ROLE OF VITAMIN D AND ADIPONECTIN IN ETHNIC-SPECIFIC DIFFERENCES IN BODY FAT DISTRIBUTION AND RISK FOR CARDIOVASCULAR DISEASE

by

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ABSTRACT

Background: Body fat distribution, in particular visceral adipose tissue (VAT), contributes to risk of cardiovascular disease (CVD). The Multicultural-Community Health Assessment Trial (M-CHAT) reported that South Asians have greater VAT than Europeans despite similar BMIs, putting them at greater risk of CVD. However, the molecular mechanisms underlying ethnic-specific differences in body fat distribution are unclear. Low circulating 25-hydroxyvitamin D (25OHD) and adiponectin concentrations are prevalent in individuals with obesity (BMI ≥ 30kg/m^2), and are associated with increased risk of CVD. Furthermore, 25OHD is inversely associated with blood pressure. Adiponectin is an adipokine that has insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties. Adiponectin circulates as isoforms low (LMW), medium (MMW), and the reported most biologically active isoform, high (HMW) molecular weight. This thesis aims to investigate ethnic-specific differences in the relationship between plasma 25OHD and adiponectin concentrations with body fat distribution and CVD risk factors.

Methods/Results: Europeans (n=171) and South Asians (n=176) from the M-CHAT cohort were assessed for demographics, plasma 25OHD, total and HMW adiponectin concentrations, and CVD risk factors. A computed tomography (CT) scan was used to quantify VAT and subcutaneous adipose tissue (SAT) deposition. South Asians had lower ($p < 0.001$) 25OHD in comparison to the Europeans (63.0 nmol/L vs. 39 nmol/L, respectively). VAT was inversely associated ($p < 0.05$) with 25OHD even after adjustment for age, sex, BMI, season of blood collection, SAT and total body fat (%). This suggests that VAT mediates the relationship between plasma 25OHD and CVD risk. Furthermore, the inverse association between systolic and diastolic blood pressure and 25OHD was no longer significant after adjustment for VAT. However, circulating plasma adiponectin concentrations remained strongly associated with

fasting plasma HDL-cholesterol, triglycerides, insulin, and HOMA-insulin resistance (HOMA-IR), even after adjustment for VAT. Interestingly, South Asians also had lower (p<0.001) total and HMW adiponectin concentrations compared to Europeans.

Conclusion: These findings demonstrate that plasma 25OHD and total and HMW adiponectin concentrations are associated with VAT deposition in Europeans and South Asians. Furthermore, plasma 25OHD and total and HMW adiponectin concentrations are lower in South Asians than the Europeans, which may contribute to elevated CVD risk in South Asians.

PREFACE

Statement of co-authorship:

This thesis has been prepared in partial fulfillment of the requirement for the degree of Master of Science in Pathology and Laboratory Medicine. I have prepared this thesis in its entirety under the direction and supervision of Dr. Angela Devlin and Dr. Scott Lear. This thesis was revised by Dr. Angela Devlin, Dr. Scott Lear, Dr. Tim Green, and Dr. John Hill.

This thesis is a cross-sectional observational study with study participants drawn from the Multi-Cultural Community Health Assessment Trial (M-CHAT), which is led by Dr. Scott Lear. Members of the Lear lab recruited and interviewed study participants to assess socio-demographic information, as well as collected blood samples. Blood analysis and body composition and body fat distribution measurements were performed at St. Paul's Hospital. I was responsible for conducting all experiments pertaining to DNA genotyping and adiponectin isoforms concentrations quantification. I performed all of the statistical analyses in consultation with Dr. Angela Devlin, Dr. Scott Lear, and Dr. Danijela Gasevic. I prepared all plasma samples for 25OHD quantification (BC Biomedical Laboratories Ltd., Surrey, BC, Canada).

I have prepared a manuscript pertaining to the findings of Aim 1 of this thesis and it has been accepted by *PLoS ONE* on July 17th, 2012.

This study was approved the Simon Fraser University Research Ethics Board (certificate: 38199) and the Providence Health/University of British Columbia Research Ethics Board (certificate: H07-00764), and the Children's and Women's Health Centre Research Ethics Board (certificate: H10-00106).

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LIST OF ABBREVIATIONS AND SYMBOLS

CVD	Cardiovascular disease
HSF	Heart and Stroke Foundation
WHO	World Health Organization
M-CHAT	Multi-Cultural Community Health Assessment Trial
CT	Computed tomography
BMI	Body mass index [mass (kg)/height^2(m^2)]
TG	Triglycerides
CHOL	Total cholesterol
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
T2DM	Type 2 diabetes mellitus
VAT	Visceral adipose tissue
FFA	Free fatty acid
25OHD	25-hydroxyvitamin D
IOM	Institute of Medicine
TAT	Total abdominal adipose tissue
SAT	Subcutaneous abdominal adipose tissue
ApoB	Apolipoprotein B
tHCY	Total homocysteine
ELISA	Enzyme-linked immunosorbent assay
SD	Standard deviation
HMW	High molecular weight
MMW	Medium molecular weight
LMW	Low molecular weight
CV	Coefficient of variability
HOMA-IR	Homeostatis model assessment-insulin resistance
GLM	General linear model
CI	Confidence interval
DBP	Vitamin D binding protein
Ca	Calcium
PTH	Parathyroid hormone

PCR	Polymerase chain reaction
FPI	Fasting plasma insulin
FPG	Fasting plasma glucose
VDR	Vitamin D receptor
RAS	Renin-angiotensin system
Ang	Angiotensin
ACE	Angiotensin-converting enzyme
GLUT4	Glucose transporter4
PPAR-α	Peroxisome proliferator-activated receptor-α
ABCA1	Adenosine triphosphate-binding cassette transported A1

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I would like to dedicate this page to express my deepest gratitude to very important individuals who have helped me throughout the journey of this Master's degree.

First and foremost, I would like to thank Dr. Angela Devlin, my supervisor and mentor, who has given me the opportunity to learn and grow in her lab for the past few years. Without her guidance, the completion of this Master's project would not have been possible.

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Last but not least, I would like to thank my closest friends who have been supportive and encouraging during the hardest parts of this journey.

DEDICATION

I would like take the opportunity to dedicate this work to my immediate family, and especially to my late mom, Siti Maryam.

Without their continuous support and unconditional love, I would not have been the courageous person that I am. From them, I learn how to strive and fully dedicate my time, energy and passion into every life journey that I endeavour.

I am greatly indebted to them and am blessed to be part of the family.

CHAPTER 1: Overview

Obesity is a risk factor for cardiovascular disease (CVD) [1,2]. According to the World Health Organization (WHO), 17.3 million deaths were attributed to CVD in 2008, accounting for 30% of deaths worldwide. This places CVD as the leading cause of death globally. In Canada, CVD is one of the top three causes of death and costs the economy $18B each year [3]. Furthermore, in the past 10-20 years the prevalence rates of overweight and obesity in adults have risen to 59% and 23%, respectively [4]. The increased prevalence of CVD has risen in parallel with the prevalence of overweight and obesity [5-7]. In United States alone the prevalence of obesity is significantly affected by ethnicity in such a way that adult non-Hispanic blacks and Hispanics have a higher prevalence, 44.1% and 38.7% respectively, in comparison to their Non-Hispanic white counterparts, 32.8% [8].

Overweight and obesity are defined as a body mass index (BMI) of 25.0 - 29.9 kg/m^2 and a BMI $\geq$ 30.0 kg/m^2, respectively. Obesity can be further stratified by sub-classifications and includes the following: mild obesity, BMI 30.0 - 34.9 kg/m^2; moderate obesity, BMI 35.0 - 39.9 kg/m^2; and severe obesity, BMI $\geq$ 40 kg/m^2. A BMI between 18.5 - 24.9 kg/m^2 is considered healthy. Despite the use of BMI to define obesity, a growing body of evidence demonstrates that BMI may not be the best predictor of CVD risk [9]. As early as 1940, it was suggested that the location where body fat accumulates (body fat distribution) is a better predictor of obesity-related diseases such as Type 2 diabetes mellitus (T2DM) and CVD, than increased total body weight (mass) [10,11].

The first scientific evidence pertaining to the importance of body fat distribution in predicting CVD risk came in 1982 when Kissebah et al. reported the association between body fat distribution and circulating glucose, insulin, and triglyceride (TG) concentrations [12]. This

study reported that women with upper body segment obesity (apple-shaped) had significantly higher plasma glucose, insulin, and TG concentrations compared to women with lower body segment obesity (pear-shaped). Waist to hip ratio (WHR) calculated from the measurement of waist and hip circumference was used to classify study participants as apple-shaped or pear-shaped obesity [12]. Furthermore, needle-biopsied subcutaneous fat cell size from the thigh showed no relationship to plasma glucose, insulin, and TG concentrations [12]. The following year, Krotkiewski et al reported that abdominal adiposity, determined by waist and hip ratio (WHR), was associated with higher fasting plasma glucose, insulin, and TG concentrations in men and women [13]. WHR was calculated by comparing waist circumference, measured in the standing position around the waist at a point one-third of the distance between the xiphoid process and the umbilicus, and hip circumference, measured in the erect position around the hip at a point 4cm below the anterior superior iliac spine [13]. Subcutaneous fat cell size obtained from the epigastric region, at the same point where waist circumference was measured, and subcutaneous fat cell size obtained from the hypogastric region, at the same point where hip circumference was measured, were associated with higher fasting plasma insulin, glucose and TG concentrations. Meanwhile, needle-biopsied subcutaneous fat cell size from the gluteal and femoral region were only associated with fasting plasma insulin, but not with fasting plasma glucose or TG concentrations [13]. The gluteal needle biopsies were obtained from the upper lateral quadrant of the gluteus maximus and the femoral region needle biopsies were obtained from a point where thigh circumferences were measured, which was at a point one-third of the distance from the anterior superior iliac spine to the patella [13]. The authors proposed that visceral adipocytes may play an important role in underlying the metabolic abnormalities associated with abdominal adiposity due to their connection to the portal circulation [13]. The

exact mechanism underlying the role of visceral adipocytes on metabolic abnormalities and CVD is not completely understood. A common hypothesis is that visceral adipocytes are in close proximity to the portal circulation causing the liver to be exposed to a high level of free fatty acids (FFA), which can cause abnormalities in hepatic metabolism contributing to hypertriglyceridemia, impaired glucose tolerance, and hyperinsulinemia [14].

Abdominal adiposity has also been associated with lower concentrations of high density lipoprotein cholesterol (HDLC) [10]. Furthermore, abdominal adiposity (determined by high WHR) has been associated with elevated diastolic blood pressure (BP) in men and women [13]. Subcutaneous fat cell size obtained from the epigastric and hypogastric regions were associated with elevated diastolic BP, while the subcutaneous fat cell size obtained from the femoral and gluteal regions were not associated with elevated BP [13]. High BP or hypertension, and hyperlipidemia, charcterized by elevated TG, low HDLC and high LDLC, are considered the major risk factors for CVD, in addition to smoking [15].

More recently, a large body of evidence has accumulated to suggest that body fat distribution plays an important role in the development of CVD and other chronic diseases such as type 2 diabetes mellitus (T2DM) [8-14]. Therefore, it is important to investigate what factors contribute to body fat distribution and how they further predict CVD risk. In particular, visceral adipose tissue (VAT) is reported to have the strongest association with cardiometabolic abnormalities associated with obesity, compared to other measures of adiposity such as total body fat and subcutaneous adipose tissue (SAT) [16,17] reviewed by [8]. Instead of using BMI as a measure of adiposity, it is more accurate to measure body fat distribution as an indicator of CVD risk [8,10,13]. At present, however, it is not fully understood what factors contribute to VAT deposition and how VAT contributes to CVD pathology.

Interestingly, researchers studying ethnic-specific differences in body fat distribution and CVD risk report that the rates and prevalence of obesity and CVD vary based on ethnicity, putting certain ethnic groups at a higher risk for cardiometabolic abnormalities [18-20]. A cross-sectional study investigating the relationship between BMI and body fat percentage reported that Singaporean Chinese, Malays, and Indians, have a higher body fat percentage compared to Europeans at a given BMI [21]. These ethnic-specific differences in body composition were also accompanied by differences in CVD risk [19,20]. A cross-sectional study in Chinese and European adults in the UK reported that the prevalence of glucose intolerance (defined in this study as fasting plasma glucose > 7.8 mmol/L) in Chinese men and women was similar or higher than their European counterparts (13.6% vs. 13.0% in men and 20.2% vs. 13.3% in women), despite lower BMIs and WHRs [20]. Futhermore, another cross-sectional study in Europeans and Indian Asians living in the UK also reported that the Indian Asians had significantly higher fasting plasma glucose and TG concentrations despite similar BMIs and WHRs than the Europeans [19].

As early as 1957, it was observed that deaths caused by CVD were higher among South Asians than other ethnic groups in various regions of the world (reviewed by [22]). A study from Singapore reported that the prevalence rates of CVD were seven times higher in Indian males than in Chinese based on an analysis of necropsy records undertaken between1950 to1954 [23]. A study from Uganda reported that 43% of deaths among South Asian men in Kempala during the period of 1956-58 were due to CVD, whereas no CVD deaths were reported in the African population [24]. In South Africa, CVD-associated mortality rates between 1955-1957 were 49% higher in Indian women compared to their European counterparts [25]. Similarly, the mortality rates between 1968 and 1977 were 45% higher in Indian men than the Europeans [26].

Furthermore, it was also reported that Indians living in Fiji had three times higher mortality rates of CVD (more specifically ischaemic heart disease) than their Melanesians counterparts during the period of 1971-80 [27]. Higher CVD-related mortality rates were also reported to be 40-60% higher among South Asians than their English and Welsh counterparts in London in the 1981 census [22]. In Canada, a cross-sectional study from Ontario confirmed that CVD prevalence rates and risk factors vary by ethnic group [18]. They reported that South Asians have the highest prevalence of CVD, 11%, compared to a prevalence of 5% in Europeans and 2% in Chinese. These ethnic-differences in CVD prevalence is a public health concern because studies from a decade ago reported that the prevalence rates of obesity among South Asians were on the rise and were around 10% in men and 20% in women, regardless of their geographical locations [28-30].

Increased adiposity has a direct consequence on the prevalence rates of CVD among South Asians [19,20]. Although VAT deposition has the strongest association with CVD risk factors, little is known about the effect of ethnicity on VAT deposition, other than reports in European and African-American men and women [31,32]. In a cross-sectional study based on a 10-year follow-up of the Coronary Artery Risk Development in Young Adults (CARDIA) longitudinal study in 190 African-Americans and 201 Caucasian Americans reported that African-American men had less VAT than their European counterparts (73.1 cm^2 vs. 99.3 cm^2) even after adjusted for total body fatness [31]. Meanwhile, African-American women had higher VAT (75.1 cm^2 vs. 58.6 cm^2) than European women, but the difference disappeared after adjustment for total body fatness [31]. Similarly, The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study reported that African-American men had significantly lower VAT than their European counterparts (74 cm^2 vs. 109 cm^2, $P < 0.0001$), but there was no

significant difference in VAT deposition among the African-American and European women in the study (67 cm^2 vs. 74 cm^2, respectively) [32]. The Multi-Cultural Community Health Assessment Trial (M-CHAT) was designed to assess the relationship between body composition and CVD risk in men and women of Aboriginal, Chinese, European, and South Asian descent [33]. One hundred men and 100 women each of Aboriginal, Chinese, South Asian, and European descent residing in Vancouver, BC were recruited such that there was equal representation of subjects with healthy, overweight, and obese BMIs within each sex/ethnic group [33]. Findings from the M-CHAT cohort revealed that South Asians have significantly higher VAT deposition compared to Europeans, even after adjustment for demographics, socio-economic status, age, sex, and BMI [34], and that VAT mediates the increased CVD risk in South Asians compared to Europeans [35].

Despite a large body of evidence suggesting VAT deposition is the strongest predictor of CVD risk, the mechanisms by which VAT deposition contributes to CVD pathology are not fully understood. As such, the goal of my thesis research is to begin to explore the biological factors that underlie the ethnic-specific differences in body composition and CVD risk reported for South Asians and Europeans in the M-CHAT cohort. Specifically, I have targeted vitamin D and adiponectin and assessed the ethnic-specific differences and the relationship between these two biological factors with body composition and CVD risk. My thesis research was conducted in collaboration with Dr Scott Lear, lead investigator of the M-CHAT cohort. I assessed previously collected blood samples from the M-CHAT participants, which had been stored for later analyses. The anthropometric, body composition, and body fat distribution measurements were previously collected in the M-CHAT participants and the initial published findings of the cohort [34-38] serve as the foundation for my thesis work.

My thesis project stemmed from the question: *How do ethnic-specific differences in VAT deposition contribute to CVD pathology?* The first target I chose to investigate was vitamin D. Several studies have reported a negative association between vitamin D status and CVD [39-41]. But most studies have been conducted in subjects of European decent and little is known about different ethnic groups. Furthermore, several recent studies have reported that adiposity is inversely associated with plasma 25-hydroxyvitamin D (25OHD), which is a biomarker for vitamin D status [42-46]. However, whether the association between circulating 25OHD concentrations and CVD is mediated by adiposity and body fat distribution, remain to be further investigated. Recently, there have been a handful of studies reporting a negative relationship between circulating 25OHD and VAT deposition in European populations [44-47]. However, little is known about this relationship in different ethnic populations.

The other biological factor that I investigated was adiponectin. This is because adipose tissue no longer serves solely as a storage organ, but rather an endocrine organ that secretes various adipocytokines involved in metabolic and inflammatory processes that can contribute to the pathophysiology of obesity-related abnormalities (reviewed by [48]). A large body of studies has reported that adiposity is inversely associated with adiponectin [49-54], and particularly with VAT [52,54]. Consequently, adiponectin has been reported to be strongly associated with fasting plasma insulin, glucose, HOMA-IR, HDL-C and TG concentrations [54-56] suggesting its insulin-sensitizing and anti-atherogenic properties. However, whether these associations are independent of body fat distribution, especially VAT, in South Asians and Europeans remain to be further investigated.

My first aim is to determine the relationship between plasma 25OHD concentrations and body composition in European and South Asian subjects from the M-CHAT cohort. Plasma

25OHD concentrations are also reported to be negatively associated with blood pressure. My second aim is to determine the relationship between plasma 25OHD concentrations and BP and whether VAT deposition mediates this relationship. Finally, my third aim is to explore the relationship between ethnic-specific differences in VAT deposition and CVD risk with adiponectin.

CHAPTER 2: Hypothesis and Aims, Study Participants, and General Assessments

2.1 Overall Hypothesis and Specific Aims

The overall **hypothesis** of my thesis is that *ethnic-specific differences in VAT deposition are accompanied by variations in plasma 25OHD and adiponectin concentrations and this contributes to CVD risk.* The following Specific Aims will address my hypothesis:

1. To determine if plasma 25OHD (vitamin D) concentrations are associated with body composition and body fat distribution in European and South Asian M-CHAT subjects.
2. To explore the relationship between plasma 25OHD concentrations and blood pressure and to determine the effect of ethnicity, body composition, and body fat distribution on this relationship.
3. To determine ethnic-specific differences in plasma total and high molecular weight adiponectin concentrations and to explore the relationship with body composition, body fat distribution, and CVD risk.

2.2 Multi-Cultural Community Health Assessment Trial (M-CHAT)

The study participants were recruited between 2004 to 2005 in Vancouver, BC as part of the M-CHAT cohort [33]. The cohort includes men and women between the age of 30 to 65 years old and are self-reported to be of either European or South Asian descent. Individuals were eligible to be part of M-CHAT if they were immigrants, or first or second generation Canadians, and have lived in Canada for > 3 years to allow acculturation. The European study participants include individuals originating from continental Europe, Ireland or the United Kindgom. The South Asian study participants include individuals originating from India, Pakistan, Bangladesh,

Nepal, or Sri Lanka. At the time of recruitment and enrollment in the study, participants had no known CVD diagnosis and must have had stable body weight in the past three months (no more than 2 kg of body weight lost or gained) [33]. As mentioned previously, an equal representation of participants with healthy, overweight, and obese BMIs in each ethnic group was ensured. Any known family history (a parent or sibling) of diabetes and CVD was also recorded. Blood tests, anthropometric, body composition, and body fat distribution measurements, and interviews were done at St Paul's Hospital, Vancouver, BC. Dr. Scott Lear is the principal investigator for the M-CHAT cohort (SFU).

2.3 General Methods

2.3.1. Biochemical Assessments

Fasting serum and plasma were obtained from participants by venipuncture following a 12-hour fasting period. Plasma total cholesterol (TC), high-density lipoprotein (HDLC), triglycerides (TG), apolipoprotein B (apo B), insulin, and glucose were quantified by the clinical lab at St Paul's Hospital using standard methods. Low-density lipoprotein (LDLC) was calculated using the Friedewald equation [35].

2.3.2. Anthropometric, body composition, and body fat distribution measurements

Body mass index (BMI) was calculated from body weight in kg divided by height in meters squared. Waist circumference was taken from an average of two readings midway between the lower point of rib margin and the iliac crest.

Total body fat and lean mass were quantified by dual-energy x-ray absorptiometry (DEXA) and the Norland XR-36 Scanner (Norland Medical Systems, White Plains, NY) [34].

Total abdominal adipose tissue (TAT) and VAT were quantified using a computed tomography (CT) scan taken from a single 10mm-thick cross-sectional slice at the lumbar 4/5 (L4/L5) of the intervertebral disc. Scan reading was done using the CTi Advantage Scanner (GE, Milwaukee, WI) and the computation of surface areas were performed using SliceOmatic v.4.2. (Tomovision, Montreal). TAT is defined as the total area in square centimeters (cm^2) within an attenuation range of (-190) to (-30) Hounsfield units for adipose tissue in the L4/L5 cross-sectional slice image. VAT is the total area in square centimeters (cm^2) within the aforementioned attenuation range that falls within the abdominal wall. Subcutaneous abdominal adipose tissue (SAT) was calculated as the difference between TAT and VAT.

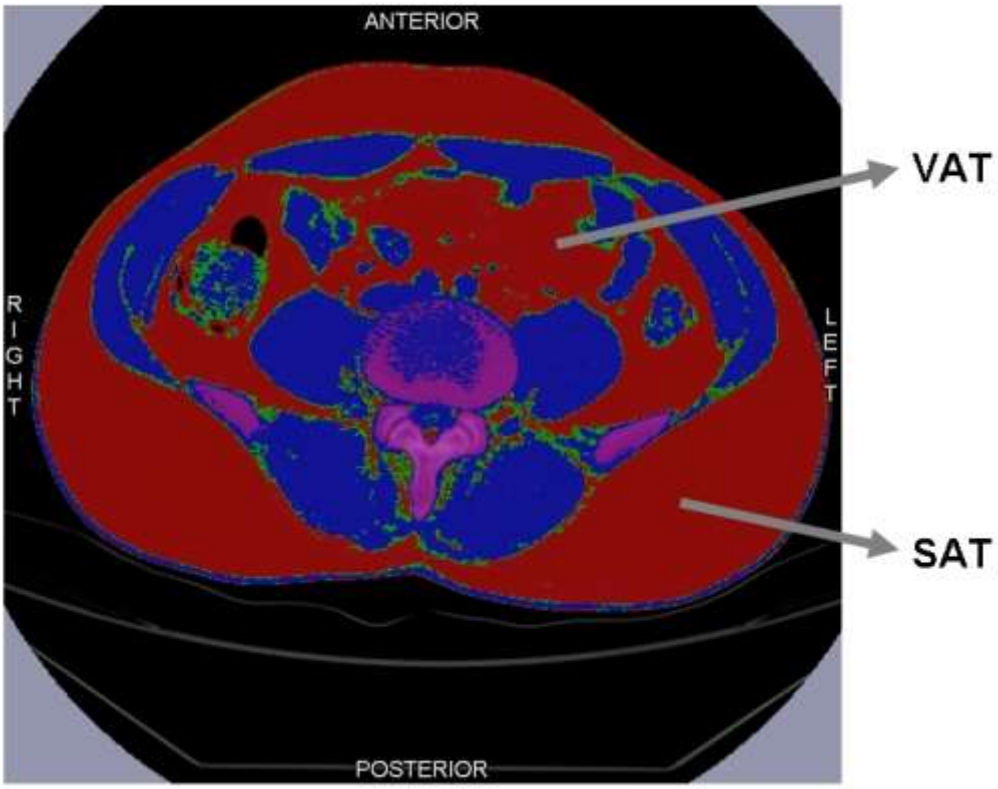


Figure 1. An example of study participant's CT-scan indicating the location of VAT and SAT (Image courtesy of Dr. Scott Lear).

2.3.3. Genomic DNA

Genomic DNA was extracted from whole blood using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). DNA concentrations were determined by spectrophotometry using a NanoVue^{TM} Plus Spectrophotometer (GE, Montreal) and the assumption that an optical density of 1.0 at 260 nm is equivalent to 50 μg/μl of genomic DNA. An A_{260}/A_{280} ratio of $\geq$1.8 was used as an indicator of genomic DNA purity.

CHAPTER 3: Role of Vitamin D in Ethnic-Specific Differences in Body Composition and Body Fat Distribution

3.1 Specific Aim 1

To determine if plasma 25OHD concentrations are associated with body composition and body fat distribution in European and South Asian M-CHAT subjects.

3.2 Background & Rationale

It is well-established that vitamin D plays a crucial role in bone metabolism [57-60]. Vitamin D deficiency leads to bone-related diseases such as rickets and osteomalacia. However, recent reports have shown that sub-optimal vitamin D status is also associated with several chronic diseases such as osteoporosis, cancer, multiple sclerosis, allergy, asthma, and CVD. Specifically, low vitamin D status has been associated with risk of CVD [35,61,62], and is negatively associated with hyperlipidemia [40] and hypertension [63,64]. An observational retrospective cohort of 10,899 participants reported that vitamin D deficiency was associated with several cardiovascular-related diseases such as hypertension and coronary artery disease [40]. Furthermore, vitamin D supplementation was reported to be associated with better survival in patients followed up by a cardiovascular practice in a medical center, especially among those who were previously vitamin D deficient [40].

Vitamin D is a fat-soluble vitamin and status is best assessed by quantifying 25-hydroxyvitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) concentrations, abbreviated as 25OHD, in plasma or serum. Vitamin D may be obtained from endogenous production by skin epithelial cells from the precursor, 7-dehydrocholesterol, and stimulation by UVB radiation [65-67]. Vitamin D can also be obtained from a small number of foods and supplements. Dietary

vitamin D is predominantly found in egg yolks, fatty fish, liver, and foods fortified with vitamin D such as milk [68]. Because vitamin D comes from skin synthesis, which is dependent on sunlight exposure, environmental factors have a major influence on vitamin D status. For example, factors that affect UVB radiation exposure reaching and penetrating the skin such as sunscreen use, skin colour, and latitude can affect vitamin D status [69-71]. Researchers in the US showed that African Americans with darker skin colour are less efficient at synthesizing vitamin D in response to sunlight exposure than their European counterparts [66]. Furthermore, to achieve maximum formation of pre-vitamin D3, individuals with darker skin colour require longer UVB radiation exposure times compared to their counterparts with lighter skin colour [66]. Living further away from the equator and above the 37° North (based on a study done in Boston, MA) or South parallels, is also associated with less UVB radiation exposure and therefore less efficient skin synthesis of vitamin D [67].

Circulating 25OHD concentrations are used to classify vitamin D status and the value associated with optimal status is actively being debated. Currently, the Institute of Medicine (IOM) suggests that that the population maintains circulating 25OHD concentrations above 50 nmol/L is for optimal bone outcomes [72]. However, it has been proposed that 25OHD greater than 75 to 80 nmol/L may be required for non-bone outcomes such as prevention of CVD and cancer [73-75]. For purposes of this thesis, I wlll define vitamin D insufficiency as circulating 25OHD less than 50 nmol/L. It is important to note that circulating 25OHD concentrations are an indicator of 25OHD available for conversion to the active form of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25OHD), as opposed to an indicator of vitamin D function. Circulating 25OHD concentrations are 1000 times more than that of the 1,25OHD concentrations [76]. Circulating 25OHD concentrations were chosen as a biomarker of vitamin D status because

circulating 25OHD concentrations are more stable and are present in the circulation at higher concentrations than 1,25OHD [76]. The half-life of 25OHD is approximately 3 weeks compared to a half-life of about 4 to 6 hours for 1,25OHD [68]. More importantly, 1,25OHD is tightly regulated under the control of PTH and ultimately serum calcium [68].

A schematic diagram illustrating vitamin D metabolism is represented by **Figure 2**.

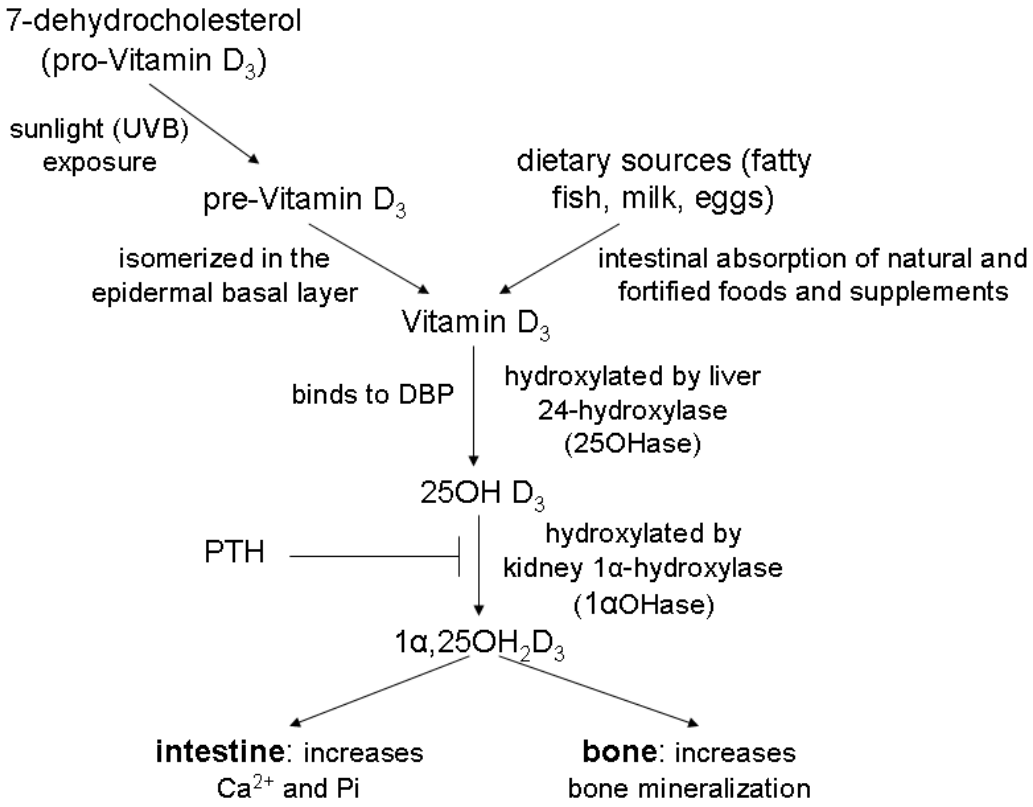


Figure 2. Vitamin D Metabolism (Adapted from [77]).

Vitamin D from endogenous and exogenous sources are converted to 25OHD in the liver by the enzyme 25-hydroxylase, which then binds to vitamin D binding protein (DBP) in the circulation, followed by transport to the kidney. In the kidney, 25OHD is converted to 1,25OH_2D, by 1α-hydroxylase under tight regulation of plasma PTH levels, as well as serum

calcium and phosphorus levels (reviewed by [78]). The biologically active form of vitamin D, 1,25OHD, is secreted into the circulation where it binds to DBP and is then transported to target tissues. The following are traditional targets for vitamin D: (i) bone, where it causes bone resorption of calcium; (ii) small intestine, where it functions to increase calcium absorption; and (iii) parathyroid glands, PTH concentrations are inversely correlated with active 1,25OHD, as PTH concentration increases it stimulates the conversion of 25OHD to active 1,25OHD in the kidney and PTH can directly affect calcium resorption in the bone causing increased concentrations of calcium in serum (reviewed by [78], [77]). Given that vitamin D status is negatively associated with several chronic diseases [41,79-81], it suggests that vitamin D has additional, as yet uncharacterized functional roles in other tissues, such as the central nervous system, cardiovascular system, immune system, and adipose tissue.

Poor vitamin D status (low circulating 25OHD concentrations) is common in obesity [42,43,82,83]. It has been suggested this is the result of low physical outdoor activity and therefore less sun exposure. However, an increasing body of evidence has reported that adiposity is inversely associated with vitamin D status independent of sunlight exposure [42-44], leading to the speculation that adipose tissue may sequester 25OHD, making it less available. In fact, recent studies have reported that VAT measured using CT-scan shows the strongest inverse association with circulating 25OHD concentrations compared with other measures of adiposity such as BMI and SAT in an American Caucasian population [44], young women in Quebec [45], and American children [84]. In addition, a cross-sectional study in African-Americans with type 2 DM reported that 25OHD concentrations were inversely associated with VAT, but not with SAT [46][85]. However, a similar study in 917 Hispanic-Americans and 439 African-Americans showed that 25OHD concentrations were inversely associated with VAT and SAT [46].

Similarly, a report in a Caucasian-American population from the Framingham cohort reported that 25OHD concentrations were inversely associated with VAT and SAT [44]. The report also highlighted that the highest rates of vitamin D deficiency, defined as 25OHD concentrations less than 50 nmol/L, was found among those with BMI $\geq$ 30 kg/m^2 and within the highest VAT deposition tertile [44]. A study among 43 women undergoing gynecological surgeries in Quebec reported that 25OHD concentrations were inversely associated with VAT, SAT, BMI, total body fat mass, and total adipose tissue [45]. Furthermore, the study also reported that dietary vitamin D intakes and plasma 25OHD concentrations were inversely associated with the omental and SAT samples obtained from the surgery [45]. Findings from the previous chapter also showed that 25OHD concentrations were inversely associated with VAT and SAT, but VAT seemed to have a stronger association with 25OHD concentrations.

In addition, findings of the Insulin Resistance Atherosclerosis (IRAS) Family Study in 917 Hispanic- and 439 African-Americans reported that 25OHD concentrations were inversely associated with BMI, SAT, and VAT (P<0.001), but were not associated with 5-year changes in adiposity [46]. A single-blinded randomized study in 33 healthy adults, reported that 25OHD concentrations were inversely associated with BMI and total body fat (measured with DEXA), and that vitamin D3 supplementation was associated with a significant increase in 25OHD concentrations in lower abdominal SAT biopsies [86]. Furthermore, a double-blinded, placebo-controlled trial investigating the role of calcium and vitamin D (100 IU) fortified orange juice on abdominal VAT deposition in 171 overweight and obese adults reported that supplementation was associated with a lowering of VAT deposition (P=0.001) [47].

A number of studies have identified that South Asians living outside their country of origin are more prone to low circulating 25OHD concentrations [87-91], presumably because of

their darker skin colour and less sun exposure. Even among healthy South Asians living in Delhi, in northern India, 25OHD concentrations were considerably low (lower than 50 nmol/L) [60]. These studies however, have been small and have not investigated other possible determinants. Other factors, such as excess VAT deposition, may contribute to low circulating 25(OH)D concentrations in South Asian populations.

To summarize, South Asians have higher prevalence rates of CVD [22-27] and low vitamin D status [87-91], and a unique phenotype of greater VAT deposition per kg of body fat [34], compared to Europeans. Given the recent reports of a negative association between VAT deposition and plasma 25OHD concentrations, I postulate that South Asians also have lower plasma 25OHD concentrations and this contributes to the CVD risk though its greater association with greater VAT depositon. In the first Aim of my thesis I will investigate whether ethnic-specific differences in body fat distribution and composition are associated with differences in plasma 25OHD concentrations in European and South Asian M-CHAT subjects.

3.3 Methods

3. 3.1 Plasma 25OHD concentrations

Plasma 25OHD was determined by BC Biomedical Laboratories Ltd (Surrey, BC) using a *DiaSorin* LIAISON® 25-OH Vitamin D TOTAL Assay, a competitive chemiluminescence immunoassay used for the quantitative determination of both $25OHD_2$ and $25OHD_3$ metabolites. BC Biomedical Laboratories Ltd. participates in the Vitamin D External Quality Assessment Scheme, an external quality control program for 25OHD measurement [92]. During the period in which plasma 25OHD concentrations were determined (April 2010) all controls were within 8% of the assigned mean value for the method.

3.3.2 Seasonality

Because of variability in plasma 25OHD concentrations due to season (and sun exposure), we stratified season of blood collection based on the estimated amount of sun exposure during these periods: (i) November to February; (ii) March to June; and (iii) July to October.

3.3.3 *GC* rs2282679 Variant Genotyping

Genetic variation may also account for differences in plasma 25OHD concentrations. A genome-wide association study reported that several genetic variants are strongly associated with plasma 25OHD concentrations [93,94]. Specifically, the rare allele of the rs2282679 variant in the gene encoding the DBP (*GC*) showed the strongest association with lower plasma 25OHD concentrations. Therefore, I genotyped the *GC* rs2282679 variant in the study participants and assessed the influence on ethnic-specific differences in plasma 25OHD concentrations.

Genotyping for the *GC* rs2282679 variant was conducted by Real-time PCR using Taqman genotyping reagents (Applied Biosystems) and TaqMan *GC* rs2282679 primers (Hs C__26407519_10). The genotyping call rate was 100%.

3.4 Statistical Analyses

T-tests were used to compare continuous variables and chi-squared tests were used to compare categorical variables between Europeans and South Asians. General linear models were used to analyze the association between plasma 25OHD concentrations and body composition (BMI, WC, and WHR) and body fat distribution (VAT, SAT, TAT (total abdominal adipose tissue)), total body fat (%)). Variables that are not normally distributed were transformed using the natural-log, as listed in **Table 1**. Subsequent regression models were further adjusted for

factors known to influence plasma 25OHD concentrations including BMI, age, sex, ethnicity, and season of blood collection. A significant interaction ($p < 0.05$) was observed between sex and season of blood collection. As such, separate models were used to assess the effects in men and women. There was, however, no interaction between VAT, plasma 25OHD and ethnicity.

3.5 Results

The baseline characteristics of the study participants are summarized in **Table 1**. In summary, South Asians had a significantly higher VAT, SAT, total body fat (%), and TAT ($p < 0.05$). This was accompanied by significantly lower ($p < 0.001$) plasma 25OHD concentrations compared to the Europeans (39.0 vs. 63.0 nmol/L, respectively).

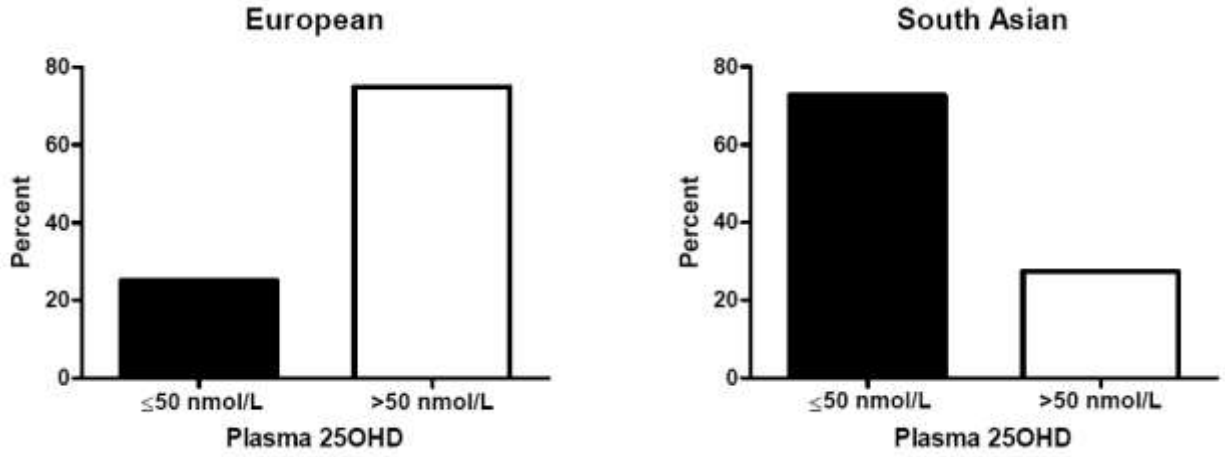
The prevalence of low vitamin D status (using the IOM definition of ≤50 nmol/L), was around 75% among South Asian participants and only 25% among the European participants.

Table 1. Baseline Study Participants Characteristics

Characteristics	**European (n=171)**	**South Asian (n=176)**	***P* Values**
Women, %	50	47	0.687
Season of blood collection (%)			0.008
Nov – Feb	32 (18)	42 (22)	
Mar – Jun	96 (53)	69 (37)	
Jul – Oct	54 (30)	77 (41)	
Age, y	50.8 ± 9.1	45 ± 8.4	<0.001
BMI, kg/m^2	27.8 ± 5.1	27.9 ± 5.0	0.867
Waist circumference, cm^2	89.7 ± 12.7	88.8 ± 12.3	0.508
Waist/hip ratio	0.88 ± 0.1	0.88 ± 0.1	0.447
Total body fat, %	32.5 ± 10.0	35.9 ± 9.3	0.001
Visceral adipose tissue, cm^2	102.1 (79.1, 145.0)	118.9 (88.3, 162.4)	0.013
Subcutaneous adipose tissue, cm^2	266.5 (198.8, 385.2)	309.4 (225.5, 389.43)	0.023
Total abdominal adipose tissue, cm^2	411.8 ± 177.0	448.9 ± 165.0	0.027
Plasma 25OHD, nmol/L	63.0 (50.0, 80.0)	39.0 (32.0, 51.0)	<0.001

Values presented are means ± SD. Subcutaneous and visceral adipose tissue, as well as plasma 25OHD were not normally distributed and were transformed using the natural log for statistical analyses; values presented are median (25^{th} percentile, 75^{th} percentile). Differences in variables between ethnic groups were assessed using t-test. Ethnic-specific differences in season of blood collection were assessed by Pearson's chi-squared test.

Figure 3. Low Vitamin D Status among European and South Asian Study Participants



Bivariate correlation analysis between plasma 25OHD and adiposity showed that plasma 25OHD was strongly negatively associated with BMI, total body fat (%), VAT, SAT, and TAT in both European and South Asian study participants (**Table 2**).

Table 2. Bivariate Correlation between Plasma 25OHD with Anthropometric Measures and Body Fat Distribution

	R-value	**Significance (P-Value)**
BMI	-0.195	<0.001
Waist circumference	-0.131	0.015
Waist/hip ratio	-0.056	0.302
Total abdominal adipose tissue	-0.312	<0.001
Ln VAT	-0.285	<0.001
Ln SAT	-0.289	<0.001
Total body fat (%)	-0.307	<0.001

Outcome: Ln (natural log transformed) circulating 25OHD.

r-Value: Pearson correlation coefficient.

Data were analyzed using unadjusted separate linear regression models.

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

A separate linear regression analysis for each measure of body fat distribution adjusted for confounding factors such as age, sex, BMI, and season of blood collection revealed that plasma 25OHD concentrations were negatively associated with TAT, VAT, SAT, and total body fat (%) in women ($p < 0.05$ for each variable) (**Table 3**). In men, plasma 25OHD concentrations were only negatively associated with VAT and total body fat (%). Ethnicity also came out significant for each measure of body fat distribution ($p < 0.05$).

Table 3. Relationship between Plasma 25OHD and Anthropometric Measures and Body Fat Distribution in Men and Women

	Women		**Men**	
	β-coefficient (standardized)	**Significance**	**β-coefficient** (standardized)	**Significance**
Waist circumference	-0.173	$p = 0.193$	-0.099	$p = 0.492$
Total abdominal adipose tissue	-0.492	$p = 0.002$	-0.199	$p = 0.067$
Ln VAT	-0.354	$p < 0.001$	-0.256	$p = 0.001$
Ln SAT	-0.258	$p = 0.042$	-0.122	$p = 0.195$
% Total body fat	-0.288	$p = 0.008$	-0.197	$p = 0.025$

Outcome: Plasma 25OHD.

Data were analyzed by separate linear regression models for men and women adjusted for ethnicity, age, BMI, and season of blood collection. Females and males were analyzed separately.

SAT, subcutaneous adipose tissue

VAT, visceral adipose tissue

$p < 0.05$ for ethnicity for all variables of body composition and body fat distribution.

In a regression analysis, it was revealed that plasma 25OHD was negatively associated with VAT in both men and women even after adjustment for sex, ethnicity, BMI, season of blood collection, along with VAT, SAT, and total body fat (%) in the same model ($p<0.05$, **Table 4**). In this model, all measures of body fat distribution were included in the same model. Ethnicity also came significant for each measure of body fat distribution ($p < 0.05$).

Table 4. Relationship of Plasma 25OHD with Body Fat Compartments in Men and Women

	Women		**Men**	
	β-coefficient (standardized)	**Significance**	**β-coefficient** (standardized)	**Significance**
BMI	0.149	$p = 0.246$	0.015	$p = 0.881$
Ln VAT	-0.289	$p = 0.005$	-0.217	$p = 0.016$
Ln SAT	-0.061	$p = 0.672$	0.020	$p = 0.865$
Total body fat (%)	-0.150	$p = 0.237$	-0.130	$p = 0.258$

Outcome: Plasma 25OHD.

Data were analyzed by linear regression models adjusted for ethnicity, age, BMI, and season of blood collection. VAT, SAT, and total body fat (%) were included in the same model. Females and males were analyzed separately.

SAT, subcutaneous adipose tissue

VAT, visceral adipose tissue

$p < 0.05$ for ethnicity for all variables of body composition and body fat distribution

To illustrate how VAT depositions are associated with plasma 25OHD concentrations, Figure 4a. and 4b. showed that increased VAT depositions (stratified into quartiles) were associated with decreased plasma 25OHD concentrations. I had separated the illustration based on ethnicity because South Asians have significantly higher VAT deposition than Europeans and therefore, the range of VAT quartiles in both ethnic groups were different. However, there was no interaction between VAT, plasma 25OHD concentrations and ethnicity. Therefore, there was no difference in the association between the VAT deposition and plasma 25OHD concentrations in Europeans and in South Asians. The only difference is that the South Asians had significantly higher VAT deposition compared to the Europeans.

Figure 4a. Plasma 25OHD Concentrations Stratified by VAT Quartiles in Europeans

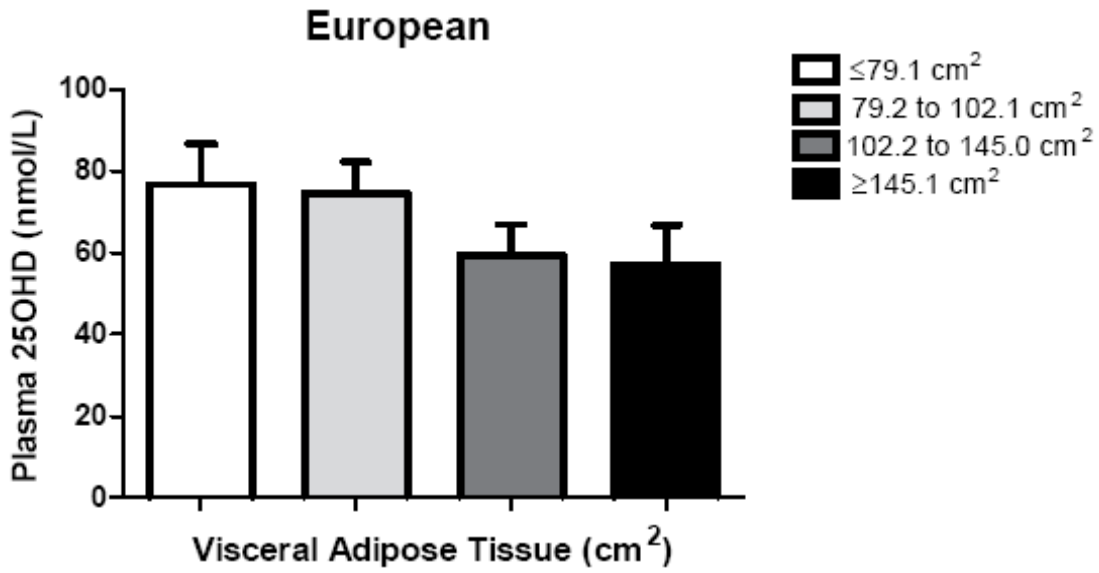


Figure 4a. illustrates the relationship between plasma 25OHD concentrations and VAT deposition in Europeans.

Figure 4b. Plasma 25OHD Concentrations Stratified by VAT Quartiles in South Asians

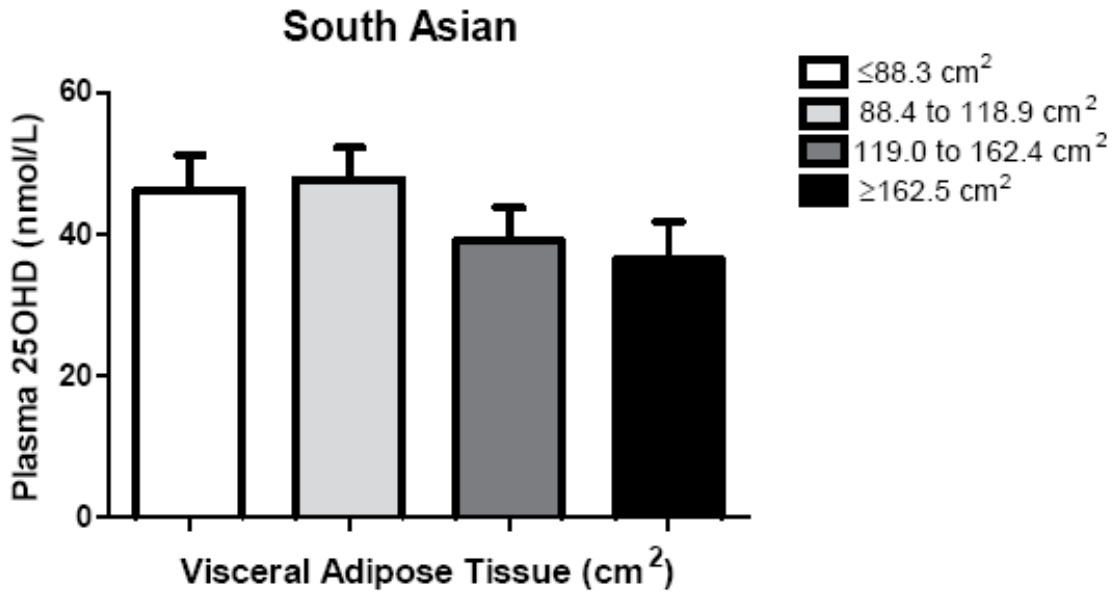


Figure 4b. illustrates the relationship between plasma 25OHD concentrations and VAT deposition in South Asians.

I also investigated the effect of *GC* rs2282679 on plasma 25OHD concentrations Europeans and South Asians. There were no significant differences on the concentrations of plasma 25OHD and *GC* rs2282679 genotype as illustrated in Figure 5. The allelic distributions of TT, GT, and GG carriers between Europeans and South Asians were almost similar such that the Europeans' was 50%, 43%, and 7%, respectively. Meanwhile, the South Asians' was 49%, 43%, and 8%.

Figure 5. Plasma 25OHD Concentrations by *GC* rs2282679 Genotype in Europeans and South Asians

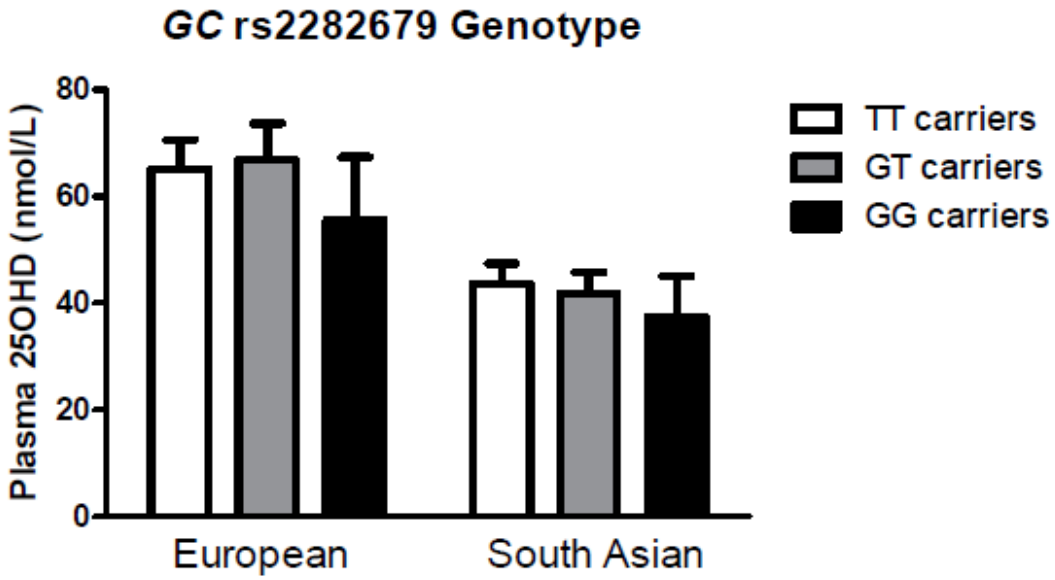


Figure 5. illustrates the concentrations of plasma 25OHD by *GC* rs2282679 genotype in Europeans and South Asians.

CHAPTER 4: Role of Visceral Adipose Tissue in the Relationship Between Plasma 25OHD Concentrations and Blood Pressure

4.1 Specific Aim 2

To explore the relationship between plasma 25OHD concentrations and BP and to determine the effect of ethnicity, body composition, and body fat distribution on this relationship.

4.2 Background & Rationale

Several studies have reported that vitamin D may play an important role in regulating BP. Findings of epidemiological studies have reported that circulating 25OHD concentrations are inversely associated with BP [64,95,96], and hypertension (self-reported) [97]. However, these studies are observational studies and do not provide evidence to discern whether low plasma 25OHD concentrations contribute to elevating BP or if elevated BP affects plasma 25OHD concentrations. Recent evidence suggests that vitamin D may play an important role in CVD risk [39,62], a phenomenon that may occur because of the effect of vitamin D on BP.

Blood pressure is tightly regulated by the renin-angiotensin system (RAS). In the kidney, angiotensinogen (AGN) is transformed by renin to become angiotensin (Ang) I, which is then further converted to become Ang II (a strong vasoconstrictor), in the presence and action of angiotensin-converting enzyme (ACE) [98]. Increased Ang II increases BP. In addition, alterations in calcium metabolism have also been shown to disturb BP homeostasis [99,100]. Because of the synergistic function of calcium and vitamin D, it is assumed that vitamin D also plays a role in the maintenance of BP [101,102]. Supplementation of vitamin D and calcium was reported to lower BP, as opposed to calcium supplementation alone in elderly women [103]. This suggests that vitamin D itself is a regulator of BP.

As for vitamin D status, BP is also affected by the environment, including reports of an increase in BP for every 10° latitude away from the equator [104], and ethnic-specific difference in BP [64,104-108]. A cross-sectional study among Europeans and Afro-Caribbeans in London reported that systolic BP was 6 mmHg higher in Afro-Caribbean than European men and 17 mmHg higher in Afro-Caribbean than European women [105]. Similarly, after adjustment for age and sex, African Americans were reported to have systolic BP 5.2 mmHg higher than Europeans [108]. Studies in the UK reported that individuals with darker skin colour had higher BP in comparison to their counterparts of European ethnicity [30,109-111]. A cross-sectional study reported that South Asians living in the UK had significantly higher age-adjusted systolic and diastolic BP compared to Europeans [30]. Similarly, a cross-sectional study based on a survey between 1991 and 1996 in London also reported that South Asians had significantly higher age-adjusted systolic and diastolic BP [111]. A longitudinal study in adolescents in the UK showed that those who self-reported being of Indian, Pakistani and Bangladeshi descents had significantly higher systolic and diastolic BP compared to their European, Afro-Caribbean and African counterparts [109]. However, another study based in the UK reported that the rates of hypertension (defined as systolic and diastolic BP > 160 and 95 mmHg, respectively) among South Asian men were similar to that of Europeans, despite the fact that South Asian men between the age of 40-49y showed higher diastolic BP than the Europeans [110]. Furthermore, compared to individuals with similar skin colour living abroad, those living in their countries of origin had lower BP and lower prevalence of hypertension [107,112,113]. This may be attributed by dietary changes (especially with sodium consumption) or lifestyle habits associated with elevated BP such as smoking and alcohol consumption (reviewed by [104]).

A study in 1980s reported that abdominal adiposity, defined as elevated WHR, was associated with elevated diastolic BP [13]. Furthermore, the study showed that SAT biopsies from the abdominal region (waist and hip) were associated with elevated diastolic BP, but the same association was not found with SAT fat cell size biopsies from the thigh region [13]. Based on the Third National Health and Nutrition Examination Survey (NHANES III) of 16,573 adults (aged ≥20 years), the inverse association between circulating 25OHD concentrations and systolic BP was weakened, although still significant but diastolic BP was no longer inversely associated with circulating 25OHD concentrations after adjustment for BMI [64]. Similarly, another study reported that following adjustment for BMI, systolic and diastolic BP were no longer inversely associated with circulating 25OHD concentrations in Hispanic-American and African-American populations [114]. Taken together, these studies suggest that BMI and adiposity may play a mediating role in the relationship between circulating 25OHD concentrations and BP.

It is unknown whether the association between 25OHD concentrations and BP is mediated by other measures of adiposity, such as VAT and SAT deposition. Circulating 25OHD concentrations are negatively associated with BP but the effect of other factors that influence circulating 25OHD concentrations, such as body composition and body fat distribution, on this relationship has not been investigated. In this Aim I will explore the relationship between plasma 25OHD concentrations and systolic and diastolic BP, and determine the effect of body composition and body fat distribution on this relationship. Given that I found 75% of the South Asians have low plasma 25OHD concentrations compared to 25% of the Europeans and I observed a negative relationship between VAT and plasma 25OHD concentrations, in Aim 2 my

goal is to assess whether body composition and body fat distribution influence the relationship between plasma 25OHD concentrations and blood pressure.

4. 3 Methods

4.3.1 Blood Pressure Measurements

Blood pressure was recorded following 10-mins of seated rest, and taken from an average of 5 successive measurements using an appropriate cuff-size and an automated oscillometric office blood pressure monitor (VSM MedTech Ltd, Coquitlam, Canada) [36].

4.3.2 *MTHFR* C677T Variant Genotyping

The C677T variant (rs1801133) in the gene encoding methylenetetrahydrofolate reductase (*MTHFR*) was genotyped. MTHFR is an enzyme that is required to convert folate to the metabolically active form, 5-methyltetrahydrofolate. Homozygosity for the *MTHFR* C677T variant was reported to be associated with hypertension in a European population [115] and a Chinese population [116], and has also been reported to be associated with increased risk of stroke [117] and coronary heart disease [118]. As such, I wanted to investigate whether this variant influenced BP in the European and South Asian M-CHAT subjects.

Genotyping of the *MTHFR* C677T variant was accomplished by Real-time PCR using TaqMan SNP Genotyping reagents and primers (assay number: C_12022883_20, ABI Life Technologies). The genotype call rate was 100%.

4.4 Statistical Analyses

T-tests were used to compare systolic and diastolic BP and chi-squared tests were used to compare categorical variables between Europeans and South Asians. Bivariate analysis was used

to investigate the association between BP and its predictors (sex, age, ethnicity, smoking, alcohol consumption, physical activity, *MTHFR* C677T genotype, BMI, VAT, and plasma 25OHD concentrations). General linear models were used to analyze the relationship between plasma 25OHD concentrations and predictors of BP, as well as the interactions between covariates (age, physical activity, BMI, VAT, and plasma 25OHD concentrations) and confounding variables (sex, ethnicity, smoking, alcohol consumption, and *MTHFR* C677T genotype). There was a sex*ethnicity interection ($p < 0.05$) and therefore, subsequent linear models stratified the data by sex and adjusted for ethnicity. In **Model 1** for systolic BP (outcome), the linear model was adjusted for age, ethnicity, smoking, alcohol consumption, and BMI. In **Model 1** for diastolic BP (outcome), the linear model was adjusted for ethnicity, smoking, physical activity, and BMI. In **Model 2** for both systolic and diastolic BP (outcomes), VAT was added as a covariate. In all models, plasma 25OHD concentration was the predictor.

4.5 Results

The baseline characteristics of study participants pertaining to BP and predictors of BP are summarized in **Table 5**. Europeans had significantly higher percentage of alcohol consumption and physical activity per week ($P<0.001$). Similarly, among Europeans, the percentage of smokers was higher than among South Asians ($P=0.028$). However, the systolic and diastolic BPs were not significantly different between Europeans and South Asians. Although the allelic frequency of *MTHFR* C677T was different between Europeans and South Asians ($p < 0.001$) this does not seem to have any consequences on the systolic and diastolic BP between the two groups. As shown in **Table 6**, *MTHFR* C677T genotype was not associated with systolic and diastolic BP.

Table 5. Baseline Characteristics for Blood Pressure and Predictors of Blood Pressure

Predictors	**European (n = 171)**	**South Asian (n=176)**	***p*-Values**
Smoker, %	7.5	2.6	0.028
MTHFR C677T genotyping: (%)			<0.001
CC	49.7	66.8	
CT	34.2	24.9	
TT	12.3	3.1	
Family history of CVD, %	49.7	44.4	
Physical activity, average minutes per week	316 (146, 508)	160 (69, 290)	<0.001
Alcohol consumption: (%)			<0.001
Never – 1 per week	42.0	78.3	
1 – 5 per week	38.3	14.8	
6 – 10 per week	19.7	6.9	
Systolic blood pressure (BP), mmHg	117 (109, 127)	118 (108, 127)	0.438
Diastolic blood pressure (BP), mmHg	77.2 ± 8.7	78.0 ± 10.6	0.424

Diastolic BP values presented are means ± SD. Activity and systolic blood pressure were not normally distributed and were transformed using the natural log for statistical analyses; values presented are median (25^{th} percentile, 75^{th} percentile). Differences in variables between ethnic groups were assessed using t-test. The categorical variables were assessed by Pearson's chi-squared test.

Bivariate correlation analyses between systolic and diastolic BP and their predictors revealed that systolic and diastolic BP were positively associated with sex, smoking, BMI, and VAT ($p < 0.05$ for each variable) (**Table 6**). Systolic BP was also positively associated with age and alcohol consumption ($p < 0.05$). Diastolic BP was also negatively associated with physical activity ($p < 0.05$). Both systolic and diastolic BP were negatively associated with plasma 25OHD in both ($p < 0.05$ and $p < 0.001$, respectively).

Table 6. Bivariate Correlation between Systolic and Diastolic Blood Pressure (BP) and Its Predictors

Predictors	Systolic BP	Diastolic BP
	r-Value	r-Value
Sex	0.115*	0.193**
Age	0.233**	0.057
Ethnicity	-0.040	0.042
BMI	0.346**	0.352**
Ln VAT	0.458**	0.452**
Smoking	0.146**	0.158**
Family history of CVD	0.078	0.024
Ln physical activity	-0.102	-0.114*
Alcohol consumption	0.121*	0.069
MTHFR C677T genotyping	-0.034	-0.079
Ln plasma 25OHD	-0.118*	-0.200**

Outcome: Ln Systolic BP.
r-Value: Pearson correlation coefficient.
*. Correlation is significant at the 0.05 level.
**. Correlation is significant at the 0.01 level.

Before further investigating the negative association between plasma 25OHD and systolic and diastolic BP using linear regression model, I first investigated the interactions between covariates (BMI, VAT, age, and physical activity) and confounding variables (sex, ethnicity, smoking status, alcohol consumption, CVD history, and MTHFR C677T genotype) using a general linear model (GLM). There was a sex*ethnicity interaction ($p < 0.05$) and therefore, the subsequent statistical analysis will be stratified by sex and adjusted for ethnicity.

Table 7 summarized the association between systolic BP and plasma 25OHD concentrations in men and women. Systolic BP was no longer negatively associated with plasma 25OHD both in men ($p = 0.064$) and women ($p = 0.182$) following adjustment with significant confounding factors obtained from bivariate correlation analysis in **Table 6**, including ethnicity, age, smoking, alcohol consumption and BMI (Model 1). In Model 1, BMI became a significant predictor of systolic BP. Further adjustment that includes VAT (Model 2) showed that not only the inverse association between systolic BP and plasma 25OHD concentrations disappeared, but also BMI was no longer a significant predictor for systolic BP. Except among women, in Model 2, in addition to VAT, BMI remained to be a significant predictor for the inverse association between systolic BP and plasma 25OHD concentrations.

Table 7. Association between Systolic BP and Plasma 25OHD Concentrations

Predictor (Plasma 25OHD)	**Systolic BP**	
	Men	**Women**
	Standardized β-Value (*p*-Value)	**Standardized β-Value (*p*-Value)**
Model 1[a]	-0.159 (0.064)*	-0.105 (0.182)*
Model 2: Model 1 adjusted for Ln VAT	-0.113 (0.198)**	-0.054 (0.490)* **

Additional predictors other than plasma 25OHD were obtained from **Table 6**.

[a]Model 1 = adjusted for ethnicity, age, smoking, alcohol consumption, and BMI.

* $P < 0.05$ for BMI

** $P < 0.05$ for Ln VAT, BMI was no longer significant.

Table 8 summarized the association between diastolic BP and plasma 25OHD concentrations in men and women. Diastolic BP was no longer negatively associated with plasma 25OHD in men ($p = 0.077$) and women ($p = 0.116$) following adjustments for significant confounding factors obtained from bivariate correlation analysis in **Table 6** such as: ethnicity, smoking, physical activity and BMI (Model 1). In Model 1, BMI became a significant predictor of diastolic BP. Further adjustment that includes VAT (Model 2) showed that not only the inverse association between diastolic BP and plasma 25OHD concentrations disappeared, but also BMI was no longer a significant predictor for diastolic BP. Except among women, in Model 2, in addition to VAT, BMI remained to be a significant predictor for the inverse association between diastolic BP and plasma 25OHD concentrations.

Table 8. Association between Diastolic BP and Plasma 25OHD Concentrations

Predictor (Plasma 25OHD)	**Diastolic BP**	
	Men	**Women**
	Standardized β-Value (*p*-Value)	**Standardized β-Value (*p*-Value)**
Model 1 [a]	-0.154 (0.077)*	-0.125 (0.116)*
Model 2: Model 1 adjusted for Ln VAT	-0.118 (0.177)**	-0.084 (0.281)* **

Additional predictors other than plasma 25OHD were obtained from **Table 6**.

[a]Model 1 = adjusted for ethnicity, smoking, physical activity, and BMI

* $P < 0.001$ for BMI

** $P < 0.005$ for Ln VAT, BMI was no longer significant

CHAPTER 5: Role of Adiponectin in Ethnic-Specific Differences in Body Fat Distribution and Risk of CVD

5.1 Specific Aim 3

To determine ethnic-specific differences in plasma adiponectin isoform concentrations and to explore the relationship with body composition, body fat distribution, and CVD risk.

5.2 Background & Rationale

Adiponectin is another factor that may play a role in the association of adiposity and body fat distribution with CVD risk. Adiponectin, encoded by *ADIPOQ,* is a 30 kDa protein mainly synthesized and secreted by adipocytes (reviewed by [48]). In 1996, a study reported that adiponectin mRNA expression in adipocytes was reduced in *db*/*db* mice, deficient in leptin receptor and a model of obesity and T2DM [119]. The *db*/*db* mice were also reported to have low circulating adiponectin concentrations, which were further lowered in those fed high-fat diet [120].

Adiponectin is a unique adipocytokine in that circulating concentrations are inversely associated with adiposity [49-54]. A cross-sectional study conducted in 87 non-obese and 57 obese subjects (obesity was defined as BMI > 26.4 kg/m^2) in Japan reported that plasma total adiponectin concentrations were significantly lower in the obese subjects compared to the non-obese subjects [49]. In both the obese and non-obese subjects, BMI had a strong negative association with plasma total adiponectin concentrations [49]. A cross-sectional study involving 486 nondiabetic Japanese women reported that circulating total adiponectin concentrations were inversely associated with BMI and body fat mass [50].

Further studies have reported association of circulating adiponectin concentrations with body fat distribution. A cross-sectional study in 76 men and 106 women of European descent reported that plasma total adiponectin concentrations were negatively associated with BMI, as well as with SAT and VAT [51], with the strongest association observed with VAT [51]. Similarly, others have reported that circulating total adiponectin concentrations are inversely associated with VAT in Caucasian American populations [52-54]. A cross-sectional study in 23 normal-weight and 26 obese adolescents reported that adolescents with a BMI greater than 30 kg/m^2 had approximately 50% lower plasma total adiponectin concentrations than adolescents with healthy BMIs and that adolecents with obesity and the greatest VAT deposition had lower plasma total adiponectin concentrations than adolescents with obesity but less VAT deposition [52]. Another cross-sectional study in 242 Caucasian subjects without T2DM also reported that high VAT deposition was a strong predictor of low plasma total adiponectin concentrations, especially among those with obesity [53]. Furthermore, a cross-sectional study in 47 subjects without and 21 subjects with T2DM reported that serum total adiponectin concentrations were negatively associated with waist/hip ratio (WHR) and trunk/leg fat mass ratio [54]. Interestingly, the high molecular weight (HMW) adiponectin concentrations had stronger associations with WHR and trunk/leg fat mass ratio [54].

Adiponectin circulates in four different isoforms, globular (monomeric), low molecular weight (LMW or trimeric), medium molecular weight (MMW or hexameric), and HMW (oligomeric) (reviewed by [48,121]). Unless otherwise stated, when a human study mentions 'adiponectin', it is usually referring to the total adiponectin. It has been reported that MMW and HMW adiponectin are the major oligomeric isoforms circulating in plasma [122]. Whereas, the globular and LMW (trimeric) isoforms were found to be at low concentrations, explained in part

by their shorter half-life [123]. Circulating globular (monomeric) and LMW (trimeric) isoforms can complex to form MMW (hexamer) isoforms, which can further be complexed to form the HMW isoform [124]. Globular adiponectin has high affinity for adiponectin receptor 1 (ADIPOR1) and less so for adiponectin receptor 2 (ADIPOR2) (reviewed by [48]). LMW (trimeric) adiponectin has intermediate affinity for the ADIPOR2 (reviewed by [48]). ADIPOR1 is ubiquitously expressed, with highest levels observed in skeletal muscle [125], whereas ADIPOR2 is predominantly expressed in liver [125]. The LMW (trimeric) and MMW (hexameric) adiponectin isoforms are also reported to have high affinity for T-cadherin [126]. T-cadherin is highly expressed in the heart and the aortic, carotid, iliac, and kidney arteries ([127], reviewed by [48]).

Upon binding to the receptor, adiponectin stimulates the adenosine monophosphate (AMP)-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPAR-α) pathways. Through these pathways, adiponectin exerts effects on glucose-insulin homeostasis and lipid metabolism (reviewed by [48]). **Figure 6** summarizes the mechanism of action of adiponectin on glucose-insulin homeostasis and lipid metabolism.

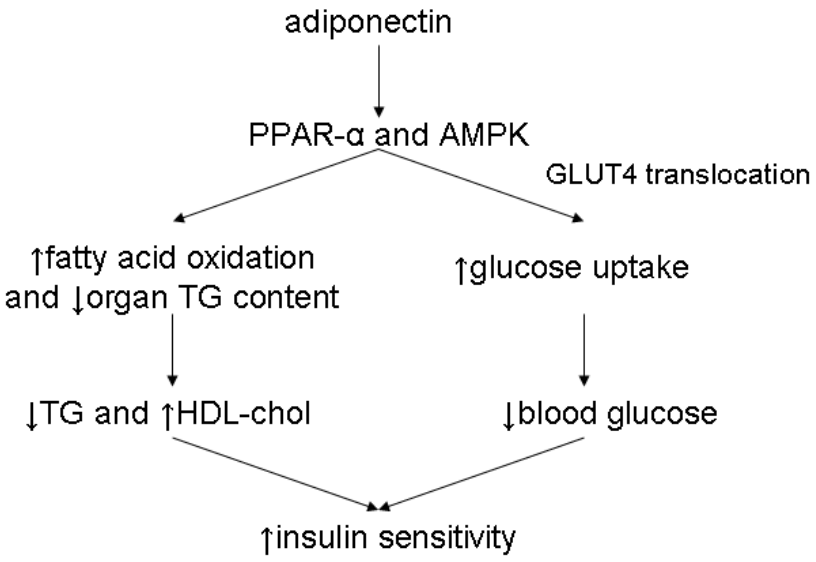


Figure 6. Adiponectin mechanism of action.

The role of adiponectin in lipid metabolism is supported by a large body of evidence in reporting a strong association between circulating adiponectin concentrations and plasma HDL-C, LDL-C, and TG concentrations [53,128-130]. For example, a cross-sectional study in 101 healthy 58-year old Swedish men with no CVD and T2DM reported that circulating plasma total adiponectin concentrations were negatively associated with LDL-C [128]. A longitudinal study among 1174 patients in Germany who were previously diagnosed with CVD, further reported that serum total adiponectin concentrations were positively associated with HDL-C and negatively with plasma TG concentrations, even after adjustment for age and sex [129]. This relationship remained significant even after further adjustments for BMI, alcohol intake, smoking status, presence of diabetes and/or hypertension, lipid-lowering drug therapy, and elevated fasting plasma glucose [129]. Similarly, a cross-sectional study in 283 adolescent Japanese children reported that circulating total adiponectin concentrations significantly predict LDL-C, HDL-C and HDL-C/TG ratio, independent of BMI, but only in girls [130]. A further

cross-sectional study in 242 Caucasian subjects without T2DM also confirmed that plasma total adiponectin concentrations are strongly associated with HDL-C and negatively associated with LDL-C and TG concentrations [53].

A role for adiponectin in the maintenance of glucose-insulin homeostasis has also been suggested [50,52,54,131]. A cross-sectional study in non-diabetic Japanese women reported that circulating plasma total adiponectin concentrations were inversely associated with insulin resistance (HOMA-IR) even after adjustment for age, diastolic BP, BMI, and serum TG concentrations [50]. Another cross-sectional study in 23 normal-weight and 26 adolescents with obesity reported that plasma total adiponectin concentrations were positively associated with peripheral and hepatic insulin sensitivity [52]. This study further showed that even after adjustment for BMI, low plasma adiponectin concentrations were strongly associated with insulin resistance and beta-cell dysfunction, as assessed by the proinsulin-to-insulin ratio [52].

One of the first studies to identify the ethnic-differences in circulating adiponectin concentrations was a cross-sectional study in 23 Caucasian subjects and 121 Pima Native American Indians. The study reported that plasma adiponectin concentrations were negatively associated with plasma fasting insulin and glucose concentrations, and positively associated with insulin sensitivity as assessed by a hyperinsulinemic clamp [131]. Interestingly, the Caucasian subjects in the study had significantly higher plasma adiponectin concentrations compared to the Pima Native American Indians and plasma adiponectin concentrations were significantly correlated with glucose tolerance in this group [131]. Another cross-sectional study in African-American and Caucasian women with and without obesity reported that plasma adiponectin concentrations were significantly higher in African-American women than Caucasian women, but only among in those subjects without obesity [132]. Furthermore, South Asian subjects living

in Ontario were reported to have significantly lower circulating adiponectin concentrations compared to their European subjects after adjustment for age, sex and BMI. Even after further adjustment for WHR and percent total body fat (measured by DEXA), adiponectin concentrations were lower in South Asians than in Europeans [133]. Little is known about the effect of VAT deposition in South Asians on circulating adiponectin concentrations. Considering that South Asians have higher VAT deposition than Europeans [34], and that VAT is inversely associated with circulating adiponectin concentrations in other ethnic groups [52-54], one would expect that the differences in adiponectin concentrations are attributed by ethnic-differences in VAT deposition.

I have investigated ethnic-specific differences in plasma adiponectin isoform concentrations and explored the relationship with body composition, body fat distribution, and CVD risk factors. I investigated plasma total and HMW adiponectin cocentrations because HMW adiponectin is one of the most abundant isoforms in the circulation [122] and is suggested to be the most highly active in the liver [134]. Furthermore, a cross-sectional study reported that circulating HMW adiponectin concentrations have a stronger relationship with fasting plasma insulin concentrations and HOMA-IR than total adiponectin concentrations [55]. Interestingly, this study also found that VAT deposition was inversely associated with circulating HMW adiponectin concentrations, but not with circulating total adiponectin concentrations [55]. Further support for analyzing circulating HMW adiponectin concentrations comes from another cross-sectional study that reported that circulating HMW adiponectin concentrations, not total adiponectin concentrations or HMW/total adiponectin ratio, are most associated with insulin sensitivity, reduced abdominal fat (measured by DEXA), and high basal lipid oxidation [54]. Moreover, this study also reported that even after adjustment for glucose disposal rate and BMI,

circulating HMW adiponectin concentrations were independently associated with HDL-C and LDL-C [54]. Therefore, in Aim 3, I investigated the role of VAT deposition in mediating the association between plasma total and HMW adiponectin concentrations with fasting plasma, insulin, HOMA-IR and lipid concentrations.

5.3 Methods

5.3.1 Plasma Total and HMW Adiponectin Quantification

Total and HMW adiponectin concentrations were quantified in plasma using a commercial ELISA (47-ADPHU-E01, ALPCO Diagnostics, Salem, NM). This ELISA kit utilizes antibodies that recognize only total (monomeric) and HMW (dimeric) adiponectin. Total adiponectin was quantified by pretreating plasma to reduce to monomers and dimers followed by quantification with the ELISA. HMW adiponectin was quantified by first pretreating plasma samples with a protease, which selectively digests LMW (trimeric) and MMW (hexameric) adiponectin. For every 96-well ELISA plate, standards from human serum were run in duplicates to make the standard curve to eliminate non-biological variability between plates.

5.5. Statistical Analyses

T-tests were used to analyze mean differences in plasma total and HMW adiponectin concentrations, and the total/HMW adiponectin ratio between European and South Asian study participants. Chi-squared was used to compare categorical variables between the two groups. All variables that are not normally distributed were naturally log transformed prior to any statistical analysis. Bivariate analysis was done to identify the correlation between total and HMW adiponectin with measures of body composition, body fat distribution and risk factors for CVD (lipid concentrations, fasting plasma insulin, glucose and HOMA-IR, and blood pressure). Prior

to doing multivariate linear regression, general linear model analysis (GLM) was performed to detect any interactions between covariates, in this case sex*ethnicity (p = 0.147). From the GLM analysis, no significant interaction was found. Therefore, subsequent multivariate linear regression analysis were not stratified by sex, but rather adjusted for age, sex, ethnicity and BMI. Following adjustment with age, sex, ethnicity, and BMI, multivariate linear regression was used to analyze the relationship between total and HMW adiponectin with measures of adiposity and risk factors for CVD. Ln VAT was later added into the list of covariates to further identify the role of Ln VAT in the association between risk factors for CVD with plasma total and HMW adiponectin concentrations.

The inter-assay and intra-assay coefficient of variability (CV) were calculated and the values were 6.15% and 2.4%, respectively. Based on the total and HMW adiponectin ELISA manufacturer, inter-assay % CVs of less than 15 are acceptable. Meanwhile, intra-assay % CVs should be less than 10%. The inter-assay % CV was calculated from the average coefficient of variation of a control between plates. In this case, the number of plates run was ten and the control being used to calculate inter-assay CV was the total adiponectin control (µg/mL).

$$\text{Plate mean (µg/mL)} = \frac{(6.80 + 6.53 + 6.00 + 6.34 + 5.52 + 4.98 + 6.09 + 5.53 + 5.68 + 5.00)}{10}$$

Plate mean (µg/mL) = 5.85

Standard deviation (SD) = 0.615

Inter-assay % CV of means = SD*100% = 6.15%, which is less than 15%.

Meanwhile, the intra-assay % CV was calculated from the average coefficient of variation between duplicates of one standard that were run in 10 plates. In this case, I used standard 2 to calculate the intra-assay % CV.

$$\text{Total intra-assay \% CV (n=10 plates)} = \frac{\text{total \% CV}}{10}$$

% intra-assay CV = (SD between duplicates/duplicates mean)*100%

% intra-assay CV of standard 2 in Plate 1 = (0.117/1.487)*100% = 7.89%

% intra-assay CV of standard 2 in Plate 2 = (0.010/1.404)*100% = 0.71%

% intra-assay CV of standard 2 in Plate 3 = (0.006/1.313)*100% = 0.48%

% intra-assay CV of standard 2 in Plate 4 = (0.030/1.339)*100% = 2.22%

% intra-assay CV of standard 2 in Plate 5 = (0.076/1.244)*100% = 6.08%

% intra-assay CV of standard 2 in Plate 6 = (0.043/1.195)*100% = 3.61%

% intra-assay CV of standard 2 in Plate 7 = (0.010/1.113)*100% = 0.89%

% intra-assay CV of standard 2 in Plate 8 = (0.016/1.093)*100% = 1.42%

% intra-assay CV of standard 2 in Plate 9 = (0.003/1.228)*100% = 0.23%

% intra-assay CV of standard 2 in Plate 10 = (0.006/1.230)*100% = 0.46%

$$\text{Total intra-assay \% CV} = \frac{\text{total \% CV}}{10}$$

$$= \frac{(7.89 + 0.71 + 0.48 + 2.22 + 6.08 + 3.61 + 0.89 + 1.42 + 0.23 + 0.46)}{10}$$

= 2.40%, which is less than 10%.

5.6. Results

In this chapter, nine European and ten South Asian study participants were excluded from the analysis because their total and HMW adiponectin concentrations were not analyzed due to limited amount of plasma available. **Table 9** summarized the concentrations of adiponectin between Europeans and South Asians, Europeans had significantly higher total and HMW adiponectin concentrations compared with South Asians ($p < 0.001$). Their HMW/total adiponectin ratio was also higher among Europeans than South Asians ($p < 0.001$).

Table 9. Adiponectin Concentrations and Subfractions Stratified by Ethnicity

Variables	**European (n=162)**	**South Asian (n=166)**	***p*-Value**
Total adiponectin, ng/mL	5.47 (3.82, 7.90)	4.42 (3.20, 6.10)	
Ln total adiponectin	1.68 ± 0.48	1.45 ± 0.48	<0.001*
HMW adiponectin, ng/mL	2.74 (1.63, 4.45)	1.83 (1.17, 3.00)	
Ln HMW adiponectin	0.88 ± 0.80	0.57 ± 0.75	<0.001*
HMW/total adiponectin ratio	0.52 (0.39, 0.60)	0.44 (0.33, 0.54)	<0.001*

Data presented are mean ± SD or median and 25^{th},7^{th} percentiles.

*****p-Value obtained by using natural log-transformed values.

Independent samples t-test was done to analyzed the significant difference between ethnic groups.

As summarized in **Table 10**, South Asians had significantly higher TG, fasting plasma glucose, fasting plasma insulin, HOMA-IR, and Hcy. They also had significantly lower HDL-chol and plasma 25OHD in comparison to their European counterparts. Overall, South Asians had worse cardiometabolic risk factors than the Europeans.

Table 10. Baseline Characteristics of Study Participants

Variables	European (n=162)	South Asian (n=166)	*p*-Value
Total cholesterol, mmol/L	5.26 ± 0.98	5.30 ± 0.98	0.710
HDL-C, mmol/L	1.30 ± 0.38	1.18 ± 0.29	0.002
LDL-C, mmol/L	3.30 ± 0.85	3.35 ± 0.85	0.557
TG, mmol/L	1.19 (0.88, 1.65)	1.39 (1.07, 2.20)	
Ln TG	0.18 ± 0.59	0.38 ± 0.55)	0.001
Glucose, mmol/L	5.1 (4.8, 5.5)	5.2 (5.0, 5.5)	
Ln glucose	1.64 ± 0.13	1.67 ± 0.12	0.036
Insulin, pmol/L	61.0 (40.0, 79.0)	76.0 (54.5, 106)	
Ln insulin	4.07 ± 0.55	4.36 ± 0.49	<0.001
HOMA-IR	1.89 (1.29, 2.75)	2.46 (1.79, 3.52)	
Ln HOMA-IR	0.66 ± 0.62	0.97 ± 0.55	<0.001
Homocysteine, umol/L	7.60 (6.40, 9.10)	8.20 (6.80, 9.95)	
Ln Hcy	2.03 ± 0.23	2.14 ± 0.31	<0.001
Total area, mm^2	19.6 (13.9, 33.0)	13.8 (11.7, 18.6)	
Ln total area	25.9 ± 17.5	17.9 ± 11.7	<0.001
Apolipoprotein B, g/L	0.98 (0.84, 1.18)	0.99 (0.87, 1.17)	0.350*

*****p-Value obtained by using natural log-transformed values. Independent samples t-test was done to analyzed the significant difference between ethnic groups.

Values presented are means ± SD. Variables that have natural log transformed values were not normally distributed and therefore values presented are median (25^{th} percentile, 75^{th} percentile). The natural log transformed values are presented as geometric means ± 95% CI.

As summarized in **Table 11**, both total and HMW adiponectin were inversely associated with different measures of body composition and body fat distribution, except for TAT and SAT.

Table 11. Correlation Analysis between Adiponectin and Body Fat Distribution

	Total Adiponectin	**HMW Adiponectin**
	r-Value	**r-Value**
BMI	-0.178**	-0.172**
WC	-.333**	-0.328**
WHR	-0.432**	-0.397**
Total body fat (%)	0.179**	0.210**
TAT	-0.097	-0.088
Ln VAT	-0.304**	-0.251**
Ln SAT	-0.023	-0.020

Data were analyzed by separate Pearson's bivariate correlation. Total and HMW adiponectin were also analyzed separately. WC: waist circumference, WHR: waist/hip ratio, TAT: total abdominal adipose tissue, Ln VAT: log (natural) transformed visceral adipose tissue, Ln SAT: log (natural) transformed subcutaneous adipose tissue

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

In **Table 12**, I investigated the bivariate correlation between both total and HMW adiponectin with CVD risk factors that previously have been shown to be associated with adiponectin such as: LDL-C, HDL-C, TG, ApoB, fasting plasma glucose and insulin, HOMA-IR, systolic and diastolic BP. Both total and HMW adiponectin were positively associated with HDL-C and negatively associated with TG and Apo B concentrations, diastolic BP, fasting plasma insulin, glucose and HOMA-IR. Total and HMW adiponectin were not significantly associated with systolic BP.

Table 12. Correlation Analysis Between Adiponectin and CVD Risk Factors

	Total Adiponectin	**HMW Adiponectin**
	r-Value	**r-Value**
LDL-C	-0.010	-0.006
HDL-C	0.496**	0.454**
Ln TG	-0.344**	-0.266**
Ln fasting plasma insulin	-0.398**	-0.297**
Ln fasting plasma glucose	-0.225**	-0.193**
Ln HOMA-IR	-0.403**	-0.305**
Ln systolic BP	-0.70	-0.056
Diastolic BP	-0.201**	-0.178**

Data were analyzed by separate Pearson's bivariate correlation. Total and HMW adiponectin were also analyzed separately.

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

HDL-C: high-density lipoprotein, LDL-C: low-density lipoprotein, TG: triglycerides, HOMA-IR: homeostatic model assessment (insulin resistance), BP: blood pressure.

Table 13 summarized the association between total and HMW adiponectin and body composition and body fat distribution after adjustment for age, sex, ethnicity, and BMI. TAT and SAT were not further adjusted as they were not significantly associated with total and HMW adiponectin in the earlier analysis. In **Table 14**, both total and HMW adiponectin were dependent variables. Meanwhile, WC, WHR, total body fat (%), and VAT are predictors (independent variables) for adiponectin.

Table 13. Regression Analysis for Measures of Body Composition and Body Fat Distribution with Total and HMW Adiponectin Adjusted for Age, Sex, Ethnicity, and BMI

	Total Adiponectin	**HMW Adiponectin**
	Standardized-β (*p*-Value)	**Standardized-β (*p*-Value)**
WC	-0.303 (0.004)†	-0.247 (0.019)†
WHR	-0.305 (0.000)†	-0.165 (0.046)†
Total body fat (%)	0.059 (0.561)	0.125 (0.221)
Ln VAT	-0.252 (0.000)†	-0.128 (0.068)†

†$p < 0.05$ for ethnicity for all variables.

Dependent variable: total or HMW adiponectin.

Total and HMW adiponectin were analyzed by separate linear regression models adjusted for age, sex, ethnicity, and BMI.

WC: waist circumference, WHR: waist/hip ratio, VAT: visceral adipose tissue.

Table 14 summarized the association between total and HMW adiponectin with CVD risk factors (HDL-C, TG, fasting plasma insulin, fasting plasma glucose, HOMA-IR, and diastolic BP) after adjustment for age, sex, ethnicity, and BMI. Systolic BP, and LDL-C were not included in this analysis because they were not significantly associated with total and adiponectin in the earlier analysis (**Table 12**). In **Table 14**, both total and HMW adiponectin were predictors (independent variables) for CVD risk factors. Meanwhile, the CVD risk factors are the dependent variables.

Table 14. Regression Analysis for Measures of CVD Risk Factors with Total and HMW Adiponectin Adjusted for Age, Sex, Ethnicity, and BMI

	Total Adiponectin	**HMW Adiponectin**
	Standardized-β (*p*-Value)	**Standardized-β (*p*-Value)**
HDL-C	0.305 (<0.001)	0.251 (<0.001)†
Ln TG	-0.275 (<0.001)†	-0.172 (0.004)†
Ln Fasting plasma insulin	-0.312 (<0.001)†	-0.185 (<0.001)†
Ln Fasting plasma glucose	-0.146 (0.015)†	-0.095 (0.110)†
Ln HOMA-IR	-0.311 (<0.001)†	-0.185 (<0.001)†
Diastolic BP	-0.102 (0.086)	-0.066 (0.264)

†$p < 0.05$ for ethnicity for all variables.

Independent variables: total or HMW adiponectin. Both total and HMW adiponectin were log (natural) transformed before statistical analysis.

Dependent variables: HDL-C, TG, fasting plasma insulin and glucose, HOMA-IR, and diastolic BP. Variables that were not normally distributed were log (natural) transformed before statistical analysis.

Total and HMW adiponectin were analyzed by separate linear regression models adjusted for age, sex, ethnicity, and BMI.

Adiponectin is known for its insulin-sensitizing and anti-atherogenic properties and although there are a few hypotheses how adiponectin could contribute to CVD risk factors, it is unclear what the exact mechanism is. To investigate if perhaps VAT mediates the association between adiponectin and CVD risk factors, multivariate regression analyses were performed and adjusted for confounding factors for CVD including age, sex, ethnicity, BMI, and VAT. Interestingly, as summarized in **Table 15**, even after adjustment for VAT, both total and HMW adiponectin still remained significantly associated with HDL-C, TG, ApoB, fasting plasma insulin and HOMA-IR ($p < 0.05$ for each variable). They were no longer significantly associated with fasting plasma glucose and diastolic BP.

Table 15. Regression Analysis for Measures of CVD Risk Factors with Total and HMW Adiponectin Adjusted for Age, Sex, Ethnicity, BMI, and VAT

	Total Adiponectin	HMW Adiponectin
	Standardized-β (*p*-Value)	**Standardized-β (*p*-Value)**
HDL-C	0.266 (<0.001)*	0.228 (<0.001)*
Ln TG	-0.207 (<0.001)*	-0.137 (0.016)*
Ln fasting plasma insulin	-0.243 (<0.001)* †	-0.149 (0.002)* †
Ln fasting plasma glucose	-0.098 (0.102)*	-0.069 (0.238)*
Ln HOMA-IR	-0.238 (<0.001)* †	-0.148 (0.002)* †
Diastolic BP	-0.051 (0.388)*	-0.041 (0.475)*

*$p < 0.001$ for VAT for all variables

†$p < 0.05$ for ethnicity

Independent variables: total or HMW adiponectin. Both total and HMW adiponectin were log (natural) transformed before statistical analysis.

Dependent variables: HDL-C, TG, fasting plasma insulin and glucose, HOMA-IR, and diastolic BP. Variables that were not normally distributed were log (natural) transformed before statistical analysis.

Total and HMW adiponectin were analyzed by separate linear regression models adjusted for age, sex, ethnicity, BMI and Ln VAT.

CHAPTER 6: General Discussion

The overall objective of my thesis was to test the hypothesis that ethnic-specific differences in VAT deposition (South Asians have higher VAT deposition than the Europeans even after adjustment for age, sex and BMI) are accompanied by variations in plasma 25OHD and adiponectin concentrations and this contributes to CVD risk. I addressed three Specific Aims to test my hypothesis. For Aim 1, I completed studies addressing ethnic-specific differences in vitamin D status and body composition. I observed that South Asians have significantly lower plasma 25OHD concentrations than Europeans and the prevalence rates of low vitamin D status (circulating plasma 25OHD < 50 nmol/L) was three times higher among South Asians than Europeans. As predicted, plasma 25OHD concentrations were inversely associated with measures of body composition and body fat distribution with the strongest relationship observed for VAT. This relationship was independent of age, BMI, season of blood collection, SAT, and total body fat (%). Ethnicity still had an effect on the inverse association between plasma 25OHD and VAT. In Chapter 4, studies pertaining to Aim 2 were reported and assessed the relationship between plasma 25OHD concentrations, BP, and body composition. I observed no differences in systolic and diastolic BP between South Asians and Europeans. I did find that systolic and diastolic BP were negatively associated with plasma 25OHD concentrations but this relationship was dependent on body composition and disappeared following adjustment for BMI and VAT. In Chapter 5, studies pertaining to Aim 3, were reported and assessed whether ethnic-specific differences in plasma adiponectin concentrations were associated with ethnic-specific differences in body composition and CVD risk. I observed that South Asians had significantly lower total, HMW and HMW/total adiponectin ratio than the Europeans. As expected, total and HMW adiponectin concentrations were inversely associated with body composition and body fat

distribution. I also found that total and HMW adiponectin concentrations were positively associated with HDL-C and negatively associated with fasting plasma insulin and glucose, HOMA-IR and TG concentrations, and diastolic BP and these relationships remained significant after adjustment for VAT. Interestingly, ethnicity remained to be a significant confounder in the inverse associations between total and HMW adiponectin concentrations with fasting plasma insulin and HOMA-IR.

6.1 Ethnic Specific Differences in Plasma 25OHD Concentrations and Adiposity

The objective of Aim 1 in Chapter 3 is to assess the relationship between plasma 25OHD with anthropometric measures and body composition in South Asian and European populations. Previous findings reported by others [46,88-91,135] showed that South Asians had significantly lower plasma 25OHD concentrations and a higher prevalence of low vitamin D status (circulating 25OHD <50 nmol/L) compared to Europeans. Similar to those, I also found that ethnicity is a predictor of plasma 25OHD concentrations as such that the South Asians had significantly lower plasma 25OHD than the Europeans (39.0 nmol/L vs. 63.0 nmol/L, respectively). My findings are in line with prior reports by others. A prevalence rate of 80% for low vitamin D status was reported in a study of 123 healthy subjects living in Delhi, the northern part of India [60]. Similarly, others have reported a high prevalence of low vitamin D status in South Asians living in the UK and New Zealand [88,89,91]. For example, a study assessing 1,416 Pakistani women living in the UK reported 94% had circulating 25OHD concentrations < 50 nmol/L [89]. A cross-sectional study in Auckland, NZ reported the mean serum 25OHD concentration in 228 South Asian women was 41.0 nmol/L [91], similar to the values I observed for the M-CHAT subjects. Furthermore, a cross-sectional study in 201 healthy adults in the UK

also reported that the South Asians had significantly lower 25OHD concentrations compared to Europeans [59], similar to my findings. As such, my findings confirm in a larger study that similar to findings reported for South Asians living in the UK and New Zealand, South Asians living in Vancouver have lower plasma 25OHD concentrations than Europeans.

Ethnic-specific differences in circulating 25OHD concentrations have been attributed to differences in skin colour [71], as well as cultural and lifestyle differences pertaining to sun exposure [91]. However, evidence has accumulated suggesting that in certain ethnic groups and geographical regions, sufficient sun exposure will not necessarily improve vitamin D status [69,136]. A prospective cohort study of 15 South Asian subjects and 109 Europeans in the UK (53.5°N) reported that an intervention of controlled UV exposure led to a 10.7 nmol/L increase in 25OHD concentrations among South Asians compared to a 26.2 nmol/L in Europeans [136]. In a cross-sectional study of adults living in Honolulu, Hawaii (21° latitude), despite abundant natural sun exposure, the mean serum 25OHD concentrations were 31.6 ng/mL (78.9 nmol/L) [69]. Moreover, 51% of study participants had low vitamin D status, using a cutoff at 75 nmol/L [69]. Although this study consisted of multi-ethnic participants (42% white Caucasians, 30% Asians, 20% multi-racial, and 8% Hawaiian/Pacific Islanders), it further proved the point that despite abundant sun exposure and regardless of skin colour, the prevalence of low vitamin D status remained high. These findings suggest that there may be other confounding variables that lead to ethnic-specific differences in circulating 25OHD concentrations other than skin colour and sun exposure. To account for the effect of sun exposure, subsequent statistical regression analyses investigating the role of body composition and body fat distribution on plasma 25OHD concentrations were controlled for season of blood collection [137].

I hypothesized that body composition and body fat distribution may also contribute to circulating 25OHD concentrations in South Asian population. Using a simple bivariate analysis, I confirm that plasma 25OHD concentrations are negatively associated with BMI, WC, WHR, TAT, VAT, SAT, and total body fat (%) in South Asians and Europeans. Even after controlling for factors that affect circulating 25OHD such as ethnicity, age, BMI, and season of blood collection, plasma 25OHD remained significantly associated with body fat distribution with the strongest association seen with VAT. To illustrate the effect of VAT on plasma 25OHD concentrations, **Figure 4a** and **4b** showed that increased VAT deposition, presented as VAT quartiles based on the 25^{th}, 50^{th} and 75^{th} percentile cutoffs, was accompanied with decreased plasma 25OHD. This was similar to that reported among American Caucasians whereby the highest prevalence of low vitamin D status (defined as circulating 25OHD concentrations lower than 50 nmol/L) was found in among those with BMI and the higher VAT deposition [44]. Similarly, an inverse association between circulating 25OHD and VAT has also been reported in women undergoing gynecological surgeries [45], American children in Pittsburg, PA (40°N) [84], Hispanic-and African-Americans [46], as well as African-Americans with T2DM [85]. To further investigate if the inverse association between plasma 25OHD and VAT is independent of other measures of body fat distribution, I included VAT, SAT and total body fat (%) in the same model with other confounding variables such as: ethnicity, age, BMI, and season of blood collection. It was revealed that revealed that plasma 25OHD concentrations remained significantly and inversely associated with VAT but not with BMI, SAT or total body fat (%). This further elucidates that the strongest body fat distribution predictor for plasma 25OHD is VAT despite the fact that VAT only accounts for only 10% to 20% of total body fat [138]. Given

that low plasma 25OHD concentrations are associated with CVD [41,44], this finding further highlights the importance of VAT as a contributing factor to CVD risk.

A previous finding of the M-CHAT cohort was that South Asians had a greater CVD risk than Europeans and VAT was the mediating factor [35]. However, the mechanism as to how VAT could contribute to elevated CVD risk is not known. VAT itself is an independent risk factor for CVD [1,17]. Findings in Chapter 3 indicated that VAT was the greatest predictor for circulating plasma 25OHD concentrations. Although low circulating 25OHD concentrations are associated with increased CVD [39,40] and that VAT is associated with increased CVD [1,17], the inverse association found between plasma 25OHD concentrations and VAT deposition in Aim 1 does not necessarily show causal-effect. The finding is an association and one cannot draw a conclusion that VAT sequesters circulating 25OHD making less available in circulation.

To address the question whether supplementation of vitamin D with calcium lowers adiposity or whether adiposity leads to decreased circulating 25OHD, two clinical trials investigated the role of vitamin D in adiposity. The first study in 63 healthy, overweight or obese women completed a 15-week weight loss intervention with daily calcium (Ca) and vitamin D intakes of 1200 mg of Ca and 400 IU of vitamin D_3 (treatment) or 600 mg and 200 IU of vitamin D_3 (placebo), showed that after 15-week of intervention, BMI did not change [139]. However, another study with Ca and vitamin D intervention reported that supplementation with Ca and vitamin D did lead to a decreased in VAT despite no change in BMI [47]. In this parallel design double-blinded, placebo-controlled study, 171 participants who received 1050 mg Ca and 300 IU of vitamin D_3/day in an orange juice for 16 weeks had around 13-cm^2 reduction in their VAT deposition despite no change in BMI compared to what in the placebo orange juice group [47]. However, this study was Ca and vitamin D and not intervention with vitamin D alone, as such to

conclude that vitamin D supplementation would lead to decreased in VAT is incorrect. Further investigation is necessary to elucidate the exact mechanism by which vitamin D could lead to VAT reduction and ultimately CVD risk.

In Aim 1, I also investigated a possible role of genetic background that could explain ethnic-differences in circulating 25OHD between South Asians and Europeans. Based on a genome-wide association study (GWAS), a single nucleotide polymorphism (SNP) in a gene encodes for vitamin D binding protein (*GC*) rs2282679 has previously been reported to be association with circulating 25OHD [93]. However, I did not find that the GC genotype affects circulating 25OHD in South Asians and Europeans and therefore, subsequent statistical analyses were not adjusted for *GC* rs2282679 genotype. Furthermore, a cross-sectional study in 52 Caucasian and 36 African American women reported that vitamin D binding protein (DBP) in plasma analyzed by ELISA were not different between the two groups despite the fact that the concentrations of plasma 25OHD were lower in 36 African Americans than their 52 Caucasian counterparts [140]. The authors also reported that DBP concentrations were unrelated to plasma 25OHD concentrations, and were not affected by ethnicity or adiposity [140].

Overall I have shown in Aim 1 that the South Asians have significantly lower plasma 25OHD concentrations than the Europeans and that plasma 25OHD concentrations are negatively associated with VAT depositions, with the lowest plasma 25OHD concentrations observed in South Asians with the highest quartile of VAT deposition. The association between VAT and plasma 25OHD concentrations was stronger than other measures of body fat distribution such as SAT, TAT, and total body fat (%), suggesting a distinct role VAT with respect to plasma 25OHD concentrations and may in part explain the lower plasma 25OHD concentrations in South Asians.

6.2 Plasma 25OHD Concentrations and Blood Pressure

Despite a large body of evidence showing ethnic-specific differences in blood pressure (not just in South Asians and Europeans) [104-106,108], I found no difference in systolic and diastolic BP between South Asians and Europeans. This finding was not entirely surprising in that to qualify for recruitment into the M-CHAT cohort subjects had to be healthy with no previous diagnosis of CVD or CVD-related diseases [33]. Previous findings on ethnic-specific difference in BP have been inconsistent. However, after reviewing the literature, it appears that people of African and Afro-Caribbean descent have the highest prevalence of elevated BP compared to European [64,105,141], Hispanics [64], and South Asians [109,111]. A cross-section study based on NHANES III data that included 16,573 participants reported that the African-Americans had systolic BP 3 mmHg higher than Europeans after adjustment for age, sex and BMI [64]. In the same study, it was also reported that Hispanics have a 1.5 mmHg higher systolic BP than Europeans [64]. A bigger ethnic-specific difference in BP was reported among Afro-Caribbean and Europeans from the UK [105]. It was reported that systolic BP was 17 mmHg higher in Afro-Caribbean than European women, whereas systolic BP in Afro-Caribbean men was 6 mmHg higher than in European men [105]. A longitudinal study based in the UK among adolescents also reported that mean diastolic BP was significantly higher among Afro-Caribbean and African descents compared to their European counterparts [109]. A cross-sectional study based on the 2002-2006 NHANES among 1984 African Americans and 5156 Caucasians showed that the difference in systolic BP between the two ethnic groups was 5.2 mmHg higher among African Americans [108]. Lastly, a systemic review compared men and women of African descents with their European counterparts and reported that the mean

differences in systolic and diastolic BP in subjects of African descent are higher than Europeans [141].

Differences in BP between South Asian and European have also been previously reported, but the differences were only at a certain age category and only with diastolic BP [109,111]. A longitudinal study reported that diastolic BP were significantly higher among South Asians adolescents compared to Europeans [109], and similarly, a cross-sectional survey based on the 1991-1996 Health Surveys in England also reported that diastolic BP was significantly higher in South Asian men > 40 years compared to European men [111]. In contrast to their findings, I did not find any ethnic-specific differences in blood pressure between South Asians and Europeans. This is in part may be due to the differences in the number of study participants. The number of study participants in my thesis is less than 400 compared to the number of participants in the previous studies which include more than 6000 study participants.

Furthermore, it is also important to note that there are conflicting arguments regarding which measure of BP is important. In general, hypertension (elevated systolic and diastolic BP) is an unfavourable condition and is a risk factor for CVD [15]. Findings from the Framingham Study 14-year follow-up reported that using a linear correlation between yearly incidences of CVD and either systolic or diastolic BP, the correlation was strongest between systolic BP and yearly incidences of CVD in all age groups (> 35 yrs of age) [142]. Interestingly, it was reported that in men between the ages of 38 to 41 yrs (age at entry), diastolic BP seemd to be the strongest predictor for CVD incidence after 14-year of follow-up [142]. An earlier study investigating the physiological differences between systolic and diastolic BP reported that the systolic BP is regulated in part by the elasticity of large vessels, whereas resistance in peripheral vessels, especially arterioles, contributes to diastolic BP (reviewed by [143]). Furthermore increased in

peripheral resistance contributes to hypertension without changes in cardiac output and suggests that diastolic BP is the underlying cause of hypertension while increased systolic BP is more related to increased rigidity of the aorta due to aging [143]. However, the authors do comment that it would be misleading to say that systolic BP does not contribute to the pathology of hypertension and CVD and suggest that it is more accurate to consider increases both systolic and diastolic BP when assessing CVD risk rather than just the clinical designation of hypertension (systolic BP ≥ 160 mmHg and diastolic BP ≥ 95 mmHg) [143]. Along this line, a prospective study following 11,150 male physicians with no history of CVD or antihypertensive treatment reported that systolic BP was a better predictor of CVD risk in those > 60 years but diastolic BP was a better predictor of CVD risk in those < 60 years [144]. These findings suggest that age is an important variable when assessing CVD risk associated with systolic or diastolic BP. It is also important to note that one BP measure is not necessarily stronger than the other, but depends on other variables besides age such as ethnicity, sex, and BMI.

In Chapter 4 (Aim 2), I explored another variable that is reported to be associated with BP. It has been suggested that ethnic-specific differences in BP are in part, due to ethnic-specific differences in vitamin D status [108,145]. A large cross-sectional study of 1984 African Americans and 5156 Caucasians reported differences in systolic BP between the two ethnic groups after adjustment for age and sex with serum 25OHD contributing to 26% of the mean differences in systolic BP between African Americans and Caucasians and 39 % when subjects receiving antihypertensive treatments were excluded [108]. Although, I did not find ethnic-specific differences in systolic and diastolic BP, I did find that plasma 25OHD concentrations were negatively associated with systolic and diastolic BP in South Asians and Europeans.

Vitamin D was initially postulated as a contributor to the ethnic-specific differences in BP because of several reports that factors affecting vitamin D status have also been reported to affect BP. Blood pressure has been reported to change with latitude, with higher levels observed in subjects living at greater distances from the equator; season, with higher levels observed in winter; and ethnicity, as discussed above. My findings suggested an important role of vitamin D on BP as both systolic and diastolic BP were negatively associated with plasma 25OHD concentrations, a relationship that remained after adjustment for age, ethnicity, smoking, physical activity, and alcohol consumption. However, when I included indicators of adiposity in the model the negative association between systolic and diastolic BP and plasma 25OHD concentrations were no longer significant. VAT came out to be the strongest mediating factor on the relationship between plasma 25OHD concentrations and BP. I included VAT and not SAT or total body fat (%) in the model because VAT is an independent predictor for CVD [1] and more importantly, VAT was the strongest predictor for plasma 25OHD concentrations (Aim 1). The role of VAT in hypertension has been suggested through the renin-angiotensin system by the contribution of angiotensin (Ang) II in the formation of large and dysfunctional adipocytes (reviewed by [98]), characteristic features of VAT. The large and dysfunctional adipocytes will then serve as positive feedback for Ang II, which is a strong vasoconstrictor (reviewed by [98]).

The findings from Aim 2 on the important role of VAT in mediating the inverse association between BP and circulating 25OHD concentrations is a novel finding because I found an effect of VAT on this relationship. A number of studies have reported an inverse association between circulating 25OHD concentrations and BP did adjust for BMI, but not VAT. These studies were conducted in several different populations including Caucasian, Hispanic and African Americans [64,114] [96] [108] [63,146]. However, my studies discussed above are

association studies and therefore no conclusions regarding causality can be drawn and it remains to be determined as to whether low circulating 25OHD concentrations cause high BP or that high BP contributes to low circulating 25OHD concentrations. However, one cannot rule out the possibility that improvement in vitamin D status could have some effects on BP. Supplementation with 600 mg of calcium and 400 IU of vitamin D_3 daily in a 8-week, double-blinded control trial reported that supplementation with calcium and vitamin D_3 was associated with a significant reductions in systolic and diastolic BP [103]. The study was done in 148 subjects in Germany and it is important to note that the mean BMIs between the supplemented group and control group were not significantly different [103]. Furthermore, the vitamin D supplementation also included calcium and therefore, it cannot be concluded that vitamin D alone would have the same beneficial effect on BP. Another double-blinded interventional study among 20 European postmenopausal women aged 50- 70 yrs in Switzerland, reported that daily supplementation with 800 IU vitamin D_3 for four months was associated with a lowering of systolic BP by 5.7 mmHg than levels observed in the control group [147]. However, a prospective, randomized, double-blind, placebo-controlled trial of 114 post-menopausal women in Madison, WI supplemented with 2500 IU of vitamin D_3 in a cookie showed no change in systolic or diastolic BP after four months of intervention [148]. Although the design was almost identical with an even higher dose of vitamin D_3, this study did not find the same outcomes in BP as study from Germany [103] discussed above. And although not significant, the treatment groups in the Germany study had lower BMI than the control group (23.2 kg/m^2 vs. 25.5 kg/m^2, respectively). Meanwhile, the treatment group of the Madison-based study had significantly (p = 0.022) higher BMI compared to the placebo group (27.1 kg/m^2 vs. 25.3 kg/m^2, respectively). The difference in BMI between groups might contribute to the different outcomes of the studies.

Furthermore, we also cannot assume that the same beneficial effect of vitamin D would be seen in other ethnic groups, in particular those who are more prone to low vitamin D status, such as African Americans, Afro-Caribbeans, and South Asians. Lastly, the two interventional studies only included women participants and therefore, the same conclusion cannot be applied to men.

The findings in Chapter 4 have important significance due to the mounting evidence showing the inverse association between circulating 25OHD concentrations and CVD incidents, which can include myocardial infarctions, stroke, heart failure, or prolonged chest pain documented by ECG changes [39]. The Framingham Offspring cohort with 1739 participants showed that vitamin D deficiency (circulating 25OHD lower than 25 nmol/L) was associated with incident CVD [39]. The incident of CVD was even higher in those who previously had hypertension [39]. A prospective study using the electronic medical record database of the integrated Intermountain Healthcare system containing 41,504 patient records reported that vitamin D concentrations were strongly associated with CVD (coronary heart disease, heart failure, myocardial infarction, and stroke), as well as with incident death, heart failure, coronary heart disease/myocardial infarction [62]. However, these studies are only association studies and one cannot assume the causal-effect of vitamin D on CVD events and mortality. Furthermore, as summarized in a systemic review and meta-analysis from 51 clinical trials, vitamin D intervention, and not circulating 25OHD concentrations, showed no significant effect on death, stroke, myocardial infarction, hyperlipidemia, as well as blood pressure [149]. However, not all of the clinical trials included in the systemic review had CVD or CVD-related events as outcomes and therefore, was difficult to measure outcomes following vitamin D intervention [149]. Moreover, the systemic review investigated vitamin D intervention as opposed to circulating 25OHD concentrations in serum or plasma. This can be misleading, as vitamin D

intakes do not necessarily correspond to circulating levels in the blood because of possible confounding factors such as ethnicity, age, sex, BMI, season, and body fat distribution as previously discussed in this thesis.

Considering the evidence on ethnic-specific differences in CVD risk in such a way that South Asians have a higher risk than other ethnic groups, Europeans, Africans, Chinese, Melanesians previously described [22-25,27], and that low vitamin D status is quite common among South Asians [87,88,90,91], it is important to investigate the possible mechanisms by which low vitamin status contributes to increased CVD risk. Despite the lack of knowledge in understanding the exact mechanisms by which vitamin D plays a role in CVD risk, some evidence showed that some of the possible mechanisms may include: (i) the suppression of the renin gene expression [150]; (ii) the presence of vitamin D receptors on aortic endothelial cells [151]; and (iii) the direct influence of vitamin D status on hypertension [64,97]. As explained in the background of Chapter 4, vitamin D plays an important role in the renin-angiotensin system (RAS) and is suggested do to its ability to provide negative feedback for RAS [150]. The study found that vitamin D receptor-null (*Vdr-/-*) mice had three times higher renin mRNA expression, assessed by quantitative Northern blot analysis, compared to their *Vdr+/+* littermates (n = 4 mice in each group) [150]. Furthermore, the *Vdr-/-* mice had 2.5 higher plasma Angiotensin (Ang) II compared to their *Vdr+/+* littermates (n = 15 in each group) [150]. Ang II is a strong vasoconstrictor and increased in Ang II led to significant increased in systolic and diastolic BP (> 20 mmHg) BP in the *Vdr-/-* group (n = 8 mice for the *Vdr-/-* group; n = 9 mice for the *Vdr+/+* group). Another mechanism by which vitamin D could potential have an affect on CVD is due to the presence of vitamin D receptors (VDR) on the aortic endothelial cells [151]. VDR (1,25-dihydroxyvitamin D_3 receptors) were shown to be expressed in the cultured bovine aortic

endothelial cells [151]. Moreover, immuno-istochemistry analysis showed that VDR were also expressed in the vascular endothelial cells of human dermal vessel [151]. A study in human umbilical vein endothelial cells (HUVEC) reported that incubation of the cells with either $1,25(OH)_2D_3$, 25OHD, or tumor necrosis-alpha (TNF-α, which has been associated with cell death or apoptosis) led to induction of endothelial intercellular adhesion molecule–1 (ICAM-1) and vascular cell adhesion molecule–1 (VCAM-1), analyzed using ELISA, in the cells incubated with TNF-α, and not in those incubated with $1,25(OH)_2D_3$, 25OHD [152]. ICAM-1 and VCAM-1 are endothelial adhesion molecules that are involved in recruiting monocytes, which can lead to further inflammation of the endothelial cells (reviewed by [153]). Another possible mechanism by which low circulating 25OHD could contribute to CVD risk is through its direct role in increasing risk for hypertension [97]. As reported in a prospective cohort study in 613 men from the Health Professionals' Follow-Up Study and 1198 women from the Nurses' Health Study, plasma 25OHD concentrations were inversely associated with increased relative risk of incident hypertension even after adjusted for covariates including: age, BMI, physical activity, race, smoking status, family history of hypertension, and intakes of alcohol, vitamin D, folate, sodium, calcium, magnesium, and potassium, as well as menopausal status in women [97].

6.3 Ethnic-specific differences in Adiponectin, Adiposity, and CVD Risk

In Chapter 5 (Aim 3), I investigated if ethnicity is associated with differences in the concentration of total and HMW adiponectin, as well as the total/HMW ratio in the South Asian and European M-CHAT subject. South Asians had significantly lower total and HMW adiponectin, as well as total/HMW ratio. Ethnic-specific differences in adiponectin have been reported in several populations including: Caucasians vs. Pima Native American Indians [131];

Europeans, South Asians, Aboriginal, and Chinese [154]; and Caucasian and African American women [132]. However, these studies only assessed total adiponectin concentrations and not HMW adiponectin or total/HMW ratio. HMW adiponectin has previously been shown to have a stronger association with cardiometabolic risk factors such as fasting plasma insulin, HOMA-IR, HDL-C, and TG concentrations [54-56]. A study in 59 obese Japanese children reported that both total and HMW adiponectin concentrations were inversely associated with fasting plasma insulin, HOMA-IR and TG concentrations, but only HMW adiponectin concentrations were negatively associated with VAT deposition [55]. In line with these studies in children, another cross-sectional study in 33 women and 35 men, reported that serum HMW adiponectin concentrations showed a stronger relationship with HDL-C, VLDL-C, LDL-C and insulin sensitivity than total adiponectin concentrations [54]. As such it is important to assess both total and HMW adiponectin concentrations when determining its association with CVD risk.

As discussed above, it is well known that circulating adiponectin concentrations are inversely associated with BMI [49-51,54], VAT [52,53,55,155], and insulin-glucose homeostasis and lipid concentrations [52,54,55,155-157]. I initially analyzed the relationship between total and HMW adiponectin concentrations with body composition and body fat distribution and found that both were inversely associated with BMI, WC, WHR, and VAT. However, I found no relationship with TAT and SAT. My findings are in line with findings by others that have reported stronger associations between total adiponectin concentrations and VAT rather than SAT in men from Quebec City [155], Japanese children with obesity [55], Caucasian adolescents [52], Korean women [157], and middle-aged Japanese men and women [158]. To further investigate the relationship between adiponectin with body composition and body fat distribution, I performed regression analysis adjusting for the following confounding variables:

age, sex, ethnicity, and BMI. Both total and HMW adiponectin concentrations were inversely associated with WC, WHR and VAT even after adjustment for age, sex, ethnicity, and BMI. In this analysis, ethnicity was also a significant confounding variable ($p < 0.05$). The data analysis was not separated by sex, because using the general linear model (GLM) I did not find significant interaction between sex and ethnicity. The findings indicate that as opposed to BMI, the other measures of body composition, WC and WHR are better predictors for total and adiponectin concentrations in Europeans and South Asians. Furthermore, the findings also suggest that VAT, and not SAT, has the strongest relationship with total and HMW adiponectin concentrations. The exact mechanism as to how increased VAT deposition could lead to reduction in total and HMW adiponectin concentrations remains to be determined.

The inverse association of adiponectin concentrations with body composition and body fat distribution may contribute to the observed 'protective' properties of adiponectin. Adiponectin has previously been shown to have insulin-sensitizing [52,54,131,157-159] and anti-atherogenic properties, through its role in the maintenance of lipid concentrations [54-56,130,155,157]. Using a simple bivariate correlation analysis, I found that both total and HMW adiponectin concentrations were positively associated with HDL-C, but negatively associated with TG concentrations. These findings are similar to others [54,55,155,155,157] and have been attributed to potential role of adiponectin in stimulating PPAR-α and AMPK pathways in the liver, which leads to increase fatty-acid oxidation, increased in HDL-C, and reduced circulating TG concentrations (reviewed by [48]). To determine if plasma total and HMW adiponectin concentrations independently affect HDL-C and TG concentrations, I performed linear regression analysis and controlled for age, sex, ethnicity, and BMI. As predicted, I found that both total and HMW adiponectin were independently and strongly associated with HDL-C and

TG concentrations. Furthermore, ethnicity was also a significant contributor to the associations between adiponectin (total and HMW) and HDL-C and TG concentrations. My findings suggest that ethnic-specific differences in in plasma total and HMW adiponectin concentrations may account for differences in HDL-C and TG concentrations observed between South Asians and Europeans.

To further explore the independent role of adiponectin on plasma lipid concentrations, I performed further linear regression analysis controlling for possible confounders such as age, sex, ethnicity, BMI, and VAT. VAT was included in the analysis in addition to BMI because BMI is not the best predictor of adiposity, and VAT is strongly negatively associated with adiponectin [52,54,55,155,157]. Interestingly, both total and HMW adiponectin concentrations remained strongly associated with HDL-C and TG concentrations. This suggests that even after taking ethnic-specific differences in VAT deposition between South Asians and Europeans, the low HDL-C and high TG concentrations among South Asians is strongly related to plasma adiponectin concentrations.

In addition to a role in the maintenance of plasma lipid concentrations, plasma total and HMW adiponectin are also involved in the glucose-insulin homeostasis. It has been suggested that via the PPAR-α and AMPK pathways, adiponectin regulates the translocation of GLUT4 to plasma membranes, which leads to increased glucose uptake and lowering circulating blood glucose levels (reviewed by [48]). Using a bivariate Pearson's correlation analysis, I found that plasma total and HMW adiponectin concentrations were inversely associated with fasting plasma glucose and insulin, and HOMA-IR. While the role of adiponectin in the maintenance of blood glucose is clear, the role of adiponectinin insulin homeostasis is less so. However, a large body of clinical research findings indicates that the role of adiponectin on insulin homeostasis is

crucial. A study in adolescents reported that plasma adiponectin concentrations were positively correlated with peripheral and hepatic insulin sensitivity and negatively associated with fasting proinsulin and the proinsulin-to-insulin ratio, as determined by a constant-rate glucose infusion and a 3-h hyperinsulinemic-euglycemic clamp [52]. Similarly, a study in 33 women and 35 men also reported that total and HMW adiponectin concentrations were strongly associated with increased insulin sensitivity as determined by the euglycemic-hyperinsulinemic glucose clamp technique [54]. Furthermore, HMW adiponectin concentrations showed a stronger association than total adiponectin concentrations suggesting a role for HMW adiponectin in maintaining insulin homeostasis. However, there have not been many studies in South Asians assessing the role of adiponectin on insulin sensitivity or resistance, and in those that have been reported the findings are inconsistent. A cross-sectional study in 30 South Asian and 22 Caucasian women reported no relationship between insulin resistance and circulating total and HMW adiponectin concentrations in South Asian women but this relationship was observed in the Caucasian women [159]. In contrast, a cross-sectional study in 150 South Asian men and women from San Francisco reported that adiponectin was strongly associated with insulin resistance, but the strength of this association was attenuated after adjustments for body composition [160]. The discrepancies in findings can be due to various reasons, one being the characteristics of study participants, whether they are BMI-category-matched or not. Another would be the participants' state of insulin sensitivity or insulin resistance. In my thesis, the study participants were recruited as apparently healthy individuals with no pre-existing CVD, T2DM, or use of antihypertensive medications.

The study participants of the M-CHAT cohort were recruited to satisfy an equal distribution of participants based on the normal, overweight and obese category using BMI [33].

Therefore, we would expect that the association of total and HMW adiponectin concentrations are not attributed to differences in BMI. In fact, after adjustment for BMI, as well as age, sex and ethnicity, total and HMW adiponectin concentrations remained negatively associated with fasting plasma glucose, insulin and HOMA-IR. In this analysis, ethnicity was also a strong confounding factor ($p < 0.05$) suggesting that differences in fasting plasma glucose, insulin and HOMA-IR between South Asians and Europeans may be attributed to differences in total and HMW adiponectin concentrations. Further adjustment with VAT also revealed that the association between both total and HMW adiponectin concentrations with fasting plasma insulin and HOMA-IR remained significant. This suggests that despite ethnic-specific differences in VAT deposition between South Asians and Europeans, ethnic-specific differences in adiponectin concentrations contribute to glucose and insulin homeostasis, independent of VAT.

Although others have shown that adiponectin might have effects on other CVD risk factors such as systolic and diastolic BP [157], I only found a negative association between both plasma total and HMW adiponectin concentrations with diastolic BP. This inverse association remained significant even after adjustments for age, sex, ethnicity, and BMI. However, once VAT was included in the model, the relationship was no longer significant suggesting VAT is the mediating factor. The association between BP and adiponectin is not well-studied. However, a study in animal models showed that adiponectin knock-out (KO) male mice (8 to 10 weeks old) had significantly higher systolic BP, measured using the tail-cuff technique with an automatic sphygmomanometer at the tail artery or using the arterial catheters in the carotid artery, than wild-type controls [161]. Furthermore, intravenous injection of adenoviral-delivered adiponectin reduced systolic BP in the adiponectin knock-out mice to the levels of the wild-type controls [161].

In summary, ethnic-specific differences in VAT deposition contribute to ethnic-specific differences in total and HMW adiponectin. South Asians with higher VAT deposition compared to the Europeans had significantly lower plasma total and HMW adiponection concentraions, which may contribute to greater CVD risk. In fact, a recent finding from the M-CHAT cohort confirmed that South Asians have elevated risk for CVD and it is attributed to their elevated VAT deposition [35].

6.4 Summary and Conclusions

The strengths of my thesis rely upon several factors. The first is the unique phenotype of the study participants. It is a privilege to workwith the M-CHAT population in which the study participants are well-characterized for their ethnicity. An additional strength of my thesis is how well-phenotyped the body composition of study participants, using the sophisticated imaging techniques such as DEXA and CT scanning to quantify discreet regions of body fat composition. This enables me to assess the association of plasma 25OHD and total and HMW adiponectin concentrations with several discrete indicators of body fat composition and distribution. Furthermore, the participants had complete demographics and biochemical data, which enable me to adjust for confounding factors.

However, there are some limitations in the interpretation of the findings reported in my thesis. It is important to note that this is a cross-sectional observational study and although, some of the mechanisms that underlie the association are well-known, the findings of this study cannot be extrapolated for causality.

Further prospective studies are required to assess the relationship of plasma 25OHD and adiponectin concentrations with body fat composition and distribution over time and how this

relates to the development of CVD in European and South Asian subjects. Furthermore, additional research is required to investigate the relationship between vitamin D and adiponectin and the possible protective effects on development of CVD. Several studies have reported a positive association between vitamin D and adiponectin [162] [163] [83][164]. However, most of these studies found that after adjustment for BMI, the positive association was no longer significant [162,163] whereas others found that the association remained following adjustment for BMI [83,164]. However, none of these studies or others has looked at the role of VAT in mediating the association. I conducted a preliminary investigation and found a significant positive association between plasma 25OHD concentrations and total and HMW adiponectin concentrations. However, the associations quickly disappeared following adjustment for BMI, age, sex, and ethnicity. Therefore, I did not include this analysis in my thesis.

6.5 Future Directions

Additional studies are required to assess mechanisms underlying the metabolism of 25OHD by different fat depots, and to determine whether changes in VAT deposition predicts circulating 25OHD concentrations or whether low circulating 25OHD concentrations are a consequence of increased VAT deposition. In addition, to investigate if vitamin D supplementation lowers VAT deposition or CVD risk or effects weight loss, adequately powered randomized control trials (RCTs) need to be conducted. Furthermore, epigenetic mechanisms, which are heritable changes in gene expression that occur without changes in the DNA sequence has been shown to play an important role in the pathology of CVD [165]. Adiponectin has been reported to be regulated by DNA methylation, an epigenetic mechanism [166]. Therefore, by analyzing the DNA methylation patterns, in particular by looking at markers for global DNA

methylation between South Asians and Europeans and CVD risk factors would further elucidate if epigenetic regulation through DNA methylation is the underlying mechanism that regulates the role of adiponectin on CVD risk factors.

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