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ULTRASOUND EVALUATION OF THE CAROTID ARTERY IN A POPULATION AT HIGH RISK OF TYPE 2 DIABETES MELLITUS

By

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MASTERS OF TECHNOLOGY: RADIOGRAPHY

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PREFACE

This thesis is submitted towards a Masters of Technology Degree in Radiography. Some of the literature presented in this thesis has already been published as referenced below:

1. **Kisten, Y**., Govender, P., Naidoo, N.G., Gihwala, D. and Isaacs, F. 2013. Duplex ultrasound: A diagnostic tool for carotid stenosis management in type 2 diabetes mellitus. *Afr J Prm Health Care Fam Med*. 5(1), Art. #414, 6 pages. http://dx.doi.org/10.4102/phcfm.v5i1.414

2. Matsha, T.E., Hartnick, M.D., **Kisten, Y**., Erasmus, R.T. and Kegne, A.P. 2013. Obesity phenotypes and subclinical cardiovascular diseases in a mixed-ancestry South African population: A cross-sectional study. *J Diabetes*. Oct 13. DOI: 10.1111/1753-0407.12089. PMID: 24028321. http://onlinelibrary.wiley.com/doi/10.1111/1753-0407.12089/full

Chapter 1 introduces the topic, background information, research problem, questions and objectives of the study. It gives the reader the author's perception of gathered information. The research is hypothesis driven. The writing style of this thesis uses a combination of styles from different learning areas and influences in keeping with CPUT guidelines. The delimitations are outlined, and the significance of the study is established. Chapter 2 reviews the related literature, and discusses concepts, which are important in understanding why the study was done, in both the national and global context. An integrated theory base is provided to demonstrate the mechanisms of atherosclerosis, its contributing risk factors and the value of ultrasound research in diabetes, cardiovascular diseases and stroke, and how this study compares with the current global trends. The potential of new knowledge generation increases in a population that is unique to the Western Cape of South Africa. Chapter 3 is the recipe. It discusses the methodology (research design, variables used and sample selection) and how the study was carried out. The inclusion and exclusion criteria, ethical guidelines and the protocols are also mentioned. Chapter 4 discusses the results, according to the research objectives of the study and chapter 5 forms part of the discussions made as a precursor to the conclusions derived from this ultrasound study.

DECLARATION

I, Yogan Shunmugam Kisten, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Yogan. S. Kisten	29 April 2015
Signed	**Date**

ABSTRACT

Interrelationship between ultrasound evaluation of the carotid artery and type 2 diabetes in a selected population

BACKGROUND: Diabetic patients are at increased risk of cardiovascular events and stroke, and its prevention is therefore the desired goal. In the arsenal of available techniques, ultrasound plays a vital role in primary healthcare. It is reliable, cost-effective and a non-invasive diagnostic tool that may prove beneficial for screening individuals at risk of cardiovascular disease (CVD) and stroke in SA.

OBJECTIVE: To determine the interrelationships between carotid ultrasound findings with glycaemia status and contributing risk factors of atherosclerosis in the selected population.

METHODS: Initially blinded by the glycaemia status, blood results, contributing risks and patient demographics, both carotid arteries were evaluated with duplex ultrasound (DUS), during July 2010 – July 2011. Using graphs, figures, frequency tables, means and standard deviations for the selected study population, univariate, multivariate and stepwise regression analysis was done to determine the association between ultrasound findings and risk factors for atherosclerosis. The hypothesis tested in this study was to determine if there is an increased incidence of carotid artery intima-media thickening (CIMT), plaque formation and stenosis in patients diagnosed with T2DM and hyperglycaemia in a very specific sub-population of mixed-ancestory, residing in Bellville South Africa (BSA).

RESULTS: Of 534 subjects, 375 were of mixed ancestry and ≥35_{yrs} of age, which met the inclusion criteria for the carotid ultrasound substudy. The glycaemic status for each individual was established, and 44% (165/375) were diagnosed hyperglycaemic, of which 66.7% (110/165) were diabetic (T2DM) and 33.3% (55/165) were pre-diabetic (Pre-DM). Majority (56%:265/375) had a normal glycaemic status. The ultrasound measurement of the carotid wall thicknesses (Mean Rt. and Lt. CIMT) revealed a statistically significant rise from normal glycaemia status to DM status for both the males **($p = 0.0115^{*}$; $p = 0.0259^{*}$)** and females **($p < 0.0001^{**}$; $p < 0.0001^{**}$)** respectively. In terms of plaques and internal carotid artery (ICA) stenosis (124/375), when grouped into normal and hyperglycaemic sub-groups, indicated plaque presence and some form of narrowing. A <50% stenotic ratio noted in 61% (76/124) of the hyperglycaemic group, that was 1.6 times higher than those with normal glycaemia (48/124). Predisposing factors demonstrated significantly higher levels in the females than in the males. The univariate multiple regression analysis after adjusted R^2 of 0.3247 for all independent variables (predisposing /contributing risk factor markers) of age ($_{yrs.}$), SBP ($_{mmHg}$), hs-CRP ($_{mg/L}$), S-Cotinine ($_{ng/mL}$) and LDL ($_{mmol/L}$) showed statistically significant positive associations with dependent variable of the mean carotid wall thickness **($p < 0.0001^{**}$, $p< 0.0001^{**}$, $p = 0.0033^{*}$, $p = 0.0409^{*}$ and $p = 0.0044)^{*}$** respectively. Statistically significant positive differences and standard error (SE), for every unit of change ($1_{yr.}$) of age $_{(yrs.)}$, as a contributing factor for atherosclerosis, there was a change in

the mean carotid wall thickness as predicted according to this model. The total contribution of independent risk factors to CIMT ultrasound measurements were calculated as 34.5% (Adjusted R^2 = 0.3247). In the multivariate stepwise regression analysis, the independent variables of age **(p < 0.0001) **** and systolic blood pressure **(p < 0.0001) **** showed the strongest positive association with carotid wall thickeness changes. The hs-CRP $_{(mg/L)}$ inflammatory markers **(p = 0.0014)*** and LDL $_{(mmol/L)}$ **(p = 0.0208)*** were the 2nd and 3rd highest positive associated contributory risk factors for carotid artery wall thickening. The hip circumference **(p = 0.0008)*** and waist circumference **(p = 0.0555)** $^{+}$ risk factors related to obesity was significant and approached significance, respectively, with the predicted increase of carotid artery wall thickening.

CONCLUSION: Subjects diagnosed with T2DM and hyperglycaemia had increased levels of CIMT, plaques and carotid artery stenosis, compared to those subjects without T2DM. Age and systolic blood pressure, inflammatory (raised hs-CRP) and LDL cholesterol changes, and central (truncal) waist circumference adiposity, were positively associated with increased carotid intima media thickness. Smoking (S-Cotinine) and gender also reflected a direct relationship with CIMT changes. The hip circumference adiposity and diastolic blood pressure measurements were not directly associated with an increase in CIMT, which are in keeping with hypertension and obesity formulas. These findings confirm the association of thickened CIMT, plaques and stenosis with 'unhealthy' T2DM subjects at higher risk of CVD and stroke. The total contribution of independent risk factors to CIMT measurements were calculated as 34.5% (Adjusted R^2 = 0.3247). The gathered information, discussion of results, and concluding statements thereby supports the recommendation of carotid artery ultrasound evaluation, for screening and diagnosis in primary health care, for 'flagging' high risk individuals at risk of stroke, so that lifestyle changes and appropriate management is early adopted.

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■■ Peninsula Technikon, now known as the Cape Peninsula University of Technology. ■■Groote Schuur and Tygerberg Hospital and its referral centres. ■■ Umhlanga Radiology ■■ Medical Research Council (MRC) ■■ Natal Technikon, Durban Institute of Technology now known as Durban University of Technology. ■■ The Nelson Mandela Scholarship Programme. ■■ The University of Wales, Bangor, United Kingdom. ■■ University of Liverpool, United Kingdom. ■■ The University of Cape Town. ■■ The University of Stellenbosch ■■ University of Johannesburg ■■EMA2SA scholarship, Karolinska Institute, Global Health Division and Department of Medicine, Unit for Clinical Therapy Research, Inflammatory Diseases (ClinTRID). Karolinska University Hospital, Stockholm, Sweden.

DEDICATION

To all the participants of this study

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CHAPTER 5

GLOSSARY

CLARIFICATION OF CONCEPTS

Acoustic Shadowing: Occurs due to failure of a sound beams passing through an object, caused by attenuation. E.g. calcified/echogenic plaques prevent sound from passing through, hence the shadowing is pronounced (Terminology and Diagnostic Criteria Committee, 2009; Sanders and Winter, 2007:34; Edelman, 2007; Kremkau, 2006).

Arteriosclerosis: A chronic disease characterised by abnormal thickening and hardening of the arterial walls with resulting loss of elasticity (Brohall, 2007; Dähnert, 2003).

Atherosclerosis: A disorder characterised by atheromatous deposits and fibrosis of the inner layer of the arteries (Pellizzon, 2008). The walls may appear sonographically echogenic/ hyperehoic, and when atherosclerotic changes occur, it could have appearances of mixed echogenicity (Myers and Clough, 2004; Dähnert, 2003).

Biomarker: A measurable biochemical indicator of a biological state, such as the progress of disease (Mitka Mike, 2012).

B-Mode: Brightness modulation: A two-dimensional display of the intensity (amplitude) of an echo by varying the brightness of a dot to correspond to echo strength on real time ultrasound scanners (Edelman, 2007; Sanders and Winter, 2007; Kremkau, 2006).

Cardio Vascular Disease (CVD): An abnormal condition of the heart and its circulation. CVD includes: arteriosclerosis, coronary artery disease, heart valve disease, arrhythmia, heart failure, hypertension, orthostatic hypotension, shock, endocarditis, diseases of the aorta and its branches, disorders of the peripheral vascular system, and congenital heart disease (Heart and Stroke Foundation, South Africa, 2006)

Carotid Intima Media Thickness (CIMT): The thickness or measurement of the inner lining of the carotid artery called intima and the intermediate layer called the media in combination with ultrasound guidelines for CIMT measurement (Conventional ultrasound CIMT is average readings of the 3 CIMT points ±2cm from carotid bifurcation).

Carotid Endarterectomy (CEA): Surgical removal of the carotid bifurcation plaque designed to prevent future strokes

Carotid Plaque Characterisation: As the progression of plaque lesions vary in echogenicies, echotextures and echopatterns, so too must the atherosclerosis disease process. Plaque characteristics are described with reference to its reflective ultrasound properties. The carotid wall surface could be regular or irregular or the plaque lesions homogenous or heterogeneous. The reflective echoes are determined by the plaque constituency, either soft (hypo-echoic or iso-echoic) or hardened (echogenic / hyper-echoic or mixed echogenicity). Plaque stability, area and volumes whenever possible, is used to compliment the plaque characterisation (Terminology and Diagnostic Criteria Committee, 2009; Dähnert, 2003: 374).

Colour Doppler: Doppler echoes are usually displayed with grey scale brightness mode. In Colour Doppler, the colours correspond to the direction of flow, either positive or negative, and Doppler shifts that are directed towards or away from the transducer. The brightness of the colour represents the intensity of echoes, to indicate the extent of spectral broadening (Terminology and Diagnostic Criteria Committee, 2009; Evans, Jensen and Nielsen, 2011).

Coronary Artery Disease (CAD): A condition, and especially one caused by atherosclerosis, that reduces the blood flow through the coronary arteries to the heart muscle and typically results in chest pain or myocardial infarction (Mitka Mike, 2012; National guidelines for stroke care, 2005).

Diabetes Mellitus: A metabolic disorder of multiple aetiologies characterised by chronic hyperglycaemia with alterations in carbohydrate, fat and protein metabolism resulting from insulin deficiency, insulin resistance, or both. The effects of uncontrolled diabetes mellitus may result in long-term vascular damage and target organ dysfunction or failure (Balasubramaniam *et al.,* 2012).

Doppler Doppler ultrasound is dependent on the insonating (transmitted) frequency (f), the velocity of moving blood cells (V) and the angle between the sound beam and direction of the moving blood cells (θ) divided by the ultrasound speed constant (c) 1540m/s. The Doppler Frequency shift formulae is $\Delta F = (2fV\cos\theta)/c$. If the sound beam is perpendicular to the direction of blood flow, there will be no Doppler shift ($\cos 90^\circ = 0$). Therefore there would be no display of flow in the vessel. The angle of the sound beam should be less than 60 degrees at all times to reduce artefacts. (Edelman, 2007; Sanders and Winter, 2007; Kremkau, 2006; Zwiebel, 2005).

Hyperlipidaemia: Elevated concentration of lipid in the blood can cause pancreatitis (Balasubramaniam, Viswanatha *et al*, 2012).

Impaired Glucose Intolerance (IGT): Impaired glucose tolerance is regarded as the intermediate stage in the natural history of diabetes mellitus. IGT is defined as two-hour glucose levels of 140 to 199 mg per dL (7.8 to 11.0 mmol) on the 75-g oral glucose tolerance test. These glucose levels are greater than normal but below the level that is diagnostic for diabetes. People with IGT have a significant risk of developing diabetes and thus are an important target group for primary prevention (MedicineNet, 2009).

Impaired Fasting Glucose (IFT): Impaired fasting glucose is the intermediate stage in the natural history of diabetes mellitus. IFT is defined as glucose levels of 100 to 125 mg per dL (5.6 to 6.9 mmol per dL in fasting patients. The glucose levels are greater than normal but below the level diagnostic for diabetes. People with IFT have a significant risk of developing diabetes and are also an important target group for primary prevention (MedicineNet, 2009).

Power Doppler: Another type of Doppler flow imaging in which the integrated power of the Doppler signal is displayed, rather than the mean frequency shift used in conventional colour Doppler (Edelman, 2007; Sanders and Winter, 2007; Kremkau, 2006).

Transducer (Probe): A device capable of converting energy from one form to another (Piezoelectric effect). Heat is given off when the sound wave is attenuated, through absorption (Zwiebel, 2005). The transducer houses the piezoelectric crystals which are the senders and receivers of sound waves that are displayed as ultrasound images

Type 1 diabetes mellitus (T1DM): Previously known as insulin-dependent or childhood-onset diabetes. It is characterised by a lack of insulin production resulting from atrophy of the islets of Langerhans and causing hyperglycemia and a marked tendency toward ketoacidosis. Also called juvenile diabetes, juvenile-onset diabetes, type 1 diabetes mellitus (T1DM).

Type 2 diabetes mellitus (T2DM): T2DM, previously known as non-insulin-dependent or adult-onset diabetes. It is caused by the body's ineffective use of insulin. It often results from excess body weight and physical inactivity (Balasubramaniam, Viswanathan et al., 2012).

Spectral Imaging and Stenosis: A display that shows the various waveforms that make up the pulsed Doppler profile. (Wood et al., 2010; Sanders and Winter, 2007; Edelman, 2007) Stenosis is regarded as the narrowing (Wood et al., 2010; Edelman, 2007) of a lumen, vessel or organ. In carotid stenosis, there is narrowing of the carotid artery usually due to plaque formation. Narrowing of the vessel increases the blood flow velocities, and at a stenosis there is higher resistance to flow

Transient Ischemic Attack (TIA): Transient paralysis of a part of the body due to temporary interference with blood supply to the brain (Sanders and Winter, 2007)

GLOSSARY

ABBREVIATIONS/ ACRONYMS

BMI	Body Mass Index
BSA	Bellville South Africa
CAD	Coronary Artery Disease
CCA	Common Carotid Artery
CEA	Carotid Endarterectomy
CIMT	Carotid Intima Media Thickness
CVD	Cardiovascular Diseases
DM	Diabetes Mellitus
ECA	External Carotid Artery
EDV	End Diastolic Velocity
ICA	Internal Carotid Artery
IFT	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IMT	Intima Media Thickness
Pre-DM	Pre-Diabetes Mellitus
PSV	Peak Systolic Velocity
TIA	Transient Ischemic Attack
TPV or TVP	Total Plaque Volume or Total Volume of Plaque
T1DM	Type 1 Diabetes Mellitis
T2DM	Type 2 Diabetes Mellitis
WC	Waist Circumference
HP	Hip Circumference

CHAPTER 1

INTRODUCTION

CHAPTER ONE

1.0 INTRODUCTION

The increasing prevalence of diabetes associated with atherosclerosis and high blood pressure makes it an ever-increasing contributor to morbidity, pre-mature mortality and healthcare costs, especially in middle and low income countries like South Africa. A common concern in the South African public healthcare setting is that patients present to specialised referral centres when their condition is already severe. Type 2 diabetes mellitus (T2DM) and other identifiable risk factors lead to atherosclerosis, and is further complicated when it causes carotid and coronary artery stenosis. Feasable measures such as ultrasound evaluation of the carotid arteries is therefore proposed to early identify these changes, in the light of preventing further complications related to coronary artery diseases (CAD) and stroke.

Atherosclerosis is a complex chronic disease characterised by the accumulation of lipids within arterial walls that eventually develop into plaques, which results in some form of stenosis and/or occlusion of arteries (Balasubramanian et al., 2012; Pellizzon, 2008). Stroke and coronary artery disease in particular, is the cause for a large proportion of deaths in South Africa (Steyn and Fourie, 2007). The exact aetiology of atherosclerosis remains unknown; however certain risk factors, either modifiable or non-modifiable, increase an individual's chance of its development (Hansson, 2005). Carotid stenosis caused by atherosclerosis can be prevented, or its development delayed, when appropriate management is adopted earlier (Hansson, 2005; Gronholdtl et al., 2001). Utrasound evaluation has a vital role to play in this regard. Some of the contributing risk factors, either dependent or independent of one another, in no particular order include:

⊙Diabetes Mellitus ⊙Hypercholesterolemia ⊙Hypertension ⊙Smoking ⊙Obesity
⊙Inactive lifestyle ⊙Age ⊙Ethnicity ⊙A family history of CVD and stroke.

Ultrasound is a reliable and cost effective imaging modality when used responsibly and as an alternative to other diagnostic modalities, where scientific evidence supports its appropriateness in healthcare (American Institute of Ultrasound in Medicine (AIUM), 2012; Kremkau, 2006; Edelman, 2007; Bots et al., 1997). Sound wave technology continues to evolve, and when used in telemedicine, further contributes to primary healthcare (Wootton et al., 2009; Crisp, 2007). However this transfer of ultrasound technology to the rural parts of middle and low-income countries, like South Africa, is not used to its full potential. The lack of human resources and infrastructure is in itself a problem in countries like South Africa, and is said to pose a major barrier in implementing preventative medical strategies (World Health Organization (WHO) Report, 2006). These include early detection and life style modification, to reduce ill health.

While physicians can only indirectly assess an individual's risk based on various predisposing risk factors, the possibility to directly depict the changes, (atherosclerosis in the vascular walls in patients with diabetes mellitus for example), adds more value to an individuals' risk assessment.

Kisten et al., (2013) also reported in a mini-study performed at a tertiary education hospital of the Western Cape that predisposing atherosclerotic risk factors significantly increased the diagnosis of carotid stenosis development in Type 2 diabetes mellitus (T2DM). Duplex ultrasound in that study proved to be an effective diagnostic tool for carotid stenosis management in T2DM. Holland et al., (2009) also showed the role of ultrasound (Carotid intima-media thickness) as a predictor of coronary artery disease in South African patients of African decent.

Apart from real-time four dimensional (4D) technology advancements, the use of Doppler principles in blood flow imaging has also evolved. Contemporary spectral Doppler signature analysis provides earlier identification and quantification of sub-clinical vascular disease. The spectral waveform characteristics and patterns of specific vessels are analysed in order to offer a more precise and accurate diagnosis (Wood et al., 2010; Bucek, et al., 2006). A combination of ultrasound imaging techniques (general, vascular ultrasound and echocardiography), good clinical evaluation and laboratory test results, provide additional benefit in early atherosclerosis detection, for more precise cardiovascular risk assessment (Taylor et al., 1985; Stein et al., 2009; Golledge and Siew, 2008). Globally, ultrasound imaging complimented by other diagnostic techniques continues to evolve and its utility value is spread across various disciplines (Steyn and Fourie, 2007). Ultrasound imaging of the Carotid Intima-Media Thickness (CIMT), coronary calcification assessed by electron-beam Computed Tomography (CT), plaque characterisation, and Magnetic Resonance Imaging (MRI) of the carotid artery walls, have all been explored to further understand the pathogenesis of atherosclerosis (Spence, 2006). Whatever the method used, it is the early detection that is of utmost importance.

The purpose of this study was to therefore establish the association of the carotid ultrasound evaluation (CIMT, plaques and stenosis) as an indicator of atherosclerosis, with T2DM, and other risk factors for atherosclerosis, in an aging ($\geq 35_{yrs}$) South African mixed Ancestry population. Whether this can be used as a motivation for offering ultrasound screening programs to improve early diagnosis and the management of individuals at risk of CAD and stroke in SA, still remains to be tested. The hypothesis tested in this study was to determine if there is an increased incidence of carotid artery intima-media thickening, plaque formation and stenosis in patients diagnosed with T2DM, in a very specific sub-population. The study was designed to answer the following questions: What is the association

between the carotid ultrasound findings and an individual's glycaemia status? What relationship do the associated risk factors of age, obesity, hypertension, smoking, hypercholesterolemia and T2DM have on the development of carotid atherosclerosis in the selected population?

Screening tests for carotid atherosclerosis are not routinely done (Lorenz et al., 2006) in the public sector of most ultrasound departments in middle and low income countries globally, let alone, in the Western Cape of South Africa. The global problem of obesity and diabetes continues to grow in South Africa (Van der Merwe and Pepper, 2002: 315). Communities affected by poor socio-economic conditions, where diet, lifestyle, illiteracy, lack of awareness and understanding of the epidemic, is in itself, a problem (Scott et al., 2002). Literature (Wetterholm, 2008; Wohlin et al., 2008; Gronholdtl et al., 2001) suggest many measures to address the epidemic, highlighting the need for prevention and more accurate diagnostic techniques that can early identify people vulnerable to atherosclerosis, T2DM, CAD and stroke. These differences also occur among people of different ethnicities or ancestral origins (Anand et al., 2000).

When referring to mixed ancestry population in the South African context, colonial history and urbanisation need to be considered. This group is also subject to a more Western lifestyle that predisposes them to greater risk of associated atherosclerotic diseases. This might indeed be exacerbated by hereditary factors subjecting them to this disposition (Kisten et al., 2013). Higher incidence of carotid stenosis in SA could also be associated with urban living (Holland et al., 2009; Kisten et al., 2013). Cultural and lifestyle changes due to urbanisation also contribute to development of T2DM, hypertension, increased blood cholesterol levels, alcohol abuse, smoking and obesity. There is a genetic link among the mixed ancestry population and South Africans from different social ethnic groups. Genome studies by de Wit *et al* (2010), using both the admixture and linkage models analysis in STRUCTURE revealed that the major ancestral components to be predominantly Khoesan (32-43%), Bantu-speaking Africans (20- 36%), European (21-28%) and a smaller Asian contribution (9-11%), depending on the model used (de Wit et al., 2010). This was consistent with historical data. While of great historical and genealogical interest, de Wit et al., (2010) also reported that this information is essential for future admixture mapping of disease genes in the mixed ancestry population (de Wit et al., 2010). There has been a marked geographical and ethnic variation in the prevalence of diabetes caused by urbanisation, demographic and epidemiological transitions that have rendered diabetes to be one of the major non-communicable diseases in South Africa (Levittt et al., 1993; Erasmus et al., 2012; Matsha et al., 2011; Erasmus et al., 2001; Motala et al., 2003; Kisten et al., 2013). The rates of chronic diseases vary substantially throughout the world and continue to increase in South African sub-urban townships.

The ultrasound carotid study was based at a research clinic in the Bellville South suburb of the Western Cape, and is home to approximately 21 536 people of mixed ancestry origin (City of Cape Town Census, 2001), who have been identified as a population at high risk of T2DM (Erasmus et al., 2012; Matsha et al., 2011). The Bellville South Township was formed in the late 1950s, historically considered to be reserved for 'coloured' people, referred to as an ethnic group of mixed Ancestry in the carotid study. A statistical record from the Bellville South Community Health Care Centre (Kasselsvlei) indicated an increased number of patients presenting to the clinic with chronic and lifestyle diseases (Keating, 2008). Also noted by Matsha et al., (2011) was that a large proportion of the Bellville South participants tested in 2008, were unaware of their diabetes status.

This ultrasound study is written up as a master's thesis based on the results of a quantitative research study entitled: 'Ultrasound evaluation of the carotid artery in a population at high risk of T2DM' which forms part of the larger Bellville South Africa (BSA) study, which seeks to understand the inter-relationships between obesity, diabetes and impaired glucose tolerance with diet, tobacco and alcohol intake, physical activity, atherosclerosis, cardiovascular risks, lipids, pro-inflammatory and oxidative markers in an urban adult mixed ancestry population.

This cross sectional cohort was made up of a sample drawn from the larger BSA study over a 12 month time frame. Both carotid arteries were evaluated ultrasonically on all the volunteers for this study, however, only those who met the inclusion criteria of the carotid ultrasound study were included for this sample. The glycaemic status, blood laboratory results, associated risk factors, anthropometric measurements, demographics and CVD risk profiles were established by the larger BSA study. However, this information was withheld from the researcher (blinded) up until the end of the ultrasound study, thus reducing biases, ensuring increased reliability and validity of the study findings.

This study is unique in terms of ethnicity and therefore excluded participants who were not of mixed ancestry. The NASCET guidelines (NASCET collaborators, 1991; Staikkov, Arnold and Mattle, 2000), recommendations made by the 'Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine (2009); Society of Radiologists in Ultrasound Consensus Conference (2003), and ultrasound vascular laboratory protocols were used for the carotid ultrasound examination. The American College of Radiology (ACR) and American Institute of Ultrasound in Medicine (AIUM) Safety and Practice guidelines (ACR-AIUM-SRU) for the performance of an ultrasound examination of the extracranial cerebrovascular system (Res. 10-2011) were also adopted. The ACR-AIUM-SRU has recently updated the safety and practice guidelines to (Res. 39-2014).

The research objectives for this study population were as follows:

- To determine the blood flow velocimetry of the common carotid arteries (CCA), internal carotid arteries (ICA), external carotid arteries (ECA) and vertebral arteries using spectral Doppler ultrasound imaging.

- To measure the common carotid wall thickness (CIMT) using B-mode ultrasound.

- To determine the characteristics and number of plaques present.

- To establish whether there is an association between the ultrasound findings and glycaemic status (normal blood glucose level, Pre-DM and T2DM)

- To establish the association between the ultrasound findings and the atherosclerotic risk factors of age, obesity (increased waist circumference, Hip circumference and BMI), hypertension, smoking, increased blood cholesterol levels (lipid profile), glycaemic status and gender.

CHAPTER 2

LITERATURE REVIEW

CHAPTER TWO

2.0 LITERATURE REVIEW

The major focus in the management of patients with T2DM is glucose control. This however should always be in the context of a comprehensive cardiovascular risk factor program, to include smoking cessation and the adoption of other healthy lifestyle habits, blood pressure control, lipid management with priority to statin medications, and, in some circumstances, antiplatelet therapy (Inzucchi et al., 2015). Reducing hyperglycemia is thought to decrease the onset and progression of microvascular and macrovascular complications. Ultrasound has a major role to play in the management of T2DM patients.

2.1 Incidence of T2DM

The key findings from the International Diabetes Federation (IDF) in 2014 revealed that 387 million people (mostly aged between 40-59 years), have diabetes, and that by 2035 is projected to rise to 592 million globally (IDF Diabetes Atlas, 2014; King et al., 2008). The United Nations has also declared Diabetes Mellitus (DM) as a chronic, debilitating and costly disease associated with severe complications, which poses severe risks for families, Member States, and the entire world (General Assembly 61st session - Agenda 113). The increasing incidence of T2DM is spreading across each region, 77% of which are located in low and middle-income countries like South Africa. Also alarming, is that 179 million of the world's population that are diabetic, are undiagnosed, indicating that 1 in 2 people with diabetes, do not know that they have it. In relation to the global regions affected, it was shown by the IDF that in Africa, 76% of deaths due to diabetes are in people under the age of 60 years, with Europe having the highest prevalence of type 1 diabetes in children. There are 1 in 10 people in regions of Middle East and North Africa that are affected by diabetes. In North America and the Caribean, 39 million are reported as having diabetes with a prevalence of 11.4%, and that more dollars were spent on healthcare for diabetes in 2014, than in any other region. In South and Central Americas, 25 million or 1 in 12 of the population (8.1% prevalence) are living with diabetes, while 25.4% are undiagnosed. In South Asia, 75 million people have diabetes, but more than half the population are undiagnosed (52.8%). In the Western Pacific, 138 million of the adult population are diabetic, which presents as the highest diabetes burdan across all regions globally (IDF Diabetes Atlas, 2014).

Urbanisation and the adoption of Western lifestyles have shown to be a consistent theme for the rise in diabetes and non-communicable diseases in sub-Saharan Africa (Kegne, et al., 2005; Mollentze, W.R. 2003). The IDF Diabetes Altas (2014) illustrates that 21.5 million

Africans have diabetes, affecting 1 in 20 adults. The diabetes prevalence being 5.1%, indicating the lowest prevalence across regions, however is also the region with the highest percentage of undiagnosed diabetics (61.5%) and with lowest diabetes-related expenditure (1% of word-wide total). African populations are believed to have undergone some genetic changes that make them prone to diabetes development. Some of the elements of 'Westernization' as described by Kegne et al., (2005) include a diet higher in total calories and fat but lower in fibre and less need to expand energy because of labour-saving devices. The various components of diet have shown a link to the prevalence and incidence of T2DM, even though the exact dietary composition that causes the greatest risk is not absolutely clear (Kegne et al., 2005). Erasmus et al. (2012) showed a 28.2% prevalence of T2DM within the Bellville South African mixed-ancestry community tested in 2008-2009, and suggested that the high prevalence of undiagnosed diabetes in South Africans may grow to epidemic proportions in the near future, especially if this condition is not early detected. Type 2 diabetes mellitis and other identified risk factors mentioned elsewhere, lead to atherosclerotic changes in the arteries which can be early detected with ultrasound. Kisten et al. (2013) found the incidence of severe stenosis that led to surgery (carotid artery endarterectomies) to be higher in those patients of European decent (male: p=0.0411; female: p=0.0458), than the other social groups sampled over a limited study period. Genetics, being overweight, a poor balanced diet, and too little exercise, increases the risk of diabetes as emphasised elsewhere (Jones, 2007; National Institute of Health, 2011). Glycaemic status and gender differences also vary between individuals.

2.2 Description of the disease

Diabetes mellitus is described as a metabolic disorder of multiple aetiologies. It is characterised by chronic hyperglycaemia with alterations of carbohydrate, fat and protein metabolism due to insulin deficiency and insulin resistance, or a combination of both (Balasubramania et al., 2012). The effects of uncontrolled diabetes mellitus include long term target organ damage, dysfunction and failure of different organs, especially eyes, kidneys, nerves, heart and blood vessels. Diabetes also has complications in pregnancy. Pre-diabetes mellitus, diabetes mellitus type 1 and type 2 are briefly described below:

Pre-Diabetes Mellitus

Individuals diagnosed with impaired glucose tolerance (IGT) do not have diabetes, but are believed to have a significant risk of developing diabetes, hence 'flagged' as pre-diabetics (Pre-DM). In a 15-year follow-up Finnish study on hyperglycemia and compositional lipoprotein abnormalities as predictors of cardiovascular mortality, also demonstrated the

risk among women with diabetes to be higher than those with non-diabetes (Niskanen et al., 1998). Therefore early detection of potential T2DM women that present with Pre-DM and atherosclerosis need also be considered. Even when adjusted for conventional risk factors, individuals with T2DM still exhibit a two- to four fold increased relative risk of occlusive vascular disease in comparison to non-diabetes mellitus subjects (Brohall, 2007; Bradshaw et al., 2007; Maiti and Agrawal, 2007; Grundy et al., 2000). Individuals with Pre-DM, are also reported to have an equal chance of lowering their glycaemia status to normal, thus are an important target group for primary prevention (Erasmus et al., 2012; Balasubramaniam et al., 2012; MedicineNet, 2009). In 1979, impaired glucose tolerance (IGT) was defined in USA by the National Diabetes Data Group as a state of disturbed glucose metabolism. Pre-DM is viewed as a transitional stage in the development from normal glucose tolerance to T2DM. This concept that Pre-DM is not necessarily a road to diabetes is supported by results from studies showing that in subjects with Pre-DM, life style modification treatments, weight reduction and increased physical exercise prevented the transition to diabetes (Lindstrom, 2006; Lindstrom, 2003; Knowler, 2002; Tuomilehto, 2001).

Impaired glucose tolerance often co-exists with a cluster of metabolic abnormalities such as; hypertension, central obesity, dyslipidaemia and insulin resistance (Reaven, 1988; Kylin, 1923). It is known that Pre-DM is associated with a risk of future CVD events (Meigs, 2002; Coutinho, 1999; Fuller, 1980). It was not clear, however, if high “non-diabetes” glucose levels were independently related to atherosclerosis (Bonora, 1999). Data from other studies indicate that IGT is a risk factor for CVD even when it is not followed by the subsequent development of diabetes (Qiao, 2003). The prevalence of Pre-DM is said to vary between different populations. In a large population-based study in Sweden, the prevalence of IGT among women at the age of 60 was reported to be 11% (Lindahl, 1999). Diabetes mellitus, as established by the National Department of Health and other research groups, further emphasises T2DM and atherosclerosis as contributing risk factors for CAD and stroke (Bradshaw et al., 2007; Maiti and Agrawal, 2007: 292; Grundy et al., 2000).

Atherothrombotic cardiovascular disease is said to be the leading cause of death worldwide despite significant progress in the management of critical risk factors (Callow, 2006). A major reason for this trend is the ongoing epidemic of obesity-induced insulin resistance and T2DM (Behn and Ur, 2006). A preventive medical strategy therefore aims to reverse this trend. There appears to be public health measures that try to address over-nutrition and lack of physical exercise as the key. However, achieving success in lifestyle changes is proclaimed to be challenging, therefore complementary approaches that identify potential therapeutic targets relevant to atherosclerosis *per se* in diabetics are needed (Bornfeldt and Tabas, 2011). Atherosclerosis progression (vulnerable plaques) in the arteries complicated by diabetes is

further emphasised as a contributor to the large proportion of CAD and stroke occurances globally (Sims and Muyderman, 2009; Spence, 2006; Dähnert, 2003: 371; Carra et al., 2003: 288). This study propagates the ultrasound surveilance of T2DM subjects affected by the disease process complicated by atherosclerosis (intima media thickening, plaques and stenosis)

Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitis previously known as insulin-dependent or childhood-onset diabetes, is also referred to as juvenile diabetes or juvenile-onset diabetes, and is characterised by a lack of insulin production resulting from atrophy of the islets of Langerhans causing hyperglycemia and a marked tendency toward ketoacidosis. Latent autoimmune diabetes in adults (LADA) accounts for about 7% of all diabetic patients in Europe and usually defined as glutamic acid decarboxylase (GAD) antibody-positive diabetes, with onset greater than 35 years of age (WHO, 1965). These disease types could be divided further into subtypes possibly representing different disease mechanism. In addition, there are less common diabetes types including maturity onset diabetes of the young (MODY), representing a monogenic form of diabetes with well-defined mutations in more than 6 different genes, as well as neonatal and secondary diabetes. These forms represent a range of genetic etiologies from the monogenic MODY variants to T2DM, which is a highly complex multigenic disease with a very strong enviromental component (DeFronzo et al., 2015).

Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus, the most common form, constituting 80-90% of all diabetics, was previously known as non-insulin-dependent or adult-onset diabetes caused by the body's ineffective use of insulin. T2DM develops when pancreatic cells can no longer increase their insulin secretion enough to compensate for increasing insulin secretion resistance imposed by increasing obesity. It often results from excess body weight and physical inactivity (Balasubramaniam et al., 2012; National Institute of Health, 2011). While T2DM onset is usually after the age of 35, it is not uncommon that T2DM is diagnosed already in adolescents in high-risk regions such as Asia, Middle East and USA (IDF Diabetes Atlas, 2014).

Being characterised by disorders of insulin resistance and insulin secretion, either of which may be the predominant feature, both are usually present at a time when diabetes is clinically manifest. Insulin levels may be normal or even elevated at a time when diabetes is diagnosed. However, in the setting of insulin resistance, these levels are inadequate to maintain normoglycaemia. This relative insulin deficiency is what differentiates diabetic insulin-

resistance individuals from normoglycaemic insulin-resistance individuals. Indeed, it is noteworthy that, to date, the majority of genes that have been associated with T2DM are related to insulin secretion and not to insulin resistance (Billings, L.K and Florez, 2010). At least intially, and often throughout their lives, these individuals do not need insulin treatment to survive (Kissbah et al., 1982). T2DM is frequently asymptomatic and undiagnosed for many years because the hyperglycaemia is often not severe enough to provoke noticeable symptoms (Harris et al., 1992). Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. T2DM is a very heterogenous disorder and there are certainly many different causes of this form of diabetes. However, on the positive side, it is likely that the number of patients placed in this catergory, may decrease in the future as identification of specific pathogenic processes and genetic defects permit better differentiation and a more definitive classification (DeFronzo et al., 2015).

2.3 Pathophysiology: Hyperglycaemia and atherosclerosis

Although there is data from human and animal studies that support a direct proatherogenic role of insulin resistance in vascular cells, the same may not be as strong for hyperglycaemia. There is evidence though, that suggest that high glucose is atherogenic, particularly at the level of the arterial endothelium (Bornfeldt and Tabas, 2011). Some studies have provided evidence that may support theories and possible mechanisms that may be involved in hyperglycaemia that promotes atherosclerosis. Abnormal factors that are relevant to atherogenisis include chronic hyperglycemia, dyslipidemia, and insulin resistance that render arteries susceptible to atherosclerosis. Multiple cell type functions are altered by diabetes including endothelium, smooth muscle cells, and platelets, indicating the extent of vascular disarray in this disease.

A correlation between suboptimal glycaemic control and cardiovascular events have been demonstrated by clinical studies that show a benefit of lowering glucose levels in T2DM patients, in order to improve coronary artery disease outcomes (Brown et al., 2010; Mazzone, 2010). Long-term follow-up studies by Brown et al., (2010) provided evidence in which intensive glucose lowering was initiated soon after being diagnosed as diabetic or before the onset of cardiovascular events. As explained by Bornfeldt and Tabas (2011) with reference to the DCCT-EDIC study (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) that showed an impressive reduction of 57%, in the risk of non-fatal myocardial infarction (MI), stroke, or death from CVD in the intensive glucose-lowering group of T1DM subjects compared to the conventionally treated group (Nathan et al., 2005). The lowering of blood glucose levels of newly diagnosed T2DM subjects also showed similar beneficial effects by studies done by Holman et al., (2008). Human post-mortem studies

at the level of the vascular walls revealed that lesions from patients with diabetes have higher microphage content, than lesions from subjects without diabetes, in a way that shows a correlation with glycated hemoglobin (HbA1c) levels rather than lipid levels (Burke et al., 2004). However, the relationship between hyperglycemia and coronary artery disease appears to be unclear (Brown et al., 2010; Mazzone, 2010). As outlined by Bornfeldt and Tabas (2011), that the unclearness may in part, be due to a number of reasons. Firstly, T2DM is associated with several cardiovascular risk factors, and hyperglycemia may provide a relatively minor contribution to the overall coronary artery disease risks. Secondly, the elevated HbA1c, used in clinical studies as a measure of glycemic control, is said not to always reflect the biological effect of hyperglycemia, because transient spikes in glucose do not result in overall HbA1c changes and/or because HbA1c levels can be influenced by genetic components unrelated to glucose (Soranzo et al., 2010). Thirdly, coronary artery disease often occurs before T2DM has developed in subjects with insulin resistance. Last, but not least, coronary artery disease development occurs over decades, whereas clinical intervention studies to lower blood glucose are carried out over shorter study periods, with exception to the positive studies like the ones outlined previously.

Using animal models to explore the effects of diabetes on atherosclerosis is usually complicated by hyperlipidemia co-existance, which is reported to override the effects of diabetes on atherosclerosis (Reaven et al., 1997; Renard et al., 2004; Kanter et al., 2007). Studies have shown in some mouse models, however, that increased blood glucose levels caused by diabetes, was not associated with increases of plasma lipids, and that a proatherogenic effect of diabetes could be observed (Kunjathoor et al., 1996; Gerrity et al., 2001; Renard et al., 2004; Vikramadithyan et al., 2005). The mouse models used for these experiments showed that diabetes accelerated the formation of early, macrophage-rich atherosclerotic lesions at sites that are susceptible in the arterial wall (Renard et al., 2004; Vikramadithyan et al., 2005) and that this effect can be prevented by insulin therapy (Renard et al., 2004; Schuyler et al., 2011; Johansson et al., 2008). In other studies, diabetes-induced models demonstrated increases in plasma low-density lipoprotein (LDL), and that very-low-density lipoprotein (VLDL) was found to be necessary for lesion progression that exhibited intra-plaque hemorrhage and rupture-prone phenotypes (Johansson et al., 2008). By collating all of the above information alike, a suggestion made by Bornfeldt and Tabas (2011) is that hyperglycemia in the setting of a nondiabetes-mediated hypercholesterolemic background, is apparently sufficient to promote the formation of early lesions, but that accelerated progression of advanced plaques in diabetic mice, as compared to observations in non-diabetic mice, requires diabetes-induced elevation of atherogenic lipoproteins (Bornfeldt and Tabas, 2011).

2.4 Risk factors

Identification of risk factors relating to T2DM poses to be challenging due to environmental, genetic and lifestyle factors that are interrelated and associated with insulin resistance and metabolic conditions. Groups that are at greater risk of diabetes development are those with strong family history of the condition, older age, obese, and those that are physically inactive, just to mention a few. The complications of atherosclerosis and its association to cardiovascular risks need also be considered. Therefore, it is important for clinical practice and management of T2DM patients, to also be aware of the atherosclerosis status (wall thickening and/or stiffness, plaques and stenosis). Although the risk factors are many, only a limited number relating to this study could be described:

Age

The initial manifestation of carotid atherosclerosis is said to be characterised by a subtle increase in vascular intima-media thickening, the progression of which leads to plaque formation and vascular narrowing (Lee et al., 2007). Atherosclerosis is reported to be part of the aging process and can present itself even in patients' that are not diabetic (Stern et al., 2003). Kisten et al. (2013) also noted in their study that older patients (≥ 52 years) had a carotid stenosis >70%, and that age was an important factor for plaque development, linking it with a higher likelihood of surgery. It is said to be exceptionally rare to document a young patient < 35 years having a carotid stenosis >70% requiring surgical intervention in clinical practice (Kisten et al., 2013). Early detection of a thickened carotid wall and or plaques in younger patients therefore raises a 'flag' for atherosclerosis development. Although gender related risks for diabetes varies from population to population, men are more at risk for atherosclerosis than women (Schulz and Rothwell, 2001). By middle age or older, a substantial amount of plaque build-up can cause signs or symptoms of hypertension due to restricted arterial blood flow. In men, after age of 45 years, the risk increases, while in women, the risk increases after the age of 55 years (Hansson, 2005; American Heart Foundation, 2011). Adiposity is also closely related to aging (Vlassopoulos et al., 2014).

Obesity

Obesity is an important and rapidly growing global health problem, and is associated with excess risk of premature morbidity and mortality, particularly from T2DM and CVD (Swinburn et al., 2011). Efforts to better characterise the disease risk associated with obesity suggest that accompanying metabolic abnormalities are not uniform in all obese persons (Matsha et al., 2013). Being overweight, due to an increased body mass; muscle,

bone, fat, and water is contributory to CVD. An increased BMI, a disproportional waist and hip circumference size, and excessive abdominal fat are in itself a risk. In 1947, it was reported that people with larger waist circumferences were at higher risk of premature CVD and death than people who had 'slimmer' waists or carried more of their weight around their hips and thighs (Vague, 1947). More than 38 years later, it was reported that so-called "abdominal (truncal) obesity" was strongly associated with an increased risk of T2DM, even after controlling for BMI (Ohlson et al., 1995). Obesity, caused by urbanisation and the aging process is well established in South Africa (Heart and Stroke Foundation South Africa, 2006; Bradshaw et al., 2007), and hence a key contributing factor in T2DM and atherosclerosis. The Baltimore Longitudinal Study of Aging (WHO, 2008), also examined the effects of weight change on changes in fat distribution (Shimokata et al., 1989). The study found that changes in waist and hip circumferences correlated directly with changes in weight, but there were differences in the pattern of change by gender. In males, waist changes were larger than hip changes, whereas in females they were similar. This resulted in weight changes in males having a larger effect on waist-hip ratio. On average, with a 4.5 $_{kg}$ weight gain, males had a 4 $_{cm}$ increase in waist circumference and a 2.5 $_{cm}$ increase in hip circumference. Comparable values for females were reported as 3.3 $_{cm}$ and 3.6 $_{cm}$, respectively. Gender differences in body fat deposition are noted as early as in the fetal stage, but become more pronounced during puberty (Wells, 2007). After adjusting for height differences, males have shown a greater total lean mass and bone mineral mass, as well as a lower fat mass than females; these differences are reported to continue throughout adult life (Wells, 2007).

Published data from the larger BSA study stated that in spite of the discrepancies in the definition of metabolic status across settings, existing studies, mostly from high-income countries, suggest each obesity phenotype to represent a sizable proportion of the general population. However, evidence on the association of each of the phenotype with CVD risk was far from conclusive, and that no known published study had assessed the prevalence of obesity phenotypes and their correlation with disease risk in Africans. Accordingly, the researchers involved, determined the prevalence of obesity phenotypes, and investigated their association with sub-clinical cardiovascular disease using echocardiography and carotid artery ultrasound techniques, in a mixed-Ancestry population in South Africa (Matsha *et al.,* 2013). The results for the above obesity phenotype and subclinical CVD study, reported the median CIMT to have been higher in the metabolically abnormal subjects among normal-weight and overweight ($p \leq 0.033$ and 0.014), but not among obese ($p = 0.23$) ^{NS}, while the Intra-ventricular septum (IVS) was higher only in the metabolically abnormal overweight subjects (1.3 vs. 1.1 cm, p=0.0001) (Matsha et al., 2013). 'The clustering of cardiometabolic abnormalities was frequent across the BMI

categories in this population, with only borderline effects on markers of subclinical CVD. Among normal weight-participants (17.1% of the total sample), up to 29.1% were metabolically abnormal, while among obese participants (53.7% of the total sample), 30.8% were metabolically healthy. This pattern was very similar to reports from the USA (Wildman et al., 2008), which, like South Africa, has high prevalence of overweight and obesity. The absence of gradient in the levels of risk factors and markers of subclinical CVD across BMI strata, and by metabolic status supports the importance of the clustering of cardiometabolic factors in conferring the disease risk associated with adiposity. Accounting for this clustering is thought to aid tailoring the prescription and intensity of risk reducing interventions in those with high sub-clinical disease burden and who will benefit the most, particularly among normal-weight and overweight subjects (Matsha et al., 2013).

Hypertension

Elevated blood pressure is constantly noted in diabetes indepandant of age, obesity and renal disease. Hypertension is related either directly and/or indirectly to CVD and stroke (Gaziano., 2011). Hypertension is considered 'more risky' if it stays at or above 140/90 mmHg over a period of time (American Heart Foundation, 2011), hence blood pressure monitoring and its regulation should be the desired goal in high-risk individuals. The prevalance of hypertension increases with the duration of diabetes and markly increased with nephropathy. It has become apparent that insulin has a variety of potentially important actions that might impact on blood pressure (Diabetes Australia, 2013; LeRoith et al., 2003).

As described by LeRoith et al., (2003) that insulin acts as a peripheral vasodilator, acting by endothelial nitric-oxide-dependant pathways. Insulin also has central actions resulting in sympathetic activation and acts as a trophic factor to vascular smooth muscle cells. Insulin also acts to increase sodium retention in the kidney. One possible cause is the increase in exchangeable sodium and enhanced cardiovascular reactivity, which occur in both T1DM and T2DM. Alternatively, increased intracellular calcium and decreased magnesium have been reported amoung diabetic hypertensive patients. Furthermore, the insulin resistance syndrome, with accompanying hyperinsulinemia, may contribute to hypertension in individuals with T2DM (LeRoith et al., 2003). Ranging from as far back as the 1960s, non-invasive Doppler spectral analysis was introduced in the evaluation of occlusive arterial disease (Strandness et al., 1966), and hypertension was established as an important modifiable risk factor for stroke (Dickinson and Thomson, 1960). The presence of hypertension in individuals with diabetes is an independent contributory risk factor for microvascular disease (coronary, cerebral and peripheral), retinopathy and nephropathy, to mention a few. Diabetes Australia (2013), reports that early detection, active treatment and frequent

review are essential if morbidity is to be reduced. Healthcare givers should aim for lower blood pressure levels in people with diabetes because their blood vessels (both macro and micro) are more susceptible to hypertensive damage (target ≤130/80 mmHg). Gaziano (2011) reported that high blood pressure is one of the most important preventable causes of premature ill health and death in the UK. It is a major risk factor for stroke, heart attack, heart failure, chronic kidney disease and cognitive decline as described previously. The article also relates that primary hypertension is diagnosed when there is no simple identifiable cause of the raised blood pressure. The hypertension may be related, in part, to obesity, dietary factor such as salt intake, and smoking, physical inactivity or genetic inheritance (Gaziano, 2011).

Smoking

Smoking is known to cause damage and 'tightening' of blood vessels (vasoconstriction); it raises the blood pressure and cholesterol levels. Smoking (broncho-constrictor) also prevents adequate redistribution of oxygen to body tissues (National Institute of Health, 2011; American Heart Foundation, 2011; Heart and Stroke Foundation South Africa, 2006). Previous studies have confirmed a statistically significant relationship between smoking and carotid artery stenosis (NASCET Collaborators. 1991; MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis, 1991; Wilson et al., 1997; Kisten et al, 2013).

The S-Cotinine $_{ng/mL}$ concentration levels is not a proxy for smoking, but rather an exposure to tobacco/nicotine (burning, oral, nicotine replacement therapies or otherwise). The most dangerous by-products of tobacco are created during the burning process, however, in Sweden and Norway, where 'snus' a form of oral packaged tobacco originated, noted a link to significantly higher risk of pancreatic cancer, mouth sores, dental cavities, heart attack, stroke, and diabetes risk; and they do deliver nicotine, which is an addictive drug. Other related studies also claim that smokeless tobacco products don't burn, but is a major cause of oral cancer and oesophageal cancer as well. Interestingly, 'snus', and or other forms of Nicotine exposure may not affect the lungs directly, but are made with a special process to help control nitrosamine levels. The 'catch' though, is that carcinogen levels in 'snus' or other similar nicotine concentrations of smokeless products may be lower than in cigarettes, but they are not low. Nicotine addiction and dependency to the habit need also be considered. Although smoking regulations are very good in Sweden as compared to other countries, like South Africa, the passive smokers also need to be considered. The question could be put whether it is the smoking/lung damage effect, and or tobacco/nicotine exposure, that provides the 'harmful' effect in

diabetes mellitus, atherosclerosis, CVD and stroke, or any other for example using serum cotinine biomarkers. The cotinine test for second hand smoking and those indirectly exposed to nicotine apparently requires no special preparation. There are also established charts for normal and abnormal concentrations of cotinine done by Neal Benowitz et al. (2009). An unhealthy lifestyle or a lack of physical activity can worsen other contributory risk factors.

Genetics and hereditary characteristics

The strong genetic link in cases of diabetes is related to a predisposition to developing the condition; however a family history of diabetes does not make its development a certainty. Genetics, ethnicity and/or lifestyle factors also relate to gradual plaque build-up in the arteries. Screening tests for carotid atherosclerosis is not routinely done (Lorenz et al., 2006) in the public sector of most ultrasound departments in middle and low income countries globally, let alone, in the Western Cape. The global problem of obesity and diabetes continues to grow in SA (Van der Merwe and Pepper, 2006). Communities affected by poor socio-economic conditions, where diet, lifestyle, illiteracy, lack of awareness and understanding of the epidemic, is in itself, a problem (Scott et al., 2002). Literature (Wetterholm, 2008; Wohlin *et al.,* 2008; Gronholdtl et al., 2001) suggest preventive measures to address the epidemic, in which ultrasound has a significant role to play. This highlights the need for preventive and more accurate diagnostic techniques in the early identification of high risk populations that are more vulnerable to atherosclerosis complicated by CVD and stroke. Conclusions made by Ruige et al., (1998) in meta-analysis of 12 international studies of fasting and/or post glucose load plasma insulin concentrations, reported that hyperinsulinaemia was a relatively weak predictor of CVD (relative risk 1.1.8, 95% confidence interval 1.08–1.29). Ethnic background emerged as a potential modifier, showing a stronger longitudinal relationship between hyperinsulinaemia and CVD in whites versus non-whites (Ruige et al., 1998). As described in the meta-analysis, differences in the relationship between insulin sensitivity and carotid intimal thickness were observed between the different ethnic groups examined. Although there were limitations to the methods that made interpretation of these studies difficult, they reiterate the view of ethnicity as a modulator of CVD risk (Stern, 1995). For example, the obese insulin resistant Pima Indians of Arizona are spared from cardiovascular diseases relative to other groups, a dose-response effect of genetic admixture being postulated for other United States groups at intermediate levels of risk (Stern, 1995). This was in contrast to the high risk of cardiovascular diseases among South Asian migrants to the United Kingdom and elsewhere (Bhopal, 2002). South Asians (who comprise one fifth of the global population) are also highly susceptible to insulin resistance and T2DM with a tendency to abdominal fat deposition, higher plasma insulin concentrations, and dyslipidaemia (Bhopal, 2002). These observations point to,

as yet unidentified factors in certain populations that may modify the relationship between insulin resistance and cardiovascular sequelae (Peyser, 1997; LeRoith et al., 2003).

A family history of cardivascular disease and stroke is a contributing risk factor that cannot be controlled or modified, however the risk increases, if a father or a brother was diagnosed with CVD prior to 55 years of age, or if a mother or a sister was diagnosed with CVD prior to 65 years of age. Although age and family history are associated with CVD, it is not obvious that an individual with a family history of CVD and stroke will necessarily develop atherosclerosis. Familial clustering of T2DM and coronary heart disease show variations in the literature (Peyser, 1997; Marenberg et al., 1994). However, with the exception of potent monogenic defects causing major inherited dyslipidaemias, such as familial hypercholesterolaemia or polymorphisms of apolipoprotein E, the genetics of predisposition to CAD in the presence or absence of T2DM, prompted further testing (Marenberg et al., 1994; LeRoith et al., 2003). Implementing lifestyle changes and appropriate management of other associated risk factors for atherosclerosis, often reduces the genetic influences on the development of atherosclerosis (Wohlin et al., 2008).

Hyperlipidemia

Hyperlipidemia is a hetrogenous group of disorders characterised by an excess of lipids in the blood stream, especially increased levels of low density lipoprotein-cholestrol (LDL-C) and Increased levels of certain fats called triglycerides that have the potential to increase the risk of CVD, particularly in women (Heart and Stroke Foundation South Africa, 2006; National Institute of Health, 2011; Hansson, 2005). These lipids include cholestrol, cholestrol esters, phospholipids and triglycerides. Lipoproteins are based on chylomicron, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Lipids are transported in the blood as large ‘lipoproteins’ (National Institute of Health, 2011). A certain amount of cholestrol is metobilized daily, however abnormal levels result due to genetics and/or diet related. The HDL assists in ‘coating’ the arteries like protective oils that prevent blockages, while the LDL when in excess can lead to blockages of arteries. Hyperlipidemia is simplified into different types. Primary hyperlipidemia is genetic based but genetic defects are known only for certain patients. Secondary hyperlipidemia may result from diseases such as diabetes, thyroid disease, renal disorders, liver disorders and Cushings syndrome, as well as obesity, alcohol consumption, estrogen administration and other drug associated changes in lipid metabolism. Hyperlipidemia to certain degree is a major modifiable risk factor for atherosclerosis, cardiovascular disease, especially in CAD (Heart and Stroke Foundation South Africa, 2006; National Institute of Health, 2011). Increased levels of VLDL and remanants of VLDL and

chylomicron metabolism represent hypertriglyceridemia, and is common among T1DM and T2DM (LeRoith et al., 2003). The VLDL elevations in diabetic subjects may be due to several possible mechanisms: a) increased free fatty acid and glucose availability to the hepatocyte leading to the overproduction of VLDL. b) Abnormalities of lipoprotein lipase result in decreased clearance both of chylomicrons and VLDL. c) Prolonged postprandial hypertriglyceridemia leads to accumulation of chylomicron remanants. Although there is still contraversy regarding the relationship between hypertriglyceridemia and atherosclerotic CVD in the non-diabetic population, studies have declared that elevated triglycerides are risk factors for atherosclerosis among individuals with T2DM, even after adjustment for other risk factors (Bornfeldt and Tabas, 2011; LeRoith et al., 2003).

Inflammation

Researchers continue to study other possible emerging risk factors for atherosclerosis and have established that high levels of C-reactive protein (CRP) in the blood (inflammatory response) may raise the risk for complicated atherosclerosis and a heart attack (National Institute of Health, 2011; Hansson, 2005). Increased levels of CRP are indicative of inflammation, which is the body's response to injury or infection; however it is not specific to the exact location of injury or infection. Damage to the intima layer or inner walls of the arteries are reported to trigger inflammatory responses. It is suggested that individuals with low CRP levels may develop atherosclerosis at a slower rate than people with high CRP levels. On-going research continues to determine whether reducing inflammation and lowering CRP levels can also reduce the risk of complicated atherosclerosis. It is believed that damage to the intima /inner layer of the arterial walls, appear to trigger inflammation, thus encouraging plaque growth and atherosclerosis. High levels of C-reactive protein (CRP) in serum are indicative of some form of infection or inflammation. Increased, highly sensitive (hs) CRP levels are also reported to raise the risk of heart disease (Hansson, 2005). Varied hs-CRP levels have been established by the American Heart Association and U.S. Centres for Disease Control and Presentation relating these CRP levels to CVD risks. C-reactive protein levels of <1mg/L is regarded as low risk, 1.0-3.0mg/L regarded as average risk and >3mg/L is associated with high risk (Lab Tests Online) of CVD. Although increased levels of CRP are the body's response to injury and/or infection, it is not specific to the exact origin or location of injury, infection/ inflammation, hence ultrasound compliments the findings.

Other factors

Apart from conventional risks mentioned above, other factors that are considered to increase an individual's risk of atherosclerosis as described by the Heart and Stroke

Foundation South Africa (2006) are: sleep apnoea which is referred to as a disorder in which the breathing stops, or gets very shallow while a person is sleeping. The National Institute of Health (2011) also describes untreated sleep apnoea to be associated with higher chances of hypertension, diabetes, and possibly a heart attack. “Stress”, is reported as the most common "trigger" for a heart attack, often due to an emotionally upsetting event. Anger is said to be a contributing instigator (National Institute of Health, 2011; Heart and Stroke Foundation South Africa, 2006). Alcohol intake is also noted to be an added factor. Heavy drinking is proposed to cause damage to the heart muscles, with the ability to worsen other risk factors of atherosclerosis. Studies from South Africa have suggested, that men having no more than two alcoholic drinks a day be regarded as negligible, while women should have no more than one alcoholic drink a day (Heart and Stroke Foundation South Africa, National Institute of Health, 2006). This however is debatable in terms of an individual’s metabolic rate and the body’s responses to alcohol toxicity and tolerance doses.

2.5 Consequences of T2DM on the heart:

Chronic cardiac failure is described as a clinical syndrome with complexities, and can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood (Hunt et al., 2001; Dokken, 2008). Systolic heart failure arises from a compromise in the contractility of the heart, which is well seen with ultrasound, and is defined as a left ventricular ejection fraction of < 45%. Echocardiography can also establish when the diastolic dysfunction interferes with the heart’s ability to relax and fill with blood (Gutierrez and Blanchard, 2004). Cardiac failure in diabetic patients may arise from myocardial damage resulting from an ischemic, thrombotic event. Endothelial dysfunction, oxidation and glycation of atherogenic lipids, and the hypercoagulability of the blood are contributors to the patient’s resulting heart failure. In many cases, however, cardiac failure in diabetic patients may have a non-thrombotic etiology and other pathophysiological factors are at play, as in the case of diabetic cardiomyopathy. Diabetic cardiomyopathy is regarded as a myocardial disease in diabetic patients that cannot be attributed to any other known CVD, such as hypertension or CAD (Marwick, 2006). Because of the changes in structure and functions that occur in diabetic cardiomyopathy, patients with diabetes are vulnerable to cardiac failure early in their disease course. At least 2 different epidemiological studies using sensitive echocardiographic diagnostic methods found the prevalence of asymptomatic diastolic dysfunction in patients with T2DM to be between 52% and 60%, despite meeting clinical criteria for acceptable glycemic control (Poirier et al., 2001; Redfield et al., 2004). Left ventricular diastolic dysfunction, characterised by impaired early diastolic filling, prolonged isovolumetric relaxation, and increased atrial filling was even noted in young patients with T1DM (Redfield

et al., 2004). Myocardial damage without CAD (macrovascular) is most likely related to microvascular dysfunction. Microvascular damage in the diabetic heart could propagate myocardial injury, fibrosis, and hypertrophy found in diabetic cardiomyopathy (Kawaguchi et al., 1997) and therefore ultrasound measurements of the myocardium and the intraventricular septum becomes more valuable. In patients with T1DM in the absence of CAD, impaired coronary flow reserve (dependent on the microvasculature) predicts dysfunction diastole, and may be related to autonomic neuropathy (Pop-Busui et al., 2004). A similar relationship as noted by Galderisi et al., (2002), between the magnitude of coronary flow reserve reduction and the degree of myocardial diastolic dysfunction was found in uncomplicated hypertension which is another condition characterised by impaired coronary microcirculation. This association is because coronary flow occurs predominantly during diastole (Galderisi, 2006), so that normal coronary flow and diastolic dysfunction are interdependent.

In addition to microvascular disease, another factor is hyperglycemia that increases the risk for the development of cardiac failure in diabetic patients. Prospective Diabetes Study in the United Kingdom showed that for every 1% increase in A1C was associated with a 12% increase in heart failure (Stratton et al., 2000). In the Strong Heart Study, the presence of T2DM was associated with left ventricular enlargement and decreased myocardial function in both genders. The extent and frequency of A1C levels were directly proportional to diastolic dysfunction (Devereux et al., 2000). The mechanisms by which hyperglycemia can contribute to the development and progression of diabetic heart failure are many that could hold true. Diastolic dysfunction in diabetic cardiomyopathy could be due to myocellular hypertrophy and myocardial fibrosis (Bell, 2003), and can be well evaluated by heart ultrasound /echocardiography. Laboratory studies have provided evidence that the decrease in cardiac efficiency in diabetics is related to the increased fatty acid utilisation, which leads to an increased production of reactive oxygen species (Boudina and Abel, 2005). The oxidative stress increase in diabetic hearts has been found to decrease nitric oxide levels, worsen endothelial function, and induce myocardial injury through stimulation of inflammatory mediators (Szabo, 2002). Cardiac hypertrophy and low-grade inflammation may precede development of the vascular dysfunction (Palmiere et al., 2003).

As summarised by Dokken (2008) that diabetic cardiomyopathy demonstrates multiple mechanisms by which diabetes affects the cardiovascular system. Microvascular disease, including endothelial dysfunction caused by diabetic autonomic neuropathy (DAN) and decreased nitric oxide bioavailability is of crucial importance. The underlying defects of oxidative stress and inflammation contribute to diastolic dysfunction, especially in the presence of poor metabolic control. Cardiovascular disease accounts for most of the mortality and morbidity associated with diabetes. Also described by Dokken (2008) was the strong

correlation between the left ventricular hypertrophy by ultrasound and markers of chronic inflammation in T2DM patients. In the Strong Heart Study, which included 1,299 adults with T2DM, showed those with left ventricular hypertrophy had higher levels of fibrinogen and C-reactive protein (both markers of chronic inflammation) and urinary albumin independent of traditional cardiovascular risk factors (Palmiere et al., 2003). An overall risk assessment therefore plays an important role in "flagging" potential CVD, especially CAD and stroke patients (Gronholdtl et al., 2001). The responsible use of echocardiography and vascular ultrasound screening is therefore being recommended.

2.6 Consequences of T2DM on the brain:

Diabetes mellitus is considered to be a major risk factor for ischaemic stroke. Diabetic patients with predisposing risk factors have a greater incidence of stroke compared to non-diabetic subjects, but consensus is yet to be reached on optimal screening methods for cerebrovascular complications. Cerebrovascular disease is associated as a cause for 20% of deaths in diabetic patients (Zwiebel, 2005). There are however some marked differences in stroke patterns between non-diabetic and diabetic subjects suffering a stroke. Diabetics are greater at risk of plaque development, especially when these plaques are vulnerable to displacement that may cause a stenosis or occlusion and eventually a stroke. Diabetic patients usually develop ischaemic strokes with an increased proportion of lacunar strokes that present as clinically silent (Tuttolomondo et al., 2008). Additionally, infratentorial infarcts are more common in people with diabetes. Conventional digital subtraction angiography is currently considered to be inappropriate as a first-line investigation for diagnosing carotid stenosis, since it is an invasive and radioactive technique. Computed tomography angiography and magnetic resonance angiography provide more reliable anatomical resolution but are not cost-effective. As the prevalence of ill-health complicated by diabetes that leads to a stroke continues to grow, so too (contradictory so) does the continuos growth and advancement of medical imaging technologies.

2.7. Medical imaging advancement in stroke: A brief history

A medical imaging history of stroke and cerebrovascular disorders stems from about 2400 years ago, when "stroke" was first recognised by Hippocrates as a condition called apoplexy, in Greek terminology meaning "struck down by violence" (Wetterholm, 2008). Up until the mid-1600s, there was no or very little understanding of this condition. Jacob Wepfer, from the University of Padua in Italy, then made a discovery, while experimenting on dissected corpses, that something disrupted the blood supply to the brains of people who died from apoplexy. In some cases, the artery blockage was noted; in others, there

appeared to have been massive bleeding into the brain tissue (Eastcott, 1994; Gurdjian, 1979; Wetterholm, 2008). A number of discoveries were made in the 1800s when the development of modern physics led to further advances in medicine.

In 1842, physicist Prof. Christian Johann Doppler introduced for the first time, the Doppler principle (Roguin, 2002). Heinrich Hertz $_{(Hz)}$ and Guglielmo Marconi's experiments on radio-waves in 1895 added value to ultrasound physics and engineering. On 8th November 1895 in the same year, William Conrad Roentgen discovered a new kind of radiation called X-Rays, at the University of Wurzburg (Crawford, 1984; Fröman, 1996). This is suggested to be the start of carotid angiography. By the 1920s, further advances in contrast angiography were made. Ultrasound studies for medical use were introduced by the late 1920s. During the 1930s, pioneers in ultrasound engineering, appeared to have got their ideas and technical solutions from the military, were they used a 'supersonic reflectoscope' built by Floyd Firestone who used soundwave properties to detect objects underwater, as would dolphins and whales by means of echolocation. In 1947, ultrasound was first used for medical diagnostic purposes by Dr. George D. Ludwig at the Naval Medical Research Institute, Bethesda, Maryland. He was able to demonstrate that gallbladder calculi could be detected by an ultrasonic pulse echo principle using amplitude mode metal flaw detectors and naval sonar (Blauw et al., 1997). The acoustic shadowing artefact is still useful in diagnostic testing of echogenic or calcified lesions, e.g. plaques (Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine, 2009; Carra et al., 2003).

During the 1950s, a surgical removal of a carotid artery blockage was performed by Michael de Bakey known today as a carotid endarterectomy (CEA) for restoration of blood flow to the brain (De Bakey et al., 1959). During that same decade, Warfarin medication as an anticoagulant was used for the first time in stroke patients (Carter, 1961). Contrast angiography techniques developed further. However, the side effects of the contrast media in combination with radiation hazards and invasive testing posed serious health risks. In the late 1950s, initial attempts to measure blood flow velocities using the Doppler concept were tested (Franklin et al., 1959), but the technique then used was invasive and not suitable for clinical use. The first clinical application of the transcutaneous non-invasive Doppler flow-meter was developed in 1958 in Japan, by Satomura and co-workers, who managed to measure arterial flow velocities (Kaneko, 1986). In the 1960s, a non-invasive more specialised Doppler technique of spectral analysis was introduced in the evaluation of occlusive arterial disease (Strandness et al., 1966), and hypertension was established as an important modifiable risk factor for stroke (Dickinson and Thomson, 1960). By the late 1960s, Strandness et al., diagnosed various arterial diseases using a

spectral analysis of the Doppler signals (Barber et al., 1974; Wood et al., 2010). In the 1970s, aspirin was introduced as a treatment to prevent stroke. Although new platelet anti-aggregation treatments have been introduced, aspirin remains the most frequently used antithrombotic drug (Thomas, 1971). Lifestyle modification (exercise and diet), and natural therapies were also alternate treatment options. Major steps were taken in improving brain imaging radiology techniques, which included computed tomography (CT), studies of brain metabolism; and positron emission tomography (PET) scans (Wetterholm, 2008). In the early 1970s, pulsed Doppler instruments were introduced by Wells and Baker that enabled non-invasive regional measurements of blood velocity. Baker and his ultrasound engineers, in the year 1974, combined pulsed Doppler with a real-time B-mode imager, to form an instrument known as a duplex scanner (Rickey and Fenster, 1996). Duplex Doppler instruments allow the Doppler angle to be determined from the B-mode image, and with the addition of spectral analysis, enabled a more accurate measurement of blood flow velocity (Rickey and Fenster, 1996; Wood et al., 2010). In the 1980s cigarette smoking was established as a major risk factor for stroke (Khaw et al., 1984). The introduction of MRI increased the diagnostic possibilities to differentiate cerebral infarction from bleeding (Wetterholm, 2008; Bluemmand et al, 1984).

By the 1990s, larger studies showed that Carotid Endarterectomy (CEA) was clinically effective in preventing stroke (MRC European Carotid Surgery Trial, 1991). In 1991 two major studies were carried out: The North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the European Carotid Surgery Trial (ECST) (NASCET Collaborators, 1991; MRC European Carotid Surgery Trial, 1991). These studies showed clear benefit of CEA in patients with significant symptomatic internal carotid artery (ICA) stenosis. Determination of the severity of stenosis was based on angiography results. Slightly different methods of measuring were used, and resulted in some difficulties, in comparing NASCET and ECST results (Staikkov et al., 2000). More recently, recalculation of a common database has contributed many important new conclusions on how to handle subgroups, e.g. patients with tandem lesions. The most important development is probably the increased understanding of the importance of early intervention to avoid recurrent embolism (Rothwell et al., 2004; Rothwell and Warlow, 2005; Wetterholm, 2008).

Currently, angiography is rarely done in high-income countries, due to the risk of its associated complications. In Sweden, about 80% of CEA are performed exclusively on the basis of carotid duplex information regarding degree of stenosis (National guidelines for stroke care, 2005). This is also common practice in other high-income countries (Wood et al., 2010). Advanced technologies in spectral Doppler signature analysis and 4D

ultrasound adds value in exact plaque composition, and stenosis recognition for management (Wood et al., 2010). Thus, it has been suggested that plaques causing at least 50% stenosis may be associated with an increased future risk of stroke in symptomatic patients (Gronholdtl et al., 2001). Optimum medical strategies have evolved to further prevent the occurrences of stroke in these symptomatic patients. Oral anticoagulants were proven effective to prevent stroke in patients with atrial fibrillation. Wetterholm and colleagues (2007) using rodent models in another study noted that, when further statins have been introduced in hypercholesterolemia therapy, it showed to significantly lower the risk of stroke. Hypercholesterolemia is also reported to be prevalent in an aging population, which are at a higher risk of CAD and stroke when affected by atherosclerosis and diabetes.

2.8. Atherogenesis

The mechanisms of atherogenesis in atherosclerotic diseases, to date, are not fully understood (Brohall, 2007). As mentioned previously, hypercholesterolemia is closely associated with atheromatous plaque development. This results when there is an elevated total blood cholesterol and low-density lipoprotein (LDL) in the blood. High levels of high-density lipoprotein (HDL) in the blood is desirable (American Heart Foundation, 2011). Other important contributors to this condition include inflammation, oxidative stress, and insulin resistance. Pellizzon (2008), also reports that foods which contain high dietary saturated fat and cholesterol (i.e. “Western-type diets”) are associated with elevations in circulating cholesterol levels (in particular LDL cholesterol), prompting the recommendation that individuals limit the intake of these dietary constituents (Pellizzon, 2008), thus reducing the risks of arterial wall damage and atherosclerosis.

2.8.1 Carotid wall thickening and the inflammatory process

The arterial wall has 3 layers called the intima, media and adventitia. The intima or internal layer is a single layer of endothelial cells that is supported by a continuous membrane and internal elastic lamina. Myers and Clough (2004) explain that the endothelial cells have 5 basic functions namely: the regulation of haemostasis and thrombosis; interaction with white blood cells; the production of growth factors; it is permeable to nutrients; and it controls the vascular tone of the artery. The media or middle layer contains smooth muscle cells (SMCs) that are responsible for the muscle contraction to maintain vascular tone and the synthesising matrix of collagen, elastin and glycoprotein’s. The adventitia or outer layer consists of fibroblasts in a matrix of elastin and glycoprotein’s (Myers and Clough, 2004).

When this 3-layered arterial wall is affected by atherosclerosis, the mechanisms involved in its transition, need to be understood, in order to relate it to the ultrasound findings, which may be depicted by its echo-patterns, vasculature and thickness.

Evolving from studies done in the 80's and 90's, the most popular hypothesis explaining the atherosclerotic process, is the response to injury theory involving cellular tissue and microcirculation (Brohall, 2007; Camejo et al., 1998; Carew et al., 1984; Falcone et al., 1984; Schwenke and Carew, 1989). The infiltration and retention of LDL-cholesterol in the arterial intima or inner wall layer, is reported to initiate an inflammatory process in the arteries, where the modification of LDL through oxidation or other mechanisms take place (Leitinger, 2003; Skalen et al., 2002; Brohall, 2007). This process is said to include the activation of endothelial cells in areas of hemodynamic strain along the arterial wall (Brohall, 2007). A critical step in the development of atherosclerosis occurs when cytokine and cell adhesion molecules, produced in the inflamed intima, induce the monocytes to enter the plaque and differentiate into macrophages (Smitt et al., 1995; Brohall, 2007). It is believed that the macrophages are thereafter further transformed to cholesterol-laden foam cells (Haberland et al., 1984). The accumulation of macrophages, migration and proliferation of smooth-muscle cells (SMCs) and the formation of fibrous tissue leads to further enlargement and restructuring of the lesion. Over time the lesion becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue. Over time, an advanced, complicated lesion is created (Ross, 1999; Brohall, 2007).

2.8.2. B-Mode ultrasound: CIMT and Plaques

On B-mode imaging the lumen of the artery is usually outlined by a hyper-echoic stripe, and depending on the transducer angle, the intimal lining of the artery is visualised (Neumyer, 1999:368). A double layer (the intima-media layer and adventitia) is visible with the use of a high frequency transducer, usually between 5-15 MHz; depending on the anatomical variation e.g. body size and structure. The posterior wall is more visible in the common carotid artery (CCA). The normal thickness of the intima-media layer is ≤ 0.8mm (Bots et al., 1997) in healthy adults.

Care is taken not to mistake reverberation artefacts for pathology (Pollex et al., 2005; Thrush and Hartshorne, 2003). Pollex et al., (2005) described early stages of carotid artery disease process to relate to the thickness and configuration of the intima-media layer. As the disease progresses, more substantial areas of atheroma can be seen, most likely to occur at the carotid bifurcation. However, in a small proportion of patients, significant disease may be seen in the CCA and may even involve the CCA origin (Lal et al., 2006). On Brightness Modulation (B-Mode) settings (Edelman, 2007); the sonographic

appearances of a plaque can be seen. Plaques can vary depending on its density and echoic properties. It can be seen as hypo-echoic (low production of reflective waves, higher wave absorbtion) or areas of low echogenicity. Image settings are important. Too low gain settings could cause plaques to appear hypoechoic and vise versa, and could result in misleading image interpretations. It is reported that large areas of atheroma are often seen in the origin of occluded internal carotid arteries (ICA). The occluded ICA may appear much smaller, as the lumen contracts over time (Myers and Clough, 2004). The hallmark of atherosclerosis is the established plaque. Sophisticated software packages have the ability to recognise stable fibrous plaques. Joakimson and colleagues (1999) noted that such plaque increases as the individual grows older, and was reported to occur in the carotid arteries of more than 50% of 60 year old subjects tested. As the process progresses, some established plaques are noted to develop into vulnerable plaques, which in general are characterised by a large lipid pool, a thin overlying fibrous cap, and an abundance of inflammatory cells in the shoulder regions of the plaque (Mann and Davies, 1996). Identification of vulnerable plaques for proper risk assessment of these individuals becomes more important. Plaque characterisation offers additional ultrasound markers for "flagging" high risk asymptomatic patients.

2.9. Ultrasound Plaque Characteristics

The ultrasound descriptions of plaque characteristics are important in establishing its potential for CVD (myocardial infarcts) and stroke. As the progression of the plaque lesions vary in echogenicies, echotextures and echopatterns, so too must the atherosclerosis disease process.

Myers and Clough classify these types of plaque as follows: A fatty streak, a fibrous plaque, an atheroma, calcified plaque and arterial thrombosis (Myers and Clough, 2004: 830-840). A fatty streak is defined as a superficial, flat intimal lesion that is characterised by the focal accumulation of sub- endothelial SMCs and lipid deposits in the artery. A fibrous plaque is a protruding lesion that has a central core of lipids and cell debris which is surrounded by SMCs, collagen, elastic fibres and proteoglycocans with fibrous cap that separates the lipid core from the lumen of the vessel. A complicated lesion is a complex fibrous plaque that undergoes degenerative changes such as calcification and haemorrhage of the plaque, rupture or ulceration of the intima or mural thrombosis (Dähnert, 2003). Unstable plaques of this nature are a threat to potential risks. A calcified plaque is due to secondary degeneration that leads to an inflammatory response with repair that develops into mixed components of fibrous tissue, and lipids with increasing amounts of calcification. These patterns are interpreted with

ultrasound by its acoustic properties. The surface appears irregular and may cause a thrombus to develop and embolise. Arterial thrombosis is caused by the rupture of the plaque and releases substances that promote coagulation. This lesion may narrow the lumen of the vessel progressively and annulally occlude the vessel (Myers and Clough, 2004).

Carra et al., (2003:) classified plaque morphology as follows: Type 1 states that the plaque is uniformly anechoic or sonolucent, type 2 is mainly hypo-echoic with less than 50% echoic areas, type 3 is mainly echogenic with less than 50% hypo-echoic areas, type 4 is uniformly iso- or hyper-echogenic and type 5 contains plaques that cannot be classified due to acoustic shadowing from the calcification or calcium in the plaque. Dähnert (2003) also classified plaques as hypo-echoic, when the echogenicity is less than that of the sternocleidomastoid muscle and when a void or disturbance in the flow pattern is visible on the colour Doppler image.

Iso-echoic plaque has an echogenicity equal to that of the muscle. A hyper-echoic plaque has an echogenicity greater than that of the muscle whilst calcified plaque is so echogenic that it casts an acoustic shadow (Dähnert, 2003). The plaque description according to its homogeneity (uniform echopattern) and heterogeneity (mixed echopatterns) properties needs further revision when establishing plaque stability. According to Dähnert (2003) plaque haemorrhage and plaque ulceration and/ or rupture, 'floating plaques' are considered to be complicated lesions that could be interpreted or considered to be more unstable forms vulnerable to detachment. This could lead to the assumption that fatty streak and fibrous plaque formations that are echogenic and homogenous, are the more stable forms of plaque present in the arteries (Dähnert, 2003).

According to Carra et al., (2003) the progression of the plaque (and thus the course of the disease) is the result of thrombosis and haemorrhage within the plaque lesion and will serve as a determinant of the acute ischemic event instead of the degree of the stenosis present within the vessel. It is also stated that an inhomogeneous or heterogeneous (mixed-echopatterns), irregular plaque is indicative of a high-risk lesion that may present symptomatically. This leads to the assumption that the inhomogeneous or heterogeneous (mixed-echopatterns), irregular and hypo-echoic plaque lesions are more 'risky' than homogeneous hyperechoic (uniform echo pattern) plaque lesions. Dähnert (2003) confirms this assumption.

2.10. Colour Doppler imaging techniques

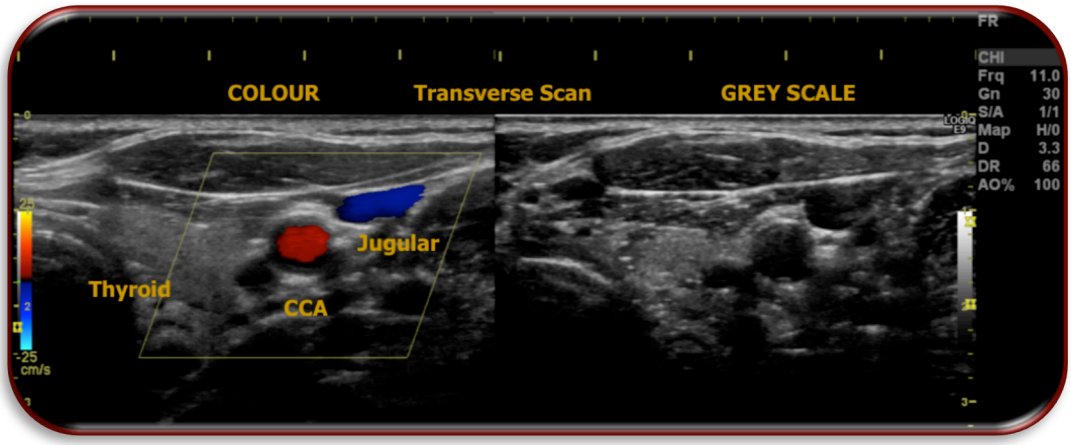


Figure 1: Original image demonstrating the transverse view of the common carotid artery (CCA) and surrounding structures in a normal individual.

Colour-coded Doppler sonography uses the combination of the anatomical information derived from pulse-echo techniques to generate colour-coded maps of tissue velocity superimposed on grey-scale images of tissue anatomy. Instead of utilising the Fast Fourier Transformation (FFT) as in pulsed wave Doppler, colour Doppler uses an autocorrelation procedure that provides information about the phase difference that is obtained as a direct measure of the mean velocity distribution in a sample volume. The results of the scan are displayed in colour coded signals. In colour mode of the normal pulsating carotid artery, forward flow is presented throughout the cardiac cycle, and when directed towards the transducer is usually depicted as colour red, and when moving away from the transducer depicted as colour blue (Colour coding for flow direction is operator dependant). The flow velocity is indicated by degrees of brightness in the coloured pixels. A bright pixel indicates high velocity, and a dark pixel slow blood flow (Evans, Jensen and Nielsen, 2011). When the appropriate Pulse Repetition Frequency (PRF) is selected, colour continually fills the vessel lumen up to the vessel walls. Flow in the ECA appears more pulsatile than in the ICA and CCA due to its anatomical variation (Ridgeway, 2004). According to Zwiebel (2005), the lack of colour filling within the vessel wall may indicate the presence of atheroma; however, he highlights the importance of ensuring that filling defects are not due to poor Doppler angle, inappropriate PRF, or due to image artefacts which could prevent the colour from being displayed (Robbin and Lockhart, 2006). Increased velocity within a stenosis or at a shunt, demonstrates a colour change before and after the narrowing. The aliasing phenomenon can be seen in spectrum analysis when the Nyquist limit is exceeded. The maximum frequency that can be displayed without

error is referred to as the Nyquist limit. High differences in flow direction and pressure often are associated with aliasing, or colour reversal, depending on the instrument settings (Edelman, 2007; Kremkau, 2006; Zwiebel, 2005; Hykes et al., 2005; Curry et al., 1990). High velocity jets may be seen at a stenosis when the path of the flow is no longer parallel to the vessel wall (disturbed and turbulent flow). The complete absence of colour Doppler filling within a vessel could indicate that the vessel is occluded but this should be confirmed by optimising the Doppler angle and colour gain for low-velocity flow detection to rule out the presence of a very tight stenosis somewhere along the vessel (Thrush and Hartshorne, 2003).

Power Doppler can be supplemented to confirm slow or no flow (Paulose, 2002). Colour and Power Doppler flow determinants for vasodilatation has also added benefit in assessing vascularisation and blood flow in inflammatory diseases e.g. enthesitis common characteristic sign and hallmark of several rheumatic diseases (Mandl et al., 2012). Studies have also shown colour flow imaging to compliment vasoconstriction determinants; to help locate the area of greatest narrowing within a diseased vessel segment using spectral Doppler (Wood et al., 2010; Gooding, 1998).

2.11. Spectral Doppler Waveforms

Spectral Doppler waveform analysis of the ECA demonstrates increased peak systolic flow velocity (PSV $_{cm/s}$) pulsatile waveforms, and low end-diastolic (EDV $_{cm/s}$) flow patterns as compared to the low resistance PSV $_{cm/s}$ waveform shape seen in the ICA (Zwiebel, 2005). The normal CCA waveform has a shape somewhere between that of the ICA and the ECA (Robbin and Lockhart, 2006). The PSV seen in the carotid arteries depend on the relative size of the vessel but are typically less than 125 $_{cm/s}$ in the normal ICA (Wood et al., 2010; Bucek et al., 2006). PSV ≥125$_{cm/s}$ within context (Robbin and Lockhart, 2006; Zwiebel, 2005; Edelman, 2007; Kremkau, 2006; Hykes et al., 2005; Curry et al., 1990) is regarded as “risky”. The presence of narrowing within the carotid arteries will lead to an increase in the velocity of the blood across the stenosis and this can be measured using spectral Doppler. Significant changes in the velocity within and just beyond a stenosis will be detected once the vessel is narrowed by a >50% reduction in diameter (Robbin and Lockhart, 2006; Neumyer, 1999: 367; Curry et al., 1990). The increase in velocity is related to the degree of narrowing. Usually low velocities can indicate the presence of disease proximal or distal to the site at which the Doppler recording is made. High - resistance waveforms, with absent EDV to reversed flow, obtained from the CCA may indicate a severe ICA stenosis or near occlusion (Scott et al., 2002).

2.12. Spectral Doppler and Carotid Artery Stenosis (CAS)

In carotid stenosis, there is a narrowing of the carotid artery lumen, often due to plaque formation (Wood et al., 2010; Edelman, 2007). Narrowing of the vessel increases the blood flow velocities, and at a stenosis there is higher resistance to flow. Furthermore, stenosis of the carotid can be classified according to the severity of narrowing. The grading of carotid artery stenosis, like other arterial diseases, are described in terms of the vessel diameter reduction. A stenosis is considered haemodynamically significant when the diameter reduction reaches 50%, corresponding to the cross-sectional area reduction of 75% (Bucek et al., 2006). The diameter reduction can be classified as normal, less than 50% stenosis, 50-69%, 70-79%, 80-90% and >90% stenosis to occlusion. Remodelling of the artery need also be considered. According to Golledge and Siew (2008), patients with carotid artery stenosis may suffer from TIA or amaurosis fugax (a form of visual disturbance). Symptoms for TIA may be temporary and the patients are said to make a full recovery within 24 hours. Patients suffering from a stroke will have symptoms lasting more than 24 hours and may not make full recovery. Symptoms include a single or multiple episodes of power loss of limbs and sensations: mono paralyses and/or hemi paralysis; slurring or speech loss (dysphasia), or visual spatial neglect (Golledge and Siew, 2008). Asymptomatic carotid disease is usually discovered clinically by the presence of a carotid bruit, however the presence of a carotid bruit may not always be due to an ICA stenosis, but could instead be related to an ECA or aortic stenosis, or no stenosis at all (Bucek et al., 2006). Spectral Doppler analysis in the subclinical diagnosis of a stenosis caused by plaques becomes more useful, when considering the most appropriate management options.

2.13. Management of severe stenosis and carotid plaques

The preferred treatment for symptomatic patients with severe ipislateral ICA stenosis >70% is a CEA (Snow et al., 2007). Surgical treatment offers a six fold reduction in the rate of stroke in the follow-up period compared to medical treatment, but benefit from surgery has not clearly been demonstrated for symptomatic patients with a moderate carotid stenosis, and in general these patients are managed conservatively. For patients with a carotid stenosis of 50% or lesser, although surgery reduces the incidence of ipislateral stroke, the benefits are comparatively small and surgery is not recommended, however close ultrasound monitoring at regular intervals, becomes more valuable in patients with vulnerable plaques (Snow et al., 2007). Ultrasound is also used for pre and post treatment monitoring. The CCA, ICA, ECA and vertebral arteries are reported to receive 15% of the cardiac output (Robbin and Lockhart, 2006); therefore, a thorough carotid ultrasound interrogation should be performed on high-risk individuals. Flow direction in the vertebral

arteries is also important to recognise severe stenosis or occlusions of the ICA. Other pathology can also be evaluated at the vertebral level. Flow reversal in the vertebral artery ipsilateral to a proximal subclavian artery stenosis was shown by Contorni (1960) and Reivich et al., (1961), and was called the subclavian steal syndrome by Fisher (1961). In the beginning, a great spectrum of symptoms were suspected for this disorder, ranging from the brainstem ischaemia and stroke and were thought to be caused spontaneously or secondary to arm exercise (Herring, 1977). Other reports questioned the significance of retrograde vertebral artery flow in producing cerebrovascular events. Similar to a low-grade internal carotid artery stenosis, the subclavian steal phenomenon was a representation of generalised atherosclerosis and thought to be a harmless haemodynamic phenomenon (Borsteien et al., 1988; Hennenci et al., 1986). Reversed flow in the vertebral artery by colour Doppler ultrasound is accepted practice and is a validated as a substitute to other invasive procedures as the first line imaging modality. Examination of the vertebral arteries using colour Doppler ultrasound is quick and ascertains vertebral artery flow reversal when present. Reversal or biphasic flow detection at rest in the vertebral artery assists in the diagnosis of subclavian steal syndrome without requiring any further investigation. Certain cases however, steal from the basilar artery does not occur at rest and blood flow increase to the arm should show vertebral flow reversal (Bendick and Glover, 1990). Great emphasis is placed on specially trained vascular sonographers (Holland et al., 2009; Kisten et al., 2013; Edelman, 2007; Kremkau, 2006; Zwiebel: 2005; Hykes et al., 2005; Curry et al., 1990). Advanced intravascular ultrasound technology is also evolving as built-in therapeutic catheter devices, dedicated to the coronary arteries. The intravascular ultrasound findings of the carotid and coronary arteries provide a more precise cardiovascular assessment.

2.14. Intravascular Ultrasound: Advanced Technology

Intravascular Ultrasound (IVUS) is a catheter-based imaging modality that is able to provide high-resolution cross-sectional images of the coronary arteries. It has the ability to image both the lumen and the outer wall of the vessel, unlike angiography, which can only display the opacified silhouette of the lumen (Bourantas et al., 2010: 1282). Using IVUS, characterisation of the plaque type inside the blood vessel can be established. Although IVUS is not routinely used in coronary angiography studies, there are instances where this approach is very useful in the diagnosis and management of vascular disease (Hoshina et al., 2010). Some sources regard IVUS as the gold standard of coronary lesion assessment. Quantitative coronary angiography (QCA) on the other hand, is an investigation often done for coronary artery disease. It uses x-ray imaging angiography principles which allows the estimation of luminal stenosis, lesion length and lumen width determination. The technique however, is often reported not accurate in cases where the artery contains diffusely diseased sections (e.g. in patients with

diabetes associated vascular disease), when the vessel is foreshortened or overlapping itself. QCA cannot provide information on the composition of intra-luminal plaque (e.g. soft, mixed or hard plaque) or its extent. It is based on 2D image acquisition and does not take into consideration the 3D nature of a blood vessel (Bourantas et al., 2010). Intravascular ultrasound provides added benefits that better assess plaque morphology, the degree and distribution of lipid and calcium deposition within atherosclerotic plaque. It offers vessel endothelium characteristics, for the assessment of mural and endothelial thickness in order to clarify the cause (intimal or mural disease) of narrowing (Shalhoub et al., 2010). The utility of IVUS provides accurate real-time information of luminal vascular dimensions allowing detection of a critical stenosis and correct balloon size selection during angioplasty (Von Segesser et al., 2002). It therefore, enhances atherectomy and stenting of peripheral, renal, and carotid atherosclerosis and provides improved visualisation of intimal thickening after angioplasty. It enables monitoring to see how well apposed stents are related to the intima and thus, guides the need for additional angioplasty to reduce separation between intima and stent (Von Segesser et al., 2002).

2.15. Contrast Enhanced Ultrasound (CEUS)

Contrast-enhanced sonography (CEUS) has only been explored in the last 30 years, and the commercial availability of the ultrasound contrast media has spread across many Asian and European countries, to further enhance the image resolution to establish a more accurate diagnosis (Catalano et al., 2007). According to Shalhoub et al., (2010), CEUS can be used to study vasculature (e.g. to exclude stenosis or atherosclerotic changes) in order to monitor the response to plaque-stabilising therapies. The micro bubbled CEUS particles remain within the vascular space, thereby improving the resolution of the vessel lumen (Shalhoub et al., 2010). Furthermore, CEUS has greatly improved the visualisation of structures (i.e. neovascularisation of CIMT, which deems to elucidate plaque instability) as well as have the ability to probe plaque biology, by targeting micro bubbles to specific ligands expressed on vascular endothelium. Micro bubble-based contrast agents which are composed of albumin or lipid shells (which are filled with air or high molecular weight gas) are injected intravenously in a dose on the order of 1ml, which translates into microliters of administered gas phase and a fraction of milligram of the shell material to enhance ultrasound images (Klibanov et al., 1999). Micro bubbles can re-circulate due to small size, whereas larger bubbles are retained in the pulmonary circulation (Shalhoub et al., 2010:382). The limitation of contrast media is the intravenous approach, and its safety regarding allergic reactions are not easily accepted by all patients. The image resolution of ultrasound equipment has evolved over the years and there are now transducers that offer superficial imaging >20 MHz. This however, may pose some concern regarding the biological

effect of high frequency imaging, especially with Doppler mode. Although CEUS and high frequency imaging is common practice (Catalano et al., 2007) in high-income countries, due to the 'benefits' outweighing the 'risks', it does not imply that ultrasound safety guidelines be neglected.

2.16. Ultrasound Safety

The ACR and AIUM Safety and Practice guidelines for the performance of ultrasound of the Extracranial Cerebrovascular System (Res. 10-2011, Ammended Res. 39-2014), recommend its responsible use by qualified practitioners. Ultrasound is perceived a safe modality with 'no known adverse effects' in human adults, but excessive Doppler use, in animal studies have proved otherwise (Shankar and Pagel, 2011). Heat is produced at the transducer-skin interface when the beam is attenuated (absorption). During cavitation, the formation of gas bubbles are at high negative pressure, and with other mechanical effects of ultrasound forces leading to 'streaming' in fluids, and stress at tissue interfaces, causing microcellular damage. Therefore it is essential that ultrasound users ensure that its use remains safe. Strict adherence to the 'As Low As Reasonably Achievable' (ALARA) principle should always be maintained (Kremkau, 2006: 352). Although some studies indicate that Doppler ultrasound may pose a potential risk to the developing fetus, no known problems have been reported in its responsible use in adult non-obstetric patients (ACR and AIUM Safety and Practice guidelines, 2011-Amended 2014; Safety Group of British Medical Ultrasound Society, 2013). Meta-analysis of ultrasonography studies that were published in 2000 showed no 'harmful' effects from ultrasonography that were statistically significant, but indicated that there was a lack of data on long-term substantive outcomes relating to neurodevelopment (Bricker et al., 2000). Even though cerebrovascular ultrasound is regarded to be safe, dwell time was kept to a minimum in this study. By adhering to the 'ALARA' principle, scanning time is reduced to prevent biological effects that may be associated with ultrasound (Kremkau, 2006: 352; Safety Group of British Medical Ultrasound Society, 2013). Swedish studies have also shown that subtle effects of neurological damage linked to ultrasound were implicated by an increased incidence in left-handedness in boys (a marker for brain problems when not hereditary) and speech delays (Salvesen et al., 1993; Kieler et al., 1998). Heikkilä et al. (2011) however could not confirm the same findings as the above study. A later study, however, performed on a larger sample of 8865 children, has established a statistically significant, albeit weak association of ultrasonography exposure and being non-right handed later in life (Salvesen, 2011). The exposure output therefore should be kept to a minimum which still gives an accurate diagnosis. In accordance with US FDA Guidelines, the overall maximum acoustic SPTA intensity for LOGIQ e is limited to 720 mW/cm^2 and mechanical index (MI) is limited to 1.9. Modes for which the thermal index (TI) does not exceed 0.1 is marked by <0.1.

CHAPTER 3

RESEARCH METHODOLOGY

CHAPTER THREE

3.0. RESEARCH METHODOLOGY

Introduction

This ultrasound study is written up as a master's thesis based on the results of a quantitative study entitled: 'Ultrasound evaluation of the carotid artery in a population at high risk of type 2 diabetes mellitus (T2DM)'. The study forms part of the larger Bellville South Africa (BSA) study, which seeks to understand the inter-relationships between obesity, diabetes and impaired glucose intolerance (IGT), with diet, tobacco and alcohol intake, physical activity, atherosclerosis, CVD risks, lipids, pro-inflammatory and oxidative markers in an urban adult mixed ancestry population from the Bellville South suburb, Tygerberg. A 'correlation' of relationships and associations of the ultrasound findings with glycaemic status and risk factors were considered when setting out the objectives.

The researcher's role in the study:

The researcher is a registered radiographer (ultrasound) with the Health Professional Council of South Africa. This study forms part of a Masters degree in Radiography. The researcher was the primary investigator for the ultrasound substudy that formed part of the 2nd phase of the larger Bellville South Africa (BSA) study. He was responsible for securing funding for the purchase of the ultrasound machine and accessories used for both the echocardiography and carotid examinations. He developed the ultrasound study design, protocols, ultrasound standard operating procedures' and secured ethical approval for the carotid substudy. He performed the carotid examinations, with the help of a sonographer research assistant.

The following chapter includes the carotid ultrasound study recipe used to meet the proposed objectives of this study in order to answer the following questions regarding a selected Bellville South mixed-ancestry population:

- What is the association between the ultrasound findings of the carotid arteries and the glycaemic status of this selected population?

- What relationship does the contributory risk factors of age, obesity (increased waist circumference, hip circumference and body mass index), hypertension, smoking, hypercholeraemia (increased blood cholesterol levels - lipid profiles) and T2DM have, on the development of carotid atherosclerosis?

Without knowing the glycaemic status, blood results and CVD risk profiles of the study population (blinded), the hypothesis that subjects diagnosed with T2DM, would have increased carotid intima media thickening, and a higher prevalence of plaques and carotid artery stenosis, than those subjects without T2DM, was tested. The NASCET guidelines (NASCET collaborators, 1991; Staikkov, Arnold and Mattle, 2000), recommendations made by the 'Terminology and diagnostic criteria committee of the Japanese Society of Ultrasonics in Medicine (2009)'; Society of Radiologists in Ultrasound Consensus Conference (2003); Stein, et al., 2009) and ultrasound vascular laboratory protocols were used for the carotid ultrasound examination (refer to **Appendix C:** Carotid Ultrasound Examination protocol). The American College of Radiology (ACR) and AIUM Safety and Practice guidelines (ACR-AIUM-SRU) for the performance of an ultrasound examination of the Extracranial Cerebrovascular System (Res. 10-2011, Ammended Res. 36-2014) were also adopted.

3.1 Study Objective

The objectives for this study population were as follows:

1. To determine the blood flow velocimetry (flow pattern, speed and direction) of the common carotid artery (**CCA**), internal carotid artery (**ICA**), external carotid artery (**ECA**) and vertebral arteries of both carotid arteries using colour and spectral Doppler ultrasound imaging.

2. To measure the common carotid wall thickness i.e. carotid intima media thickness (**CIMT**) using B-mode ultrasound.

3. To determine the characteristics, number and size (area/volume) of plaques present.

4. To establish whether there is an association between the ultrasound findings and glycaemic status [i. normal glycaemia; ii. pre-diabetes (**pre-DM**) – impaired glucose tolerance (**IGT**) or impaired fasting glucose tolerance (**IFGT**); and iii. **T2DM**)].

5. To establish the association between the ultrasound findings and atherosclerotic risk factors of age, obesity (increased waist circumference, hip circumference and BMI), hypertension, smoking, increased blood cholesterol levels (Lipid profile), glycaemia status and gender.

3.2 Assumptions

Participants with **Pre-DM** and **T2DM** (hyperglycaemia) will have increased **CIMT** measurements of > 0.8mm.

3.3 Study design, setting and sampled population

This carotid ultrasound substudy was a cross sectional study cohort consisting of a sample selected over the study period July 2010 to July 2011. The sample was selected from participants of the larger BSA study. The latter study used random sampling method in the Bellville South community. The carotid ultrasound substudy used convenient sampling method: volunteers of the BSA study were recruited when they visited the BSA study's clinic in Bellville South, Western cape, South Africa. An initial sample size of 534 was recruited, however only those that met the inclusion criteria over the study period, were used for the carotid study analysis. All volunteers that attended the ultrasound clinic who provided consent were scanned, as this was also a community service provided by the larger BSA study clinic. Bilateral carotid arteries were evaluated ultrasonically. The glycaemic status (screen detected and self-reported), blood results, contributing risks, anthropometric measurements, demographics and CVD risk profiles were established by the larger study, however, this information was withheld from the researcher until the end of the ultrasound study, to prevent research bias, thus ensuring increased validity of the results.

Inclusion Criteria:

Ethical guidelines as described below have been adopted throughout the study ensuring that only those volunteers that have signed informed consent were included in the study cohort. This study population is unique in terms of ethnicity and had therefore only included those of mixed ancestry, identified as high risk for T2DM and ≥35 years of age.

Exclusion Criteria:

Incomplete data with missing variables (have not been tested for glycaemic status, blood pressure, lipid profiles, anthropometric measurements, contributing risks and demographic profiles), and those not of mixed ancestry, were excluded from this carotid ultrasound study sample.

3.4. Carotid artery ultrasound equipment and methods used for the study

The carotid ultrasound examination was carried out by one of two qualified sonographers. All subjects were examined using a portable B-mode and spectral Doppler ultrasound scanner equipped with cardiovascular imaging software which was the main data collection tool. The LOGIQ E high performance multipurpose colour compact ultrasound system, included new imaging CrossXBeam technologies with a multifrequency linear wide band vascular transducer (8L-RS 4.0-12 MHz Linear Probe) used for improved diagnostic confidence and imaging clarity for the carotid arteries. Although some studies indicate that Doppler ultrasound may pose a potential risk to the developing fetus, no known problems have been reported in its responsible use in adult non-obstetric patients (ACR and AIUM Safety and Practice guidelines, 2011-Amended 2014; Safety Group of British Medical Ultrasound Society, 2013). Even though cerebrovascular ultrasound is regarded to be safe, dwell time was kept to a minimum in this study. The ultrasound examinations done have adhered strictly to the 'ALARA' principle to reduce dwell time and biological effects that may be associated with ultrasound (Kremkau, 2006: 352; Safety Group of British Medical Ultrasound Society, 2013). The exposure output was kept to a minimum which still gave an accurate diagnosis. In accordance with US FDA Guidelines, the overall maximum acoustic SPTA intensity for LOGIQ e is limited to 720 $_{mW/cm}^{2}$ and mechanical index (MI) is limited to 1.9. Modes for which the thermal index (TI) does not exceed 0.1 is marked by <0.1. The ultrasound findings of bilateral carotid arteries were documented on ultrasound reports. These measurements and images were also recorded as still/real time video clips and archived using the unique reference identification number as coded by the larger BSA study, onto the ultrasound machine and external hard drive for backup and reviewing purposes.

3.5. The ultrasound evaluation included:

3.5.1 Spectral Doppler blood flow velocities

Bilateral peak systolic velocities (**PSV** $_{cm/s}$) and end diastolic velocities (**EDV** $_{cm/s}$) of the proximal, middle and distal-CCA; and the proximal internal and external carotid arteries (**ICA** and **ECA**) were recorded. Spectral Doppler velocimetry included pattern recognition of the vertebral arteries, and were evaluated for its patency and flow direction, which was recorded as normal, absent or reversed flow, with the help of colour Doppler imaging. Vertebral arteries usually show abnormal flow when the **ICA** is severely stenosed or occluded and then serve as collateral flow with a change in direction.

3.5.2 Bilateral Carotid Intima Media Thickness (CIMT) measurement (mm).

The maximum thickness and total mean of the far wall, of the carotid intima and media layers (**CIMT** $_{mm}$) were measured in longitudinal section, 2 $_{cm}$ from the carotid bifurcation, at 3 consecutive end points, 5-10 $_{mm}$ apart. The **CIMT** of bulbs were evaluated, however not measured in this study (Figure 3.1).

3.5.3 Plaque characteristics

Images of plaques were recorded and measured according to its sonographic appearances in terms of echogenicity and homogeneity (the characteristics of plaques described for this study were in keeping with guidelines of the 'Terminology and diagnostic criteria committee of the Japanese Society of Ultrasonics in Medicine (2009)' and ultrasound vascular laboratory protocols (refer to appendix D). The location, size (area/volume), stability (vulnerability of detachment e.g. 'floating') and amount of obvious plaques were recorded.

3.5.4 Stenosis

The **PSV** $_{cm/s}$ in the carotid arteries where relative to the size of the vessel and in this study **<125** $_{cm/s}$ **was regarded to be normal**, and **≥125**$_{cm/s}$ **as abnormal ICA flow** (NASCET collaborators, 1991, Curry et al., 1990; Society of Radiologists in Ultrasound Consensus Conference (2003); Neumyer, 1999). The percentage stenosis of the patent carotid blood flow velocities were measured using the **PSV** $_{cm/s}$ of **ICA** divided by the **PSV** $_{cm/s}$ of the distal-CCA as a guiding parameter for **ICA** stenosis, with reference to the criteria of ratio <2 as normal according to NASCET and vascular laboratory guidelines (NASCET collaborators, 1991; Staikkov, Arnold and Mattle, 2000; Society of Radiologists in Ultrasound Consensus Conference (2003); Stein et al., 2009; Neumyer, 1999 and vascular laboratory protocols). The presence of carotid plaques (>1_{mm}^2 or >1_{mm}^3) were also considered in calculating subtle or severe percentage stenosis of <50%, 50-69% and >70%. **ECA** stenosis was recorded when noted.

A written report was done in duplicate – the original report was checked and inserted in the participant's folder. The research physician present at the clinic was made aware of sonographic findings with direct access to the participant's folders for further clinical management or referral where necessary. All ultrasound data (images, video-clips and measurements) were stored according to the participants' unique reference identification number as coded by the larger BSA study, onto the ultrasound machine, and was verified by an independent sonographer for post-processing and validation of results. The ultrasound reports were captured onto the carotid ultrasound database stored on an M S Excel

spreadsheet, using the unique reference identification number. At the end of the study period, the reports and images saved on the ultrasound machine were retrieved for audit. The independent ultrasound researcher and supervisors inspected the carotid ultrasound reports and database, as part of the checking process. Only after the supervisors approval, was the validified ultrasound data merged with the larger study database. The principle investigator and sonographer research assistant of the carotid ultrasound study remained blinded to the glycaemic status, blood results, contributing risks, anthropometric measurements, demographics and CVD risk profiles up until the data analysis stage.

3.6 Data Collection and Analysis

The sample selected for analysis were only those that met the inclusion criteria over the carotid ultrasound substudy period July 2010 to July 2011; and excluded all those with missing variables, not of mixed-ancestry, below 35 years of age, and those not residing in the Bellville South region.

Data of blood flow patterns and velocities (**PSV** $_{cm/s}$ and **EDV** $_{cm/s}$) of both right (**Rt.**) and left (**Lt.**) sides of the **CCA, ICA** and **ECA** were computed as non-parametric variables and the vertebral arteries as parametric variables in statistical format. A severe stenosis or occlusion usually results in reversed vertebral flow. Non-parametric testing was used due to no prior knowledge about the population or the parameters, but was still suitable to test the overall hypothesis that higher levels of CIMT, plaques and stenosis will be present in diabetics as compared to non-diabetics, hence mann-Whitney test used to calculate statistical significance for non-parametric testing. The null hypothesis was free from parameters and the test statistics arbitrary, and applied both variable and attributes, however not as powerful as the parametric tests. This was also because this method was only available for norminal scale data. Other tests done were made using parametric variables. Parametric testing was made on the information about the population completely known by means of its parameters and distribution, with the test applicable only for variable and t-test, f-test, z-test and ANOVA statistics possible. A specific assumption was made regarding the population as mentioned above and null hypothesis made on parameters of the population distribution.

The means ± standard deviations (SD) for the **Rt.** side only, **Lt.** side only and the combined mean of **Rt. and Lt.** sides of all of the above carotid vessels were calculated in order to meet the 1st objective.

Variables used for **CCA** were:

- right (**Rt.**) **Rt. CCA (PSV)** $_{cm/s}$**; Rt. CCA (EDV)** $_{cm/s}$ and
- left (**Lt**.) were **Lt. CCA (PSV)** $_{cm/s}$**; Lt. CCA (EDV)** $_{cm/s}$

- And for both CCA's the mean was used: **Mean Rt.andLt CCA (PSV)** $_{cm/s}$ and **Mean Rt.andLt CCA (EDV)** $_{cm/s.}$

Variables used for **ICA** were:

- right (**Rt.**) **Rt. ICA (PSV)** $_{cm/s}$**; Rt. ICA (EDV)** $_{cm/s}$ and
- left (**Lt**.) were **Lt. ICA (PSV)** $_{cm/s}$**; Lt. ICA (EDV)** $_{cm/s}$
- And for both ICA's the mean was used: **Mean Rt.andLt ICA (PSV)** $_{cm/s}$ and **Mean Rt.andLt ICA (EDV)** $_{cm/s.}$

Variables used for **ECA** were:

- right (**Rt.**) **Rt. ECA (PSV)** $_{cm/s}$**; Rt. ECA (EDV)** $_{cm/s}$ and
- left (**Lt**.) were **Lt. ECA (PSV)** $_{cm/s}$**; Lt. ECA (EDV)** $_{cm/s}$
- And for both **ECA**'s the mean was used: **Mean Rt.andLt ECA (PSV)** $_{cm/s}$ and **Mean Rt.andLt ECA (EDV)** $_{cm/s.}$

The carotid intima media thickness was measured using B-mode ultrasound. **CIMT** variables: CIMT1, CIMT2 and CIMT3 of both **Rt. and Lt.** carotid arteries were documented (Figure 3.1). The mean and maximum/thickest CIMT measurement was determined on the far wall of the **CCA** for each side, and the combined mean of **both CIMT Rt. and Lt.** sides were established. A mean **CIMT of ≤0.8mm was used as a normal reference value**.

Variables used for **CIMT** were:

- right were **Mean Rt. CIMT** $_{(mm)}$ and the widest diameter **Thickest Rt. CIMT** $_{(mm)}$
- left were **Mean Lt. CIMT** $_{(mm)}$ and the widest diameter **Thickest Lt. CIMT** $_{(mm)}$
- And for both **CIMT's** the mean was used **Mean Rt.andLt. CIMT** $_{(mm)}$ and the mean widest diameter **Thickest Rt. or Lt. CIMT** $_{(mm)}$

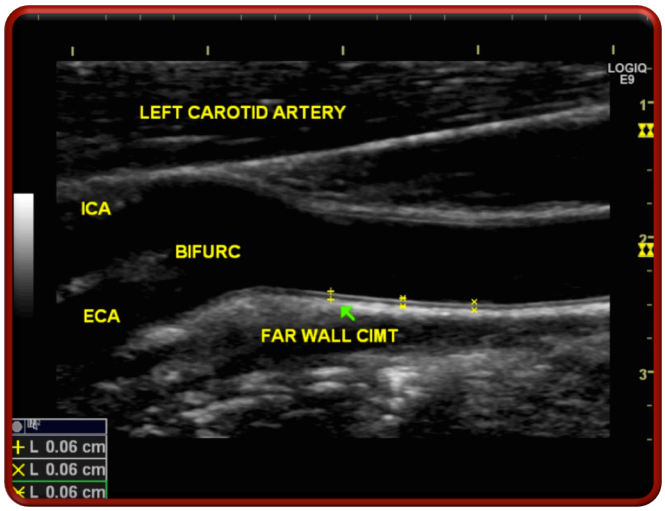


Figure 3.1: Original image of the Carotid Intima Media thickness (CIMT) measurement of left carotid artery of a normal individual.

The number and characteristics of plaques were documented. The presence, size (area/volume), amount and characteristics of plaques recorded, were categorised for analysis as follows: Plaque echogenicity: Hypo-echoic, Iso-echoic, and hyper-echoic were compared to the echogenicity of the sternocleidomastoid muscle. Calcified echogenic plaques were referred to as hyper-echoic. Plaques with mixed echo patterns were referred to as heterogeneous.

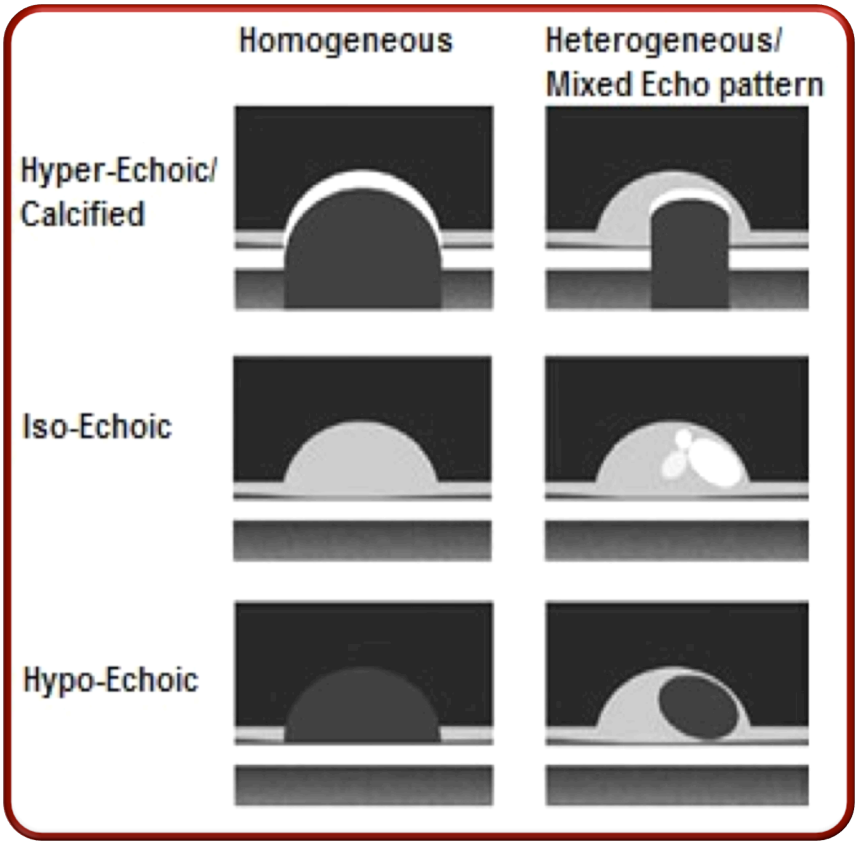


Figure 3.2: Plaque characteristics (Adapted with permission from the terminology and diagnostic criteria committee of the Japanese Society of Ultrasonics in Medicine, 2009).

The stability of plaques was established as either stable or unstable (vulnerable).

The formula for calculating plaque area (mm^2) was length (mm) X width (mm). Plaque volume (mm^3) was calculated by multiplying the length (mm) X width (mm) X depth (mm) of measurable plaques. The accumulative amount of plaques present was also established bilaterally. Subjects with no plaques, unilateral or bilateral plaques were then identified.

Variables used for **stenosis** were:

- Right were: **Rt. ICA/CCA Ratio (PSV)**
- Left were: **Lt. ICA/CCA Ratio (PSV)**
- Both left and right were: **Mean Rt.andLt. ICA/CCA Ratio**

In order to establish whether there was an association between the ultrasound findings and glycaemic status of the study population, the carotid artery ultrasound findings (Spectral Doppler velocimetry); CIMT measurements, plaques and stenosis findings were then grouped according to glycaemic status of normal, **Pre-DM** (**IGT/IFGT**) and diabetes (**T2DM**) in order to test the hypothesis that subjects diagnosed with **T2DM**, would have increased carotid intima media thickening, and a high prevalence of plaques and carotid artery stenosis, than those subjects without **T2DM**.

Variables and abbreviations used for glycaemic status: refer to definitions in glossary under clarification of concepts:

- **Normal glycaemia**
- **Pre-DM =** Pre-Diabetes Mellitus
    - **IGT =** Impaired Glucose Tolerance
    - **IFG =** Impaired Fasting Glucose
    - **IFG/IGT =** Impaired Fasting Glucose Tolerance
- **DM =** Diabetes Mellitus
- **Known DM =** Known with DM
- **New DM =** Newly diagnosed
- **Pre-DM + DM =** Hyperglycaemia

To establish the association between the ultrasound findings and atherosclerosis risk factors, data collected included age, obesity (increased waist circumference, hip circumference and BMI), hypertension, smoking, increased blood cholesterol levels (Lipid profile), glycaemic status and gender.

Variables used for ultrasound findings (detailed above):

- Carotid artery wall thicknesses (**Mean Lt. and Rt. CIMT),** Plaques and ICA stenosis

Variables used for contributing risk factors:

- Age $_{yrs}$ =years
- Anthropometric measurements in relation to obesity
    - Waist Circumference =**WC** $_{cm}$ = centimetres
    - Hip Circumferance **=HP** $_{cm}$ = centimetres
    - Body Mass Index =**BMI** $_{Kg/m^2}$ = Kilogram/meter squared

- Hypertension:
    - Systolic Blood pressure **=SBP** $_{mmHg}$ = millimetres of Mercury
    - Diastolic Blood Pressure **=DBP** $_{mmHg}$ = millimetres of Mercury
- Inflammatory markers
    - High sensitive C-reactive protein **=hs-CRP** $_{mg/L}$ = milligrams per litre
- Smoking
    - Serum Cotinine =**S-Cotinine** $_{ng/ml}$**=** nanograms per millilitre
- Increased blood cholesterol levels, lipid profiles and liver function test
    - High-Density Lipoprotein **=HDL** $_{mmol/L}$ **=** concentration millimole per litre
    - High-Density Lipoprotein **=LDL** $_{mmol/L}$ **=** concentration millimole per litre
    - Total Cholesterols **=TC** $_{mmol/L}$ **=** concentration millimole per litre
    - Triglycerides **=TG** $_{mmol/L}$ **=** concentration millimole per litre
    - Gamma-Glutamyl Transpeptidase =**GGT** $_{IU/L}$ = International Units per litre
- Glycaemia status
    - Glycated haemoglobin **=HbA1C % =** percentage
    - Fasting Blood Glucose =**FBG** $_{mmol/L}$ **=** concentration millimole per litre
- Gender of the mixed ancestry study population
    - Males
    - Females

The carotid study cohort was made up of participants scanned between July 2010-July 2011 of which was used for this analysis. The larger BSA study team leaders categorised the carotid study cohort into glycaemic status groupings, after ensuring that there was no data mismatch or missing variables. All data required for the carotid study was made available by the researcher and supervisors for data analysis using STATISTICA 9 (IBM SPSS and StatSoft Inc. 1984–2009) in order to meet the five objectives of the ultrasound study. The larger study principal investigator and a senior member of the research team with statistical experience, grouped the larger study results with the carotid study data and provided the researcher support in the statistical calculations. The type of statistical tests to be done, was determined by a consultant statistician's suggestions made at the onset of the carotid study at the proposal stage in 2008, of which was used for the analysis.

Descriptive statistics was done using the frequency tables, means and standard deviations for the selected study population. The Mann-Whitney U-test was used for independent data. Chi square test and univariate analysis were used to determine the association of the ultrasound findings between the different groupings of the participants' glycaemic status and the relationships of the ultrasound findings (CIMT, plaques and stenosis) with other contributory risks factors (biochemical markers, obesity and hypertension) were determined, to include

stepwise multivariate regression analysis. The researcher designed all graphs, figures and tables into catergories to illustrate the findings. Data was represented as mean ± SD (standard deviation), unless stated otherwise. Statistical significance for comparison was regarded when the probability value: **$p < 0.0500$*** as significant; **$p < 0.0001$**** as highly significant, and **$p < 0.1000$ +**, as approaching significant; or not significant (ns). The researcher was responsible for the interpretation and write up of statistical results of this carotid study.

3.7. Ethical considerations

Ethics approval was obtained from the Research Ethics Committee of the Faculty of Health and Wellness Sciences, CPUT, prior to the commencement of the carotid study (CPUT/HW- REC 2010/H008). Informed consent was obtained from the BSA study for each participant, who was given the prerogative to withdraw from the study at any given time. Patient confidentiality continues to be maintained in order to uphold the research participants' rights in terms of respect for human dignity, thus safeguarding their confidentiality (Bryant et al., 2006). A numeric code was used as a reference to the participants' identification in order to link the results of the carotid study to that of the larger study, which was kept separate in a secure, lock up cupboard, in the research laboratory of the larger BSA study.

The carotid ultrasound data was only accessible, with permission from the larger study team leaders, principle and primary investigators. (Refer to **Appendix B** for informed consent, **Appendix E** larger study acknowlegement, **Appendix F** for ethics certificate and **Appendix G** for permission of information used from the Japan Society of Ultrasonics in Medicine)**.**

CHAPTER 4

RESEARCH RESULTS

CHAPTER FOUR

4.0 RESEARCH RESULTS

From the initial BSA study population of 534 subjects, 157 were excluded due to age, ethnicity, geographic location and/or missing data and those not within the study period July 2010-July 2011. This resulted in the final analytic carotid ultrasound study sample reduced to 377 mixed ancestry subjects ≥35_{yrs} old (N = 377).

4.1. Ultrasound findings of study population (N = 377) according to gender are summarised in Table 4.1:

Blinded by the larger study results, the carotid ultrasound study reported 105 males and 272 females, indicating a lower proportion of males than females. Data was represented as mean ± standard deviation (SD), unless stated otherwise. The Mann-Whitney *U*-test was used for non-parametric data. Statistical measurement differences and comparison between genders (Male 27.9%: Female 72.1%) was made using P-Values of statistically significant **(p < 0.0500)***, highly significant **(p< 0.001) ****, approaching significance **(p < 0.1000) $^{+}$** and not significant (^{ns}).

4.1.1 Ultrasound findings (Spectral Doppler Velocimetry: Blood Flow Velocities and Waveforms)

The peak systolic velocities **(PSV)** $_{cm/s}$ and end diastolic velocities **(EDV)** $_{cm/s}$ of common carotid artery (**CCA),** internal carotid artery **(ICA)** and external carotid artery (**ECA)** were recorded according to vascular ultrasound laboratory protocols.

Common Carotid Arteries (CCA)

The **PSV** $_{cm/s}$ and **EDV** $_{cm/s}$ of the common carotid arteries showed no statistical differences between male (27.9%) and female (72.1%) measurements of the **Rt. CCA** (p = 0.1498; p = 0.1728) ^{ns} and **mean Rt. and Lt CCA** (p = 0.0595; p = 0.1395) ^{ns}. Interestingly the **Lt. CCA (PSV)** in males (85.67 ± 18.53) $_{cm/s}$ demonstrated a significantly higher **(p = 0.0474)* PSV** value than the females (81.58 ± 18.70) $_{cm/s}$ (refer to Table 4.1)

Internal Carotid Arteries (ICA)

The ICA (PSV) $_{cm/s}$ of **<125$_{cm/s}$** was used as a normal reference value and the ICA (EDV)$_{cm/s}$ of **≥40$_{cm/s}$** was referenced as abnormal (Society of Radiologists in Ultrasound Consensus Conference (2003); Neumyer, 1999 and vascular laboratory protocols). Comparison of both internal carotid arteries between male (105/377) and female

(272/377) subjects showed no statistical differences in **PSV** $_{cm/s}$ measurements of **Rt. ICA** (p = 0.1082)^{ns}, **Lt. ICA** (p = 0.2707)^{ns} and **mean Rt. and Lt ICA** (p = 0.1045)^{ns}. Interestingly, **the Rt. ICA (EDV)** $_{cm/s}$ **(p = 0.0998)** $^{+}$ and **mean Rt. and Lt. ICA (EDV)** $_{cm/s}$ **(p = 0.0864)** $^{+}$ in males (22.62 ± 6.60; 23.12 ± 5.87) $_{cm/s}$, respectively, approached lower ICA **(EDV)** $_{cm/s}$ velocities than the females (24.35 ± 8.25) $_{cm/s}$ and (24.67 ± 7.11) $_{cm/s}$ (refer to Table 4.1).

External Carotid Arteries

Pattern recognition for increased PSV $_{cm/s}$ >200 $_{cm/s}$ were used as a guide for abnormal ECA peak systolic velocities. No statistical differences seen in **PSV** $_{cm/s}$ **and EDV** $_{cm/s}$ measurements of the **mean Rt. and Lt. ECA** (p = 0.8910, p = 0.1193)^{ns} of males (80.84 ± 20.02; 31.84 ± 7.64) $_{cm/s}$ as compared to the females (79.61 ± 17.01; 33.08 ± 7.42) $_{cm/s}$. (refer to Table 4.1)

Vertebral Arteries

The bilateral vertebral arteries were evaluated for direction of flow (antegrade/forward or retrograde/reversed) and flow characteristics (normal patency, abnormal or absent flow). All 377 (100%) participating subjects demonstrated normal forward patent flow. A common finding that was noted when evaluating the vertebral arteries was that one vertebral artery appeared to be larger than the other, with the left often larger than the right.

4.1.2 Ultrasound findings (Carotid Intima Media Thickness)

The results of the 2nd objective was obtained for both common carotid arteries using the maximum thickness and mean CIMT measurements 2 $_{cm}$ from the carotid bifurcation, at 3 consecutive end points, 5-10 $_{mm}$ apart. The CIMT variables: CIMT1, CIMT2 and CIMT3 of **both Rt. and Lt. Carotid arteries** were compared between males and females. **The maximum thickness and mean CIMT** of ≤0.80$_{mm}$ was regarded as being within the normal range. The average mean and SD of the three **Rt; Lt;** and **mean Rt. and Lt. CIMT** measurements were 0.96 ± 0.31; 1.03 ± 0.34 and 0.99 ± 0.30 $_{mm}$ in the males respectively, and in females measured 0.84 ± 0.25; 0.85 ± 0.26 and 0.85 ± 0.24 $_{mm}$. Interestingly, the males demonstrated highly statistically significant increases for all CIMT categories: **Mean Rt. CIMT** $_{mm}$ **(p = 0.0002)*, Mean Lt. CIMT** $_{mm}$ **(p < 0.0001) **** and **Mean Rt. and Lt. CIMT** $_{mm}$ **(p < 0.0001) **.** The maximum and SD of the **thickest CIMT** measurements of the **Rt; Lt;** and **mean thickest Rt. or Lt. CIMT** in males was 1.02 ± 0.38; 1.07 ± 0.34 and 1.07 ± 0.35 $_{mm}$ respectively, and in females measured 0.87 ± 0.26; 0.89 ± 0.28; 0.90 ± 0.26 $_{mm}$ respectively. Interestingly, all CIMT categories for maximum thickness demonstrated a highly statistically significant increase of carotid intima media thickening in the males as compared to the females: **Thickest Rt. CIMT** $_{mm}$ **(p = 0.0003)*,**

Thickest Lt. CIMT $_{mm}$ (p < 0.0001),** and **Thickest Rt. or Lt. CIMT $_{mm}$ (p < 0.0001)**.**

4.1.3 Ultrasound findings (Plaques and stenosis)

In keeping with the guidelines of the Society of Radiologists in Ultrasound Consensus Conference (2003), the presence of plaques in the ICA was "flagged" as an indication of some form of flow disturbance or stenosis. Of the 377 mixed ancestry population selected for this carotid ultrasound study, 2 male subjects were excluded due to missing variables, resulting in a sample N=375. Of these subjects who were ultrasonically evaluated for plaques and stenosis, 33% (124/375) were diagnosed with either unilateral or bilateral carotid plaques, with a plaque area >1 $_{mm}^{2}$ or a plaque volume of >1$_{mm}^{3}$.

The ICA/CCA ratio was used in combination with plaques to determine the percentage of stenosis by dividing the **PSV of ICA over PSV of CCA.** The ICA/CCA ratio of <2 was established as normal. Among the male and female categories in Table 1, the mean stenotic ratios and SD for the **Rt. ICA/CCA Ratio (PSV); Lt. ICA/CCA Ratio (PSV)** and the combined **Mean Rt. and Lt. ICA/CCA Ratio** for males were (0.81 ± 0.20; 0.84 ± 0.38 and 0.82 ± 0.27) respectively, and (0.88 ± 0.23; 0.88 ± 0.23 and 0.88 ± 0.19) respectively in females. The males demonstrated a statistically significant lower stenotic ratio for categories: **Rt. ICA/CCA Ratio (p = 0.0019)*; Lt. ICA/CCA Ratio (p = 0.0022)*** and **Mean Rt. and Lt. ICA/CCA Ratio (p = 0.0001)***as compared to the females (Refer to Table 4.1).

Using all of the above information collectively the stenotic ratios were grouped into categories of normal, <50% or > 50% ICA stenosis. The **ICA/CCA Ratio < 2** with plaques, was regarded as some form of stenosis, in the category <50% stenosis. Interestingly, in this selected sample, there were no subjects with **ICA/CCA Ratio >2** with a stenosis >50%. The presence of plaques and percentage stenosis was also used for objectives 4 and 5.

Table 4.1: Ultrasound findings of study population according to gender

Data represented as mean ± standard deviation (SD), unless stated otherwise. Statistical significance when P-Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000) +**, Not significant (^{ns}). Mann-Whitney *U*-test.

SAMPLED (N = 377) (Ultrasound Findings)		Male (n = 105) (Mean ± SD)	Female (n = 272) (Mean ± SD)	P-Value
Common Carotid Arteries	**Rt. CCA (PSV) cm/s**	84.28 ± 17.34	81.91 ± 16.96	0.1498 ^{ns}
	Lt. CCA (PSV) cm/s	85.67 ± 18.53	81.58 ± 18.70	**0.0474***
	Mean Rt.andLt CCA (PSV) cm/s	84.97 ± 16.80	81.75 ± 16.36	**0.0595^{+}**
	Rt. CCA (EDV) cm/s	22.67 ± 6.14	23.80 ± 6.35	0.1728 ^{ns}
	Lt. CCA (EDV) cm/s	23.66 ± 6.53	24.85 ± 7.68	0.1782 ^{ns}
	Mean Rt.andLt CCA (EDV) cm/s	23.16 ± 5.71	24.32 ± 6.31	0.1395 ^{ns}
Internal Carotid Arteries	**Rt. ICA (PSV) cm/s**	66.54 ± 14.87	69.96 ± 16.68	0.1082 ^{ns}
	Lt. ICA (PSV cm/s	68.87 ± 22.19	70.12 ± 17.84	0.2707 ^{ns}
	Mean Rt.andLt ICA (PSV) cm/s	67.70 ± 16.44	70.04 ± 14.35	0.1045 ^{ns}
	Rt. ICA (EDV) cm/s	22.62 ± 6.60	24.35 ± 8.25	**0.0998^{+}**
	Lt. ICA (EDV) cm/s	23.62 ± 8.62	24.96 ± 8.67	0.1253 ^{ns}
	Mean Rt.andLt ICA (EDV) cm/s	23.12 ± 5.87	24.67 ± 7.11	**0.0864^{+}**
External Carotid Arteries	**Rt. ECA (PSV) cm/s**	80.50 ± 19.65	81.63 ± 21.06	0.4113 ^{ns}
	Lt. ECA (PSV) cm/s	81.34 ± 25.77	78.24 ± 21.62	0.5010 ^{ns}
	Mean Rt.andLt. ECA (PSV) cm/s	80.84 ± 20.02	79.61 ± 17.01	0.8910 ^{ns}
	Rt. ECA (EDV) cm/s	20.72 ± 7.93	21.58 ± 9.58	0.7400 ^{ns}
	Lt. ECA (EDV) cm/s	21.52 ± 8.44	23.94 ± 15.42	0.5214 ^{ns}
	Mean Rt.andLt. ECA (EDV) cm/s	31.84 ± 7.64	33.08 ± 7.42	0.1193 ^{ns}
Carotid Intima Media Thickness	**Mean Rt. CIMT (mm)**	0.96 ± 0.31	0.84 ± 0.25	**0.0002***
	Mean Lt. CIMT (mm)	1.03 ± 0.34	0.85 ± 0.26	**< 0.0001****
	Mean Rt.andLt. CIMT (mm)	0.99 ± 0.30	0.85 ± 0.24	**< 0.0001****
	Thickest Rt. CIMT (mm)	1.02 ± 0.38	0.87 ± 0.26	**0.0003***
	Thickest Lt. CIMT (mm)	1.07 ± 0.34	0.89 ± 0.28	**< 0.0001****
	Thickest Rt. or Lt. CIMT (mm)	1.07 ± 0.35	0.90 ± 0.26	**< 0.0001****
ICA / CCA Stenotic ratios	**Rt. ICA/CCA Ratio (PSV)**	0.81 ± 0.20	0.88 ± 0.23	**0.0019***
	Lt. ICA/CCA Ratio (PSV)	0.84 ± 0.38	0.88 ± 0.23	**0.0022***
	Mean Rt.andLt. ICA/CCA Ratio	0.82 ± 0.27	0.88 ± 0.19	**0.0001***

ABBREVIATIONS

Rt. = Right, **Lt**. = Left, **CIMT** = Carotid Intima Media Thickness, **CCA** = Common Carotid Artery, **ICA** = Internal Carotid Artery, **ECA** = External Carotid Arteries, **PSV** = Peak Systolic Velocity, **EDV** = End Diastolic Velocity, **mm** = millimetres, **cm/s** = centimetres per second, **N** = Sample size, **n** = sample group.

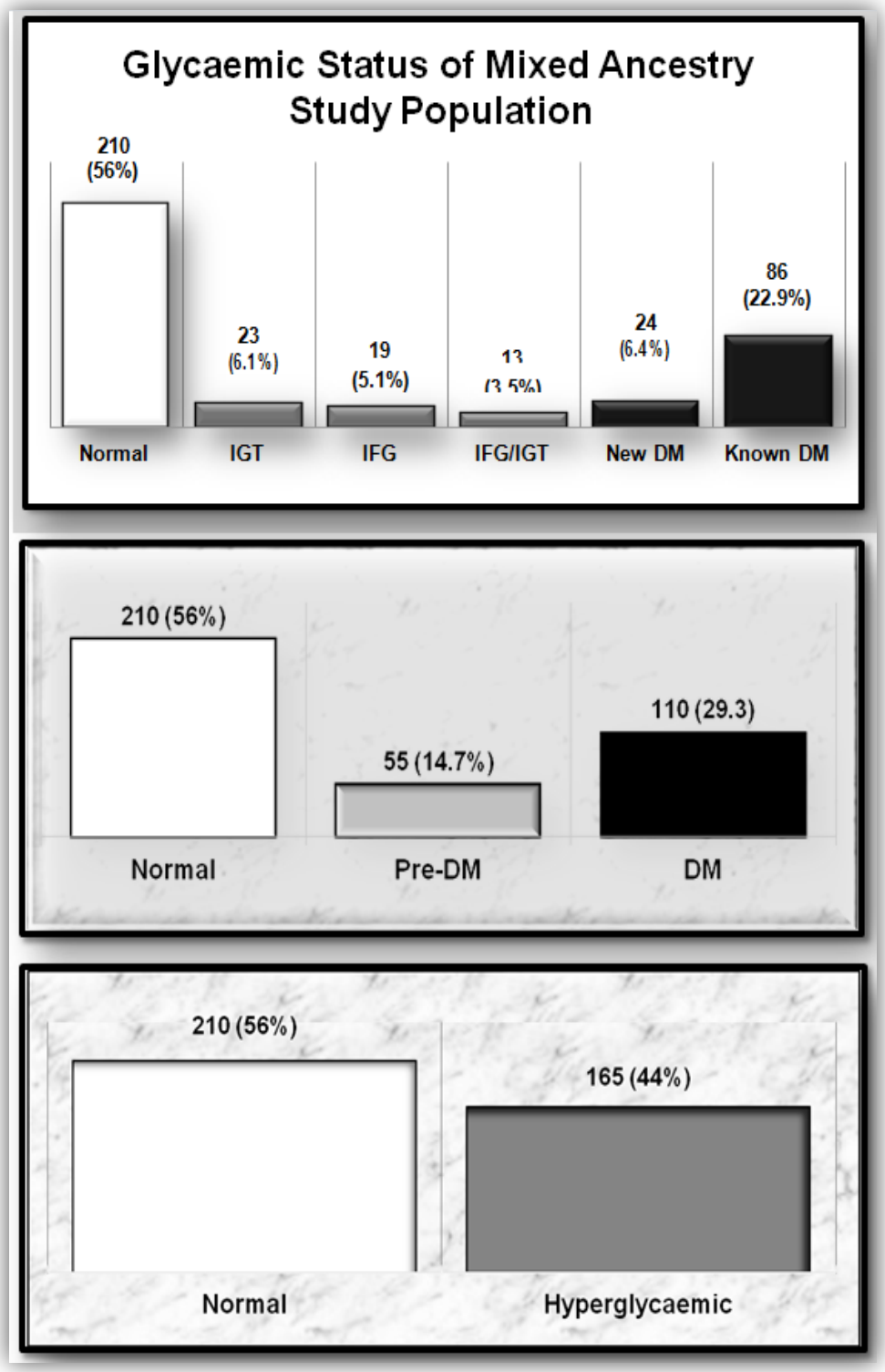
4.1.4 Association of ultrasound findings with glycaemia status (Normal glycaemia, Pre-DM and T2DM)

The glycaemia status of the study population was then made available to meet the 4th objective, which established an association between the ultrasound findings and glycaemia status (normal glycaemia, Pre-DM and T2DM) of the Bellville South subjects selected for the study. The carotid spectral Doppler velocimetry readings; CIMT measurements; the presence of plaques and stenotic ratios, were grouped according to glycaemia status in order to test the hypothesis that subjects diagnosed with T2DM, would have higher levels of CIMT, plaques and stenosis, than those subjects without T2DM.

4.2. Study population glycaemia status distribution (Figure 1.1)

According to the larger BSA study survey, 2 male subjects (105-2) were further excluded due to missing variables, causing a further 0.53% (377-2) reduction in the glycaemia status sub-sample to N= 375. The glycaemia level distribution among the study population was categorised according to WHO guidelines (World Health Organisation Report 2006 of WHO/IDF consulation, 2006), into normal glucose tolerance **(Normal)** 54% (210/375), impaired glucose tolerance **(IGT)** 6.1% (23/375), Impaired Fasting Glucose **(IFG)** 5.1% (19/375), and Impaired Fasting Glucose Tolerance **(IGT/IFT)** 3.5% (13/375) and diabetes mellitus **(DM),** which comprised 29.3% (110/375) of the study population**.** Those with IGT, IFG and IGT/IFT (14.7%, 55/375) were grouped as pre- diabetes mellitus **(Pre-DM).** Of the **DM** group, a 6.4% (24/375) proportion was identified as newly diagnosed **(New DM)** with diabetes, and 22.9% (86/375) was known of having diabetes **(Known DM).** The study population was further grouped into normal glycaemia and hyperglycaemia. Fifty six percent (210/375) were reported with normal glycaemia **(Normal)**, while 44% (165/375) of the study population was **hyperglycaemic** (refer to Figure 4.1).

The interrelationship between the Ultrasound measurements of the **CIMT** $_{mm}$ and the 3 glycaemia groups of **Normal, Pre-DM** and **DM** was thereafter determined among males and females in the carotid study population (refer to Table 4.2a and 4.2b and Figure 4.2a and 4.2b).



Abbreviations:
IGT = Impaired Glucose Tolerance; **IFG =** Impaired Fasting Glucose; **IFG/IGT =** Impaired Fasting Glucose Tolerance; **DM =** Diabetes Mellitus; **Known DM =** Known with DM; **New DM =** Newly diagnosed; **Pre-DM =** Pre-Diabetes Mellitus; **Pre-DM + DM =** Hyperglycaemia

Figure 4.1: Study population glycaemia status distribution.

4.3. Descriptive statistics and Inter-relationship of CIMT measurements and glycaemia status in male and female study population (N = 372):

Of the 375 participating subjects grouped according to their glycaemia status by the larger BSA study, 3 female subjects (272-3) were further excluded due to missing variables and incomplete data. The subsample adjusted for this study analysis resulted in N = 372. The study population comprised of 27.7% (103/372) males and 72.3% (269/372) females. The mean age of the males (57.2 ± 12.3) $_{yrs.}$ and the females (55.8 ± 12.0) $_{yrs.}$ showed no statistical age differences (p = 0.2870) ^{ns} between them. In the male catergory, 61.2% (63/103) were screen detected or self-reported with normal glycaemia, 13.6% (14/103) as Pre-DM and 25.2% (26/103) with DM. In the female catergory, 53.5% (144/269) were reported with normal glycaemia, 15.2% (41/269) as Pre-DM and 31.2% (84/269) with DM. The inter- relationship of CIMT ultrasound measurements and glycaemia status were then further analysed into gender groups separately.

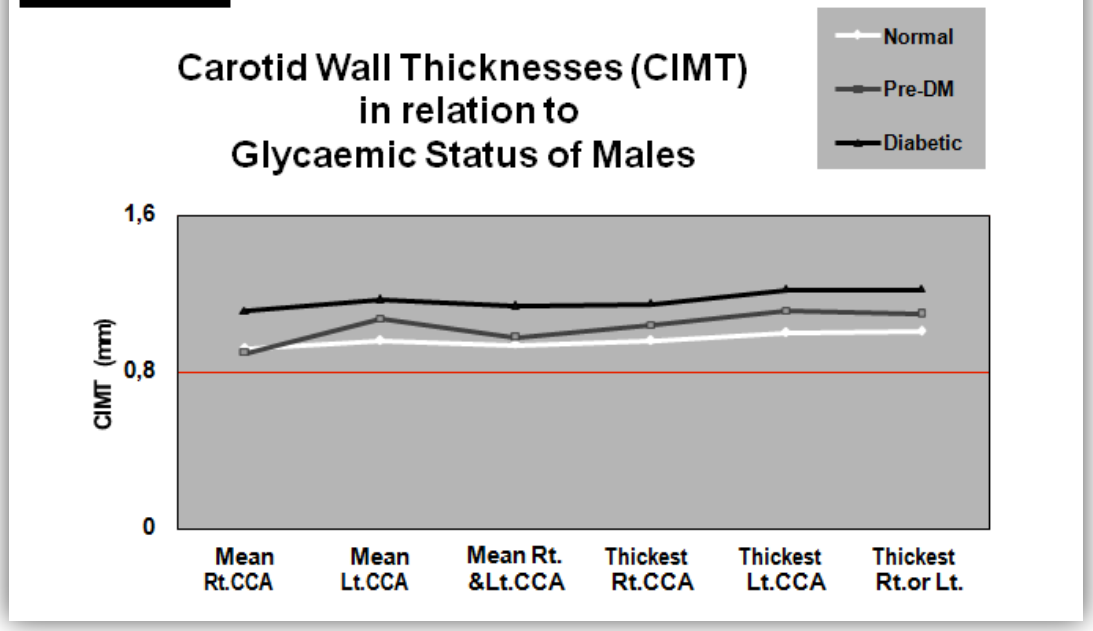
4.3.1 Inter-relationship of CIMT ultrasound measurements and glycaemia status in male population (n= 103)

The mean of 3 CIMT measurements according to the study protocol of the Rt. and Lt. carotid arteries (**Mean Rt. CIMT $_{mm}$; Mean Lt. CIMT$_{mm}$ and Mean Lt. and Rt. CIMT$_{mm}$)** in males with normal glycaemia, Pre-DM and DM were analysed. No significant change (p = 1.0000) ^{ns} of **Mean Rt. CIMT $_{mm}$** measurement in those with normal glycaemia (0.92 ± 0.29) $_{mm}$ as compared to those with Pre-DM (0.90 ± 0.29) $_{mm}$ was noted. There was however, a statistically significant increase **(p = 0.0181)*** of **Mean Rt. CIMT $_{mm}$** measurement from normal glycaemia status (0.92 ± 0.29) $_{mm}$ to DM status (1.11 ± 0.32) $_{mm}$. The levels of **Mean Rt. CIMT $_{mm}$** wall thickening increased from Pre-DM to DM status approaching statistical significance **(p = 0.0981) $^{+}$**. A similar trend was demonstrated in all other CIMT measurements from normal glycaemia to Pre-DM status in the males. Interestingly, the **Mean Lt. CIMT** and **Mean Lt. and Rt. CIMT$_{mm}$** measurements of those with DM status (1.17 ± 0.33; 0.94 ± 0.28) $_{mm}$ demonstrated significantly higher levels of **Mean Lt. CIMT$_{mm}$ (p =0.0230)*** and **combined Mean Lt. and Rt. CIMT $_{mm}$ (p =0.0115)*** as compared to those with normal glycaemia status (0.96 ± 0.31; 0.94 ± 0.28) $_{mm}$. The **thickest Lt, thickest Rt. and thickest Rt. or Lt. CIMT $_{mm}$** measurements showed no statistical differences between normal and Pre-DM subjects, however an approach to significantly thicker **(p = 0.0744) Rt. CIMT $_{mm}$** from normal glycaemia to DM was noted. The thickest **Lt. CIMT$_{mm}$ (p = 0.0222)*** and **thickest Rt. or Lt. CIMT$_{mm}$ (p =0.0259)*** demonstrated statistically higher levels of thickening in DM subjects than those with normal glycaemia status in the male group. A mean CIMT of ≤0.8mm was used as a normal reference value (Refer to Table 4.2a and Figure 4.2a).

Table 4.2 a: Inter-relationship of CIMT ultrasound measurements and glycaemia status in male study population (N = 103/372):

Data represented as mean ± standard deviation (SD), unless stated otherwise. Statistical significance when P-Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000) +**, Not significant (^{ns}). Mann-Whitney *U*-test.

CIMT (mm) MALES (N = 103)	Normal (n = 63) Mean ± SD A	Pre-DM (n = 14) Mean ± SD B	DM (n = 26) Mean ± SD C	P-Value A&B	P-Value A&C	P-Value B&C
Mean Rt. CIMT	0.92 ± 0.29	0.90 ± 0.29	1.11 ± 0.32	1.0000	0.0181*	0.0981
Mean Lt. CIMT	0.96 ± 0.31	1.07 ± 0.41	1.17 ± 0.33	0.8236	0.0230*	1.0000
Mean Rt. & Lt. CIMT	0.94 ± 0.28	0.98 ± 0.32	0.94 ± 0.28	1.0000	0.0115*	0.3205
Thickest Rt. CIMT	0.96 ± 0.31	1.04 ± 0.59	1.15 ± 0.37	1.0000	0.0744	1.0000
Thickest Lt. CIMT	1.00 ± 0.31	1.11 ± 0.40	1.22 ± 0.33	0.7940	0.0222*	1.0000
Thickest Rt. or Lt. CIMT	1.01 ± 0.33	1.10 ± 0.42	1.22 ± 0.32	1.0000	0.0259*	0.8610



Abbreviations: DM = Diabetes Mellitus; **Pre-DM** = Pre-Diabetes Mellitus; **Pre-DM + DM** = Hyperglycaemia; **Rt.** = Right, **Lt.** = Left, **CIMT** = Carotid Intima Media Thickness, **CCA** = Common Carotid Artery, **mm** = millimetres, **0,8mm** (Red line) = Normal CIMT measurement, **N** = Sample size, **n** = sample group

Figure 4 . 2a: Mean values of CIMT $_{(mm)}$ and its comparison with glycaemia status (Normal, Pre-DM and DM) in Male

4.3.2 Inter-relationship of CIMT ultrasound measurements and glycaemia status in female population (n= 269)

The maximum thickness and mean CIMT measurements of the female subjects were calculated and analysed using the same CIMT protocol as done for the males. The CIMT (**Mean Rt. CIMT** $_{mm}$; **Mean Lt. CIMT**$_{mm}$ **and Mean Lt. and Rt. CIMT**$_{mm}$**)** measurement from normal glycaemia status to Pre-DM demonstrated no significant CIMT differences in mean values. However, a statistically significant **(p = 0.0102)*** increase in **Mean Lt. CIMT**$_{mm}$ measurement **(0.83 ± 0.27; 0.97 ± 0.28)** $_{mm}$ from **Pre-DM** to **DM** was noted respectively. An increased measurement that approached statistical significance **(p = 0.0627)** in **Mean Lt. and Rt. CIMT**$_{mm}$ measurement **(0.85 ± 0.28; 0.96 ± 0.25)** $_{mm}$ from **Pre-DM** to **DM** was also identified respectively. The **thickest Lt.** (p = 0.5439) ^{ns} **and thickest Rt. or Lt. CIMT** $_{mm}$ (p = 0.2485) ^{ns} measurements showed no statistical differences in normal and Pre-DM subjects, however a statistically significant increase **(p = 0.0267)*** in the **thickest Rt. CIMT** $_{mm}$ measurement from normal glycaemia subjects (0.80 ± 0.22) $_{mm}$ to Pre-DM subjects (0.98 ± 0.25) $_{mm}$ was noted. The thickest **Lt. CIMT** $_{mm}$ **(p =0.0159)*** and **thickest Rt. or Lt. CIMT**$_{mm}$ **(p = 0.0496)*** respectively, demonstrated statistically higher levels of thickening from **Pre-DM** (0.88 ± 0.28; 0.91 ± 0.28) $_{mm}$ to **DM** (1.02 ± 0.30; 1.02 ± 0.27) $_{mm}$.

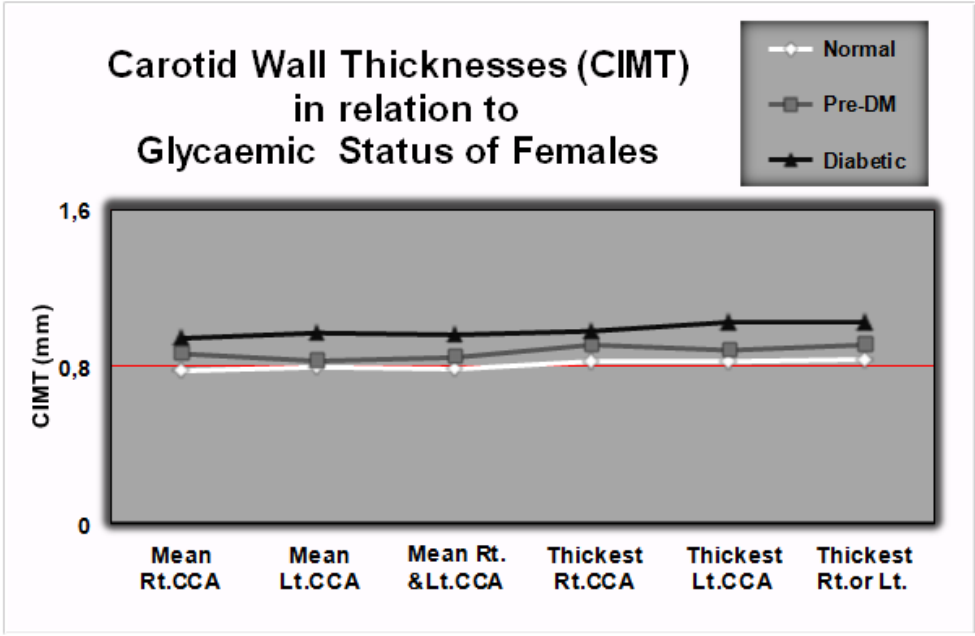
High statistically significant increase **(p < 0.0001) ****** of CIMT measurements was noted from normal glycaemia female subjects **(p < 0.0001) ****** to DM female subjects **(p < 0.0001) ******** for all CIMT categories respectively: **Mean Rt. CIMT**$_{mm}$ **(**0.77 ± 0.22; 0.94 ± 0.25)**; Mean Lt. CIMT**$_{mm}$ (0.79 ± 0.22; 0.97 ± 0.28)**; Mean Lt. and Rt. CIMT**$_{mm}$ (0.78 ± 0.21; 0.96 ± 0.25) **; thickest Rt. CIMT**$_{mm}$ (0.80 ± 0.22; 0.98 ± 0.25)**; thickest Lt. CIMT**$_{mm}$ (0.82 ± 0.23; 1.02 ± 0.30)**; thickest Rt. or Lt. CIMT**$_{mm}$ (0.83 ± 0.22; 1.02 ± 0.27) (Refer to Table 4.2b and Figure 4.2b)

The above results so far portrays a trend that subjects with T2DM, have a highly statistically significant increase of carotid intima media thicknesses **(p < 0.0001)**** than subjects without DM, for all CIMT categories of the selected Bellville South mixed ancestry population ≥ 35 years of age.

Table 4.2 b: Inter-relationship of CIMT ultrasound measurements and glycaemia status in the female study population (N = 269/372):

Data represented as mean ± standard deviation (SD), unless stated otherwise. Statistical significance when P-Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000)** [+], Not significant ([ns]). Mann-Whitney *U*-test.

CIMT (mm) FEMALE (N = 269)	Normal (n = 144) Mean ± SD A	Pre-DM (n = 41) Mean ± SD B	DM (n = 84) Mean ± SD C	P-Value A&B	P-Value A&C	P-Value B&C
Mean Rt. CIMT	0.77 ± 0.22	0.87 ± 0.30	0.94 ± 0.25	0.0670	< 0.0001**	0.4551
Mean Lt. CIMT	0.79 ± 0.22	0.83 ± 0.27	0.97 ± 0.28	0.8298	< 0.0001**	0.0102*
Mean Rt. & Lt. CIMT	0.78 ± 0.21	0.85 ± 0.28	0.96 ± 0.25	0.2281	< 0.0001**	0.0627
Thickest Rt. CIMT	0.80 ± 0.22	0.91 ± 0.31	0.98 ± 0.25	0.0267*	< 0.0001**	0.3861
Thickest Lt. CIMT	0.82 ± 0.23	0.88 ± 0.28	1.02 ± 0.30	0.5439	< 0.0001**	0.0159*
Thickest Rt. or Lt. CIMT	0.83 ± 0.22	0.91 ± 0.28	1.02 ± 0.27	0.2485	< 0.0001**	0.0496*



Abbreviations: **DM** = Diabetes Mellitus; **Pre-DM** = Pre-Diabetes Mellitus; **Pre-DM + DM** = Hyperglycaemia; **Rt.** = Right, **Lt.** = Left, **CIMT** = Carotid Intima Media Thickness, **CCA** = Common Carotid Artery, **mm** = millimetres, **0,8mm** (Red line) = Normal CIMT measurement, **N** = Sample size, **n** = sample group

Figure 4 . 2b: Mean values of CIMT [(mm)] and its comparison with glycaemia status (Normal, Pre-DM and DM) in females

4.4. Descriptive statistics of ultrasound plaque and stenosis in association with normal and hyperglycaemic status (N = 375)

Figures 4.3 and 4.3b are diagrammatic representations of plaque and stenosis in the normal and hyperglycaemic population. All those with plaques (>1_{mm}^2 or >1_{mm}^3) regarded as some form of stenosis. Of the 375 mixed ancestry population (N=375), 44% (165/375) were diagnosed with hyperglycaemia and 56% (210/375) as normal. Ultrasound detected 33% (124/375) of plaque presence demonstrating some form of ICA stenosis in the total population. All those identified with plaques were also identified as having an ICA stenosis <50%. None of the subjects presented with a ≥50% ICA stenosis. Further grouping of the population, indicated 67% (251/375) without plaques. Of those that did not have plaques, 65% (162/251) were diagnosed with normal glycaemia and 35% (89/251) were hyperglycaemic. In the group (n=124), that presented with plaques and some form of ICA stenosis, 61% (76/124) were hyperglycaemic, while 39% (48/124) were not.

The above results proved that subjects with hyperglycaemia (76/124) have a 1.6 times higher (76/48) occurrence of plaque and ICA stenosis than those subjects without hyperglycaemia (48/124) in the selected Bellville South mixed ancestry population ≥ 35 years of age.

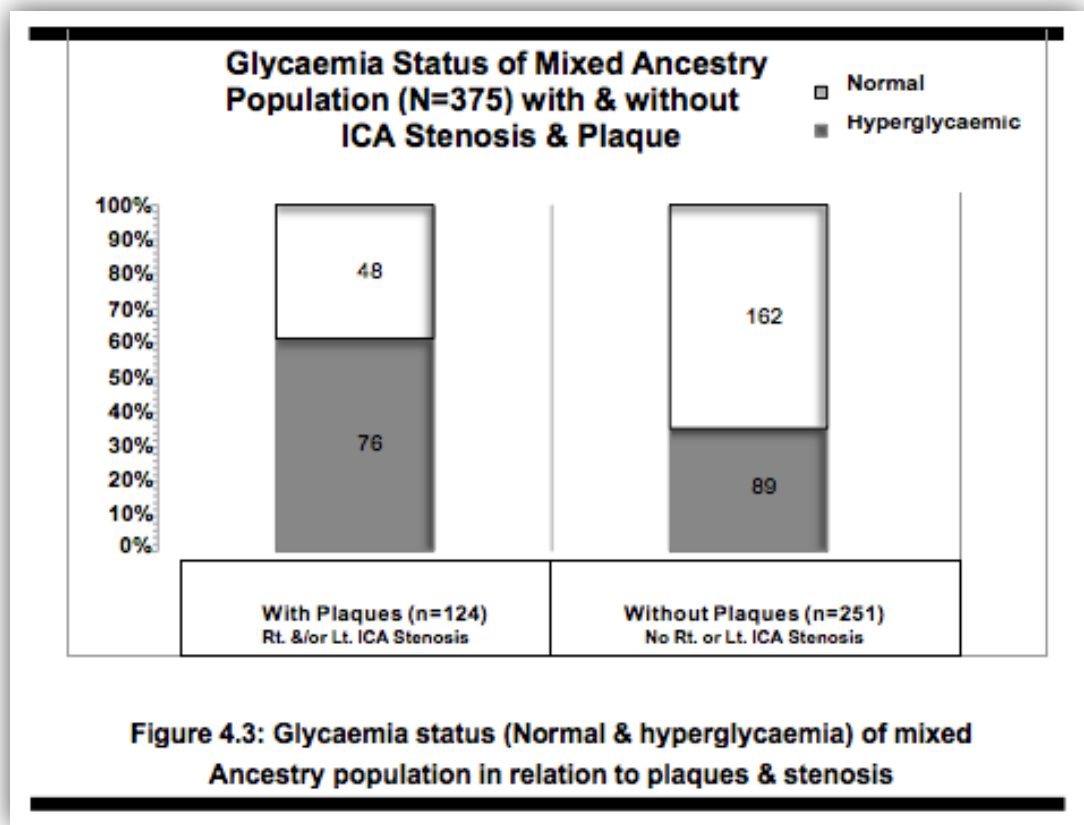


Figure 4.3: Glycaemia status (Normal & hyperglycaemia) of mixed Ancestry population in relation to plaques & stenosis

4.5: Association of ultrasound with contributing risk factors

The results of the 5th objective established the association between the ultrasound findings (**Mean Lt. and Rt. CIMT, Plaques and ICA stenosis**) and contributing atherosclerosis risk factors of **age**; anthropometric measurements in relation to obesity (**WC**, **HP** ratio and **BMI**); hypertension (**SBP** and **DBP**); Inflammatory markers **(hs-CRP)**; smoking (**S-Cotinine**); increased blood cholesterol levels and lipid profiles (**HDL, LDL, TC and TGC**); glycaemia status (**HbA1C and FBG**) and gender of the mixed ancestry study population.

4.5.1 Statistical analysis of contributing risk factors between males and females in the selected study population (N = 377) (Table 4.3)

The mean (± SD) **age$_{(yrs.)}$** of the study population was 57.2$_{yrs.}$ (± 12.3) for males, and 55.8$_{yrs.}$ (± 12.0) for females, hence no statistical age differences (p = 0.2870)^{ns} noted between genders. In terms of obesity, the body mass index $_{(Kg/m^2)}$ was significantly higher in females than in males **(p < 0.001) ****, The **BMI** mean was 27.76$_{Kg/m^2}$ (± 7.09) in males and 32.50$_{Kg/m^2}$ (± 8.25) in females. The adiposity around the waist by tape measure, showed a mean waist circumference **(WC $_{cm}$)** of 95$_{cm}$ (± 14.92) in males and 96$_{cm}$ (± 13.86) in the females, indicating no statistical waist circumference differences (p = 0.4827) ^{ns} between genders. There also appeared to be no statistical blood pressure differences (p = 0.3046) ^{ns} between genders. The mean systolic blood pressure **(SBP $_{mmHg}$)** was 139.96 $_{mmHg}$ (± 24.28) in males and 139.17 $_{mmHg}$ (± 27.74) in females (p = 0.3046) ^{ns}. The mean diastolic blood pressure **(DBP $_{mmHg}$)** in males was 85.72$_{mmHg}$ (± 12.67), and 84.57 $_{mmHg}$ (± 14.62) in females (p = 0.3046) ^{ns}. Inflammatory marker **hs-CRP $_{mg/L}$** levels 7.39 $_{mg/L}$ (± 11.62) in the males were significantly lower **(p = 0.0499)*** than in the females: 8.88 $_{mg/L}$ (± 16.92). The liver function/infection/alcohol/toxic exposure **(GGT $_{(IU/L)}$)** showed a statistically significant increase **(p = 0.0004)*** in concentration levels in the males: 47.10 $_{IU/L}$ (± 45.26) as compared to the females: 39.32 $_{IU/L}$ (± 44.9)**.** The **S-Cotinine** $_{ng/ml}$ levels of males: 138.19 $_{ng/ml}$ ± (176.56) compared to females 127.55 $_{ng/ml}$ (±178.82) showed no statistically significant (p = 0.2601) ^{ns} differences in smoking or tobacco (Nicotine) exposure (smoking status). The blood cholesterol **LDL** $_{mmol/L}$ (p = 0.5234) ^{ns}**, TC** $_{mmol/L}$ (p = 0.3301) ^{ns} and triglyceride **(TGC** $_{mmol/L}$, p = 0.1780) ^{ns} concentration of males were similar to that of females. There was however a significantly **(p = 0.0042)*** lower concentration of the mean **HDL** cholesterol concentration level in males (1.32 ± 0.49) $_{mmol/L}$ as compared to the females (1.42 ± 0.43) $_{mmol/L}$. The blood glucose average of 6.28% (± 1.49) of total haemoglobin **HbA1c$_{\%}$ (p = 0.0006)*** and 6.10 $_{mmol/L}$ (± 2.43) fasting blood glucose concentrations **FBG** $_{mmol/L}$ **(p = 0.0003) **** was significantly lower in the males as compared to the females**:** 6.62 % (± 1.64) and 6.48 $_{mmol/L}$ (± 3.09) respectively (Table 4.3

Table 4.3: Contributing risk factors according to gender

Data represented as mean ± standard deviation (SD), unless stated otherwise. Statistical significance when P-Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000)** [+], Not significant ([ns]). Man Whitney U test.

SAMPLED (N = 377) (Un-Blinded)		Male (n = 105) (Mean ± SD)	Female (n = 272) (Mean ± SD)	P-Value
Age	≥ 35 yrs	57.2 ± 12.3	55.8 ± 12.0	0.2870 [ns]
Anthropometric Measurements	BMI (kg/m2)	27.76 ± 7.09	32.50 ± 8.25	< 0.0001**
	WC (cm)	94.67 ± 14.92	96.12 ± 13.86	0.4827
Blood Pressure	SBP (mmHg)	139.96 ± 24.28	139.17 ± 27.74	0.3046 [ns]
	DBP (mmHg)	85.72 ± 12.67	84.57 ± 14.62	0.2636 [ns]
Inflammatory Marker selected	hs-CRP (mg/L)	7.39 ± 11.62	8.88 ± 16.92	0.0499*
Smoking	S Cotinine (ng/mL)	138.19 ± 176.56	127.55 ± 178.82	0.2601 [ns]
Liver Function test selected,	GGT (IU/L)	47.10 ± 45.26	39.32 ± 44.92	0.0004**
Lipids & Cholesterols	TC (mmol/L)	5.50 ± 1.03	5.64 ± 1.13	0.3301 [ns]
	TG (mmol/L)	1.69 ± 1.13	1.50 ± 0.82	0.1780 [ns]
	HDL (mmol/L)	1.32 ± 0.49	1.42 ± 0.43	0.0042*
	LDL (mmol/L)	3.42 ± 0.96	3.52 ± 1.01	0.5234
Blood glucose tests selected	HbA1c (%)	6.28 ± 1.49	6.62 ± 1.64	0.0006**
	FBG (mmol/L)	6.10 ± 2.43	6.48 ± 3.09	0.0003**

ABBREVIATIONS: BMI = Body Mass Index, **Kg/m²** = Kilogram/meter squared, **WC** = Waist Circumference, **cm** = centimetres, **SBP** = Systolic Blood Pressure, **DBP** = Diastolic Blood Pressure, **mmHg** = millimetres of Mercury, **hs-CRP =** high sensitive C-reactive protein, **mg/L** = milligrams per litre, **GGT** = Gamma-Glutamyl Transpeptidase, **IU/L** = International Units per litre, **S Cotinine** = **ng/ml=** nanograms per millilitre**, mmol/L =** concentration millimole per litre**, TC =** Total Cholesterols**, TG =** Triglycerides**, HDL =** High-Density Lipoprotein**, LDL =** Low-Density Lipoprotein, **HbA1c =** Glycated haemoglobin**, % =** percentage**, FBG =** Fasting Blood Glucose**. N =** Sample size**, n =** sample group

4.5.2 Ultrasound findings and contributing risk factors according to gender

The B-mode ultrasound carotid wall thicknesses **(CIMT $_{mm}$)** bilaterally demonstrated a significant difference in measurement between genders, for all **CIMT** categories (refer to tables 1, 2a, 2b and figures 2a and 2b). At the PSV of the CCA and ICA, significant differences **(p = 0.0474)*** in the **Lt. CCA (**Males: 85.67 ± 18.53 and Females: 81.58 ± 18.70) $_{cm/s}$ was noted. The **mean ICA/CCA ratio** of combined sides demonstrated the most significant differences in velocities and vessel narrowing **(p = 0.0001)*** between genders and was considered when measuring stenosis.

The presence of plaques in the total population was determined, and regarded as a contributory risk factor for stenosis. A total of 124 mixed ancestry subjects presented with plaques >1 mm^2 or 1 mm^3 with some form of narrowing (< 50%). Sixty one percent (76/124) were hyperglycaemic, while only 39% (48/124) were diagnosed with normal glycaemia (refer to 4.4 below). None of the subjects (0%) in this sample, presented with a >50% ICA stenosis. Interestingly, there were 35% (89/251) of the hyperglycaemic subjects that did not have plaques and stenosis, and demonstrated 0.54 times lesser occurrence than the normal glycaemic (162/251) without plaques and stenosis (65%) in the selected Bellville South mixed Ancestry population ≥ 35 years of age.

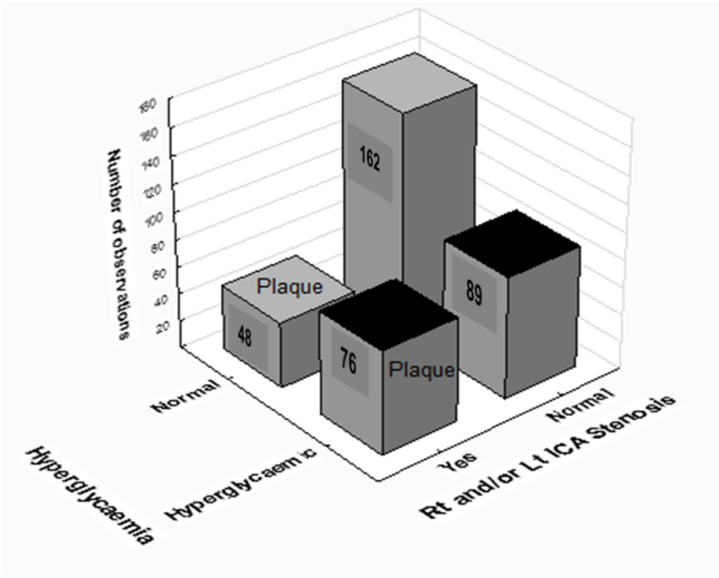


Figure 4.4: Plaques as a contributory risk factor for ICA stenosis in the normal and hyperglycaemic mixed ancestry study population (n = 375)

The average of all 6 CIMT measurements were calculated, and the mean of the Rt. and Lt. CIMT measurement was used as the dependant variable for the univariate multiple regression and correlation analysis to determine the relationship of CIMT ultrasound with other variables as contributory risk factors.

4.5.3 Univariate multiple regression analysis for dependent variable (Mean of Right and Left CIMT) in total study population (Adjusted $R^2 = 0.3247$) (Table 4.4)

The mean **Rt. and Lt. CIMT** ultrasound measurement was used as the dependent variable, and was tested against contributing risk factors for atherosclerosis as described in Table 4.4. The contributing atherosclerosis risk factors for CVD and stroke were determined by age, anthropometric measurements (obesity), blood pressure readings, inflammatory markers, smoking, blood cholesterol levels and lipid profiles, and glycaemia status for the selected mixed ancestry study population ≥35yrs of age.

All those with missing variables (114/377) were excluded. A sub-sample of 248 from 263 was selected in this univariate multiple regression analysis after adjusted R^2 of 0.3247 to include results for all independent variables used in this mixed ancestry population. Independent variables (contributing risk factor markers) of **age** (yrs.), **SBP** (mmHg), **hs-CRP** (mg/L), **S-Cotinine** (ng/mL) and **LDL** (mmol/L) showed statistically significant positive associations with dependent variable **Mean Rt. and Lt. CIMT** (mm), **($p < 0.0001$**, $p < 0.0001$**, $p = 0.0033$*, $p = 0.0409$* and $p = 0.0044$)*** respectively. In contrast, **HP** (cm), **DBP** (mmHg) and **HbA1c** (%) showed statistically significant inverse associations with the dependent variable **Mean Rt. and Lt. CIMT** (mm), **($p = 0.0079$*, $p = 0.0070$* and $p = 0.0466$)*** respectively. No significant association of CIMT ultrasound measurements in **WC** (cm), **GGT** (IU/L), **TG** (mmol/L), **HDL** (mmol/L), **postBG** (mmol/L) and **FBG** (mmol/L) was noted for this sub-sample.

Statistically significant positive differences and standard error (SE), for every unit of change ($1_{yr.}$) of **age** (yrs.), as a contributing factor for atherosclerosis, there was a change of **(0.3028 ± 0.0649) mm** in the **mean Rt. and Lt CIMT** as predicted according to this model (refer to Table 4.4). The similar mathematical formulae of change in CIMT measurements ± SE, would be noted when a unit of change for other contributing risk factors are calculated as follows: **SBP_{mmHg} (0.4002 ± 0.0861) mm; Hs-CRP** $_{mg/L}$ **(0.1570 ± 0.0529)** $_{mm}$**; S-Cotinine** $_{ng/mL}$ **(0.1119 ± 0.0545)** $_{mm}$**;** and **LDL** $_{mmol/L}$ **(0.1600 ± 0.0557) mm.** A negative change of CIMT measurement was predicted for every unit of change in **HP** $_{cm}$ **(-0.2259 ± 0.0844)** $_{mm}$**; DBP** $_{mmHg}$ **(-0.2320 ± 0.0854)** $_{mm}$**;** and **HbA1c % (-0.1692 ± 0.0846)**

mm. The total contribution of independent variables (contributing risk factor markers) to CIMT ultrasound measurements was calculated as 34.5% (Adjusted R^2 = 0.3247) refer to (Table 4.4). The combined results for carotid intima media thickening, plaques and stenosis demonstrate that the glycaemic status and contributory atherosclerotic risk factors mentioned above, are either directly or indirectly related to the ultrasound findings. A stepwise regression analysis was done to demonstrate the highest associated risk factors, to the CIMT.

4.5.4 Multivariate stepwise regression analysis of contributing risk factors with mean Rt. and Lt. CIMT ultrasound measurement (Table 5)

Table 5 demonstrates the relationship of all the contributing risk factors used in table 4 which were significantly associated with the mean Rt. and Lt. CIMT ultrasound measurement, as a stepwise regression, from the highest association to the lowest. This was calculated by computing all the variables (Contributing risk factor readings) in the first table and then in the 2nd table, a stepwise regression was done, and the computer chose the highest associated risk factors (refer to Table 4.5). In the multivariate analysis: independent variables used in the regression model demonstrated that **age** yrs. **(p < 0.0001) **** and **SBP** mmHg **(p < 0.0001)****, showed the strongest significantly positive associations with changes in mean **Rt. and Lt. CIMT** measurements by **0.2785 ± 0.0625** mm and **0.4314 ± 0.0847** mm respectively.

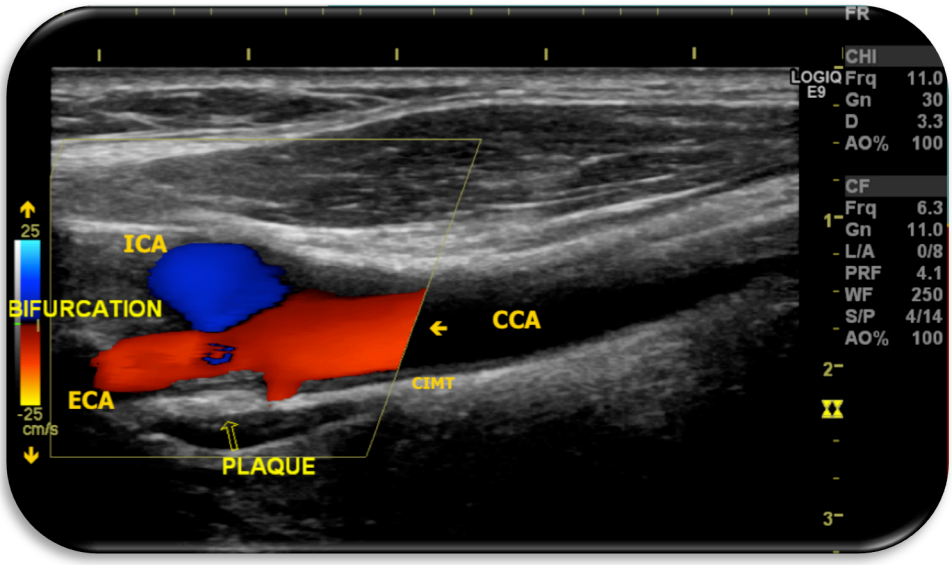


Figure 4.4 B: Original image of subtle plaque in the carotid bulb using colour Doppler mode.

Table 4.4: Univariate multiple regression analysis for dependent variable (Mean of Right and Left CIMT) in total study population (Adjusted R^2 = 0.3247)

Data represented as b* (per unit change of independent variable with dependant variable Lt. and Rt. CIMT) and ± standard error/ SE (Std.Err -b*), unless stated otherwise. Statistical significance when P- Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000) +**, Not significant (^{ns}).

Independent Variables (Contributing Risk Factors)		b*	Std.Err. - of b*	b	Std.Err. - of b	t(248)	P-Value
Intercept				0.6030	0.2283	2.6411	0.0088
Age	Age (yrs)	0.3028	0.0649	0.0067	0.0014	4.6687	< 0.0001**
Anthropometric Measurements	WC (cm)	0.1307	0.0885	0.0023	0.0016	1.4776	0.1408
	HP (cm)	-0.2259	0.0844	-0.0045	0.0017	-2.6763	0.0079*
Blood Pressure	SBP (mmHg)	0.4002	0.0861	0.0040	0.0009	4.6473	< 0.0001*
	DBP (mmHg)	-0.2320	0.0854	-0.0042	0.0015	-2.7171	0.0070*
Inflammatory marker selected	hs-CRP (mg/L)	0.1570	0.0529	0.0040	0.0014	2.9683	0.0033*
Liver Function test selected	GGT (IU/L)	-0.0110	0.0539	-0.0001	0.0003	-0.2033	0.8391
Smoking	S-Cotinine (ng/mL)	0.1119	0.0545	0.0002	0.0001	2.0554	0.0409*
Cholesterols	TG mmol/L	0.0518	0.0594	0.0155	0.0178	0.8714	0.3844
	HDL mmol/L	0.0204	0.0622	0.0111	0.0338	0.3285	0.7428
	LDL mmol/L	0.1600	0.0557	0.0415	0.0144	2.8731	0.0044
Blood glucose	HbA1c (%)	-0.1692	0.0846	-0.0696	0.0348	-2.0000	0.0466
	PostBG (mmol/L)	0.1157	0.0776	0.0104	0.0070	1.4920	0.1370
	FBG (mmol/L)	0.0557	0.0767	0.0110	0.0151	0.7264	0.4683

ABBREVIATIONS: WC = Waist Circumference, **cm** = centimetres, **SBP** = Systolic Blood Pressure, **DBP** = Diastolic Blood Pressure, **mmHg** = millimetres of Mercury, **hs-CRP =** high sensitive C-reactive protein, **mg/L** = milligrams per litre, **GGT** = Gamma-Glutamyl Transpeptidase, **IU/L** = International Units per litre, **S-Cotinine = ng/ml** = nanograms per millilitre, **mmol/L** = concentration millimole per litre, **TC** = Total Cholesterols, **TG** = Triglycerides, **HDL** = High-Density Lipoprotein, **LDL** = Low-Density Lipoprotein, **HbA1c**=Glycated haemoglobin, **%** = percentage, **HP** = Hip circumference, **FBG** = Fasting Blood Glucose, **PostBG** = Post Blood Glucose.

The **hs-CRP** (mg/L) inflammatory markers **(p = 0.0014)*** and **LDL** (mmol/L) **(p = 0.0208)*** were the 2nd and 3rd highest positive associated contributory risk factors for CIMT increase of **0.0166 ± 0.0515** mm and **0.1193 ± 0.0513** mm respectively. In contrast, **HP** (cm) **(p = 0.0008)*** and **DBP** (mmHg) **(p = 0.0055)*** showed statistically significant inverse associations in **Mean Rt. and Lt. CIMT** (mm) measurement changes **-0.02699 ± 0.0798** mm and **-0.2356 ± 0.0842** mm respectively. The **WC** cm **(p = 0.0555)**+ risk factor of obesity approached significance with the predicted increase of **mean Rt. and Lt. CIMT** difference of **0.1541 ± 0.0841** mm (Refer to table 4.5).

Table 4.5: Stepwise multivariate regression of contributing risk factors (N = 263)

Data represented as b* (per unit change of independent variable with dependant variable Lt. and Rt. CIMT) and ± standard error/SE (Std.Err -b*), unless stated otherwise. Statistical significance when P- Value **(p < 0.0500)***, **(p < 0.001) ****, and approaching significance **(p < 0.1000)** +, Not significant (ns).

Independent Variables (Contributing Risk Factors)	b*	Std.Err. - of b*	b	Std.Err. - of b	t(248)	P-Value
Intercept			0.4593	0.1610	2.8527	0.0047 *
Age (yr)	0.2785	0.0625	0.0062	0.0014	4.4563	< 0.0001 **
SBP (mmHg)	0.4314	0.0847	0.0043	0.0008	5.0930	< 0.0001 **
hs-CRP (mg/L)	0.1666	0.0515	0.0043	0.0013	3.2329	0.0014 *
HP (cm)	-0.2699	0.0798	-0.0053	0.0016	-3.3800	0.0008 *
DBP (mmHg)	-0.2356	0.0842	-0.0042	0.0015	-2.7980	0.0055 *
LDL (mmol/L)	0.1193	0.0513	0.0305	0.0131	2.3251	0.0208*
WC (cm)	0.1541	0.0801	0.0027	0.0014	1.9239	0.0555+

ABBREVIATIONS: WC = Waist Circumference, **HP** = Hip Circumference, **cm** = centimetres, **SBP** = Systolic Blood Pressure, **DBP** = Diastolic Blood Pressure, **mmHg** = millimetres of Mercury, **hs-CRP** = high sensitive C-reactive protein, **mg/L** = milligrams per litre, **mmol/L** = concentration millimole per litre, **LDL** = Low-Density Lipoproteins.

4.6: Summary of significant findings.

Gender comparisons regarding flow velocities of common and internal carotid arteries, showed the peak systolic velocities (PSV) of the Lt. CCA in males to be significantly higher **(p =**

0.0474)* than the females. There was also an approach to significantly lower **(p = 0.0864)** [+] Rt. and Lt. ICA end diastolic velocities (EDV) in men than in women. For all CIMT categories concerning maximum carotid wall thickness, a highly statistically significant increase in the males as compared to the females was demonstrated (Thickest Rt. CIMT $_{mm}$ **(p = 0.0003)***, Thickest Lt. CIMT $_{mm}$ **(p < 0.0001)****, and Thickest Rt. or Lt. CIMT $_{mm}$ **(p < 0.0001)****). The ICA/CCA ratio was used in combination with plaques to determine the percentage of stenosis, by dividing the PSV of ICA over PSV of CCA. Although there were no stenotic ratios ≥2 , the women showed significantly narrower carotid vessels than the men (Rt. ICA/CCA Ratio **(p = 0.0019)***; Lt. ICA/CCA Ratio **(p = 0.0022)*** and Mean Rt. and Lt. ICA/CCA Ratio **(p = 0.0001)***). The predisposing factors that showed significant differences across genders were also highlighted. In terms of obesity, the body mass index $_{(Kg/m^2)}$ was significantly higher in women than in men **(p < 0.001). ****** Inflammatory markers, hs-CRP $_{mg/L}$ levels in the males were significantly lower **(p = 0.0499)*** than in the females. The liver function/infection/alcohol/toxic exposure (GGT $_{(IU/L)}$) was higher **(p = 0.0004)*** in concentration in the males, as compared to the females. There was also a significantly **(p = 0.0042)*** lower concentration of the mean HDL cholesterol levels in men, however the blood glucose average of the total haemoglobin HbA1c$_{\%}$ **(p = 0.0006)*** and fasting blood glucose concentrations FBG $_{mmol/L}$ **(p = 0.0003) ****** in women were significantly higher.

Ultrasound carotid wall thicknesses (CIMT) measurement distribution among the glycaemic groups (Normal glycaemia, Pre-DM and DM) demonstrated a statistically significant increase **(p = 0.0181)*** of Mean Rt. CIMT $_{mm}$ measurement from normal glycaemia status to DM status in the males. There were higher levels of Mean Rt. CIMT $_{mm}$ wall thickening, that approached statistical significance **(p = 0.0981)**[+] from Pre-DM to DM. A similar trend was observed in all other CIMT measurements from normal glycaemia to Pre-DM status in the males. In the females however, a statistically significant **(p = 0.0102)*** increase in Mean Lt. CIMT$_{mm}$ measurement and an increased measurement that approached statistical significance **(p = 0.0627)** in Mean Lt. and Rt. CIMT$_{mm}$ from Pre-DM to DM, was evident in women. The Mean Lt. **(p =0.0230)*** and the combined Mean Lt. and Rt. CIMT$_{mm}$ **(p =0.0115)*** measurements of those with DM status, revealed significantly higher levels of thickening than normal for the men. For maximum carotid wall thickening among the females, a statistically significant increase **(p = 0.0267)*** in the thickest Rt. CIMT $_{mm}$ measurement from normal glycaemia status to Pre-DM was also revealed. The thickest Lt. CIMT $_{mm}$ **(p =0.0159)**[*] and thickest Rt. or Lt. CIMT$_{mm}$ **(p = 0.0496)***, demonstrated statistically higher levels of thickening from Pre-DM to DM among the women, but this was not so for the men. Therefore, regarding the change of carotid wall thickening from normal glycaemic levels to DM in both the males and females, showed a high statistically significant increase **(p < 0.0001) ****** for all CIMT measurements in this category. In terms of plaques and ICA stenosis (124 out of 375), when grouped into normal glycaemic and hyperglycaemic groups, showed a plaque

presence and <50% stenosis prevalence of 61% (76/124) in the hyperglycaemia group, that was 1.6 times higher than those without hyperglycaemia (48/124). The majority of those without plaques and any form of stenosis, were those that were not hyperglycaemic (65%: 162/251).

The univariate multiple regression analysis after adjusted R^2 of 0.3247 for all independent variables (predisposing /contributing risk factor markers) of age $_{(yrs.)}$, SBP $_{(mmHg)}$, hs-CRP $_{(mg/L)}$, S-Cotinine $_{(ng/mL)}$ and LDL $_{(mmol/L)}$ showed statistically significant positive associations with dependent variable Mean Rt. and Lt. CIMT $_{(mm)}$, **(p < 0.0001**, p< 0.0001**, p = 0.0033*, p = 0.0409* and p = 0.0044)*** respectively. In contrast, HP $_{(cm)}$, DBP $_{(mmHg)}$ and HbA1c $_{(\%)}$ showed statistically significant inverse associations with the dependent variable Mean Rt. and Lt. CIMT $_{(mm)}$, **(p = 0.0079*, p = 0.0070* and p = 0.0466)*** respectively. Statistically significant positive differences and standard error (SE), for every unit of change ($1_{yr.}$) of age $_{(yrs.)}$, as a contributing factor for atherosclerosis, there was a change in the mean Rt. and Lt CIMT as predicted according to this model. A negative change of CIMT measurement was predicted for every unit of change in HP $_{cm}$; DBP $_{mmHg}$ and HbA1c % **(-0.1692 ± 0.0846)** $_{mm}$. The total contribution of independent variables (contributing risk factor markers) to CIMT ultrasound measurements was calculated as 34.5% (Adjusted R^2 = 0.3247).

In the multivariate stepwise regression analysis, the independent variables of age **(p < 0.0001)**** and systolic blood pressure **p < 0.0001)**** showed the strongest positive association with Rt. and Lt. CIMT measurement changes. The hs-CRP **$_{(mg/L)}$** inflammatory markers **(p = 0.0014)*** and LDL **$_{(mmol/L)}$** **(p = 0.0208)*** were the 2nd and 3rd highest positive associated contributory risk factors for carotid artery wall thickening. In contrast, HP **$_{(cm)}$** **(p = 0.0008)*** and DBP **$_{(mmHg)}$** **(p = 0.0055)*** showed statistically significant inverse associations in Mean Rt. and Lt. CIMT **$_{(mm)}$** measurement changes respectively. The WC **$_{cm}$** **(p = 0.0555)$^{+}$** risk factor related to obesity approached significance, with the predicted increase of carotid artery wall thickening. The above results from highest to lowest significance can therefore be summarised as follows: Age and systolic blood pressure, inflammatory and LDL cholesterol changes, and central waist circumference adiposity, were positively associated with the measurement increase of the carotid intima media thickness (mean Rt. and Lt. CIMT). The hip circumference adiposity and diastolic blood pressure measurements showed a negative change in association with the right and left carotid intima media thickening. These relationships will be further discussed within context, in the following chapter.

CHAPTER 5

DISCUSSION

CHAPTER FIVE

5.0 DISCUSSION

A stastically significant increase in CIMT, plaques and stenosis in the T2DM and hyperglycaemic groups, as compared to the non-diabetic population was noted; thus proving the carotid ultrasound study hypothesis to be true.

The carotid artery wall thickness measured by B-Mode ultrasound using the CIMT criteria, showed statistically significant thickening in both the males and females that were diabetic, 29.7% (110/372), as compared to those 55.6% (207/372) that were not diabetic (as shown in tables 4.2a, b and figures 4.3a, b).

It is also emphasised that greater than normal levels of intima-media thickening is a surrogate marker for atherosclerosis (Ito et al., 2010; Holland et al., 2009). The gender comparison, as seen throughout this study, demonstrated a significantly higher **($p < 0.0001$) **** mean CIMT measurement in the males as compared to the females for all CIMT categories (as illustrated in Table 4.1). This complies with literature that men are more at risk of atherosclerosis than women, and whether this is due to anatomical, biophysical and/or hormonal variation between genders, is debateable (Schulz and Rothwell, 2001). The women showed to have narrower ICA vessels, demonstrated by significantly higher **($p = 0.0001$)*** bilateral peak systolic velocities and mean (± SD) ICA/CCA ratio of 0.88 (± 0.19) as compared to the males 0.82 (± 0.27). Severe narrowing or stenosis was established if ICA/CCA ratio is >2.0 (refer to Table 4.1). It was pleasing to note that none of the participants of this cohort had severe ICA stenosis. Vascular remodelling and blood pressure readings were considered for this population, in order to establish whether hypertension related vasoconstriction be the cause. Further observations of the left and the right sides of the extra cranial circulation were also not symmetrical for all participants, and some of the carotid arteries had different origins.

Although the mean (± SD) for Rt. and Lt. CIMT $_{mm}$ was 0.99 (± 0.30) $_{mm}$, for males, and 0.85 (± 0.24) $_{mm}$ for females, both these averages were interestingly plotted above the normal CIMT range >0.80$_{mm}$. As emphasised elsewhere that higher levels of carotid intima-media thickening than normal, is a surrogate marker for atherosclerosis, and a road to vascular complications, flags this mixed ancestry population ≥35$_{yrs.}$ of age, as high-risk (as reflected in Table 4.1). Although a normal value of ≤0.80$_{mm}$ is relevant globaly, one has to also consider other factors specific to a particular sample, and in this context ethnicity and an

aging population. An analogy of grey hair being normal for old age, the same may apply for vessel wall thickening regarding atherosclerosis as part of the normal aging process. Distinguishing between vessel wall hardening and swelling may be of added value pertaining to inflammation. Therefore it was important when drawing conclusions related to the carotid intima-media wall thickness measurement, that it should not be seen in isolation, but rather in combination, with age, gender, glycaemia status, ethnicity, inflammation and other contributory risk factors.

The mean (±SD) for age in this study population was 57.2 (± 12.3) $_{yrs.}$ in males and 55.8 (± 12.0) $_{yrs.}$ in the females, indicating an elderly (aging) sample population, with similar age differences between genders (as noted in table 4 . 3). Independent of the diabetic status, the age related risks for atherosclerosis resulted, in the regression analysis, as the strongest dependent variable **(p < 0.0001)**** (R^2 of 0.3247), positively associated with carotid intima-media thickening in this aging population. The initial manifestation of carotid atherosclerosis is said to be characterised by a subtle increase in vascular intima-media thickening, the progression of which leads to plaque formation and vascular narrowing. The Bellville South African carotid ultrasound study population was therefore regarded as an 'elderly', high risk T2DM population, with an average mean (±SD) age of 56.5 (± 12.15) $_{yrs.}$.

In relation to plaques and stenosis, the ultrasound findings proved useful, in identifying the population group that were highest and lowest at risk of developing cerebrovascular complications. This finding was dependent on the presence of plaques/stenosis, when distributed into normal and hyperglycaemic population groups (as noted in figures 4.3 and 4.4). The results of this carotid study further justifies that ultrasound imaging adds value to early detection of carotid atherosclerotic disease, due to its ability to quantify plaques formed, and estimate the degree of carotid stenosis present. The technique enabled determination of the severity of carotid stenosis by evaluating the extent of atherosclerotic changes and echo patterns of plaques that were within the vessel, using real-time B-mode ultrasound coupled with colour and spectral Doppler-imaging techniques (Duplex ultrasound or DUS). In general, DUS is reported as reliable, for delineating atherosclerotic plaques with or without calcification (Alexandrov et al., 2011; Kisten et al., 2013). The findings of this study highlighted how useful carotid US imaging was, in determining the severity of obstruction, the intima and media thickness, and the anatomical site of carotid plaques and/or atherosclerotic involvement, and is supported by these study findings (as demonstrated in previous chapter).

The carotid plaques >1mm^3 and 1mm^2 were regarded as some form of narrowing (<50%).

None in this sample had a >70% stenosis. Of the 44% (165/375) with hyperglycaemia, and the 56% (210/375) with normal glycaemia, 33% (124/375) of the combined population were ultrasound diagnosed with the presence of plaques and some form of stenosis <50%. Further grouping of this population, reflected 67% (251/375) without plaques. Of those that did not have plaques, 65% (162/251) were diagnosed with normal glycaemia and 35% (89/251) were hyperglycaemic. In the group (n=124) that presented with plaques and some form of ICA stenosis, 61% (76/124) were hyperglycaemic, while 39% (48/124) were not. Interestingly, the 35% (89/251) of the hyperglycaemic subjects that did not have plaques and stenoses, demonstrated 0.54 times lesser level than those without hyperglycaemia (162/251: 65%). These observations suggest that those with normal glycaemia, no stenosis and plaques could be grouped as individuals with the lowest stroke risk, as compared to those hyperglycaemic with plaques and stenosis, that where the highest at risk. This may also suggests that 'healthy' hyperglycaemic without plaques are not necessarily higher at risk of developing a stroke than 'unhealthy' normal glycaemics with plaques. 'Pre-DM' again 'rings a bell' as a sample that can perhaps prevent the transition to T2DM. Therefore, physical activity and lifestyle changes for this population are propogated. The following further discusses the relationship of ultrasound and contributory risk factors on the development of carotid atherosclerosis.

Factors of obesity; physical inactivity; hypertension; smoking and lifestyle related risks are supported by the ultrasound findings, demographics and laboratory analysis of serum for this selected population. Females, as noted in this carotid study, demonstrated a substantially greater adiposity than the males, and therefore these whole body fat compositions by gender differences are complemented by major variation in tissue distribution. Obesity as a contributing risk factor was explored in this carotid ultrasound study, and by recordings of the anthropometric (weight, height, waist and hip circumference measurements), together with visual observations, showed that the males in general appeared to have had a greater arm muscle mass, possibily larger and stronger bones, less limb fat and a relatively greater central distribution of fat. Females however, demonstrated more peripheral distribution of fat that could have developed in early adulthood, is an assumption that may be interesting for future studies regarding brown fat composition. Derby et al. (2006) explains that gender differences in body composition are primarily attributable to the action of sex steroid hormones, which drive the dimorphisms during pubertal development. In men, a reduction in free testosterone levels is said to be associated with an increase in fat mass and reduction in muscle mass, and both total and free testosterone levels are inversely associated with obesity (Derby et al., 2006). Efforts to better characterise the disease risk associated with obesity suggest that the accompanying metabolic abnormalities are not uniform in all obese persons. 'Indeed, there are

substantial variations in the clustering of CVD risk factors among individuals with similar BMI, and accumulating evidence are suggesting that CVD risk associated with obesity may depend essentially on co-morbid cardiometabolic abnormalities' reports Matsha et al., (2012). The BSA study group also reports in the above publication that the concepts of 'metabolically healthy-' and 'metabolically abnormal-' normal- weight, overweight and obese have been used to characterise the six phenotypes resulting from the cross classification of individuals according to the BMI and metabolic status (Wildman et al., 2008). The obesity phenotypes and subclinial ultrasound findings of the heart and carotid vessels in the Bellville South mixed ancestary population identified above, provided the larger study with some of the following important observations.

The anthropometric measurements of this carotid study population revealed an average BMI mean (±SD) of 30.13 (± 7.67) Kg/m^2 and a waist circumference of 95.40 (± 14.39) cm. According to the WHO (2008) and the International Diabetes Federation (2006), A BMI ≥30 Kg/m^2 is generally regarded as obese, while a WC < 80 cm for females and < 94 cm for males is regarded as normal. Ethnicity is also a factor that was included when calculating normal reference ranges for this specific population. The average mean BMI and WC measurements therefore revealed an obese mixed ancestry population cohort for the entire sample (N=377). The mean BMI ±SD was significantly higher **(p < 0.0001) **** in the females 32.50 (± 8.25) Kg/m^2 than in the males 27.76 (± 7.09) Kg/m^2 however when separated into gender specific BMI Kg/m^2 and WC cm reference ranges for obesity (WHO, 2008; International Diabetes Federation, 2006), the females were regarded as obese, and the males as overweight (as shown in Table 3). The hip circumference (HP) and waist-hip circumference ratios were also calculated by the larger BSA study. The carotid study regression analysis also revealed that the WC $_{cm}$ as a risk factor related to obesity approached significance **(p = 0.0555)$^{+}$**, with the predicted increase of carotid artery wall thickening. In contrast, the decrease of the HP $_{(cm)}$ measurement showed a statistically significant **(p = 0.0008)*** increase of the carotid artery wall thickening. This was relevant to the WC/HP $_{(cm)}$ ratio measurement for obesity and CIMT changes. Diet and physical activity is an important factor that appears to be neglected among individuals, yet it is an obvious modifiable risk factor for primary prevention of ill-health. Reports from elsewhere on the heterogeneity in the clustering of cardiometabolic risk factors across the continuum of BMI extend to South Africa, and adds to the controversy surrounding the association of different obesity phenotypes with disease risk' (Matsha et al., 2013). Kisten et al., (2013) also demonstrated that increased BMI and hypertension were contributing risk factors, in selected T2DM Western Cape South Africans, that ended up with surgery (carotid endarctectomy). This further highlights the need for objective methods like carotid Doppler sonography, to further assist in accurate diagnosis and patient management in T2DM patients at risk of cardio-and-cerebro-vascular complications. Ultrasound is able to diagnose and monitor vasconstriction

and high resistance to blood flow, which is known to cause complications that affect blood pressure. Hypertension also causes stress on the vascular supply and blood circulation, with a domino-effect to the entire system.

The carotid ultrasound study, demonstrated no statistical blood pressure differences (p = 0.3046) ^{ns} between genders. However both males and females were diagnosed with pre-hypertension and hypertension, well above the normal range of 120/80 $_{mmHg}$. Blood pressure SBP (120-139) $_{mmHg}$ and DBP (80-89) $_{mmHg}$ is regarded as pre-hypertensive and >139/89 $_{mmHg}$ as hypertensive. This was alarming, for the Bellville South mixed ancestry cohort, in that the mean systolic blood pressure (SBP $_{mmHg}$) ± SD was 139.96 (± 24.28) $_{mmHg}$ in the men and 139.17 (± 27.74) $_{mmHg}$ in women. The mean diastolic blood pressure (DBP $_{mmHg}$) in males was 85.72 (± 12.67) $_{mmHg}$, and 84.57 (± 14.62) $_{mmHg}$ in females. The univariate multiple regression analysis of SBP $_{(mmHg),}$ after adjusted R^2 of 0.3247 for all independent variables, indicated statistically significant **(p< 0.0001)**** positive associations with carotid artery wall thickening which was also stepwise regressed as the highest associated contributor **p < 0.0001)**** to CIMT change. In contrast, DBP **$_{(mmHg)}$** showed statistically significant **(p = 0.0055)*** inverse associations in carotid artery wall thickening measurement changes. This was relevant in that the decrease of diastolic blood pressure causes the increase in total blood pressure (hypertension) which is associated with the increase in CIMT. Tissue elasticity can also be measured with ultrasound, and shown to be affected by blood pressure in relation to vascular remodeling. Vasocontriction caused by toxic stimulants such as smoking, also affects oxygen and nutrient supply to the various organs and is risk factor for numerous illnesses. This mixed ancestry Bellville South population are already at high risk due to increased CIMT, plaques, stenosis, increased age, obesity and until now, hypertention. Smoking was the next predisposing/ contributing factor for discussion.

In the current carotid ultrasound study population, both questionnaires (self-reported) and serum cotinine levels were used to confirm smoking status and exposure to tobacco/nicotine concentrations. The carotid study used the S-cotinine levels to compliment questionnaires on smoking or exposure to tobacco. Levels of cotinine were established for all subjects, and identified all those with abnormal Cotinine levels, and distribution of smoking status. Passive smokers may be an interesting sample. Two cigarettes a day is apparently regarded as negligible, however, the strength of the tar and nicotine concentration also was taken into account. A Cotinine level on the other hand gives more an estimation of exposure to nicotine, irrespective of the number of cigarettes smoked. Studies have shown that heavy passive smoking, detected by serum cotinine concentration can be compared to 'light smokers' and cotinine levels of some 'social' smokers be regarded as

negligible. The S-Cotinine **ng/ml** levels of males for the current study was higher (138.19 ± 176.56) compared to females (127.55 ±178.82) and showed abnormally high concentrations of smoking or tobacco exposure **(**smoking status) among this high risk mixed ancestry population. The regression analysis demonstrated a statistically significant positive difference and standard error (SE), for every unit of change (1**yr.**) of age (yrs.), as a contributing factor for atherosclerosis. For every year changed, there was a change of **(0.3028 ± 0.0649) mm** in the mean Rt. and Lt CIMT as predicted according to this model (as shown in Table 4). The similar mathematical formulae of change in CIMT measurements ± SE, was noted for tobacco exposure as a contributing factor for changes per unit of S-Cotinine ng/mL **(0.1119 ± 0.0545)mm** levels. Also an observation was that not all participants reported their true smoking status of which was clearly noted by their cotinine levels. Also an observation was other forms of smoking apart from cigerettes. Smoking is known to affect cells that line the respiratory and digestive systems, and indirectly affects the vascular walls as well. The response to injury theory is again emphasised as outlined elsewhere. Smoking has been shown to be a contributor to many infammatory diseases, and is no exception for vascular diseases as well. This suggests that carotid wall inflammation does have a close relationship with smoking status. The measure of inflammatory changes using serum was tested in this study and its relationship on vessel wall thickening were observed.

Alarming, was that in the carotid ultrasound study, the mean hs-CRP values of the average mixed Ancestry population were abnormally high in both the males and females. The women of the study revealed values (8.8 ± 16.2) that were significantly higher **(p = 0.0499)*** than the men (7.39 ± 11.62) indicating that the Bellville South mixed Ancestry population were prone to possible inflammatory disorders as well, increasing CVD risks. The univariate multiple regression analysis after adjusted (R^2 of 0.3247) for independent variable (inflammatory indicator: hs-CRP (mg/L), showed a statistically significant **(p = 0.0033)*** positive association with dependent variable Mean Rt. and Lt. CIMT (mm). The hs-CRP **(mg/L)** inflammatory markers were also calculated as the 2nd most significant **(p = 0.0014)*** contributor to the change of carotid artery wall thickening. This confirms CRP's high association to carotid artery wall inflammation for this population. The limitation of using CRP markers in isolation, is that it is not specific to the source of inflammation. The raised levels of hs-CRP could also have been a result of current infection or some sort of active inflammatory diseases. Ongoing research continues to determine whether reducing inflammation and lowering CRP levels, have the ability to reduce the risk of CVD and stroke. Hereditary and ethnic variations among population groups have also shown promising results (Stern, 1995; Ruige et al., 1998; Bhopal, 2002; Peyser, 1997; Marenberg et al., 1994; LeRoith et al., 2003; de Wit et al., 2010).

Noticeably, (self-reported) the high unemployment rate and 'social drinking' among the mixed ancestry participants of the carotid ultrasound study was observed. It is also not uncommon for pregnant mothers to consume excessive alcohol, and fetal alcohol sydrome has been regarded a problem in the Western Cape. In a publication done by London (1999), it was reported that a high prevalence of alcohol abuse existed among the farm workers (mixed-ancestry) in the Western Cape, who lived and worked under adverse conditions that were the legacy of apartheid policies. Despite its official prohibition, the arrangement by which workers were paid by alcohol as a benefit of employment was known as the 'dop' system (London, 1999). Heavy alcohol consumption was reported to not only be directly injurious to the health of the farm workers and their families, but placed them at risk to various social, environmental and health hazards (London, 1999). Although levels of alcohol intake were measured by the larger study previously, it was not used for the carotid study analysis, however serum levels of gamma-glutamyl transpeptidase (GGT $_{IU/L}$) was tested due to the high alcohol consumption amoung this population. Increased gamma-glutamyl transpeptidase concentration levels are usually related to liver disease (alcohol toxicity related, jaundice, chirrosis etc). Poor lifestyle a contributing factor. A significant increase **(p = 0.0004)*** of GGT concentration levels was seen in males as compared to the females, however the stepwise multivariate regression analysis with CIMT and GGT showed no direct associations, but could have confounded for other risk factors, which was relevant for this analysis. Although not tested, a high fat diet, alcohol abuse and liver disease may have an indirect effect on the billary circulation and gall bladder function in relation to cholestrol levels.

The low-density lipoprotein cholesterol (LDL $_{mmol/L}$) levels in this study, were not statistically different between genders, but woman showed statistically significant **(p= 0.0042)*** higher concentrations (1.42 ± 0.43 $_{mmol/L}$) of HDL cholesterol than men (1.32 ± 0.49) **$_{mmol/L}$**. Although the women presented with higher levels of HDL-C than men, they also presented with higher LDL-C levels too. The total blood cholesterol (TC $_{mmol/L}$: p = 0.3301) ^{ns} and triglyceride (TGC $_{mmol/L,}$ p = 0.1780) ^{ns} concentration of males were similar to that of females. However, in the multivariate stepwise regression analysis, the independent variable LDL **$_{(mmol/L)}$**, was calculated as the 3rd highest **(p = 0.0208)*** positively associated contributing risk factor for carotid artery wall thickening. A close relationship between LDL and plaques has been shown in the literature. Although diet and nutrition was not tested in this study, it would be interesting to find out how it relates to the ultrasound findings and contributing T2DM risk factors in this population as well.

Biomarkers for blood glucose concentrations were relative to carotid artery wall thickening for this mixed ancestry population. The blood sugar average percentage of total haemoglobin HbA1c$_{\%}$ **(p = 0.0006)*** and fasting blood glucose concentrations FBG $_{mmol/L}$ **(p = 0.0003) **** showed significantly lower levels in the males as compared to the females (as seen in table 3).

Fasting blood glucose or postchallenge plasma glucose and glycaemic spikes was more strongly associated with atherosclerosis than that of fasting glucose or HbA1c% levels. A negative change of CIMT measurement **(-0.1692 ± 0.0846)** $_{mm}$ was predicted for every unit of change in HbA1c %. This was unexplained for this population; however the glycaemic status and carotid artery ultrasound measurements showed clear relationships.

The association of ultrasound findings of the carotid intima media thicknesses, with glycaemia status (Normal, Pre-DM and T2DM) for the male and female population showed interesting results. The Rt. and Lt. mean CIMT was used as the average measurement for all CIMT categories. Both males (26/103) and females (84/269) with T2DM demonstrated the highest levels of mean CIMT measurements for all categories, in contrast to the normal glycaemic males (63/103) **(p = 0.0115) *** and females (144/269) **(p > 0.0001) ****. The thickest Lt. $CIMT_{mm}$ **(p = 0.0222)*** and thickest Rt. or Lt. $CIMT_{mm}$ **(p =0.0259)*** demonstrated statistically higher levels of thickening in T2DM subjects than those with normal glycaemia status in the male group. The step up of CIMT levels from Pre-DM to T2DM status also approached significance **(p = 0.0627)** $^{+}$ in the females. There was no significant change of CIMT levels from Pre-DM to T2DM status in the males. This is an interesting group to study. Pre-DM is not necessarily a road to diabetes, and is supported by literature reports that suggest that Pre-DM individuals that undergo life style modification treatments, weight reduction and increased physical exercise have the ability to prevent the transition of Pre-DM to T2DM (Lindstrom et al., 2006; Lindstrom et al., 2003; Knowler et al., 2002; Tuomilehto et al., 2001). Carotid artery wall thickening related to age, gender, glycaemia status and contributing atherosclerotic risk factors showed positive associations in this high risk T2DM mixed ancestry population ≥35yrs of age.

As described by Spence in 2006, when it became clearer to researchers, that CIMT, coronary calcification and carotid plaque, reflected biologically and genetically, the different aspects of the atherosclerotic process, and was said to respond differently to therapy. The intima-media thickness of arteries represented mainly hypertensive medial hypertrophy. The CIMT measurement, as stated in another study by Spence (2002), was more predictive of stroke than of myocardial infarction, and was only associated with traditional coronary risk factors. Carotid plaque characterisation, however, has shown to have a stronger association with traditional risk factors, and is more predictive of myocardial infarction than of stroke. “When an individual has excess carotid plaque not explained by traditional contributing risk factors called ‘unexplained atherosclerosis,’ it is an even more powerful tool for genetic research, because age, which is accountable for baseline plaque, has

shown to have a much less influence on the rate of plaque progression" (Spence, 2006 : 611). The contributing risk factors of atherosclerosis apart from age related risks were shown to co-exist between atherosclerosis and diabetes mellitus as demonstrated in our study. Age, was also shown to be the highest associated contributing risk factor to increased CIMT in our study as seen in the stepwise regression analysis above. The mini research ultrasound study entitled 'Duplex ultrasound: A diagnostic tool for carotid stenosis management in type 2 diabetes mellitus' done prior to the current study by Kisten et al., (2013), reported a high prevalence of carotid artery stenosis in T2DM South Africans of European descent. The above mini research study included a selected high-risk group diagnosed with T2DM, from a specialised referral vascular clinic in Cape Town, and hence did not reflect the total Western Cape regional population (Kisten et al., 2013). The results however correlated with supported literature that ultrasonography has a significant role to play in monitoring patients with T2DM (Kisten et al., 2013; Lorenz et al., 2006; Balasubramaniam et al., 2012; Bradshaw et al., 2007; Maiti and Agrawal, 2007: 292; Grundy et al., 2000; Wohlin et al., 2008; Hansson, 2005; Bonora et al., 2000; Eastcott, 1994; Uusitupa et al., 1985; Paulose et al., 2002).

CHAPTER 6

CONCLUSIONS

CHAPTER SIX

6.0 CONCLUSION

In conclusion, this carotid artery ultrasound study proved useful in demonstrating the positive associations between the ultrasound findings (CIMT, plaques and stenosis) of the carotid arteries and a participant's glycaemia status. It also revealed the direct and indirect relationship of the carotid ultrasound measurements, with the contributory risk factors of age, obesity (increased waist circumference, hip circumference and body mass index), hypertension, smoking, hypercholeraemia (increased blood cholesterol levels - lipid profiles) and T2DM to have, on the development of carotid wall hypertrophy and atherosclerosis in the selected Bellville South mixed ancestry population ≥35yrs of age. The hypothesis that T2DM and hyperglycaemic subjects to have increased levels of CIMT, plaques and stenosis than those without T2DM, proves true, in this carotid ultrasound study population of Bellville South Africa. The average individual of this study population was ±56yrs old, hyperglycaemic, female, smoker, obese and hypertensive with an increased CIMT.

Study limitations and recommendations

The limitations of the study is the lack of the correlation and logistic regression analysis, which could have added a more predictive nature of the ultrasound finding; however these tests are still possible for future analysis. For the implication of ultrasound practice, the sensitivity, specificity and predictive values would also carry more weight if tested using biochemistry or other reliable markers as reference comparisons, but a possibility for further studies. The strength of this ultrasound study is its reliability, objectivity and ability in subclinical diagnosis. Images stored on the ultrasound machine and backed up on external hard-drive is also an advantage for follow-up studies, with the consent of the participants. In clinical practice, this information is vital, especially in patients with plaques. The study is limited to the incidence of plaque detection; however the exact characteristics and echogenicity of plaques would have been more suggestive for future adverse events. Although this plaque characterisation was measured and recorded for all participants, it was not included in the carotid study analysis, but definitely a recommendation for follow-up. Confounders for the study results may also be worth investigating in greater detail. Ultrasound of the kidneys may have also an added benefit to establish renal involvement, especially for severe T2DM and a suggestion for future studies.

An important recommendation is also to assist these participants in improving their current health status, such as encouraging smoking cessation, promoting physical activity and exercise, and a healthy diet. Suggestions for further research would be a 5-year follow-up of this population, using the same methodology, with the inclusion of CIMT automated software analysis. However, those that have been on treatment be identified, so that this be considered

when determining the treatment effect on comparative ultrasound measures. Plaque characteristics are suggested to be investigated and analysed in further detail, with exact description relative to plaque types and vulnerability. Ultrasound evaluation (Echocardiography) of the heart as a compliment, offers greater CVD risk profiling. Specialised ultrasound software for post-processing of images should be used for greater precision. Vascular elastography and four dimensional quantification measures are also suggested.

The gathered information, discussion of results, and concluding statements thereby supports the recommendation of carotid artery ultrasound evaluation, for screening and diagnosis in primary health care, for 'flagging' high risk individuals at risk of stroke, so that lifestyle changes and appropriate management is early adopted.

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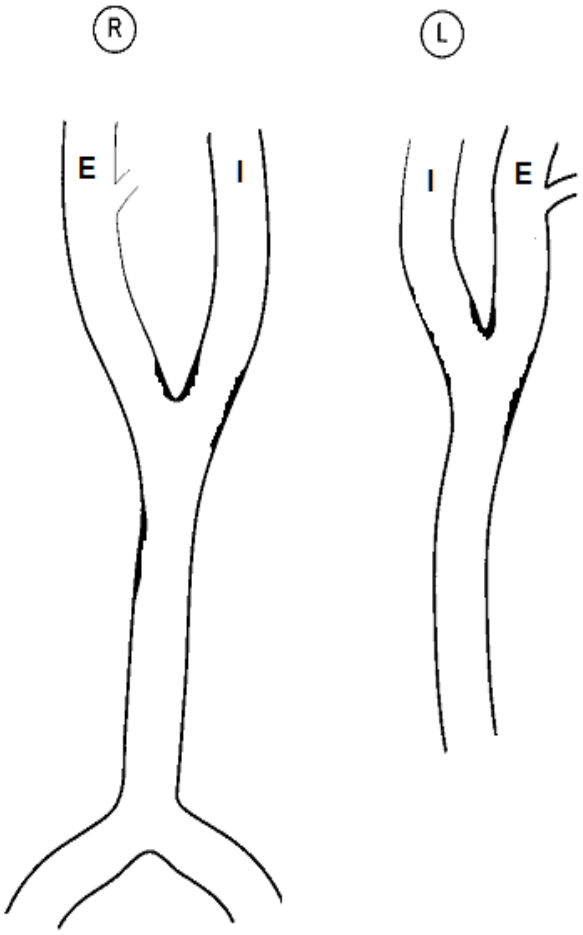
APPENDIX A: CAROTID ULTRASOUND DOPPLER STUDY: Ultrasound Report

CPUT
Faculty of Health and Wellness Sciences
Bellville South Population
Western Cape

Please attach participant research no.

Ultrasound Carotid Assessment



Summary

RIGHT Carotid

	CCA	**ICA**	**ECA**
PSV (cm/s)			
EDV (cm/s)			
ICA/CCA			
% Stenosis			
Plaque characteristics Echogenicity TPV			
IMT Measurement			
	Vertebral		
PSV (cm/s)			
Direction			

LEFT Carotid

	CCA	**ICA**	**ECA**
PSV (cm/s)			
EDV (cm/s)			
ICA/CCA			
% Stenosis			
Plaque characteristics Echogenicity TVP			
IMT			
	Vertebral		
PSV (cm/s)			
Direction			

COMMENTS :

SIGNATURE:
Print Name :

DATE:

Adapted from Edelman, 2007

APPENDIX B

BELLVILLE SOUTH INFORMED CONCENT

We kindly seek your permission to be part of this ultrasound research study which is towards a Masters of Technology in Radiography: Ultrasound. This consent form allows you to freely choose whether you want to join this study or not. We will explain to you what your participation will entail, as well as answer any questions that you may have about this research.

Voluntary Participation

You may choose to be part of this study or not. You may withdraw from the study at any time without any form of victimisation.

What is this study about?

Being part of the larger Bellville South Africa (BSA) diabetic study, you would have already had tests done to check your diabetic status. Previous research studies have shown that people with high blood sugar levels (diabetes mellitus) together with other risk factors like smoking, lack of exercise, unhealthy diet and high blood pressure, have a high chance of developing blood vessel problems in the neck which could result in a stroke. Carotid (ka-ROT-id) Doppler ultrasound is a safe test that uses high-frequency sound waves to form pictures of the insides of the two large arteries, one on each side of your neck. These arteries, called carotid arteries, supply blood to your brain. The severe blockage of this artery can lead to a stroke. By using ultrasound, the wall thickness (CIMT) of the carotid artery and its blood flow can be measured. If the CIMT or carotid artery wall thickness is ≤ 0.8 mm, then your chances of developing atherosclerosis is low, however, if the wall thickness is >0.8 mm, then your chances of developing atherosclerosis is high. This study will also be beneficial to you if you already have atherosclerosis, so that its severity can be determined. A blockage of the carotid artery of >70% will require special referral to a blood vessel and heart doctor, so that you could be treated further to reduce your chances of severe heart problems and/ or a stroke.

Entrance into the Study

We are asking the participants of the Bellville South Diabetic Study to take part in this Carotid Ultrasound Doppler Study. You will be asked some questions to determine if you can take part or not.

Ultrasound Examination

This study offers all selected participants an ultrasound examination of the neck (carotid artery Doppler). This test takes about 30 minutes. It is a painless examination and will require the participants to lie on their backs while a small instrument called a transducer is moved across both sides of the neck. The person doing your scan will always ask you how you feel throughout the procedure. If you are uncomfortable, please let the people doing the scan know, so that they can make sure that you are okay. A water-based gel will be applied to the skin so that the transducer will have good contact and enable easy movements. This hypo allergic gel

(known not to cause any allergic reactions) will be wiped off at the end of this procedure. The blood flow and wall measurements of the neck vessels will be recorded on the hard drive of the ultrasound machine, and on ultrasound reports for your research folder, and will only be used for the purpose of this study with your permission. This information will be kept confidential, and will only be revealed to the research doctor, so that he can explain to you what you will need to do, or refer you to the community clinic/ hospital, in order to make sure that you get appropriate management, needed for your condition should that be required. You may choose to see your own doctor if you want to. Our research doctor will refer you to a specialist doctor who deals with blood vessel problems at Tygerberg hospital, or a hospital/clinic that you want to go to. If your ultrasound results are normal, you will also be informed by the person doing your scan, making sure that all your information is kept locked away in a safe place so that is not accessible to anyone but the people involved in this study. Even those involved in the study will not know whom the results belong to. You will have a special unique identification number that will be used as a code for the study.

Confidentiality

All information about you will be kept confidential, by using numbers that is coded.

If you have further questions about the research you may ask the study nurse, doctor or researchers involved. If you have questions about your rights as a research participant, you can contact the Research Ethics Committee at CPUT or the principal investigator for this study (Yogan Kisten) at 0727995190/ yogankisten@gmail.com

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Participant's statement:

This study has been explained to me and I understand what it involves. I volunteer to take part in this research. I have had a chance to ask questions and may ask more should I want to.

I, Mr/Mrs/Ms **Name:**_____ **Surname:** _____

Am fully aware of the procedures involved together with the reasons why this study will be carried out. I am also aware that I have the choice of leaving the study whenever I want to without any form of victimisation, and will not be compelled to stay till the end of the research study. I hereby grant the researcher permission to include me in the study

_____ _____

PARTICIPANT'S SIGNATURE **DATE**

WITNESS: _____ **RESEARCHER**:_____

SIGNATURE:_____DATE_____ SIGNATURE_____ DATE_____

Appendix C: Carotid Ultrasound Examination Protocol

All participants were examined using a B-mode and spectral Doppler ultrasound scanner equipped with cardiovascular imaging software and a 5 – 12 MHz multifrequency linear transducer. The portable GE ultrasound LOGIQ e Instrumentation was used for this study.

The ultrasound examinations have adhered strictly to the 'As Low As Reasonably Achievable' (ALARA) principle to reduce biological effects that may be associated with ultrasound. The exposure output of the machine was kept to a low value with the thermal index <1, which still gave an accurate diagnosis (Kremkau, 2006: 352).

The ultrasound findings of both left and right carotid arteries were documented and recorded as real time images/video clips and were stored onto the hard drive of the ultrasound machine as well as onto an external hard drive for backup and follow up purposes. The ultrasound reports where checked and signed by the research sonographer after confirming the ultrasound findings with the research study doctor.

A written report was done in duplicate – the original copy was checked and inserted in the participant's folder. The data documented on the ultrasound reports were thereafter punched into the carotid ultrasound data base. The ultrasound database was checked by the Researchers, and their supervisors. The cleaned data was then merged with the larger study database.

Instrument Settings

The ultrasound machine was calibrated every morning and afternoon as part of quality assurance testing. Vascular ultrasound carotid pre-sets were defined for the GE LOGIQ e portable ultrasound device. The instrument settings were set at the start and maintained throughout the study. The ultrasound machine was installed with the GE multifrequency, linear transducer set at a frequency of 5MHz for scanning the common carotid artery of larger participants and adjusted accordingly when scanning the bulbs, intima media, plaques and the internal carotid arteries. Using the carotid pre-sets, the dynamic range was 66, high resolution, edge enhancement (E/A) 3/3, map B, Average 2, Focal point (position of focal zones: mid artery).

Range-gated Doppler was adjusted to the middle position of the vessel examined and range gate (3mm) within the area of interest. Audio output Doppler was set to audible range. The ultrasound machine recording device was installed with digital archiving capabilities.

The longitudinal and transverse images of the common carotid, carotid bulb, the external and internal carotid artery were magnified. CIMT and plaque measurements were also recorded to enable post-processing. Read magnification functions and overall gain was manipulated for optimum image resolution. The image magnification was switched "ON." Image depth altered accordingly. The "ZOOM" button pressed, and the trackball maneuverer to region of interest and set to desired image resolution on display. The GE XBeam function was set "ON" for all participants.

Prior to the commencement of the scan, the participants' details: Name, D.O.B, Gender, image presets etc. were entered onto the ultrasound machine. A unique reference number was given to each participant. Those participants that had the same reference number were detected on the ultrasound machine which would alert the user of a repeat code entered.

Participant preparation

Prior to the ultrasound examination, participants' were informed of what the ultrasound study entailed (refer to **Appendix B**: Informed consent) and were advised to wear clothing that exposed their neck region. Necklaces were removed by themselves and kept in their possession. Participants were asked to lie in a supine position on the examination couch and made comfortable, covered with a linen blanket. They were asked to raise their chin and informed about the cold ultrasound gel that was adequately smeared over the neck region in the area of the carotid arteries. The commonly used ultrasound gel is a trans-sonic material or medium which eliminates the air interface between the transducer and the skin. While enabling good contact, it also allowed easy mobility of the transducer over of the area of interest.

In the supine position the participants' shoulders were lowered to increase access to the neck, especially in participants with short neck stature. Some participants were asked to reach for their feet in order to better evaluate the neck. For easier carotid ultrasound assessment, they were asked to turn their heads slightly away from the side being examined. In many participants, the pillow was removed from underneath the head, however in some participants; the pillow was folded to enable the head to be hyper extended in order to demonstrate better blood flow. Many sonographers scan according to their own preferences, however in this study the sonographers position was next to the patients' right side. Some sonographers prefer to sit at the top of the patient's head and table, scanning facing downward from the patients' head to their shoulders. This approach is also modified by each institution with respect to their clinical settings and requirements.

Extra-cranial (Carotid) ultrasound sites examined

The purpose of the initial scan was to orientate the research sonographer to the participants' carotid anatomy. The sonographer had to locate the bifurcation and distinguish which vessel was the internal and which was the external carotid artery. The site of maximal wall thickening in the near or far wall, in the bulb or internal carotid artery was also established. Colour and pulse-wave Doppler was used as identification aids. After proper orientation, blood flow velocimetry, stenotic ratios, plaque quantification and CIMT measurements were documented. The left and the right sides of the extra cranial circulation are not symmetrical, and the carotid arteries have different origins.

On examination, the linear transducer was set to carotid pre-sets and the study began by evaluating the right carotid artery. It was first examined in transverse section, using colour Doppler mapping from the innominate or brachio-cephalic artery where it originated from. Blood flow velocities and plaques were also continuously assessed. The left carotid artery however, varied in its origin, by emerging straight from the aortic arch (Neumyer, 1999: 367). The ultrasound evaluation continued and the anatomical location of the vessels also documented. The common carotid arteries (CCA) were noted to ascend from its origins through the mediastinum and coursed through the sternocleidomastoid muscles, which were positioned posterior and medial to the internal jugular veins (IJV) (Polak, 1992). The sternocleidomastoid muscle was also used as a control for pattern recognising plaque characteristics. The echo-texture similar to that of the sternocleidomastoid muscles was referred to as iso-echoic. Echo-textures above that of the muscle was hyper-echoic (calcified/ echogenic) and that lower to echo-textures of the sternocleidomastoid muscles was referred to as hypo-echoic plaques. Lesions of many echo-textures were referred to as mixed-echoic plaques. Plaques (observer dependant) according to homogeneity (homogenous or heterogeneous) and stability (stable or unstable) also established. The CCA was then evaluated further cephalic to just below the mandible and was monitored as it terminated at the carotid bifurcation. This region was magnified sonographically, as it is the area of great interest commonly known as the carotid bulb.

The ultrasound transducer was still in transverse section, interrogating the carotid bulb for plaque and vessel wall damage as the CCA widened (Thrush and Hartshorne, 2003). Distal to the bulb of CCA, it was noted to bifurcate into the internal and external carotid artery. The ICAs and ECAs were seen to originate at the upper edge of the thyroid cartilage (the 3rd and 5th cervical vertebra). The ICA in most participants appeared to raise postero-lateral to the ECA in 90% of the individuals (Kaufman and Nesbit, 2004: 119). The ICA was seen as branchless below the skull and showed to communicate with the

internal maxillary artery and other arteries that branch off the ICA; however, this was not a common site due to anatomical variations and image resolution. MRI and angiography techniques is better used to show the ophthalmic artery which supplies the eye, and further divides into the anterior and middle cerebral arteries that supply the brain (Kaufman and Nesbit, 2004: 119; Neumyer, 1999: 367). The ECA unlike the ICA is known to have branches below the skull that supplies the muscles, facial structures and scalp. The transducer angle in this carotid ultrasound study was rotated interchangeably into longitudinal and oblique planes as part of the evaluation.

The ECA has 8 named branches, and although few are visible with duplex imaging, the branches play an important part in collateral circulation. In the presence of a severe ICA stenosis or occlusion, the body reroutes the blood supply to avoid a deficiency in blood flow to the brain, which can result in a stroke or TIA (Sanders and Winter, 2007: 536). The posterior circulation of the brain is mainly supplied by the left and right vertebral arteries, via the basilar artery.

A common finding that was noted when evaluating the vertebral arteries was that one vertebral artery appeared to be larger than the other, with the left often larger than the right. The two vertebral arteries join at the base of the skull to form the basilar artery. The circle of Willis connects both ICAs and the basilar artery: the circle of Willis provides a communication between these vessels that may allow adequate blood flow to the brain even when a severe stenosis of total occlusion exists in one of the extra cranial carotid arteries (Sanders and Winter, 2007:537).

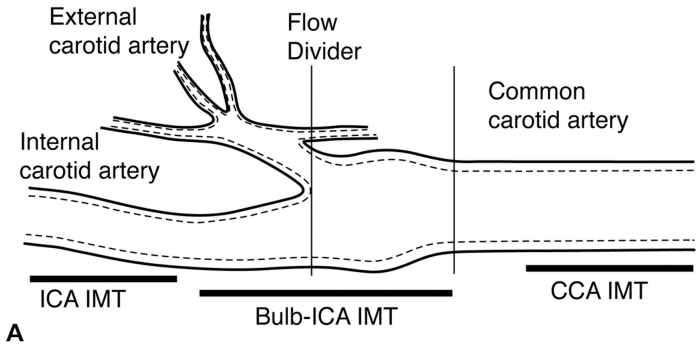


Figure 2. Carotid Vessels.

Polak, Pencina, Meisner, Pencina, Brown, Wolf, D'Agostino (2010)

Imaging Sequence

The images acquired above were obtained in real-time or a cine loop sequence of each arterial segment that was captured on hard drive. Magnification was turned "ON" with the "Zoom" button when images of the common carotid, bulb and internal carotid artery were recorded. Magnification was turned "OFF" during the transverse sweep and pulse-wave Doppler measurement. Cine loops were captured by selecting the cine-loop button and were "frozen" using the freeze button. The sonographer then cycled through the cine loop images to select the one image that best displayed the intimal walls. It was important that this image was always obtained in the latter part of the cardiac cycle, as close to end diastole as possible. End diastole is the portion of the cardiac cycle when the carotid lumen is smallest and the vessel shows the least movement. Arterial walls are typically more clearly displayed on this image. The static images were also recorded for reviewing purposes.

Methods of imaging

Two - dimensional ultrasound

At the start of the examination, as described above, the carotid arteries were observed from its origin in transverse section, and the image on the screen was seen with the participants right side presented on the left side of the image on the display/monitor. The screen was split in two, with the proximal, middle and distal CCA images magnified and recorded with and without Colour flow. The proximal, middle and distal CCA was also evaluated in its longitudinal section, seen with the cephalic region of the CCA on the left and the caudal part of the artery on the right of the image displayed.

Colour Doppler method

On examination of the carotid vessels in colour Doppler mode, it was made sure that the appropriate pulse repetition frequency was selected, so that the colour continually filled the vessel lumen in order to demonstrate its patency. Zwiebel (2005) confirms that a lack of colour filling within the vessel wall may indicate the presence of atheroma. The colour Doppler angle less than ≥60˚, and the appropriately PRF settings were maintained throughout the study, to reduce image artefacts that could prevent the colour from being displayed. The colour Doppler gain settings were also manipulated to eliminate the aliasing artefact, which is described as the mix of colour, often appearing yellowish orange, which could offer a false positive impression of turbulent flow, if the above settings are not adjusted accordingly (Zwiebel: 2005). The colour Doppler mode was also set according to the blood flow direction, towards and away from the transducer. The colour

red (warm colour) was set for the blood flow approaching or moving towards the transducer and the colour blue (cold colour) for the blood flow leaving or moving away from the transducer (refer to image Fig.3 below). This colour Doppler method was useful in assisting with flow direction, stenosis and occlusions of the carotid vessels examined.

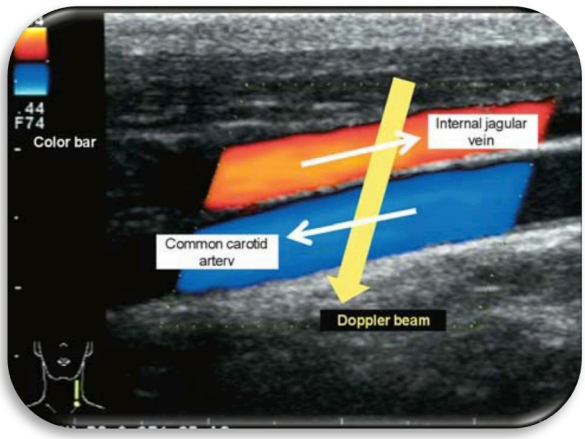


Figure 3. Colour Doppler Method.

Used with permission granted from the Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine (2009)

Pulse Doppler method

The pulse Doppler signature analyses were also interpreted and waveforms pattern recognized for CCA, ICA, ECA and vertebral arteries (Wood et al., 2010). The direction of blood flow velocities were also determined by analysing the pulse Doppler wave characteristics, which was interpreted relative to the baseline of the Doppler flow. The blood flow approaching or moving towards the transducer was depicted above the baseline (the positive side) while the blood flow leaving or moving away from the transducer was depicted below the baseline (the negative side).This decision did not apply if the orientation of blood flow was specified on the image.

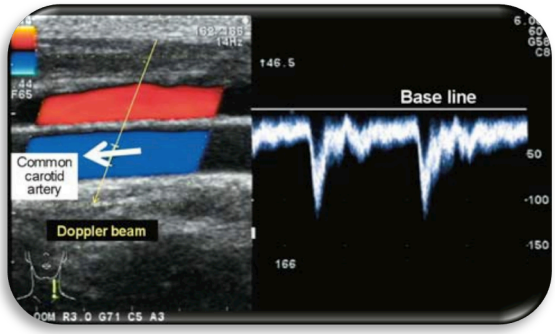


Figure 4. Pulse Doppler Method

Used with permission granted from the Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine (2009)

Scanning Approach

As mentioned above, a two-dimensional evaluation of the carotid arteries involved the transverse and longitudinal view. The lateral and oblique views were also carried out to further assess and identify difficultly located vessels. In the transverse section, vascular lesions (plaques etc.) were evaluated and measured when detected. The transverse images of the arteries were made in two directions, i.e., anterior and lateral (posterior) approaches so that inadequate depiction in one direction, was overcome by the depiction of the artery in another direction (refer to image fig. 5 below).

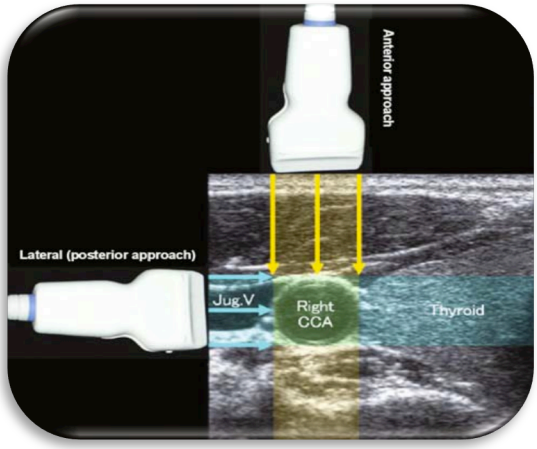


Figure 5. Transverse view. Anterior and Lateral approach

Used with permission granted from the Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine (2009).

Appendix D: Parameters used in the carotid evaluation

Carotid Intima Media Thickness

Atherosclerotic lesions were continuously searched for throughout the carotid examination, and the maximum intima-media thickness of the common carotid artery, bulbus and internal carotid artery on the right and left side were thoroughly examined. The walls could vary in thickness even across a distance of 1cm, especially in elderly and those with atherosclerosis, therefore the maximum/thickest part of the carotid intima and media layers were measured in longitudinal section, 2 cm from the carotid bifurcation, at 3 consecutive end points, 5-10 mm apart. In longitudinal section, the CIMT measurement was taken at the far wall of the distal CCA, 2 cm from the bifurcation, in which the cursors placed at 3 locations of the CIMT 5-10mm apart and measured. The mean of the 3 CIMT measurements were also documented. The CIMT of bulbs were evaluated, however not measured for this study.

Percent stenosis

The percent stenosis of the patent blood flow velocities and the area of the common carotid artery were measured as an indispensable parameter, and were documented with reference to the ICA/CCA ratios (Wood et al., 2010). The percent stenosis of the arterial diameter was additionally measured as required. When checking for lesions of the internal carotid artery, the percent stenosis was further documented according to the criteria of the North American Symptomatic Carotid Endarterectomy Trial (NASCET) as a primary parameter, which was accompanied as needed, by measuring the percent diameter narrowing and percent stenosis of the arterial diameter according to the criteria of the European Carotid Surgery Trial (ECST). In addition, the blood flow (maximum peak systolic and end diastolic velocities) through the stenotic lesion when present was also measured. For this study, the presence of plaques also considered for <50%, 50-69% and >70% stenosis categories.

Measurement of CIMT

Using high resolution B-mode imaging techniques, the intima media complex (IMC) was evaluated. The image was taken in true longitudinal section, in magnification, and zoomed, without compromising the axial resolution, thus reducing the measurement error, when measured. Measurements of the maximum/thickest portion of the posterior wall of the CIMT was taken, when the two echoic layers were noted 2cm from the carotid bifurcation, i.e., the hyper-echoic layer closer to the vascular lumen was composed of two layers, i.e.,

the hyper- echoic layer closer to the vascular lumen and the hypo-echoic layer. It is known as described previously in the literature that an increase in the carotid intima media thickness, correlates with atherosclerotic diseases such as cerebral infarction, myocardial infarction and lifestyle- related diseases, which are risks for atherosclerotic diseases (Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine, 2009). Prior to obtaining the best CIMT image for measurement, both sides of bulb were evaluated. CIMT was not measured at the bulb in this study, but on the posterior walls 2cm from the carotid bifurcation, at 3 points 1cm apart. The CIMT of the ICA and ECA was not measured in this study. In cases where the artery was obstructed or calcified, and a proper measurement unobtainable, it was reported as 'suboptimum.' Although the anterior/near wall was not measured in this study, it has been noted to be more difficult to obtain because of the characteristics of ultrasound, depiction of the CIMT along the anterior wall. CIMT measurement of ≤0.8mm was regarded as normal. The mean CIMT measurement was taken at both the right and left common carotid artery, as per protocol mentioned above. The technique of calculating the average readings of the 3 CIMT points was used in this study. Imaging departments equipped with advanced ultrasound accessories can use automated CIMT measurement software methods for measuring the mean CIMT and plaques.

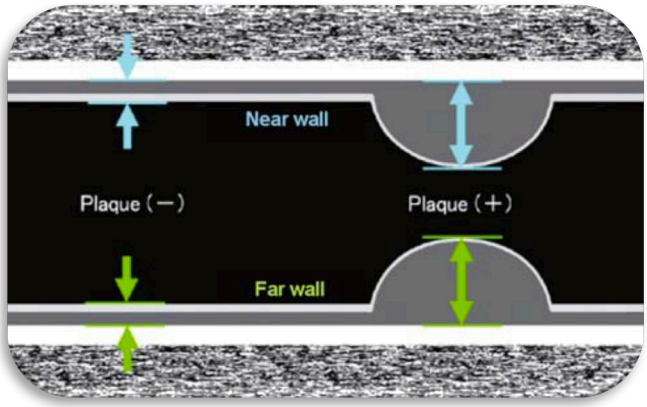


Figure 7. CIMT complex. Used with permission granted from the Terminology and diagnostic criteria committee of the Japanese society of ultrasonics in medicine (2009); Stein et al., 2009.

Measurement of plaques

Plaques for this study were referred to as sonographically visible localised elevated lesions, having a point of echoic attenuation on the surface of the intima media complex. In this study, when plaques were noted, images where taken in longitudinal and transverse sections in order to be able to measure its volume (Length x Height x Width) $_{mm}{}^{3}$. Those measured in transverse section, included area (Length x Height) measurements. The plaques identified that were >1mm^2 or >1mm^3 were related to some form stenosis or altered flow.

Parameters and properties of plaques

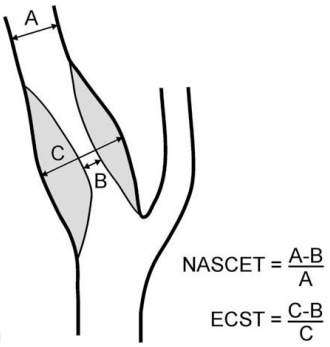
As part of the carotid artery ultrasound evaluation of plaques, the following parameters and properties were measured: the size of plaques, the surface morphology, internal properties and its mobility in terms of it being stable or unstable. Describing the nature of plaque parameters and properties are useful for evaluation, treatment and follow-up of atherosclerotic lesions. The plaque characteristics for this study were described as hyper- echoic or echogenic (Calcified), hypo-echoic (echo-lucent and/ anechoic), iso-echoic (same echogenicity) and mixed echo-patterns. It was further identified whether the plaques were heterogeneous or homogenous. The evaluation of the echo-texture of plaques was compared to the echogenicity of the sternocleidomastoid muscle. The echo-texture similar to that of the sternocleidomastoid muscles was referred to as iso echoic. Echo-textures above that of the muscle was hyper-echoic (echogenic/calcified) and that lower to echo-textures of the sternocleidomastoid muscles was referred to as hypo-echoic plaques. Lesions of many echo-textures were referred to as mixed echoic plaques. Each type of plaque was further subdivided into homogenous type (uniform echogenicity inside the plaque) and heterogeneous type (non-uniform echogenicity). In total, there are six types (homogenous hyper-echoic type, heterogeneous hyper-echoic type, homogenous is-echoic type, heterogeneous iso-echoic type, homogenous hypo-echoic type and heterogeneous hypo- echoic type). The mobility or stability of the plaques was thoroughly assessed. Mobile plaques are sometimes seen, and those plaques that float are often regarded as unstable and were ‘flagged’ for the doctor’s attention, when suspected for this study.

Mobile plaques have a variety of echo-patterns and shapes and some are pedunculated substances, fibrous capsules covering hypo-echoic substances and entire or partial plaques may be mobile. These represent thrombi or vulnerable plaques to which attention should be drawn as plaques prone to cause embolism.

The diagnostic criteria of the Japanese society of ultrasonics in medicine (2009) were used

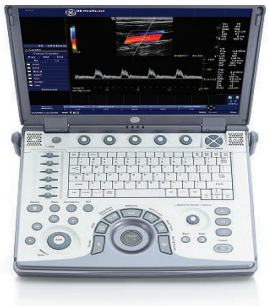
in this carotid study, in keeping with NASCET and vascular ultrasound laboratory guidelines.

- Carotid Intima Media Thickness (CIMT) measurement of ≤0.8mm regarded as normal.
- Peak systolic velocity (PSV) and end diastolic velocity (EDV) of the common, internal and external carotid artery (CCA, ICA and ECA). The vertebral arteries also evaluated to determine its patency and flow direction.
- ICA/CCA ratio
- ICA stenosis (%); a stenosis of > 70% is regarded as significant for surgical intervention



(Refer to Appendix 1: Ultrasound Report)

Ultrasound Machine Specifications



http://www.uk-ireland.bcftechnology.com/~/media/PRODUCTS/Vet%20Imaging%20Support/Ultrasound/GE%20Logiq-e%20product%20resources/Logie%20e%20BT11%20Advanced%20Ref%20Manual.pdf

Appendix E: Letter from larger BSA study leader

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23/03/2010

Chair,
Research Committee,
Faculty of Health and Wellness Sciences

Dear Sir,

This is to confirm that Mr Yogan Kisten is a member of our research team which is studying the inter-relationships between obesity, diabetes and impaired glucose tolerance with diet, tobacco and alcohol intake, physical activity, atherosclerosis, cardiovascular risk, lipids, pro-inflammatory and oxidative markers in an urban adult population from the Bellville South suburb of Tygerberg.

Rajiv T Erasmus MBBS, FMC.Path(Nigeria), FWACP(W.Africa), FACB(USA), DABCC(Am Board Certified), DHSM(Natal), FCPath(S.A)

Professor and Head, Chemical Pathology,
Department of Pathology, NHLS, University of Stellenbosch,
Cape Town, S Africa

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South Africa / Suid-Afrika
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Appendix F: Ethics Certificate for Carotid studies

Cape Peninsula
University of Technology

14 July 2010
CPUT/HW-REC 2010/H008

P.O. Box 1906 • Bellville 7535 South Africa •Tel: +27 21 442 6162 • Fax +27 21 447 2963
Symphony Road Bellville 7535

OFFICE OF THE CHAIRPERSON:
HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

At the meeting of the Health and Wellness Sciences-REC on 4 June 2010 approval was granted to Yogan Shunmugam Kisten, pending amendments that have now been received and reviewed. This approval is for research activities related to an MTech: Radiography at this institution.

TITLE:

Ultrasound evaluation of the carotid artery in a population at high risk of type two diabetes mellitus

INTERNAL SUPERVISOR: Ms F Isaacs

INTERNAL CO-SUPERVISOR: Prof P Engel-Hills

EXTERNAL SUPERVISOR: Prof RT Erasmus

Comment:

Research activities are restricted to those detailed in the revised proposal and application submitted in July 2010.

Approval will not extend beyond 13 July 2011. An extension must be applied for should data collection for this study continue beyond this date.

Prof PENELOPE ENGEL-HILLS
CHAIR: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE

e-mail: *engelhillsp@cput.ac.za*

Appendix G: Letter of permission for use of images and protocols from the Japan Society of Ultrasonics in Medicine

Permission Form

To The Japan Society of Ultrasonics in Medicine

Ochanomizu Center Bullding 6th floor, 2-23-1,
Kanda-awajicho, Chiyoda, Tokyo, 101-0063 Japan
E-mail. noguchi@jsum.or.jp

I am preparing a write up towards a thesis submitted in fulfilment of the requirements for the degree MASTERS OF TECHNOLOGY: RADIOGRAPHY in the Faculty of Health and Wellness Sciences at the Cape Peninsula University of Technology, Cape Town, South Africa

Title: ULTRASOUND EVALUATION OF THE CAROTID ARTERY IN A POPULATION AT HIGH RISK OF TYPE 2 DIABETES MELLITUS

I am requesting permission to use the material described below, in the original and subsequent editions and/or translations:

1. Author(s): Terminology and Diagnostic Criteria Committee, Japan Society of Ultrasonics in Medicine
2. Journal title: J Med Ultrasonics DOI 10.1007/s10396-009-0238-y **Volume**: 30 October 2009
Article title: Standard method for ultrasound evaluation of carotid artery lesions
3. Material to be used: Images Figures: 2,3,4,7,9,11 and 12.
4. Page(s): https://www.jsum.or.jp/committee/diagnostic/pdf/carotid_artery.pdf

5. Publisher: Springer
6. Year of Publication: 2009

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If you will kindly grant permission please sign below and return one form. The duplicate is for your files. Thank you.

Date: 10 October 2014

Requested by: Yogan Kisten
Address: Karolinska Institute, Department of Medicine, (ClinTRID), D1:00, Karolinska University Hospital, Solna, 171 76

Permission granted:

Date: 15 Month: 10 Year: 2014

PERMISSION

The Japan Society of Ultrasonics in Medicine Editor-in-Chief
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