LoghsCRP for children with greater LDL-C (-0.918 ± 0.569) and children with lesser LDL-C (-0.656 ± 0.756); t (62) = -1.467, p = 0.147 (two-tailed). The magnitude of the differences in the means (mean difference = -0.262, 95% CI: -0.620 to 0.095) was small (eta squared = 0.034).

An independent samples t-test was also used to compare brachial FMD in children with LDL-C equal to and greater than 130 and children with LDL-C lesser than 130

mg/dl (Table 59). There was no significant difference in brachial FMD for children with greater LDL-C (5.21 ± 2.54%) and children with lesser LDL-C (5.44 ± 2.23%); t (62) = -0.315, p = 0.754 (two-tailed). The magnitude of the differences in the means (mean difference = -0.22%, 95% CI: -1.65 to 1.20%) was very small (eta squared = 0.002). An independent samples t-test was used to compare LoghsCRP in children with LDL-C equal to and greater than 130 and children with LDL-C lesser than 130 mg/dl. There was no significant difference in LoghsCRP for children with greater LDL-C (-0.918 ± 0.569) and children with lesser LDL-C (-0.656 ± 0.756); t (62) = -1.467, p = 0.147 (two-tailed). The magnitude of the differences in the means (mean difference = -0.262, 95% CI: -0.620 to 0.095) was small (eta squared = 0.034).

Diagnostic codes were used to compare lipids. An independent samples t-test was used to compare lipids in FH and FCH children (Table 60). There was a significant difference (Table 61) in total cholesterol for FH children (282.2 ± 80.7 mg/dl) and FCH (229.3 ± 40.8 mg/dl), t (48.0) = 3.337, p = 0.002 (two-tailed). The magnitude of the differences in the means (mean difference = 52.9 mg/dl, 95% CI: 16.1 to 20.6 mg/dl) was large (eta squared = 0.152). There was a significant difference in triglycerides for FH (89.8 ± 45.4 mg/dl) and FCH children (163.1 ± 73.0 mg/dl); t (62) = -4.856, p = 0.000 (two-tailed). The magnitude of the differences in the means (mean difference = -73.3 mg/dl, 95% CI:-103.4 to -43.1 mg/dl) was large (eta squared = 0.276). There was a significant difference in LDL-C for FH children (218.4 ± 82.2 mg/dl) and for FCH (158.4 ± 40.1 mg/dl; t (47.0) = 3.745, p = 0.000 (two-tailed). The magnitude of the differences in the means (mean difference = 60.0 mg/dl, 95% CI: 27.8 to 92.7 mg/dl) was large (eta squared = 0.184). There was a significant difference in HDL-C for FH children (49.8 ±

10.9 mg/dl) and for FCH children (43.6 ± 10.6 mg/dl); t (62) = 2.289, p = 0.026 (two-tailed). The magnitude of the differences in the means (mean differences = 6.2 mg/dl, 95% CI: 0.8 to 11.6 mg/dl) was moderate (eta squared = 0.078). There was a significant difference in nonHDL-C for FH children (232.4 ± 81.5 mg/dl) and for FCH children (185.7 ± 39.5 mg/dl); t (46.9) = 2.943, p =0.005 (two-tailed). The magnitude of the differences in the means (mean differences = 46.7 mg/dl, 95% CI: 14.8 to 78.6 mg/dl) was moderate (eta squared = 0.123).

Diagnostic codes were used to compare age, BMI and fasting blood glucose in FH children and FCH children (Table 62). There was a significant difference in age for FH children (11.5 ± 2.4 years) and FCH children (13.0 ± 3.0 years); t (62) = -2.213, p = 0.031 (two-tailed) (Table 63). The magnitude of the differences in the means (mean difference = -1.5 years, 95% CI: -2.9 to -0.1 years) was moderate (eta squared = 0.073). There was a significant difference in BMI for FH children (19.9 ± 3.0 kg/m²) and FCH children (24.3 ± 6.7 kg/m²); t (40.7) = -3.383, p = 0.002 (two-tailed). The magnitude of the differences in the means (mean difference = -4.5 kg/m², 95% CI: -7.1 to -1.8 kg/m²) was large (eta squared = 0.156). There was no significant difference in fasting blood glucose for FH children (85.3 ± 7.3 mg/dl) and FCH children (84.4 ± 10.8 mg/dl); t (52.5) = 0.396, p = 0.694 (two-tailed). The magnitude of the differences in the means (mean difference = 0.9 mg/dl, 95% CI: -3.7 to 5.6 mg/dl) was small (eta squared = 0.003).

In addition, an independent samples t-test was used to compare brachial FMD and LoghsCRP in FH children and FCH children (Table 64). There was no significant difference in brachial FMD for FH children (5.29 ± 2.56%) and FCH children (5.24 ±

2.37%); t (62) = 0.077, p = 0.939 (two-tailed). The magnitude of the differences in the means (mean difference = 0.05% 95% CI: -1.19 to 1.28%) was very small (eta squared = 0.000). There was no significant difference in LoghsCRP for FH children ((-0.893 ± 0.577) and FCH children (-0.809 ± 0.680); t (62) = -0.531, p = 0.597 (two-tailed). The magnitude of the differences in the means (mean difference = -0.083, 95% CI: -0.398 to 0.231) was very small (eta squared = 0.005).

Using gender as an independent variable, an independent samples t- test was used to compare boys' and girls' lipids (Table 65). There was no significant difference in total cholesterol for boys (260.4 ± 49.8.1mg/dl) and girls (253.2 ± 83.5 mg/dl), t (54.8) = 0.422, p = 0.675 (two-tailed). The magnitude of the differences in the means (mean difference = 7.2 mg/dl, 95% CI: -27.8 to 42.1 mg/dl) was very small (eta squared = 0.003). There was no significant difference in triglycerides for boys (120.6 ± 62.9 mg/dl) and girls (129.4 ± 77.0 mg/dl); t (62) = 0.621, p = 0.628 (two-tailed). The magnitude of the differences in the means (mean difference = -8.8 mg/dl, 95% CI: -44.2 to 26.6 mg/dl) was very small (eta squared = 0.006). There was no significant difference in LDL-C for boys (194.8 ± 51.6 mg/dl) and for girls (184.4 ± 85.9 mg/dl); t (55.0) = 0.596, p = 0.553 (two-tailed). The magnitude of the differences in the means (mean difference = 10.4 mg/dl, 95% CI: -24.6 to 45.4 mg/dl) was very small (eta squared = 0.006). There was no significant difference in HDL-C for boys (44.7 ± 9.13 mg/dl) and girls (48.6 ± 12.5 mg/dl); t (60.0) = -1.407, p = 0.165 (two-tailed). The magnitude of the differences in the means (mean differences = -3.8 mg/dl, 95% CI: -9.3 to 1.6 mg/dl) was very small (eta squared = 0.031). There was no significant difference in nonHDL-C for boys (215.6 ± 47.5 mg/dl) and girls (204.6 ± 82.9 mg/dl); t (53.7) = 0.658, p =0.514 (two-tailed). The

magnitude of the differences in the means (mean differences = 11.0 mg/dl, 95% CI: -22.4 to 44.4 mg/dl) was very small (eta squared = 0.007).

In addition, an independent samples t-test was used to compare age, BMI and fasting blood glucose in boys and girls (Table 66). An independent-sample t-test was conducted to compare age in boys and girls. There was no significant difference in age for boys (12.2 ± 2.6 years) and girls (12.2 ± 3.0 years); t (62) = -0.055, p = 0.956 (two-tailed). The magnitude of the differences in the means (mean difference = -0.0 years, 95% CI: -1.5 to 1.4 years) was very small (eta squared = 0.000). There was no significant difference in BMI for boys (22.6 ± 4.1 kg/m²) and girls (21.6 kg/m²); t (62) = 0.752, p = 0.455 (two-tailed). The magnitude of the differences in the means (mean difference = 1.1 kg/m², 95% CI: -1.8 to 3.9 kg/m²) was very small (eta squared = 0.009). There was no significant difference in fasting blood glucose for boys (83.7 ± 9.1 mg/dl) and girls (85.8 ± 9.1 mg/dl); t (62) = -0.931, p = 0.355 (two-tailed). The magnitude of the differences in the means (mean difference = -2.1 mg/dl, 95% CI: -6.7 to 2.4 mg/dl) was small (eta squared = 0.014).

An independent samples t-test was used to compare brachial FMD and LoghsCRP in boys and girls (Table 67). There was no significant difference in brachial FMD for boys (5.41 ± 2.33%) and girls (5.15 ± 2.58%); t (62) = 0.419, p = 0.676 (two-tailed). The magnitude of the differences in the means (mean difference = 0.26%, 95% CI: -0.98 to 1.49%) was very small (eta squared = 0.003). There was no significant difference in LoghsCRP for boys (-0.922 ± 0.566) and girls (-0.791 ± 0.675); t (62) = -0.839, p = 0.405 (two-tailed). The magnitude of the differences in the means (mean difference = -0.132, 95% CI: -0.442 to 0.179) was small (eta squared = 0.011).

Analysis of Hypotheses

There are six research questions to be addressed in the following discussion.

1. What are the levels of atherogenic lipid profile, calculated by non HDL-C?

   Answer: The study group had non HDL-C 209.8 ± 68.3 mg/dl (Table 3), a level higher than ANLAEP 2011's diagnostic level (non HDL-C ≥ 190 mg/dl) for FH (Daniels et al., 2011).

2. What are the levels of endothelial function assessed by brachial FMD?

   Answer: The study group had FMD 5.27 ± 2.45 % (Table 7), a level lower than healthy children as reported by various groups listed in Table 41.

3. What are the levels of the inflammation measured by hs-CRP?

   Answer: The study group had hsCRP 0.390 ± 0.731 mg/dl which was much higher than the national level of healthy children for ages 3-16 (0.122 ± 0.0066 mg/dl) in the NHNES 1999-2004 (Dowd, Zajacova, & Aiello, 2010) or for ages 3 -17 (male 0.14 ± 0.02 mg/dl, female 0.17 ± 0.02 mg/dl) in the NHNES 1999-2004 (Table 70).

4. Is there an association between non HDL-C and brachial FMD?

   Answer: There was an inverse relationship between nonHDL-C and brachial FMD. Although not statistically significant, a higher nonHDL-C level was associate with lower brachial FMD ($r = -0.024$, $p = 0.853$). Based on calculated Person Correlation Coefficients, none of the lipid parameter had statistically significant correlation with brachial FMD.

5. Is there an association between nonHDL-C and hs-CRP?

Answer: In children with hyperlipidemia, nonHDL-C had weak association with hsCRP. But, nonHDL-C was not statistically significantly associated with hsCRP. Higher nonHDL-C level was associated with lower level hsCRP ($r = -0.239$, $p = 0.058$). In addition, there was a statistically significant association between LoghsCRP and nonHDL-C. Increased nonHDL-C was weakly associated with lower level LoghsCRP ($r = -0.282$, $p = 0.024$).

6. Is there an association between brachial FMD and hs-CRP in children with familial hyperlipidemia?

   Answer: In this study group, FMD had a weak association with hs-CRP ($r = -0.095$, $p = 0.458$). Although not statistically significant, an increased brachial FMD was associated with lower hs-CRP level.

Other Findings

Further statistical analyses done with independent samples t-tests have revealed some interesting points about our dependent variables brachial FMD and hsCRP. As a dependent variable, brachial FMD was consistently higher in the older age group from age 9 to age 12 years. Although not significantly correlated brachial FMD was higher in the children with fasting blood glucose less than 84 mg/dl.

As an independently variable, hsCRP was consistently lower in the older age group and became statistically significant at age 10. In our study group, higher hsCRP was associated with lower total cholesterol, lower LDL-C, and lower nonHDL-C. The inverse relationships between hsCRP and lipids are untraditional findings. However, further independent T-tests show how total cholesterol, LDL-C and nonHDL-C varied in

different age groups. The effect of age on hsCRP might complicate the association between these two variables.

From age 9 to 12, HDL-C was consistently higher in the younger age group and reached statistical significance at 11 years. Group means of total cholesterol, LDL-C and nonHDL-C were higher in 9 years old but became lower among 10, 11, 12 and 13 year-old children. Group means of total cholesterol, LDL-C and nonHDL-C were again higher in the older age groups and became equalizing in the 15 and 16 years old children.

Using different cutoff points of nonHDL-C and LDL-C, higher brachial FMD was associated with lower nonHDL-C levels. High LoghsCRP level was associated with lower nonHDL-C levels. Similar trends were observed using different LDL-C cutoff points. A higher LDL-C level was associated with lower brachial FMD and lower LoghsCRP levels.

Children with different diagnostic code had significant differences in lipid distributions. Children with FH had significantly higher total cholesterol, lower triglycerides, higher LDL-C, lower HDL-C and higher nonHDL-C. Also, children with FH were significantly younger and skinner. Despite these differences, brachial FMD and hsCRP were no significant differences in FH and FCH children

In our study group, there was no significant difference in total cholesterol, triglycerides, LDL-C, HDL-C and nonHDL-C between boys and girls. Also, there were no gender differences in age, BMI, fasting blood glucose. Lastly, there was no significant difference in brachial FMD and hsCRP levels between boys and girls.

CHAPTER 5: DISCUSSION

Meaning of findings

Lipids and Brachial FMD

To define atherogenic lipids in children with FH and FCH has been challenging, as evident in this report. Based on ANLAEP 2011 diagnostic criteria, 62% children had LDL ≥ 160 mg/dl, 47% children had LDL-C ≥ 190 mg/dl and 59% study children had nonHDL-C ≥ 190 mg/dl. Clinically, 30 children in the study group (47%) are eligible for lipid lowering medication therapy. Although FH and FCH children in our study were found to have lower than normal brachial FMD (Table 68), there was no clear association between brachial FMD and nonHDL-C. In FH and FCH children, abnormal high total cholesterol, LDL-C, and nonHDL-C were negatively correlated with hsCRP . In our analyses, lipid abnormality in FH and FCH children was not significantly associated with brachial FMD.

Since 1990's, investigators began to report an inverse relationship between lipoprotein (a) and flow-mediated dilation on homozygous and heterozygous FH children (Sorensen et al., 1994). However, the flow-mediated dilation was assessed on femoral artery not on brachial artery. Subsequently, investigators reported impaired brachial flow-mediated dilation to have an inverse correlation with LDL-C in eleven years old FH children (Aggoun et al., 2000). But, we are unable to identify any significant association between brachial FMD and LDL-C or nonHDL-C in children with FH and FCH. However, our finding of inverse relationship between HDL-C and increased age was similar to a report done in 333 British children (Leeson et al., 1997).

With reasonable sample size, our analysis using T-test with cutoff points at age 12, 13 and 14 years provided possible explaination of the dissociation of brachial FMD and atherogenic lipids. While group means of brachial FMD were statistically significant higher in the older children, group means of LDL-C were lower in the children 13 years or older group but higher in the children 14 years or older. Group means of total cholesterol were lower in the children 13 years older but higher in the children 14 or older. Group means of nonHDL-C were lower in children 13 or older but higher in children 14 or older. Especially, group means of brachial FMD had no significant difference when compared with different lipid criteria.

In the inferential analyses, atherogenic lipids including nonHDL-C 145, nonHDL-C 190, LDL-C 160, LDL-C 155, LDL-C 135 and LDL-C 130 showed little effects of the mean differences in children with familiar hyperlipidemia. Although nonHDL-C was believed a better biomarker than LDL-C in estimating apolipoprotein B (Sniderman, McQueen, Contois, Williams, & Furberg, 2010) and was associated with metabolic syndrome (Li et al., 2011), our data did not show its superiority in identifying attenuated brachial FMD.

The dissociation between brachial FMD and LDL-C and nonHDL-C in FH and FCH children may be due to the change of lipid components during maturation. Among children and adolescents aged 8- 18 years, Altwaijri and colleagues reported a significant impacts of sexual maturation on total cholesterol (Altwaijri et al., 2009). In our analyses, group means of total cholesterol were higher in children 9 years or older but were lower in children 10 – 13 and again were higher in children 14 or older. Our findings were similar to reports of significant differences between 6 to 11 years and 12 to 17 years

made by Ford et al. (2009) and Freedman et al (2010). Although not surveyed, the variation in cholesterol might have very likely been caused by sexual maturation. Since sexual maturation can't be defined by age, the dissociation between brachial FMD and total cholesterol may be justified.

In analyses of all the recommended lipid criteria, our findings were not able to support the sensitivity and specificity for atherogenic lipid levels. The levels of hsCRP in different LDL-C and nonHDL-C were not significantly different. This result may be explained by an inverse association between hsCRP and LDL-C and nonHDL-C in our analyses. This uncommon finding may be the results of a small sample size or other unknown factors.

Age and Brachial FMD

Our finding of age as an independent predictor for brachial FMD in children with FH and FCH was uncommon. Unlike classical findings, our study found older children to have higher brachial FMD. In a classical paper, attenuation of brachial FMD was associated with older age in 238 healthy subjects aged 15 to 72 years old (Celermajer et al., 1994). Age as a determinant factor was further confirmed by an analysis done by Herrington and colleagues that included 4,040 subjects 14 to 98 years (Herrington et al., 2001). A recent study of arterial shear rate further illustrated age-related attenuation in brachial FMD (Thijssen et al., 2009). Among three age groups, children aged 9-10 years had the highest brachial FMD (10.7 ± 4.9%) compared to young adults aged 20-41 (7.5 ± 3.1%) (mean ± sd) and older adults aged 50-66 years (6.0 ±2.9%).

In our study, children with FH or FCH aged 7-19 years had brachial FMD 5.27 ± 2.45%. But, children younger than 11 years old had brachial FMD 4.75 ± 2.85%.

Compared to the reported brachial FMD in Table 68, our findings were comparable to Aggoun and colleagues' report on 30 children with FH (Aggoun et al., 2000). In the general population, asymptomatic healthy 9- 11 years children were reported to have brachial FMD from 4.73 ± 4.38% (Leeson et al., 1997) to 10.0 ± 4.4 % (Hopkins et al., 2011). Such wide distribution of brachial FMD in children aged 9-11 years may indicate the importance of a better control in confounding factors.

In addition to shear rate, arterial diameter can be a significant confounding factor for brachial FMD. As reported by Pahkala and colleagues (2008), boys tended to have larger arterial diameters but lower brachial FMD than girls. The impact of arterial diameter on brachial FMD has been widely reported in the adult population as well (Herrington et al., 2001) in the past decade. Due to insufficient data, our study was not able to control both fluid shear rate and arterial diameters.

High Sensitivity C Reactive Protein

In our study, total cholesterol, LDL-C, nonHDL-C, age, BMI and glucose were identified to be significantly correlated with inflammation measured by hsCRP. However, only glucose and nonHDL-C were significant predictors for LoghsCRP in the linear regression analyses. The positive association between BMI and hsCRP in FH children was also reported by Ueland and colleagues (2006). Our finding of inverse correlation between hsCRP and nonHDL-C was uncommon. Traditionally, positive correlation between total cholesterol and hsCRP has reported in children with FH and FCH (Guardamagna et al., 2009).

Our finding of glucose as significant predictors for hsCRP in FH and FCH children has not been reported commonly. In our analysis, increased fasting blood

glucose was associated with increased inflammation when nonHDL-C was controlled. In additional independent samples t-tests, increased fasting blood glucose above 84 mg/dl was associated with decreased brachial FMD and increased hsCRP. In addition, the effect of fasting blood sugar was significant and moderate in children 15 years and older.

Even with a small sample size like ours, the effects of fasting blood glucose on brachial FMD and hsCRP were statistically significant and moderate. The role of increased fasting blood glucose on endothelial function in familiar hyperlipidemia children has not been well studied. In children with type 1 diabetes, endothelial dysfunction was detected in children with higher LDL-C concentration, but not in children with normal LDL-C concentration (Jarvisalo et al., 2004). Increased LDL-C concentration with increased fasting blood glucose may have a synergic effect on atherogenesis. Further investigation will certainly verify our findings and improve our knowledge about controlling hyperlipidemia and hyperglycemia in children

In our study, children with FH had different lipid abnormality than children with FCH. Despite significantly increased total cholesterol, LDL-C, and nonHDL, children with FH also had significantly lower triglycerides and higher HDL-C. Children with FH were also significantly younger, thinner and had higher fasting blood glucose. Compared with children with FCH, children with FH had slightly higher brachial FMD. However, the difference in mean was not statistically significant. Although children with FH had slightly lower LoghsCRP, the difference in means was not statistically significant. The lower concentration of triglycerides, higher concentration of HDL and lower BMI seemed to have protective effects on endothelial function and inflammation. However, our data might be biased by an 18 years old girl with FCH who had BMI of 48 kg/m^2.

Due to our small sample size, further analysis without taking out subjects with extreme value was not conducted.

Significance

The results of our observation of the impact of fasting blood glucose on hsCRP and brachial FMD are intriguing. In the simple linear regression model, blood glucose showed a significantly positive correlation with hs-CRP. Blood glucose also showed a negative correlation with FMD. The association between high fasting blood glucose and high inflammation and low FMD in children with familial hyperlipidemia has not widely reported. In the clinical application, this finding suggests the importance of fasting blood sugar in hyperlipidemia children. Since familial hyperlipidemia children are already at increased risk of cardiovascular disease, increased fasting blood sugar may have a synergic effect to promote atherogenesis.

In our analyses, we found evidence of age-related variation in all lipid components. The variation of lipids might have distorted the associations between lipids and brachial FMD and hsCRP. As the impact of maturation on brachial FMD is not known in the subject children, our report of positive linear regression between age and brachial FMD needs to be interpreted with caution.

Limitation

There are a few notable limitations in this study. First, we studied 64 children with two different types of diseases. Two different diagnoses have shown different impacts on lipids distributions. Each lipid component may have different impacts on brachial FMD and hsCRP. With a small sample size, subgroup analysis may not be fruitful. Secondly, the study group consisted of children aged 7-19 years with undefined

physical maturation. Based on epidemiological studies, sexual maturation is a known contributory factor for biological variations in lipid. The current study did not assess children's sexual maturation, which has significantly compromised the validity of the findings. Third, brachial FMD is sensitive to small arterial diameters. In the current study, baseline arterial diameters were not completely assessed. The missing baseline arterial diameter might impact our analysis of brachial FMD.

Implications for Nursing

Children with familial hyperlipidemia are at increased risk for premature CHD. With a better understanding of FH and FCH, nurses can disseminate evidence-based knowledge to the community and improve public awareness. Especially, American children and adolescents are known to have increased lipid abnormality and obesity. As a complex disease, atherosclerosis is likely to advance faster in children with multiple CVD risk factors. Early detection can facilitate timely intervention. As a major player in the health care delivery system, educated nurses can appropriately address current shortfall in under diagnosing and under treatment of children and adolescents with lipid disorders.

In addition, a research approach that combines non-invasive vascular assessment with validated biomarkers can be easily adopted by nursing scientists. The non-invasive assessment of vascular reactivity can be frequently employed to detect and monitor children at risk for CVD. The method has been validated and proven reproducibility. With proper training, this methodology can be readily adopted and utilized in future scientific investigations.

Future Research

This research project led to some very important observations. Children with FH and FCH have significantly different lipid presentations and should not be analyzed as a group. The atherogenic lipid profiles in children with FH and FCH may not be fully assessed by traditional values of total cholesterol, LDL-C, HDL-C and triglyceride. A new measure for atherogenic lipid will be helpful. Biological variation of lipid and vascular reactivity in children can be significant, while repeat measures may improve accuracy. Children in the same age group may not have the same growth rate, so the maturation stage should be assessed repeatedly.

Since children with FH and FCH are at higher risk for advanced atherosclerosi, a comprehensive assessment of their vascular health is crucial. Non-invasive ultrasound assessment of carotid intima thickness and pulse wave analysis and pulse wave velocity analysis may be incorporated into such a comprehensive assessment. A better understanding of the pathological mechanisms of atherosclerosis can enhance CVD prevention. Better prevention can translate into a significant reduction in CVD related mortality and morbidity.

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Table 1.

Frequency Analysis of Diagnosis

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	FH	33	51.6	51.6	51.6
	FCH	31	48.4	48.4	100.0
	Total	64	100.0	100.0	

Note. FH-familial hypercholesterolemia; FCH-familial combined hyperlipidemia

Table 2.

Frequency Analysis of Genders

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	30	46.9	46.9	46.9
	Female	34	53.1	53.1	100.0
	Total	64	100.0	100.0	

Table 3.

Descriptive Analysis of Lipids

		TC	TG	LDL-C	HDL-C	nHDL-C
N	Valid	64	64	64	64	64
	Missing	0	0	0	0	0
Mean		256.5625	125.2813	189.2969	46.7656	209.7813
Median		251.5000	113.5000	186.0000	45.0000	209.0000
Std. Deviation		69.37244	70.31075	71.50351	11.14656	68.33937
Minimum		132.00	39.00	72.00	22.00	85.00
Maximum		468.00	410.00	410.00	70.00	417.00

Note. TC- total cholesterol; TG- triglycerides; LDL-C-low density lipoprotein cholesterol; HDL-C- high density lipoprotein cholesterol; nonHDL-C- non high density lipoprotein cholesterol

Table 4.

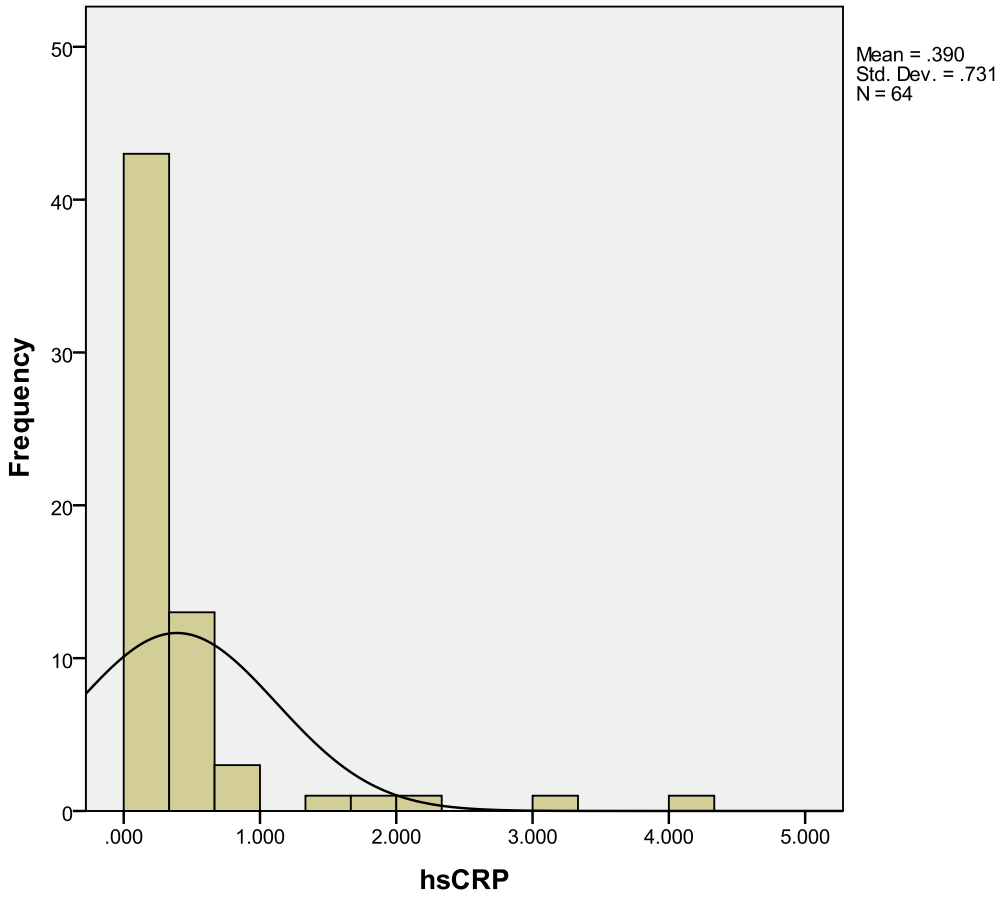
Descriptive Analysis of Non Lipids

		Age	BMI	Sys BP	Dia BP	HR	Glucose
N	Valid	64	64	64	64	64	64
	Missing	0	0	0	0	0	0
Mean		12.188	22.0466	114.945	59.164	73.969	84.8281
Median		11.500	21.1750	114.500	57.500	71.500	85.0000
Std. Deviation		2.8165	5.58409	10.7612	9.4403	15.1406	9.09309
Minimum		7.0	14.64	83.0	37.0	51.0	62.00
Maximum		19.0	48.28	140.0	86.0	115.0	103.00

Note. BMI- body mass index; Sys BP-systolic blood pressure; Dia BP-diastolic blood pressure; HR-heart rate; Glucose-fasting blood glucose

Table 5.

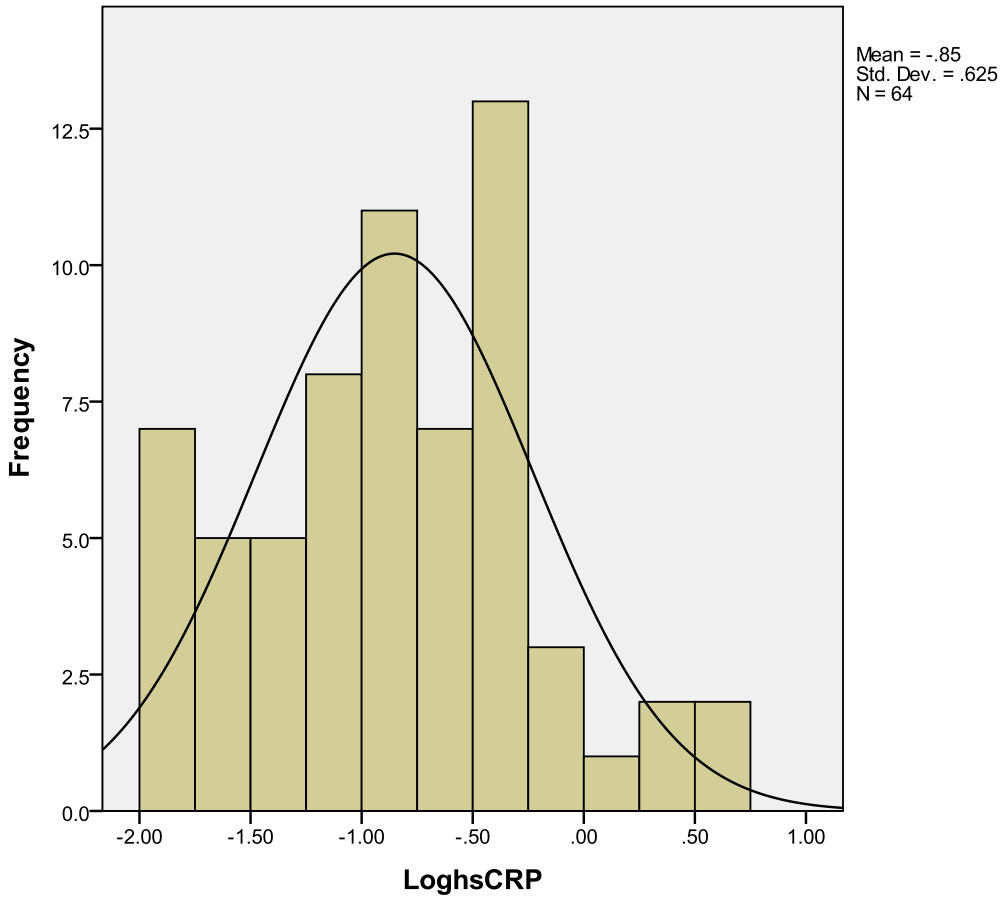
Frequency Histogram - hsCRP



Note. hsCRP-high sensitivity C reactive protein

Table 6.

Frequency Histogram - Log 10 hsCRP



Note. LoghsCRP- log10 high sensitivity C reactive protein

Table 7.
Descriptive Analysis of FMD, and hsCRP

		%FMD	HsCRP	LoghsCRP
N	Valid	64	64	64
	Missing	0	0	0
Mean		5.2684	.39030	-.8523
Median		4.8800	.11950	-.9226
Std. Deviation		2.44927	.730781	.62508
Minimum		.39	.017	-1.77
Maximum		10.30	4.270	.63

Note. %FMD-brachial flow-mediated dilation

Table 8.
Bivariate Pearson Correlations of FMD, HsCRP, Log10hsCRP,and nHDL-C

		%FMD	hsCRP	LoghsCRP	nHDL-C
%FMD	Pearson Correlation	1	-.095	-.147	-.024
	Sig. (2-tailed)		.458	.245	.853
	N	64	64	64	64
HsCRP	Pearson Correlation	-.095	1	.735**	-.239
	Sig. (2-tailed)	.458		.000	.058
	N	64	64	64	64
LoghsCRP	Pearson Correlation	-.147	.735**	1	-.282*
	Sig. (2-tailed)	.245	.000		.024
	N	64	64	64	64
nHDL-C	Pearson Correlation	-.024	-.239	-.282*	1
	Sig. (2-tailed)	.853	.058	.024	
	N	64	64	64	64

Note. **. Correlation is significant at the 0.01 level (2-tailed);
*. Correlation is significant at the 0.05 level (2-tailed).

Table 9.
Bivariate Pearson Correlations of FMD and Lipids

		%FMD	TC	TG	LDL-C	HDL-C
%FMD	Pearson Correlation	1	-.028	-.070	-.011	-.031
	Sig. (2-tailed)		.825	.585	.931	.808
	N	64	64	64	64	64
TC	Pearson Correlation	-.028	1	-.123	.969**	.171
	Sig. (2-tailed)	.825		.332	.000	.177
	N	64	64	64	64	64
TG	Pearson Correlation	-.070	-.123	1	-.193	-.462**
	Sig. (2-tailed)	.585	.332		.127	.000
	N	64	64	64	64	64
LDL-C	Pearson Correlation	-.011	.969**	-.193	1	.082
	Sig. (2-tailed)	.931	.000	.127		.518
	N	64	64	64	64	64
HDL-C	Pearson Correlation	-.031	.171	-.462**	.082	1
	Sig. (2-tailed)	.808	.177	.000	.518	
	N	64	64	64	64	64

Note. **. Correlation is significant at the 0.01 level (2-tailed).

Table 10.
Bivariate Pearson Correlations of Log 10 hsCRP and Lipids

		LoghsCRP	TC	TG	LDL-C	HDL-C
LoghsCRP	Pearson Correlation	1	-.271*	-.087	-.282*	.040
	Sig. (2-tailed)		.030	.492	.024	.752
	N	64	64	64	64	64
TC	Pearson Correlation	-.271*	1	-.123	.969**	.171
	Sig. (2-tailed)	.030		.332	.000	.177
	N	64	64	64	64	64
TG	Pearson Correlation	-.087	-.123	1	-.193	-.462**
	Sig. (2-tailed)	.492	.332		.127	.000
	N	64	64	64	64	64
LDL-C	Pearson Correlation	-.282*	.969**	-.193	1	.082
	Sig. (2-tailed)	.024	.000	.127		.518
	N	64	64	64	64	64
HDL-C	Pearson Correlation	.040	.171	-.462**	.082	1
	Sig. (2-tailed)	.752	.177	.000	.518	
	N	64	64	64	64	64

Note. *. Correlation is significant at the 0.05 level (2-tailed);
**. Correlation is significant at the 0.01 level (2-tailed).

Table 11.

Bivariate Paerson Correlations of FMD and Age

		%FMD	Age
%FMD	Pearson Correlation	1	.273*
	Sig. (2-tailed)		.029
	N	64	64
Age	Pearson Correlation	.273*	1
	Sig. (2-tailed)	.029	
	N	64	64

Note. *. Correlation is significant at the 0.05 level (2-tailed).

Table 12.

Bivariate Pearson Correlations of Log10hs CRP, Age, BMI, and Glucose

		LoghsCRP	Age	BMI	Glucose
LoghsCRP	Pearson Correlation	1	-.259*	.258*	.375**
	Sig. (2-tailed)		.039	.039	.002
	N	64	64	64	64
Age	Pearson Correlation	-.259*	1	.250*	-.268*
	Sig. (2-tailed)	.039		.046	.032
	N	64	64	64	64
BMI	Pearson Correlation	.258*	.250*	1	.255*
	Sig. (2-tailed)	.039	.046		.042
	N	64	64	64	64
Glucose	Pearson Correlation	.375**	-.268*	.255*	1
	Sig. (2-tailed)	.002	.032	.042	
	N	64	64	64	64

Note. *. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 13.
Linear Regression Model Summary – Dependent Variable FMD

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.273[a]	.074	.059	2.37542

Note. a. Predictors: (Constant), age

Table 14.
Linear Regression ANOVA Table – Dependent Variable FMD

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	28.088	1	28.088	4.978	.029[a]
	Residual	349.843	62	5.643		
	Total	377.931	63			

Note. a. Predictors: (Constant), age;
b. Dependent Variable: %FMD

Table 15.
Linear Regression Coefficients Table – Dependent Variable FMD

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.379	1.329		1.791	.078
	Age	.237	.106	.273	2.231	.029

Note. a. Dependent Variable: %FMD

Table 16.
Linear Regression Model Summary – Dependent Variable LoghsCRP

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.375[a]	.140	.126	.58423
2	.481[b]	.232	.207	.55676

Note. a. Predictors: (Constant), glucose;
b. Predictors: (Constant), glucose, nHDL-C

Table 17.
Linear Regression ANOVA Table – Dependent Variable LoghsCRP

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.453	1	3.453	10.116	.002[a]
	Residual	21.162	62	.341		
	Total	24.615	63			
2	Regression	5.707	2	2.853	9.205	.000[b]
	Residual	18.909	61	.310		
	Total	24.615	63			

Note. a. Predictors: (Constant), glucose;
b. Predictors: (Constant), glucose, nHDL-C;
c. Dependent Variable: LoghsCRP

Table 18.
Linear Regression Coefficients Table – Dependent Variable LoghsCRP

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-3.036	.691		-4.397	.000
	Glucose	.026	.008	.375	3.181	.002
2	(Constant)	-2.549	.682		-3.736	.000
	Glucose	.027	.008	.391	3.477	.001
	nHDL-C	-.003	.001	-.303	-2.696	.009

Note: a. Dependent Variable: LoghsCRP

Table 19.

T-Test Lipids by Age 9

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 9.0	61	257.5574	69.71956	8.92668
	< 9.0	3	236.3333	71.59842	41.33737
TG	>= 9.0	61	126.5902	71.09580	9.10288
	< 9.0	3	98.6667	54.50076	31.46603
LDL-C	>= 9.0	61	190.4918	71.94573	9.21171
	< 9.0	3	165.0000	69.54854	40.15387
HDL-C	>= 9.0	61	46.2131	11.08169	1.41886
	< 9.0	3	58.0000	5.56776	3.21455
nHDL-C	>= 9.0	61	211.3279	68.28048	8.74242
	< 9.0	3	178.3333	75.43430	43.55201

Table 20.

T-Test BMI and Fasting Blood Glucose by Age 9

	Age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 9.0	61	22.2202	5.59536	.71641
	< 9.0	3	18.5167	4.83589	2.79200
Glucose	>= 9.0	61	84.9016	9.26590	1.18638
	< 9.0	3	83.3333	5.03322	2.90593

Table 21.
T-Test FMD and LoghsCRP by Age 9

	age	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 9.0	61	5.4292	2.38194	.30498
	< 9.0	3	2.0000	1.39431	.80501
LoghsCRP	>= 9.0	61	-.8838	.61813	.07914
	< 9.0	3	-.2113	.44555	.25724

Table 22.
Leven's Test FMD by Age 9

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
%FMD Equal variances assumed	1.379	0.245	2.461	62	0.017
%FMD Equal variance not assumed			3.984	2.614	0.036

Table 23.
T-Test Lipids by Age 10

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 10.0	52	255.5192	69.37748	9.62093
	< 10.0	12	261.0833	72.24511	20.85537
TG	>= 10.0	52	125.6154	72.63909	10.07323
	< 10.0	12	123.8333	62.02468	17.90498
LDL-C	>= 10.0	52	188.4038	71.62902	9.93316
	< 10.0	12	193.1667	73.98751	21.35835
HDL-C	>= 10.0	52	45.8077	10.82125	1.50064
	< 10.0	12	50.9167	12.06391	3.48255
nHDL-C	>= 10.0	52	209.7115	68.11217	9.44546
	< 10.0	12	210.0833	72.37712	20.89347

Table 24.
T-Test BMI and Fasting Blood Glucose by Age 10

	Age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 10.0	52	22.3981	5.77056	.80023
	< 10.0	12	20.5233	4.59187	1.32556
Glucose	>= 10.0	52	84.0000	9.14373	1.26801
	< 10.0	12	88.4167	8.28425	2.39146

Table 25.
T-Test FMD and LoghsCRP by Age 10

	Age	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 10.0	52	5.3935	2.31178	.32059
	< 10.0	12	4.7267	3.03069	.87488
LoghsCRP	>= 10.0	52	-.9429	.59508	.08252
	< 10.0	12	-.4598	.62377	.18007

Table 26.
Leven's Test LoghsCRP by Age 10

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
LoghsCRP Equal variances assumed	0.071	0.790	-2.513	62	0.015
LoghsCRP Equal variance not assumed			-2.439	15.954	0.027

Table 27.

T-Test Lipids by Age 11

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 11.0	45	246.6667	69.58219	10.37270
	< 11.0	19	280.0000	64.68385	14.83949
TG	>= 11.0	45	128.2444	77.10800	11.49458
	< 11.0	19	118.2632	51.91215	11.90946
LDL-C	>= 11.0	45	180.2444	72.28987	10.77634
	< 11.0	19	210.7368	66.56062	15.27005
HDL-C	>= 11.0	45	44.4444	10.52174	1.56849
	< 11.0	19	52.2632	10.90281	2.50128
nHDL-C	>= 11.0	45	202.2222	68.92878	10.27530
	< 11.0	19	227.6842	65.19803	14.95745

Table 28.

Leven's Test HDL-C by Age 11

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
HDL-C Equal variances assumed	0.235	0.629	-2.687	62	0.009
HDL-C Equal variance not assumed			-2.648	32.861	0.012

Table 29.

T-Test Lipids by Age 12

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 12.0	32	248.3125	76.69060	13.55711
	< 12.0	32	264.8125	61.30539	10.83736
TG	>= 12.0	32	125.5938	76.94064	13.60131
	< 12.0	32	124.9688	64.23871	11.35591
LDL-C	>= 12.0	32	180.2188	80.33588	14.20151
	< 12.0	32	198.3750	61.37129	10.84901
HDL-C	>= 12.0	32	45.0625	10.42929	1.84366
	< 12.0	32	48.4688	11.73631	2.07471
nHDL-C	>= 12.0	32	203.2500	75.57479	13.35986
	< 12.0	32	216.3125	60.75835	10.74066

Table 30.

T-Test Lipids by Age 13

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 13.0	28	242.6786	68.09419	12.86859
	< 13.0	36	267.3611	69.36452	11.56075
TG	>= 13.0	28	129.1429	80.63898	15.23934
	< 13.0	36	122.2778	62.13654	10.35609
LDL-C	>= 13.0	28	173.8929	71.75490	13.56040
	< 13.0	36	201.2778	69.95411	11.65902
HDL-C	>= 13.0	28	44.6786	10.70844	2.02370
	< 13.0	36	48.3889	11.35726	1.89288
non HDL-C	>= 13.0	28	198.0000	67.87952	12.82802
	< 13.0	36	218.9444	68.22482	11.37080

Table 31.
T-Test Lipids by Age 14

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 14.0	19	262.1053	71.15312	16.32364
	< 14.0	45	254.2222	69.28692	10.32868
TG	>= 14.0	19	137.1579	84.32560	19.34562
	< 14.0	45	120.2667	63.89636	9.52511
LDL-C	>= 14.0	19	193.4737	73.37375	16.83309
	< 14.0	45	187.5333	71.46601	10.65352
HDL-C	>= 14.0	19	44.3158	9.42251	2.16167
	< 14.0	45	47.8000	11.74270	1.75050
non HDL-C	>= 14.0	19	217.7895	67.92936	15.58406
	< 14.0	45	206.4000	68.99190	10.28470

Table 32.
T-Test Lipids by Age 15

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 15.0	12	254.2500	80.33354	23.19029
	< 15.0	52	257.0962	67.46423	9.35561
TG	>= 15.0	12	146.3333	96.78217	27.93861
	< 15.0	52	120.4231	62.91399	8.72460
LDL-C	>= 15.0	12	180.2500	82.46335	23.80512
	< 15.0	52	191.3846	69.46466	9.63302
HDL-C	>= 15.0	12	45.0000	11.08644	3.20038
	< 15.0	52	47.1731	11.22798	1.55704
non HDL-C	>= 15.0	12	209.2500	75.78813	21.87815
	< 15.0	52	209.9038	67.30681	9.33377

Table 33.
T-Test Lipids by Age 16

	Age	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 16.0	9	255.3333	88.18305	29.39435
	< 16.0	55	256.7636	66.80041	9.00737
TG	>= 16.0	9	169.4444	101.57523	33.85841
	< 16.0	55	118.0545	62.13498	8.37828
LDL-C	>= 16.0	9	178.6667	91.69515	30.56505
	< 16.0	55	191.0364	68.53708	9.24154
HDL-C	>= 16.0	9	41.6667	9.59166	3.19722
	< 16.0	55	47.6000	11.23750	1.51526
non HDL-C	>= 16.0	9	213.6667	83.54041	27.84680
	< 16.0	55	209.1455	66.42133	8.95625

Table 34.
Leven's Test Triglycerides by Age 16

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
TG Equal variances assumed	1.986	0.164	2.086	62	0.041
TG Equal variance not assumed			1.473	0.175	0.175

Table 35.
T-Test FMD and LoghsCRP by Age 11

	age	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 11.0	45	5.4858	2.25952	.33683
	< 11.0	19	4.7537	2.84929	.65367
LoghsCRP	>= 11.0	45	-.9438	.61584	.09180
	< 11.0	19	-.6355	.60820	.13953

Table 36.
T-Test FMD and LoghsCRP by Age 11

	age	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 12.0	32	5.8106	2.43917	.43119
	< 12.0	32	4.7262	2.37379	.41963
LoghsCRP	>= 12.0	32	-.9876	.63242	.11180
	< 12.0	32	-.7170	.59690	.10552

Table 37.
T-*Test FMD and LoghsCRP by Age 13*

	age	N	Mean	Std. Deviation	Std. Error Mean
% FMD	>= 13.0	28	5.9525	2.52623	.47741
	< 13.0	36	4.7364	2.28241	.38040
LoghsCRP	>= 13.0	28	-.9539	.63336	.11969
	< 13.0	36	-.7733	.61576	.10263

Table 38.
Leven's Test FMD and Log10hsCRP by Age 13

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
%FMD Equal variances assumed	0.632	0.430	2.018	62	0.048
%FMD Equal variance not assumed			1.992	55.050	0.051
LoghsCRP Equal variances assumed	0.378	0.541	2.268	62	0.255
LoghsCRP Equal variances not assumed			2.290	57.371	0.257

Table 39.
T-Test FMD and LoghsCRP by Age 14

	age	N	Mean	Std. Deviation	Std. Error Mean
% FMD	>= 14.0	19	6.2489	2.72670	.62555
	< 14.0	45	4.8544	2.22651	.33191
LoghsCRP	>= 14.0	19	-1.0271	.65912	.15121
	< 14.0	45	-.7785	.60245	.08981

Table 40.
Leven's Test FMD & Log10hsCRP by Age 14

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
%FMD Equal variances assumed	1.413	0.239	2.139	62	0.036
%FMD Equal variance not assumed			1.969	28.633	0.059
LoghsCRP Equal variances assumed	1.025	0.315	-1.467	62	0.147
LoghsCRP Equal variances not assumed			-1.414	31.343	0.167

Table 41.
T-Test FMD and LoghsCRP by Age 15

	age	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 15.0	12	6.2600	2.82788	.81634
	< 15.0	52	5.0396	2.32398	.32228
LoghsCRP	>= 15.0	12	-1.0989	.74415	.21482
	< 15.0	52	-.7954	.58789	.08153

Table 42.
T-Test FMD and LoghsCRP by Age 16

	age	N	Mean	Std. Deviation	Std. Error Mean
% FMD	>= 16.0	9	5.4411	2.71984	.90661
	< 16.0	55	5.2402	2.42838	.32744
LoghsCRP	>= 16.0	9	-.9244	.78407	.26136
	< 16.0	55	-.8405	.60312	.08132

Table 43.
T-Test BMI and Fasting Blood Glucose by Age 11

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 11.0	45	22.8578	5.95189	.88726
	< 11.0	19	20.1253	4.12326	.94594
Glucose	>= 11.0	45	84.0444	9.71165	1.44773
	< 11.0	19	86.6842	7.32615	1.68073

Table 44.
T-Test BMI and Fasting Blood Glucose by Age 12

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 12.0	32	23.1178	6.22608	1.10063
	< 12.0	32	20.9753	4.71559	.83361
Glucose	>= 12.0	32	82.9063	9.33381	1.65000
	< 12.0	32	86.7500	8.56098	1.51338

Table 45.
T-Test BMI and Fasting Blood Glucose by Age 13

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 13.0	28	23.6379	6.33775	1.19772
	< 13.0	36	20.8089	4.64116	.77353
Glucose	>= 13.0	28	83.1071	9.72716	1.83826
	< 13.0	36	86.1667	8.46337	1.41056

Table 46.
T-Test BMI and Fasting Blood Glucose by Age 14

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 14.0	19	24.0816	7.08215	1.62476
	< 14.0	45	21.1873	4.64597	.69258
Glucose	>= 14.0	19	81.6842	11.04059	2.53289
	< 14.0	45	86.1556	7.90269	1.17806

Table 47.
T-Test BMI and Fasting Blood Glucose by Age 15

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 15.0	12	24.1492	8.24554	2.38028
	< 15.0	52	21.5613	4.75127	.65888
Glucose	>= 15.0	12	79.8333	9.63736	2.78207
	< 15.0	52	85.9808	8.65344	1.20002

Table 48.
Leven's Test Fasting Blood Glucose by Age 15

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
Glucose Equal variances assumed	0.392	0.534	-2.172	62	0.034
Glucose Equal variance not assumed			-2.029	15.359	0.06

Table 49.
T-Test BMI and Fasting Blood Glucose by Age 16

	age	N	Mean	Std. Deviation	Std. Error Mean
BMI	>= 16.0	9	25.4578	9.16540	3.05513
	< 16.0	55	21.4884	4.65589	.62780
Glucose	>= 16.0	9	81.0000	9.34077	3.11359
	< 16.0	55	85.4545	8.98315	1.21129

Table 50.
Leven's Test BMI by Age 16 Years

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
BMI Equal variances assumed	2.951	0.091	2.025	62	0.047
BMI Equal variance not assumed			1.273	8.688	0.236

Table 51.
T-Test FMD and Log10hsCRP by Glucose 84 mg/dl

	glucose	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 84.00	40	4.6685	2.38119	.37650
	< 84.00	24	6.2683	2.26894	.46315
LoghsCRP	>= 84.00	40	-.7193	.61369	.09703
	< 84.00	24	-1.0739	.59128	.12069

Table 52.
Leven's Test FMD and Log10hsCRP by Glucose 84 mg/dl

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
%FMD Equal variances assumed	0.045	0.833	-2.648	62	0.010
%FMD Equal variance not assumed			-2.680	50.450	0.010
LoghsCRP Equal variances assumed	0.105	0.747	2.268	62	0.027
LoghsCRP Equal variances not assumed			2.290	50.018	0.026

Table 53.
T Test Lipid by Glucose 84 mg/dl

	Glucose	N	Mean	Std. Deviation	Std. Error Mean
TC	>= 84.00	40	260.3500	73.14634	11.56545
	< 84.00	24	250.2500	63.58647	12.97953
TG	>= 84.00	40	121.9500	59.75418	9.44796
	< 84.00	24	130.8333	86.22804	17.60123
LDL-C	>= 84.00	40	192.0750	75.45091	11.92984
	< 84.00	24	184.6667	65.69340	13.40961
HDL-C	>= 84.00	40	47.1750	12.11248	1.91515
	< 84.00	24	46.0833	9.52761	1.94482
nHDL-C	>= 84.00	40	213.1500	72.85058	11.51869
	< 84.00	24	204.1667	61.16099	12.48443

Table 54.
T-Test FMD and Log10hsCRP by nonHDL-C 145 mg/dl

	nHDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 145.00	51	5.1661	2.50876	.35130
	< 145.00	13	5.6700	2.24734	.62330
LoghsCRP	>= 145.00	51	-.8704	.61660	.08634
	< 145.00	13	-.7814	.67844	.18816

Table 55.
T-Test FMD and Log10hsCRP by nonHDL-C 190 mg/dl

	nHDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 190.00	38	5.1774	2.61431	.42410
	< 190.00	26	5.4015	2.22958	.43726
LoghsCRP	>= 190.00	38	-.9098	.55327	.08975
	< 190.00	26	-.7683	.72057	.14131

Table 56.
T-Test FMD and LoghsCRP by LDL-C 160 mg/dl

	LDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 160.00	40	5.1652	2.59875	.41090
	< 160.00	24	5.4404	2.22054	.45327
LoghsCRP	>= 160.00	40	-.9371	.59261	.09370
	< 160.00	24	-.7109	.66435	.13561

Table 57.
T-Test FMD and Log10hsCRP by LDL-C 155 mg/dl

	LDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 155.00	43	5.1395	2.54868	.38867
	< 155.00	21	5.5324	2.26860	.49505
LoghsCRP	>= 155.00	43	-.9173	.58747	.08959
	< 155.00	21	-.7191	.69157	.15091

Table 58.
T-*Test FMD and Log10hsCRP by LDL-C 135 mg/dl*

	LDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 135.00	48	5.2123	2.53777	.36630
	< 135.00	16	5.4369	2.23059	.55765
LoghsCRP	>= 135.00	48	-.9179	.56918	.08215
	< 135.00	16	-.6556	.75557	.18889

Table 59.
T-Test FMD and Log10hsCRP by LDL-C 130 mg/dl)

	LDL-C	N	Mean	Std. Deviation	Std. Error Mean
%FMD	>= 130.00	48	5.2123	2.53777	.36630
	< 130.00	16	5.4369	2.23059	.55765
LoghsCRP	>= 130.00	48	-.9179	.56918	.08215
	< 130.00	16	-.6556	.75557	.18889

Table 60.

T Test – Lipids by FH and FCH

	DX	N	Mean	Std. Deviation	Std. Error Mean
TC	FH	33	282.1818	80.70295	14.04858
	FCH	31	229.2903	40.84866	7.33664
TG	FH	33	89.7879	45.39876	7.90291
	FCH	31	163.0645	72.95338	13.10281
LDL-C	FH	33	218.3636	82.22029	14.31272
	FCH	31	158.3548	40.09035	7.20044
HDL-C	FH	33	49.7576	10.93169	1.90296
	FCH	31	43.5806	10.63571	1.91023
nHDL-C	FH	33	232.3939	81.51723	14.19033
	FCH	31	185.7097	39.47505	7.08993

Table 61.
Leven's Test Lipids by FH and FCH

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
TC Equal variances assumed	10.598	0.002	3.275	62	0.002
Equal variance not assumed			3.337	48.025	0.002
TG Equal variances assumed	3.861	0.054	-4.856	62	0.000
Equal variances not assumed			-4.789	49.639	0.000
LDL-C Equal variances assumed	10.975	0.002	3.673	62	0.001
Equal variance not assumed			3.745	47.034	0.000
HDL-C Equal variances assumed	0.226	0.637	2.289	62	0.026
Equal variance not assumed			2.291	61.919	0.025
nHDL-C Equal variances assumed	12.281	0.001	2.886	62	0.005
Equal variances not assumed			2.943	46.856	0.005

Table 62.
T-Test Age, BMI, & Glucose by FH and FCH

	DX	N	Mean	Std. Deviation	Std. Error Mean
Age	FH	33	11.455	2.4379	.4244
	FCH	31	12.968	3.0164	.5418
BMI	FH	33	19.8885	2.97858	.51850
	FCH	31	24.3439	6.74110	1.21074
Glucose	FH	33	85.2727	7.32834	1.27570
	FCH	31	84.3548	10.76584	1.93360

Table 63.
Leven's Test Age, BMI, and Glucose by FH and FCH

	Leven's Test for Equality of Variances		t-test for Equality of Means		
	F	Sig	t	df	Sig. (2-tailed)
Age Equal variances assumed	1.445	0.234	-2.213	62	0.031
Equal variance not assumed			-2.199	57.733	0.032
BMI Equal variances assumed	9.325	0.003	-3.456	62	0.01
Equal variances not assumed			-3.383	40.729	0.02
Glucose Equal variance assumed	5.855	0.018	0.401	62	0.690
Equal variance not assumed			0.396	52.479	0.694

Table 64.

T-Test FMD and LoghsCRP by FH and FCH

	DX	N	Mean	Std. Deviation	Std. Error Mean
%FMD	FH	33	5.2915	2.56151	.44590
	FCH	31	5.2439	2.36596	.42494
LoghsCRP	FH	33	-.8927	.57673	.10040
	FCH	31	-.8093	.67972	.12208

Table 65.
T-Test Lipids by Gender

	1-Male, 2-Female	N	Mean	Std. Deviation	Std. Error Mean
TC	1	30	260.3667	49.83939	9.09939
	2	34	253.2059	83.54595	14.32801
TG	1	30	120.6000	62.89762	11.48348
	2	34	129.4118	76.96560	13.19949
LDL-C	1	30	194.8333	51.56120	9.41374
	2	34	184.4118	85.86004	14.72488
HDL-C	1	30	44.7333	9.13475	1.66777
	2	34	48.5588	12.52196	2.14750
nHDL-C	1	30	215.6000	47.50579	8.67333
	2	34	204.6471	82.91430	14.21968

Table 66.
T-Test Non-Lipid by Gender

	1-Male, 2-Female	N	Mean	Std. Deviation	Std. Error Mean
Age	1	30	12.167	2.6008	.4748
	2	34	12.206	3.0329	.5201
BMI	1	30	22.6073	4.12594	.75329
	2	34	21.5518	6.63562	1.13800
Glucose	1	30	83.7000	9.09016	1.65963
	2	34	85.8235	9.11366	1.56298

Table 67.
T-Test FMD and LoghsCRP by Gender

	1-Male, 2-Female	N	Mean	Std. Deviation	Std. Error Mean
%FMD	1	30	5.4060	2.33039	.42547
	2	34	5.1471	2.57830	.44217
LoghsCRP	1	30	-.9223	.56612	.10336
	2	34	-.7906	.67520	.11580

Table 68.

Brachial FMD in Children

Age, mean ± SD	No. of subjects	Baseline Brachial Artery Diameter (mm ± SD)	Brachial FMD, Mean % Change ± SD	Reference
13 13 13 13 13 13	95 male 79 male 79 male 77 female 107 female 46 female	2.99 ±0.32 3.03 ± 0.29 3.07 ± 0.32 2.81 ±0.27 2.72 ±0.28 2.74 ± 027	9.1 ± 4.3 (sedentary) 9.4 ±4.1 (moderately active) 10.1 ± 4.5 (active) 10.0 ± 4.2 (sedative) 9.3 ± 4.2 (moderately active) 10.6 ± 4.8 (active)	Pahkala, et al. (2008)
11 (Range 9-16)	59 male	3.1 ± 0.3	7.7 ± 4.0	Jarvisalo et al. (2002a)
11.1 ± 3.0	27 male 30 FH	3.0 ± 0.4 3.0 ± 0.5	9.3 ± 3.1 4.2 ± 2.9	Aggoun et al. (2000)
11	229 (53% male)	2.9 ± 0.3	9.10 ± 3.88	Kallio et al. (2007)
10.5 ± 0.9 10.4 ± 0.9 10.6 ± 1.6	40 (22 male) 20 (7 male) 19 (10 male)	3.0 ± 0.3 3.0 ± 0.3 3.1 ± 0.4	9.1 ± 4.4 (CRP < 0.1 mg/l) 7.8 ± 3.3 (0.1 ≤ CRP ≤ 0.7 mg/l) 6.5 ± 2.6 (CRP > 0.7 mg/l)	Jarvisalo et al. (2002b)
Range 10 - 11	116 (70female, 46 male)	2.8±0.4 (June) 2.8±0.4 (November)	10.0 ± 4.4 (June) 7.9 ±3.9 (November)	Hopkins et al. (2011)
10	2176 (51% male)	2.7 ± 0.4	8.3 ± 3.1	Charakida et al. (2010)
10	282 (40% male)	2.7 ± 0.5	9.7 ±2.5	Charakida et al. (2005)
Range 9 - 11	333(168 male)	2.74 ± 0.32	4.73 ± 4.38	Leeson et al. (1997)
Range 8-11	229 (54% boys)	2.9 ± 0.3	9.10 ± 3.88%	Kallio et al. (2007)

Table 69.
NHANES 2005-2008, Lipids in Children

	Age 4-11 years	Age 12-19 years
Total Cholesterol (mg/dl)	Mean 164.5 Male 163.8 Female 165.2	Mean 159.2 Male 156.3 Female 162.3
LDL-C (mg/dl)		Mean 88.5 Male 87.1 Female 89.9
HDL-C (mg/dl)	Mean 54.7 Male 55.6 Female 53.6	Mean 51.6 Male 49.3 Female 54.0
TRIGLYCERIDES (mg/dl)		Mean 87.8 Male 87.2 Female 88.5

Note. Roger et al., 2011

Table 70.
NHANES - HsCRP in Children

NHANES 1999-2000 AGE 3 – 17 YEARS (Ford, 2003)	Male 0.14 ± 0.02 mg/dl	Female 0.17 ± 0.02 mg/dl
NHANES 1999-2004 AGE 3 – 16 YEARS (Dowd et al., 2010)	Mean 0.122 mg/dl	

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