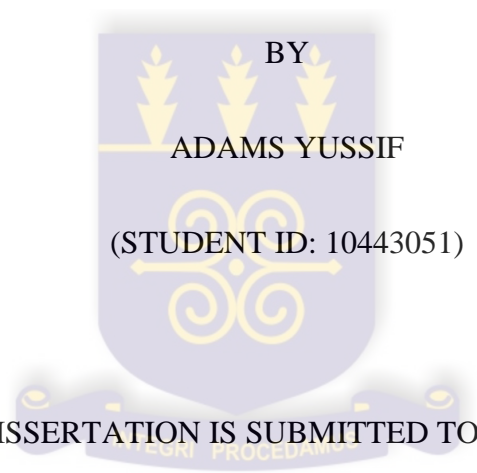UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

SERUM LEPTIN AND C-REACTIVE PROTEIN LEVELS IN OBESE

GHANAIANS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS AT

KORLE-BU TEACHING HOSPITAL (KBTH), ACCRA

BY

ADAMS YUSSIF

(STUDENT ID: 10443051)

THIS THESIS/DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF

GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE AWARD OF MPHIL CHEMICAL PATHOLOGY DEGREE

JULY, 2015

DECLARATION

I, **ADAMS YUSSIF** do hereby declare that with the exception of references to other people's work, which have been duly acknowledge, this thesis is the outcome of my own research conducted at the Department of Chemical Pathology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, and National Diabetic Management and Research Centre (NDMRC) at Korle-Bu Teaching Hospital under the supervision of Dr. Seth D. Amanquah and Dr. Henry Asare-Anane. Neither all nor parts of this thesis have been presented for another degree elsewhere.

…………………………………... Date: ..…………………

Adams Yussif

(Student)

……………………………….. Date: ………………….

Dr. Seth D. Amanquah

(Principal supervisor)

……………………………… Date: ……………………

Dr. Henry Asare-Anane

(Supervisor)

ii

DEDICATION

I dedicate this work to my family most especially to Alhaji Iddrisu Abubakar, who has been my source of motivation in reaching higher height. I also dedicate this work to Sarah Kai Bortey (a mother, a sister and a course mate) who through an untimely death pass on to the land of living.

ACKNOWLEDGEMENT

ABSTRACT

The prevalence of obesity is increasing in Ghana. Obesity is associated with increased risk of many chronic diseases especially type 2 diabetes mellitus. Researching into diagnostic markers is very important. Leptin and C-reactive protein have been implicated in the pathogenesis of diabetes related disorders such as obesity. The aim of this study was to compare serum leptin and C-reactive proteins concentration in obese Ghanaians with and without type 2 diabetes mellitus. Eighty (80) obese (BMI > 30 Kg/m^2) Ghanaians with age ranging from 30-55 years were recruited for this study. There were grouped into diabetic subjects (cases group) and non-diabetic subjects (controls). Fasting lipid profile, serum leptin, high sensitive C-reactive protein (hs-CRP) and fasting blood glucose (FBG) levels were measured by standard methods. Relevant anthropometric indices (BMI, percentage body fat, percentage muscle mass, and visceral fats) and blood pressures were also measured. The case group recruited from the National Diabetic Management and Research Centre and the control group were both 40 each comprised of 15 (33.3%) males and 25 (66.7%) females. The mean age of both case and controls (49.65 ± 5.73 and 47.28 ± 7.82) were matched and there was no significant difference (p = 0.125). Serum leptin and hs-CRP levels in cases (13.84 ± 4.76 ng/ml and 0.18 ± 0.09 ng/ml) was significantly lower than that in controls (17.92 ± 5.51 ng/ml and 0.23 ± 0.11 ng/ml). Serum leptin was strongly and positively correlated with percentage body fat (r = 0.561; P = 0.000) and weakly with body mass index (r = 0.300; P = 0.007) in all the subjects. Furthermore, serum leptin showed negative and significant correlation with triglycerides (r = -0.251; P = 0.025) and very low density lipoprotein (r = -0.253; P = 0.024) in both cases and controls. In conclusion, obese non-diabetic subjects had higher levels of serum leptin and this may suggest a possible link between obesity, insulin resistance, and type 2 diabetes mellitus.

LIST OF ABBREVIATIONS

% BODY FAT ……………………………………………………… Percentage Body Fat

% MUSCLE MASS………………………………………………. Percentage Muscle Mass

ACE Inhibitor………………………………Angiotensin-Converting Enzyme Inhibitor

ADA……………………………………………………American Diabetic Association

ApoB……………………………………………………………Apolipoprotein B

ARB……………………….…………………………Angiotensin Receptor Blockers

BIA…………………………………………………Bioelectrical Impedance Analyzer

BMI…………………………………………………………………..Body Mass Index

CNS……………………………………………………………Central Nervous System

CRP……………………………..………………………………… C-reactive protein

CT………………………………………………………………… Computed Tomograph

DBP…………………………………………………………. Diastolic Blood Pressure

DM……………………….…………………………………………..Diabetes Mellitus

DXA………………………………………………….. Dual-Energy X-ray Absorptiometry

ELISA…………………………………….…….Enzyme Linked Immuno-Sorbent Assay

FBG…………………………………………………………. Fasting Blood Glucose

FFAs ……………………………………………..……………………….. Free Fatty Acids

GDHS……………………………..……………… Ghana Demographic and Health Survey

HbA1c…………………………………………………………….. Glycated Hemoglobin

HC…………………………………………………………………. Hip Circumference

HDL………………………………………………………….High Density Lipoprotein

HRP………………………………………………………….Horseradish Peroxidase

IDF………………………………………………….. International Diabetic Federation

IGT…………………………………………………………. Impaired Glucose Tolerance

IL-6………………………………………………………………………. Interleukin 6

KBTH………………………………………………….Korle-Bu Teaching Hospital

LDL…………………………………………………………… Low Density Lipoprotein

MRI………………………………………………………. Magnetic Resonance Imaging

NDMRC……………………………. National Diabetes Management and Research Centre

Ob gene…………………………………………………………………Obesity gene

Obese DM…………………………………………………… Obese Diabetes Mellitus

Obese NDM…………………………………………………..Obese Non-Diabetes Mellitus

Ob-R…………………………………………………………………Obese Receptor

Ob-Ra…………………………………………………………………...Obese Receptor-a

Ob-Rb…………………………………………………………………...Obese Receptor-b

Ob-Rc………………………………………………………….. Obese Receptor-c

Ob-Rd……………………………………………………………… Obese Receptor-d

Ob-Re…………………………………………………………………...Obese Receptor-e

Ob-Rf …………………………………………………………………...…Obese Receptor-f
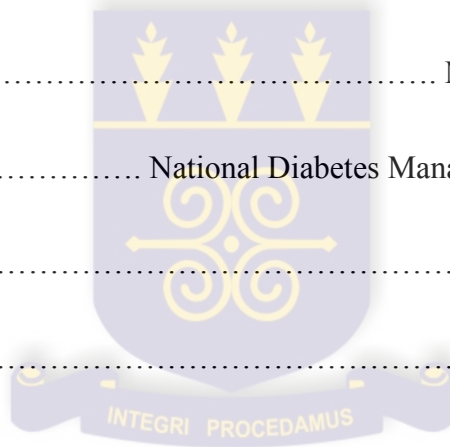
OGTT…………………………………………………… Oral Glucose Tolerance Test

PEG…………………………….. Polyethylene Glycol-coupled Cholesterol Esterase

P-value……………………………………………………….Probability Value

r………………….…………………………………Correlation Co-efficient

RBG……………………………………………….....Random Blood Glucose

SAD……………………………………………….. Sagittal Abdominal Diameter

SBP……………………………………………………….Systolic Blood Pressure

SPSS…………………………………………… Statistical Package for Social Sciences

T1DM …………………………………………………..Type 1 Diabetes Mellitus

T2DM………………………………………………….. Type 2 Diabetes Mellitus

TMB……………………………………………….3'3'5'5'-tetramethylbenzidine

TNF-α …………………………………………….Tumour Necrosis Factor Alpha

VLDL……………………………………………. Very Low Density Lipoprotein

WC……………………………………………………….....Waist Circumference

WHO…………………………………………………… World Health Organization

WHR………………………………………………………..Waist to Hip Ratio

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1.0 INTRODUCTION

1.1   BACKGROUND

Obesity has reached epidemic proportion globally, and is increasing in an alarming rate in Ghana (Benkeser *et al.,* 2012; Biritwum *et al.,* 2005). A study conducted in Accra and its surrounding communities, Ghana by Amoah *et al.,* (2003), indicated that, one quarter of the adult population were overweight and one in seven adults were obese of which the prevalence of overweight and obesity were higher in urban than the rural Ghana. The prevalence of obesity in Ghana as at 2008 was reported to be 7.5% (Ghana Demographic and Health Survey, 2009).

Coincidence with the high rates of obesity, the prevalence of type 2 diabetes mellitus is also escalating. Reports by International Diabetes Federation, (2013), have shown that, 440,000 Ghanaians aged 20 – 79 years have diabetes mellitus with eighty percent (80%) yet to be diagnosed. Approximately 80% of subjects with type 2 diabetes are obese (Dixon and O'Brien, 2002; Mehmet *et al.,* 2004). Type 2 diabetes mellitus has been defined based on a person's resistance state to insulin which is associated with hyperglycaemia (American Diabetes Association, 2012). Therefore, measurement of biochemical markers for detection of type 2 diabetes mellitus in risk groups such as obese Ghanaian subjects aged is necessary (Garber *et al.,* 2008; Dehghan *et al.,* 2007). Adipose tissue secrete leptin (Zhang *et al.,* 1994) and leptin has been shown to circulate at level of 5 – 15ng/ml in lean individuals (Sinha *et al.,* 1999). Leptin increases in humans with increasing body fats and it is thought to be involved in regulating food intake, energy storage and lipid metabolism (Attele *et al.,* 2002). Leptin is a potential determinant of obesity and its complications since it correlate strongly with adiposity

1

(Mohammedzadeh and Zarghami, 2013). Higher levels of leptin (>15ng/ml) in an individual increases the risk of type 2 diabetes mellitus through weight gain (Marguerite *et al.,* 1999). Some studies have hypothesized that, leptin is related to obesity and insulin resistance, and therefore between obesity and type 2 diabetes mellitus (Mohammedzadeh and Zarghami, 2013; Freddy *et al.,* 2011; Bradley *et al.,* 2001; Mohamed-Ali *et al.,* 1999).

Inflammation is a crucial element in pathogenesis of diabetes mellitus. Larger body fat mass of evidence suggest that, on-going low-grade inflammation may be implicated in insulin resistance and type 2 diabetes mellitus (Chan-Hee *et al.,* 2012; Pickup, 2004). Diabetes mellitus is also recognized as a state of low-grade inflammation (Ruth and Lori, 2005). Inflammatory molecules secreted by adipose tissue and visceral adiposity may pose an endocrine effect leading to insulin resistance (Tataranni and Ortega, 2005; Pickup 2004). Some studies have shown that, in the obese state, the adipocytes serves as an important source for producing inflammatory molecules, primarily due to accumulation of macrophages (Jiao and Xu, 2008) with a downstream stimulation of the liver to produce C-reactive protein (Chan-Hee *et al.,* 2012; Schuster, 2010). Studies by Shemesh *et al.*, (2007), and Santos *et al.*, (2005), showed a strong positvie association between C-reactive protein and measures of obesity, such as BMI and waist circumference. Other epidemiological studies have also shown that, increased C-reactive protein (CRP) levels above the normal reference range (0.0 – 0.3ng/ml) predict the development of type 2 diabetes mellitus (Dehghan *et al.,* 2007; Tabak *et al.,* 2010; Wannamethee *et al.,* 2008; Thorand *et al.,* 2007).

Leptin and C-reactive protein have been implicated in pathophysiology of obesity related insulin resistance (Hajer *et al.,* 2008; Martin *et al.,* 2008). Several studies have confirmed that, elevated levels of both leptin and C-reactive protein are common

features of obesity (Hoffler *et al.,* 2009; Zeman *et al.,* 2009). This presents an ideal diagnostic assay to determine type 2 diabetic subjects as well as predict high risk of future development of type 2 diabetes mellitus in obese subjects. The study therefore, aims to compare serum leptin and C-reactive protein concentration in obese subjects with and without type 2 diabetes mellitus at Korle-Bu Teaching Hospital (KBTH), Accra.

1.2   PROBLEM STATEMENT

The prevalence of obesity has been reported to increase in Ghana (International Diabetes Federation, 2013; Benkeser *et al.,* 2012; Biritwum *et al*., 2005; Amoah *et al.,* 2003). Obesity constitutes a critical risk factor for the development of many life threatening diseases, particularly in insulin resistance and type 2 diabetes mellitus (Jiao and Xu, 2008; Ceriello *et al.,* 2001; Meyre and Froguel, 2006). Obese individuals produced more leptin due to larger fat mass (Mohammedzadeh and Zarghami, 2013; Mehmet *et al*., 2004; Wei *et al.,* 1997). Coleman and Hermann, (1999), in a study reported that, insulin regulates leptin concentration and that, leptin resistance in the β-cells of the pancreas leads to inhibition of insulin secretion resulting in development of type 2 diabetes mellitus (Moran and Phillip, 2003). Obesity is also known to be an inflammatory condition and C-reactive protein levels have been shown to increase in obese persons' with insulin resistance and type 2 diabetes mellitus (Freeman *et al.,* 2002; Festa *et al.,* 2000). Leptin and C-reactive protein levels as determinant of obesity related cause of type 2 diabetes mellitus has not been determined in obese Ghanaians. Little has been done to predict the possibility of occurrence of type 2 diabetes mellitus.

1.3   JUSTIFICATION

Reports by International Diabetes Federation (2013) have shown that, 382 million people worldwide have diabetes mellitus, 19 million in Africa, and 440,000 in Ghana with an estimated 2% death rate. Obese individuals with little or no physical activity accounts for around 60% of the burden for type 2 diabetes mellitus (World Health Organization, 2007). Studies have shown that, approximately eighty percent (80%) of population with type 2 diabetes are either obese or overweight (Rajesh *et al.,* 2013; World Health Organization, 2007; Mehmet *et al*., 2004). Delaying the onset or preventing the occurrence of type 2 diabetes mellitus in risk individuals is one strategy that can be used to reduce the global burden (Diabetes Prevention Program Research Group, 2002). However, reducing type 2 diabetes mellitus burden in obese individuals has progressed minimally, especially in African countries and Ghana is no exception. Whereas effective therapy options are being sought for, specific and sensitive diagnostic markers are needed to pre-diagnose type 2 diabetes mellitus more especially in obese Ghanaians. Type 2 diabetes mellitus being a metabolic disorder and considered as a pro-inflammatory condition. Leptin and C-reactive protein levels have been implicated in insulin resistance and type 2 diabetes mellitus and measurement of these markers (leptin and C-reactive protein) might improve the prediction of type 2 diabetes in risk group such as obese Ghanaian subjects. Little or no data is available for the obese diabetic Ghanaian using leptin and C-reactive protein levels as diagnostic markers in diabetes investigation. Based on the current trends, researching into diagnostic marker is necessary to address the growing concern of obesity and obesity related type 2 diabetes mellitus.

1. 4   AIM OF STUDY

The study aims to compare serum leptin and C-reactive proteins concentration in obese subjects with and without type 2 diabetes mellitus at Korle-Bu Teaching Hospital (KBTH), Accra.

1.5   SPECIFIC OBJECTIVES

The objectives of the study are;

- To determine whether serum leptin and C-reactive protein levels vary in obese Ghanaians with or without type 2 diabetes mellitus.

- To find the correlation between the markers (serum leptin and C-reactive protein) and anthropometric indices (such as BMI, percentage body fat, percentage muscle mass, and visceral fats) that have been implicated in diabetes mellitus.

- To determine whether there is an association between the markers (serum leptin and C-reactive protein) and lipid profiles of the study subjects.

- To determine whether there is an association between the lipid profiles and anthropometric indexes.

1.6   HYPOTHESIS

There is no significant difference between levels of serum leptin and C-reactive protein in obese Ghanaians with or without type 2 diabetes mellitus

CHAPTER TWO

2.0   LITERATURE REVIEW

2.1.0   OBESITY

Obesity is defined as excessive accumulation of fats in adipose tissues to a stage that health and well-being is impaired (World Health Organization, 2000). The aetiology of obesity has been related to several conditions including excessive caloric food intake, sedentary lifestyle, and lack of physical activity (World Health Organization, 2007).

Obesity is strongly associated with a wider range of conditions such as back pains, sleep apnoea, reproductive and mental health problems (American Diabetes Association, 2012). Adolescents and children who are obese and/or overweight often face some of the same health conditions as adults and this may affect their peer-group relationship as well as self-esteem (Amy and Erinn, 2009). Obesity is a known risk factor of many health complications including type 2 diabetes mellitus, cardiovascular disease, osteoarthritis, and some cancers (American Diabetes Association, 2012).

2.1.1   PREVALENCE OF OBESITY

Report from World Health Organization (2006) have shown that, the rate of increased in the prevalence of obesity in developing countries particularly in urban areas is more rapid than in developed countries. In developed countries, obesity is shown to be similarly high in men and women (Seidell and Flegal, 1997). However, it is not the same in Africa as a review of obesity prevalence in West Africa between 2000 and 2004 reported 10% of adults' obesity, with women having the higher prevalence (Abubakari and Bhopal, 2008; Oti *et al.,* 2013). The World Health Organization (2005) estimated that, as much as 20-50% of urban populations in Africa were either overweight or

obese, three quarters of the obese population worldwide are projected to be in non-industrialised countries by 2025. Urbanization and westernization are the two popular cited theories for rapid increase in obesity and overweight in (Benkeser *et al.,* 2012). Urbanization has led to a cheaper means of personal transport and multiple mechanical and electrical aids which reduces physical effort in homes and at work places (James, 2008; Parkinson *et al.,* 2010). Reduced physical activity and sedentary life style with high caloric food intake are some of the contributing factors to increasing obesity.

In Ghana, obesity prevalence is increasing in an alarming rate in recent times (Benkeser *et al.,* 2012; Biritwum *et al.,* 2005). The "adult obesity country rankings" by International Association for the study of Obesity rated Ghana 100th out of 142 countries in the percentage global obesity prevalence (adult obesity category) based on the female obesity prevalence in 2008 (9.3%) (Ghana Demographic Health Survey, 2009). Again, the adult prevalence of obesity in Ghana was 7.5% in 2008 (Ghana Demographic Health Survey, 2009). Obesity is less common in the northern part of Ghana than the southern part. Among the ten (10) administrative regions, obesity prevalence has remained persistently high in Accra (Greater Accra) which is most urbanised region in Ghana. Some of the reasons are attributed to levels of urbanization and differences in lifestyle behaviours. Amoah *et al.,* (2003) reported that, overweight and obesity were higher in subjects living in urban centres than those in rural communities, subjects with tertiary education than those who did not have formal education, and Akans and Ga tribes than other tribes. Women obesity was also reported to higher than men obesity in the urban Ghana (Biritwum *et al.,* 2005; Rosemary *et al.,* 2007). The percentage of overweight and obese women between the ages of 15 – 49 years rose from 25% to 30% between 2003 and 2008 with highest value among urban women (Ghana Demographic Health Survey, 2009).

2.1.2   TECHNIQUES FOR ESTIMATIONS OF TOTAL BODY FATS

The development of imaging technologies has help in assessing body composition / adipose tissue distribution. There are numerous techniques available; computed tomography (CT), multi-compartment models, such as underwater weighing, dilution techniques and dual-energy X-ray absorptiometry (DXA) are all reliable methods to obtain accurate measures of total body fat (Snijder *et al.,* 2006) (table 2.1).

Table 2. 1: Techniques in estimating total body fat and fat distributions

Method	Capable of measuring total body fat	Capable of measuring fat distribution	Application in large population studies
CT	Moderate	Very high	Low
MRI	High	Very high	Low
DXA	Very high	High	Moderate
Densitometry	Very high	Very low	Low
Dilution technique	High	Very low	Moderate
BIA	Moderate	Very low	High
Anthropometry			
BMI	Moderate	Very low	Very high
WC, HC, WHR	Low	High	Very high
Skinfolds	Moderate	Moderate	High

CT (computed tomography); MRI (magnetic resonance imaging); DXA (dual- energy X-ray absorptiometry); BIA (bioelectrical impedance analysis); BMI (body mass index); WC (waist circumference); HC (hip circumference); WHR (waist-to- hip ratio); SAD (sagittal abdominal diameter) (Snijder et al., *2006).*

With computed tomography (CT), values associated with adipose tissue, muscle mass, and bone tissues can be scan to generate cross-sectional images where one can clearly

distinguish the three (adipose tissue, muscle mass and bone tissue) and calculate their respective cross sectional area (André and Jean-Pierre, 2013).

Bioelectrical impedance analyser (BIA) can also be used to measure central obesity and adiposity. BIA is monitor and scale that estimates the body mass index (BMI), body fat percentage (% body fat), skeletal muscle percentage (% muscle mass), and visceral fat by the bioelectrical impedance method. This monitor and scale send an extremely weak electrical current of 50 kHz and less than 500 µA through the body to determine the amount of water in each tissue (muscle, blood, bones and body tissues) (Omron model, Omron Health Care, USA).

However, because lack of these equipment, time and cost, these method are not routinely use in the clinics, hence, the most common method being body mass index (BMI) (quetelet index).

Body mass index (BMI) is a statistical measure which compares a persons' weight in kilograms over height in meters square. Individuals with BMI higher or equal to 30 kg/m^2 are considered obese whiles those with BMI of 25kg/m^2 but less than 30 kg/m^2 are considered overweight or pre-diabetes (World Health Organization, 2000; Seidell and Flegal, 1997). Although body mass index is a useful clinical tool that correlates with obesity, it has its limitations, thus, BMI does not differentiate between weight associated with fat and weight associated with muscle mass (Chan and Woo, 2010). For example, well-trained body builders have a very low percentage of body fat, but their BMI may be in the overweight range because of their large muscle mass (Snijder *et al.,* 2006).

2.2.0   DIABETES MELLITUS

Diabetes mellitus is a group of metabolic disorder characterized by chronic higher blood glucose (hyperglycemia) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2012). The characteristic symptoms include; thirst, polyuria, polydipsia, sometimes polyphagia, blurring of vision, and weight loss (International Diabetes Federation, 2003). Long-term complication of diabetes mellitus include; retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, and Charcot joint, and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction (American Diabetes Association, 2014).

2.2.1   CLASSIFICATION OF DIABETES MELLITUS

Based on the etiology of diabetes mellitus, it is grouped into type 1, type 2, gestational and other forms. Type 1 diabetes mellitus is an autoimmune disease which involves destruction of beta-cells of the pancreas usually leading to absolute insulin deficiency. Whiles type 2 diabetes mellitus is a metabolic disorder that results from the body's inability to make effective use of the insulin produced (American Diabetes Association, 2012). Type 2 diabetes mellitus is much more common than type 1 diabetes mellitus and account for about 90% diabetes worldwide. The risk of developing type 2 diabetes mellitus increases with age, obesity, and lack of physical activity (American Diabetes Association, 2012; Garber *et al.*, 2008; Dehghan *et al*., 2007).

2.2.2 PREVALENCE OF DIABETES MELLITUS IN GHANA

Diabetes mellitus is increasing steadily and it's gradually becoming a global epidemic; it is estimated that, diabetes will be the seventh cause of death by 2035 (International Diabetes Federation, 2013).

In 2003, the International Diabetes Federation estimated that, approximately 194 million people around the world had diabetes and this figure was projected to rise to 333 million by 2025. However, within 10 years, the figure had increased to about 382 million people worldwide reported to have diabetes mellitus with a prevalence rate of 8.3% and 46% of persons yet to be diagnose (International Diabetes Federation, 2013). Type 2 diabetes mellitus is much more common than type 1 diabetes mellitus and account for about 90% diabetes worldwide (American Diabetes Association, 2012).

Continentally, Africa recorded the lowest prevalence with highest mortality rate (76%) of diabetes in 2013 (International Diabetes Federation, 2013).

The prevalence of diabetes mellitus in Ghana as determined by International Diabetes Federation (2013) standard was 3.35% of which 82.6% of diabetic cases yet to be diagnose. Amoah et al., (2002) recorded 6.4% prevalence rate for diabetics and 10.7% for impaired glucose tolerance (IGT) in a community in Accra, Ghana. GDHS survey recorded 8.0% prevalence in 2008 (Ghana Demographic Health Survey, 2009).

2.2.3 DIAGNOSIS OF DIABETES MELLITUS

Random blood glucose (RBG), urine glucose and urine ketone bodies are usually done to screen for diabetes mellitus. But the common laboratory tests for diagnosing diabetes mellitus in Ghana are fasting blood glucose (FBG), Random blood glucose (RBG), oral glucose tolerance test (OGTT) and glycated haemoglobin (HbA$_{1C}$). The criteria for the

diagnosis of diabetes mellitus are based on the clinical signs and symptoms of hyperglycemia and fasting plasma glucose values of ≥ 7.0 mmol/L (> 6.1 mmol/L in whole blood), or random plasma glucose value of ≥ 11.1 mmol/L, and/or 2-hour plasma glucose ≥ 11.1 mmol/L during an oral glucose tolerance test (OGTT) (World Health Organization, 2005).

2.3   BODY FAT

Fats in the body can be classified into; essential fat which is stored in small amounts to protect the body, and stored fat which stocked for energy during physical activity. The distribution of body fat differs in males and females; in females, excess body fat is usually distributed as subcutaneous fat and mainly peripherally (thighs, buttocks, breasts) whiles in males, there is a relative excess of body fat stored in the abdominal cavity and as abdominal subcutaneous fat (Seidell and Flegal, 1997). Susan et al., (2000) in a study reported that, subcutaneous fat secretes more leptin compared with visceral fats.

Traditionally, fat cells (adipose tissues) was seen as energy storage depot, however, current studies have shown that, adipose tissue exerts important endocrine functions. Several hormones and biologically active peptides, including leptin and other adipocytokines (adiponectin, resistin, artrial natriuretic peptide, and angiotensinogen) are synthesized in the adipose tissue (Mohammedzadeh and Zarghami, 2013; Bradley *et al.,* 2001; Mohamed-Ali *et al.,* 1999).

Leptin was initially shown to have direct action on the central nervous system (CNS) however, it is now clear that, it action can be found on other peripheral organs such as

the pancreas, liver, skeletal muscle and gastrointestinal system (Tritos and Mantzoros, 1997; Mantzoros and Moschos, 1998).

Matsuda and Shimomura (2013) found that, fats cells are the main source of reactive oxygen species, which may contribute to the development of obesity-associated insulin resistance leading to development of type 2 diabetes and its associate complications.

In most studies, estimation of body fat is done by measuring waist circumference (WC), body mass index (BMI) and waist to hip ratio (Huffman *et al.,* 2010; Bray *et al.,* 2008; Freeman *et al.,* 2002) but this indexes are not able to distinguish between subcutaneous fat and visceral fat.

2.4   VISCERAL FAT

Visceral fats are fats found in the abdomen and surrounding vital organs. The distributions of fats in the body vary among men and women. Men have more of visceral fats and women has more of subcutaneous abdominal fat at both computed tomography (CT) cross-sectional measurement (Bray *et al.,* 2008).

Metabolically, visceral fats are considered to be more active in producing free fatty acids (FFAs) (Susan *et al.,* 2009). Some studies have shown that, too much visceral fat is thought to be closely linked to increase levels of free fats in the bloodstream, which may lead to conditions such as high cholesterol, heart disease and type 2 diabetes mellitus (Rebrin *et al.,* 1996). Furthermore, the more free fatty acids (FFAs) sent to the liver, the increased in hepatic production of glucose, and this results in decreased hepatic insulin clearance, which in turn leads to insulin resistance (Rebrin *et al.,* 1996; Mittelman and Bergman, 2000).

Several epidemiological studies have proposed a strong association between visceral fat and metabolic risk factors than subcutaneous abdominal fat (Despres *et al.,* 2008; Nicklas *et al.,* 2003). Ross *et al.,* (2002) reported a markedly different levels of insulin resistance and glucose tolerance in individuals matched for subcutaneous abdominal fat but with different degrees of visceral fat. Surgical removal of abdominal subcutaneous fat in obese subjects did not show any changes in metabolic improvements (Klein *et al.,* 2004) but removal of visceral fat led to metabolic improvements (Thorne *et al.,* 2002). Adipose tissue has been shown to secrete a number of inflammatory mediators (Lemieux *et al.,* 2001) but a much higher quantities of these inflammatory cytokines are secreted by the visceral fat (Lemieux *et al.,* 2001; Festa *et al.,* 2001). Hence visceral fat may be the distinguishing factor separating metabolically healthy obese individuals from obese individuals who are not metabolically healthy (Susan *et al.,* 2009).

Association between obesity and type 2 diabetes has been reported and well documented, and some of the studies have hypothesized that, metabolic effects of visceral adiposity may explain the association (Despres, 1993). Pouliot *et al.,* (1992) and Despres *et al.,* (1990) in their studies found that, obese individuals with low levels of visceral fats had low levels of glucose tolerance compared with lean controls. André and Jean-Pierre, (2013) found an increased in glycemic and insulinemic in obese subjects with high levels of visceral fats in response to an oral glucose challenge, indicating that, these subjects were truly at high risk of developing type 2 diabetes. These implies that, obese individuals with high levels of visceral adiposity are predisposed to risk of developing type 2 diabetes mellitus.

There are several techniques in estimation of visceral fats. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are some of the techniques (Snijder *et al.,* 2006) that are used, however, these two methods are expensive and are obviously not

suitable for large-scale epidemiological studies. Bioelectrical Impedance Analyser (BIA) is another tool that can be employ to measure visceral fats and with bioelectrical impedance analyser, visceral fats value greater than 9 (> 9) is considered high (Omron model, Omron Health Care, USA). The common and most widely used is the ratio of waist to hip circumference (WHR). The waist and hip circumferences is measured with a tape measure to the nearest 0.5cm. A waist-hip ratio (WHR) greater than 1.0 in men and greater than 0.85 in females defines obese individual (Han *et al.,* 1997; Snijder *et al.,* 2006).

2.5   ETHNICITY IN DIABETES MELLITUS

There are significant ethnic differences in diabetes risk even though obesity is a known risk of type 2 diabetes mellitus. Cleland and colleagues (2005) reported that, South Asians with lower body mass index (BMI) develop type 2 diabetes mellitus decades earlier in life than that of white Europeans (Cleland and Sattar, 2005). A cross-sectional study of 490,288 subjects using Biobank data from the United Kingdom (UK) also shared similar claims where "South Asians with BMI of 22 kg/m^2 had equal prevalence of type 2 diabetes to white Europeans with BMI of 30 kg/m^2" (Sattar and Jason, 2014). In china, the corresponding BMI for both men and women were 26 and 24 kg/m^2 respectively (Sattar and Jason, 2014; Ntuk *et al.,* 2005). Some studies hypothesized that, South Asians have higher levels of liver fat than Europeans (Anand *et al.,* 2011; Petersen *et al.,* 2006) and this may predisposed them to the risk of type 2 diabetes mellitus. These may give an idea as to why prevalence rate of type 2 diabetes mellitus increases in countries such as China and India as obesity prevalence increases.

15

In Ghana, Amoah *et al.,* (2003) reported higher prevalence of obesity among Ga and Akan tribes than other tribes. The population of Accra is predominantly Ga's. Traditionally among the Ga tribe, *kenkey,* a high carbohydrate food, is the prominent diet (Collins *et al.,* 2014). Increasing car ownership and use of public transport (popularly known as '*trotro'*) among populaces in Accra has also contributed to low physical activity patterns. These environmental factors decreased energy expenditure, thus contribute to obesity among this ethnic groups.

2.6   AGEING, OBESITY AND TYPE 2 DIABETES MILLITUS

As the world's population grows older due to advances in medical technology, health and nutrition, age-related diseases are becoming a major concern. "Two of the greatest crisis that civilisation faces in the 21st century are the predicted rapid increases in the ageing population and levels of metabolic disorders such as obesity and type 2 diabetes mellitus" (Rana *et al.,* 2014). This may be due to the fact that, as human beings ages, peripheral tissues become less sensitive to the actions of the insulin secreted from the pancreas in response to post-prandial increases in blood glucose (Amati *et al.,* 2009).

Moreover, as the individual ages, total body composition also changes (Snijder *et al.,* 2006). It has been proposed that, optimal BMI increases with age (Jackson *et al.,* 2002). This may be due to environmental factors such as reduce physical activity and high caloric-dietary intake. Studies by Anand *et al.,* (2011) have shown that, at any given body mass index (BMI), average older adults have more fat than younger adults.

As people grow older, more fat accumulates in the abdomen and less fat at the extremities and these accounts for more visceral fat in older persons than in younger persons per the absolute levels of waist circumference (Snijder *et al.,* 2006). Type 2

16

diabetes mellitus is common among all the health problems of obesity. Higher body mass index (BMI) and waist circumference (WC) are significantly related to the incidence of type 2 diabetes in men and women (Chan and Woo, 2010).

2.7   ADIPOCYTOKINES

Adipocytokines are bioactive molecules secreted by the adipose tissue. Adipose tissue serves as the major storage site for fuel, primarily in the form of triglycerides. Adipose tissue also plays an active role in many homeostatic processes including energy expenditure, appetite regulation, and glucose regulation (Schuster, 2010).

Fat cell size is usually enlarged 2-4 times in obese individuals and in extreme obesity hyperleptinaemia could be an independent risk marker for type 2 diabetes mellitus after adjustment for body fat mass (Seidell and Flegal, 1997).

Elevated free fatty acids are commonly seen in obesity and unmanaged diabetes mellitus (Bullo *et al.,* 2003). Increased levels of circulating free fatty acids are inhibitory to beta cells and lead to reduced beta cell function with diminished insulin secretion (Jiao and Xu, 2008). Free fatty acid (FFA) and tumour necrosis factor alpha (TNFα) activate regulators of inflammation which is thought be involved in the development of obesity-related insulin resistance (Jiao and Xu, 2008) with downstream increased C-reactive protein levels by the liver in the systematic circulation (Schuster, 2010; Bullo *et al.,* 2003).

Fats may be distributed peripherally or intra-abdominal in the body. The adipose tissue in intra-abdominal fat (visceral fat) functions as a metabolically active hormonal "organ"; a source of macrophages production as well as secretes adipocytokines (Tataranni and Ortega, 2005). These adipocytokines include tumour necrosis factor

17

alpha (TNF-α), interleukin-6 (IL-6) (Tataranni and Ortega, 2005). Adipocytokines contribute to increased risk of vascular complications in patients with type 2 diabetes mellitus by affecting inflammatory process (Matsuda *et al.,* 2002). These inflammatory markers (interleukin-6 and tumour necrosis factor alpha) contribute to changes in systemic metabolism of obese subject which result in insulin resistance (Mohamed-Ali *et al.,* 1998) (figure 1).

Figure 2. 1: Shows the adverse effects of excessive accumulation of fats in the adipose tissues.
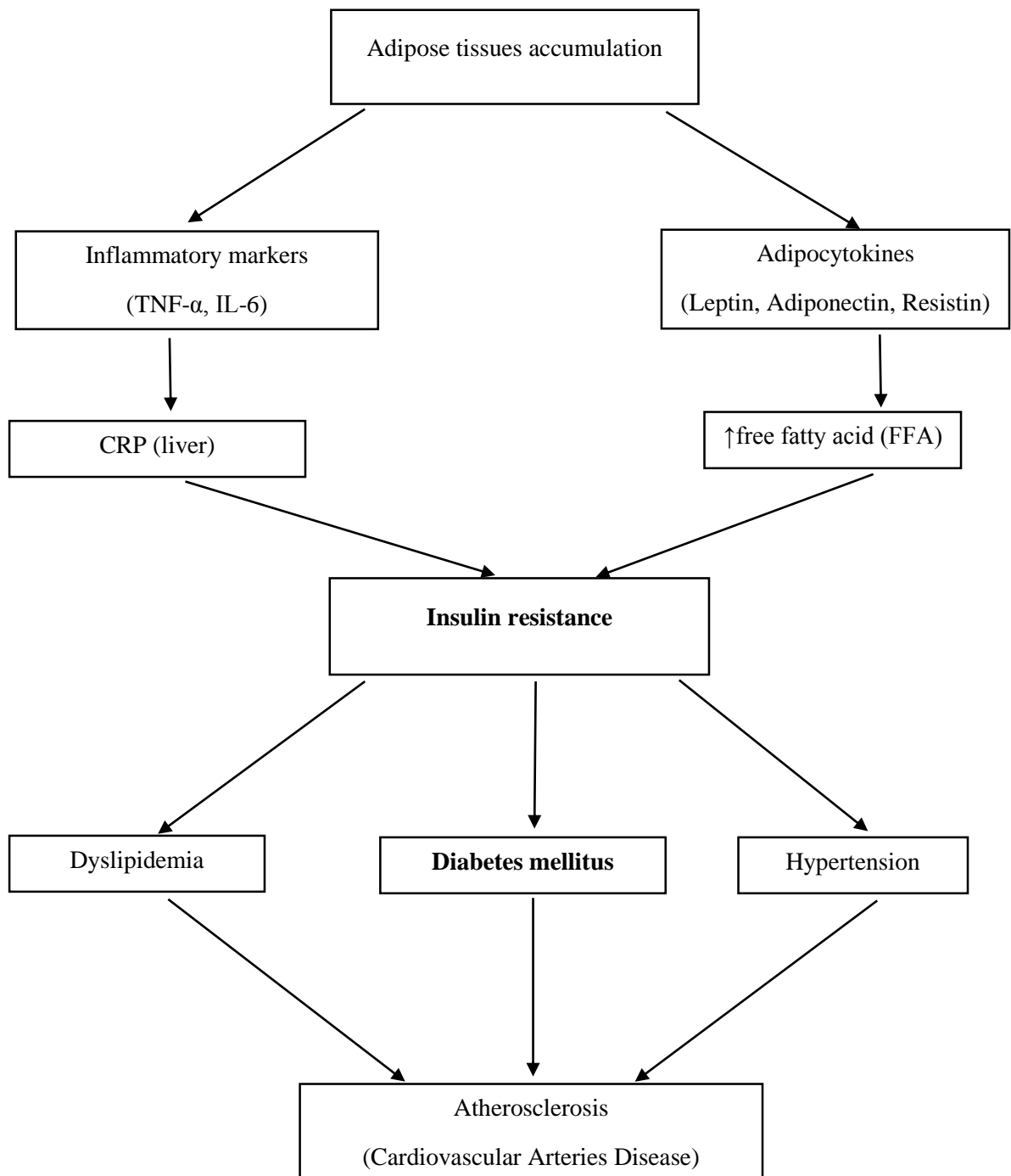
TNF-α (tumour necrosis factor alpha), IL-6 (interleukin-6), CRP (C-reactive protein)
*Source: (*Deepa *et al.,* 2007*;* Matsuda *et al.,* 2002*;* Mohamed-Ali *et al.,* 1999*)*

2.8   LEPTIN

Leptin, a 16 kDa protein, is produced by adipose tissues and circulates at a level of 5 –
15 ng/ml in lean subjects (Sinha *et al.,* 1999). Leptin, a product of obese (*ob*) gene, was
identified by a positional cloning of the *ob* gene which determines obesity in *ob/ob*
mice (Zhang *et al*., 1994).

Six different isoforms of leptin receptors (*Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re and
Ob-Rf*) have been identified with this transmembrane protein spanning the cell
membrane once (Tartaglia, 1997; Mukesh *et al.,* 2010). Most of the biological effect of
leptin appears to be mediated by the *Ob-Rb* because it is highly expressed in the
hypothalamus (Mukesh *et al.,* 2010). By binding to hypothalamic receptors, Leptin has
been found to influence several neuropeptides in the hypothalamus (Alexander *et al*.,
2013) leading to suppression of the appetite in humans, thus regulating food intake,
energy storage and lipid metabolism (Attele *et al*., 2002).

Leptin levels reduce in the fasting state and increases several days of overfeeding. That
may explain the strong correlation between serum leptin levels and body fat mass found
in obese individuals (Ren, 2004; Mukesh *et al.,* 2010).

Studies have shown that "obesity may be the consequences of leptin resistance"
(Emanuelli *et al.,* 2001) since leptin is a potential determinant of obesity and its
complications (Wei *et al.,* 2004). Leptin correlate strongly with adiposity
(Mohammedzadeh and Zarghami, 2013; Wei *et al.,* 1997) since its concentration in

blood correlate with body mass index (Mehmet *et al*., 2004) and high levels above the upper reference limits (>15 ng/ml) increases risk of developing type 2 diabetes through obesity (Marguerite *et al*., 1999). Furthermore, in obese human subjects, leptin concentrations are directly proportional to body fat mass, signifying leptin resistance, rather than leptin deficiency (Al-Daghri *et al.,* 2003).

Leptin has the ability of regulating insulin secretion from the pancreatic islet cells (Ceddia *et al.,* 1999). Moreover, receptors of insulin are located in the same key hypothalamic areas as leptin receptors. Whereas insulin secretion is stimulated acutely in response to meals, leptin secretion is not (Javad and Doudi, 2005). Insulin is a main candidate because it is the major regulator of energy utilization and adipose tissue metabolism. Studies have shown that β-cell of the pancreas has receptors for leptin (Kieffer *et al.,* 1996) increasing the possibility that, leptin may modulate insulin secretion. Moran and Phillip, (2003) indicated that, resistance to leptin in β-cell might cause inhibition of insulin secretion resulting in hypoinsulinaemia leading to development of type 2 diabetes mellitus. However, some studies have shown an inverse association between leptin receptor levels and the risk of developing type 2 diabetes independent of serum leptin levels (Sun *et al*., 1998).

Serum leptin levels are also reported to be affected by gender. Gender differences in serum leptin may be related to difference in sex hormones (van Gaal *et al.,* 1999); thus the inverse relationship between leptin and testosterone levels in males (Wei et al., 1997; Tasaka *et al.,* 1997) and the stimulation of leptin mRNA production by 17β-estradiol which is one of the females' sexual hormones (Sweeney, 2002).

2.9   C-REACTIVE PROTEIN (CRP)

C-reactive protein is an acute phase protein synthesized in the liver and increases in concentration following infection, inflammation or trauma. CRP was discovered in human by Tillett and Frances in 1930 (Tillett and Francis, 1930). This protein is called C-reactive protein because it is able to effect precipitation of somatic C-polysaccharide of *Streptococcus pneumonia.* It has been proposed that, CRP aid in complement activation, influence phagocytic cell function, and augment cell mediated cytotoxicity. In humans, C-reactive protein are present in trace constituent of serum at levels less than 0.3mg/ml (Eckersal, 2000).

Metabolic imbalances of obesity are associated with inflammation. Obesity is considered as a state of chronic inflammatory, as it can be seen by increased levels of pro-inflammatory cytokines including interleukine-6 (IL-6), interleukine-1 (IL-1), and tumour necrosis factor-α (TNF-α), with a downstream stimulation of liver to produce C-reactive protein and other cytokines (Chen *et al*., 2012; Shamsuzzaman *et al.*, 2004; Trayhurn and Beattie, 2001). The local response of inflammation is seen as an increased C-reactive protein levels in systematic circulation (Schuster, 2010).

Circulating C-reactive protein has been seen to be elevated in human with insulin resistance and type 2 diabetes mellitus (Freeman *et al*., 2002). According to Freeman *et al.,* (2002), raised C-reactive protein levels in the systemic circulation is a predictor of the development of diabetes mellitus in middle-aged men independent of established risk factors including fasting plasma triglyceride, BMI, and glucose. In addition, the same study also indicated that, men with C-reactive protein levels >4.18 mg/l have greater than three-times risk of developing diabetes mellitus compared with those with C-reactive protein <0.66 mg/l after adjustment for all other variables. Other studies demonstrate similar observation in women (Pradhan *et al.,* 2001). Some studies also

21

showed strong positive correlation between C-reactive protein and insulin resistance (Festa *et al.,* 2000). In rats, an experiment conducted indicated that, a single dose of human C-reactive protein when administered intravenously caused a profound insulin resistance in the liver *in vivo*, as assessed by euglycemic hyperinsulinaemic clamp (Liang Xi *et al.,* 2011).

Serum C-reactive protein (CRP), a marker of chronic low grade inflammation, is a reliable predictor of cardiovascular disease and a novel risk factor for diabetes (Dehghan *et al*., 2007; Ridker *et al*., 2000). Studies have shown that, pro-inflammatory cytokines including tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are elevated in patients with diabetes mellitus (Chen *et al*., 2012) and regulates genes expressed by C-reactive protein (Shamsuzzaman *et al.*, 2004; Trayhurn and Beattie, 2001). These inflammatory cytokines are secreted by adipocytes.

Overweight (BMI between 25 - 30 kg/m^2) and obese (BMI > 30 kg/m^2) individuals have large and more adipocytes and hence high level of baseline C-reactive protein (Dehghan *et al*., 2007). Overweight and obesity are known risk factors for type 2 diabetes mellitus and according to Huffman *et al*., (2010), there is a strong and independent association of C-reactive protein levels with measures of body fat and triglycerides.

CHAPTER THREE

3.0   METHODOLOGY

3.1   STUDY DESIGN

This was a cross sectional study with matched controls and it was carried out on clinically confirmed obese diabetic subjects attending the National Diabetes Management and Research Centre in Korle-Bu, and obese non-diabetic subjects. The study was carried out between the months of August 2014 and July 2015.

3.2   STUDY SITE

The study was conducted at National Diabetic Management and Research Centre (NDMRC) in Korle-Bu Teaching Hospital (KBTH) and the Department of Chemical Pathology Laboratory of the School of Biomedical and Allied Health Sciences, Public Health Reference Laboratory, Korle-Bu Teaching Hospital. Korle-Bu Teaching Hospital (KBTH) is situated in the nation's capital, Accra, Ghana, and it is the leading tertiary hospital, and the major referral centre in the country. KBTH also serves as a teaching hospital for College of Health Sciences, University of Ghana. The National Diabetic Management and Research Centre at Korle-Bu Teaching Hospital is one of the biggest tertiary healthcare centres in Ghana. Most of the complicated diabetic cases within the nation are referred to this department. Patients attending the National Diabetic Management and Research Centre are from different social and ethnic groups as well as geographical distinct areas. Thus, the demographics of the study participants were not limited to a specific social group.

3.3   SAMPLING TECHNIQUE

Purposive and simple random sampling procedures were employed. Purposive sampling was used because the study involved obese group and simple random sampling because each respondent in this group had equal chance of being selected. With obese diabetic subjects (case group), data was obtained when subjects came for a review at the National Diabetic Management and Research Centre, Korle-Bu Teaching Hospital. This was done daily over the period of data and sample collection. The case group were matched with BMI for non-diabetic subjects (control group). Obese non-diabetic subjects were randomly recruited among workers of the hospital and at Korle-Gono, nearby community to equal the female to male ratio of 2:1. Both obese diabetic and obese non-diabetic subjects who met the inclusion criteria and had agreed to participate were interviewed and fasting blood samples were collected for analysis.

3.4   SUBJECTS/STUDY POPULATION

The study population consisted of obese subjects who were non-diabetic working at the Korle-Bu Teaching Hospital and nearby community (Korle-Gorno) and obese subjects with type 2 diabetes mellitus attending the NDMRC. One hundred and sixty (160) participants were recruited of which 110 were found eligible to participate in the study. A total of one hundred (100) consented, 20 later withdrew from the study. The remaining eighty (80) participants were categorized into cases comprising of forty (40) clinical confirmed obese diabetic subjects and controls (40 obese non-diabetic subjects, apparently health persons group). The subjects were aged between 30–55 years.

Inclusion criteria

  ➢ Obese type 2 diabetic subjects between the ages of 30–55 years

  ➢ Apparently healthy obese non-diabetic subjects between the ages of 30–55 years

24

Exclusion criteria

➢ Gestational diabetic subjects, pregnant women or lactating mother

➢ Subjects who were habitual smokers

➢ Type 2 diabetic subjects with chronic illness, recent myocardial infarction (<6 months), stroke, or lower limb (s) amputation

3.5   SAMPLE SIZE DETERMINATION

The minimum number of the study participant that were enrolled for this study was obtained using the formula;

$$n \geq \frac{Z^2 \, (P) \, (1 - P)}{e^2}$$

n = minimum sample size, P = is the adult prevalence of obesity in Ghana taken as 7.5% (Ghana Demographic and Health Survey, 2009). At 95% confidence level, Z-score is 1.96 and at an allowable error of 10%, a minimum sample size was obtained;

ie

$$n \geq \frac{1.96^2 \, (0.075) \, (1 - 0.075)}{0.1^2}$$

$$n \geq 26.65 \approx 27$$

Hence, 40 subject (cases) group and 40 control (diabetic) group. Therefore, a total of 80 study participant were recruited for this study.

3.6   ETHICAL APPROVAL

The study was approved (Protocol Identification Number: MS-Et/M.6–P3.2/2014-2015) by the Institutional Ethics and Protocol Review Committee of School of

Medicine and Dentistry, College of Health Sciences, University of Ghana. On the field, detailed explanations of the purpose of the study, risk and benefits were made known to participants. Written informed consent was obtained from all participants.

3.6   STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA) was used for most of the statistical and analytical work. Descriptive statistics such as mean, median and standard deviation were used to describe the data. The obese non-diabetic group (case group) was compared to the obese diabetic group (control group) using a two-tailed student's t-test. Correlation between markers (leptin and hs-CRP) and anthropometric indices was tested by the Pearson test. For comparison of mean levels of the markers (leptin and hs-CRP) between males and females, the Mann-Whitney test was carried. Odds ratios was used for predicting the risk of developing type 2 diabetes mellitus in obese non-diabetic subjects. A probability level less than 5% (p-value < 0.05) was considered statistically significant.

3.7   DATA HANDLING/CONFIDENTIALITY

Subjects were given unique codes to correspond with the data codes. The information was accessible to only the principal investigator. Data were stored in files while in-use until the end of the study. This information will be archived for 5 years to enable the researcher respond to queries about the integrity of the study.

3.8.0 DATA COLLECTION

3.8.1 QUESTIONNAIRE

Structured questionnaires were administered to assess demographic characteristics (such as age, gender, marital status, education, occupation, and ethnicity), anthropometric measurement (such as height, weight, BMI, % body fat, % muscle mass, visceral fat, blood pressure, and pulse), medical history and medications, family history of diabetes mellitus, socioeconomic status and practice of leisure-time physical activities. The questionnaires were pre-tested at the Korle-Bu Teaching Hospital among ten obese subjects who met the criteria for the main study to determine the validity and clarity, and to also eliminate possible ambiguity. Participants were interviewed at home and work places by the principal investigator and scheduled for the clinical examination at National Diabetic Management and Research Centre in Korle-Bu Teaching Hospital.

3.8.2 ANTHROPOMETRIC MEASUREMENT

Height and weight were measured with the subjects at standing position. The height of all participants were measured using a stadiometer (Secca, Germany) and weight were measured using a Full Body Sensor Body Composition Monitor and Scale (Omron model HBF-516, Omron Healthcare, USA). The Omron body fat analyser estimates the body mass index (BMI), body fat percentage (% body fat), skeletal muscle percentage (% muscle mass), and visceral fat by the bioelectrical impedance method (BIA). The principle of the Omron body fat analyser is that, it sends an extremely weak electrical current of 50 kHz and less than 500 µA through the body to determine the amount of water in each tissue (muscle, blood, bones and body tissues) (Omron model HBF-516, Omron Healthcare, USA). Throughout the study all body measurements were made

27

according to standard protocols and cut-offs proposed by the World Health Organization (WHO).

3.8.3   MEASUREMENT OF BLOOD PRESSURE

OMRON blood pressure cuff (OMRON Healthcare, USA) was used to measure the blood pressure. This device was designed based on the oscillometric technique. The oscillations create pressure in the sphygmomanometer and gradually deflate the cuff, with the point of maximum oscillation corresponding to the mean intra-arterial pressure. The blood pressure measurement were taken from the left arm of the subjects and controls in a sitting position after at least five minutes rest by a qualified nurse.

3.8.4   BLOOD SAMPLE COLLECTION, TRANSPORT AND STORAGE

After 10 to12 hours of fasting (overnight fasting), 5ml of venous blood sample after explaining to participants was drawn from the cubical fossa of each participant. Two millilitres (2ml) of the blood sample was dispensed into a fluoride and 3ml into a gel separator tubes. Blood samples were centrifuged at 300rpm for 5minutes. Serum and plasma were separated from the cells, put into ependolph tubes and stored at -20^{0}C until assayed.

3.8.5   MEASUREMENT OF FASTING GLUCOSE

Fasting plasma glucose concentration was measured by BS 120 Mindray spectrophotometer (Mindray Medical International Limited, China) using glucose oxidase reagent (ELITech Clinical System, France) within two hours of blood collection.

Test Principle: Glucose oxidase catalyses the oxidation of glucose to give hydrogen peroxide (H_2O_2) and gluconic acid. The presence of peroxidase break down hydrogen peroxide and the oxygen released reacts with 4-aminophenazone and phenol to produce a dye [4-(p-benzoquinone-monoimme)-phenazone] whose intensity relates to concentration of glucose in plasma.
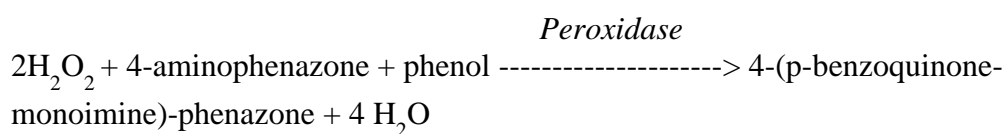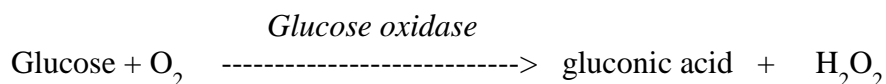
$$\text{Glucose} + O_2 \xrightarrow{\textit{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\textit{Peroxidase}} \text{4-(p-benzoquinone-monoimine)-phenazone} + 4\,H_2O$$

Assay Procedure: This is a modification of Trinder's (1969) method. Three hundred microliters (300µl) of glucose reagent (ELITech Clinical Systems, France) was pipetted into a cuvette and three microliters (3µl) of patient's serum was added. The content was well mixed and incubated for 5 minutes at room temperature. The intensity of the colour formed was measured at 500nm and the readings (absorbance) recorded.

3.8.6   MEASUREMENT OF SERUM TOTAL CHOLESTEROL

Test Principle: Cholesterol esterase hydrolyses cholesterol esters to free cholesterol and fatty acids. The free cholesterol is oxidized to cholesten-3-one and hydrogen peroxide by cholesterol oxidase. Peroxidase catalyses the reaction on hydrogen peroxide with 4-aminoantipyrine and phenol to produce a red dye (quinoeimine product).

$$\text{Cholesterol ester} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{cholesterol} + \text{fatty acid}$$

Cholesterol oxidase

Cholesterol + O_2 ----------------------------> cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol --------------------> 4-(p-benzoquinone-monoimine)-phenazone + $4 H_2O$
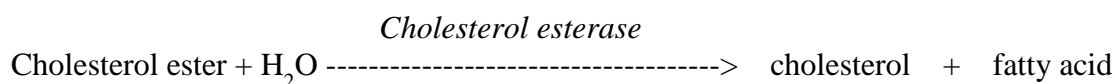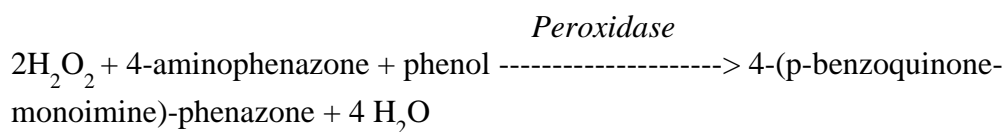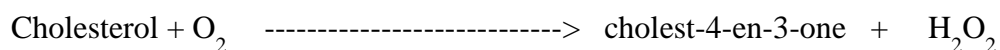
Assay Procedure: The enzymatic method described above and used in this assay is a modification of Roschlau, et al., (1974) and Allain *et al.,* (1974). Three hundred microliters (300µl) of cholesterol reagent (ELITech Clinical Systems, France) was pipetted into a cuvette and three microliters (3µl) of patient's serum was added. The content was well mixed and incubated for 5 minutes at room temperature. The intensity of the pink colour formed was measured at 500nm and the readings (absorbances) recorded.

3.8.7   MEASUREMENT OF SERUM TRIGLYCERIDE

Test Principle: Triglycerides were measured enzymatically in serum using a series of coupled reactions in which triglycerides are hydrolysed to produce glycerol. Glycerol is then oxidized using glycerol oxidase to dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase catalyses the reaction on hydrogen peroxide with 4-aminoantipyrine and phenol to produce a dye [4-(p-benzoquinone-monoimine)-phenazone].

Lipase

Triglycerides   + $3H_2O$ ------------>   glycerol   +   fatty acids

Glycerokinase

Glycerol   +   ATP ----------------------->   glycerol-3-phosphate   +   ADP

Glycerophosphate oxidase

Glycerol-3-phosphate + O2 -----------------------------> dihydroxyacetone phosphate + H_2O_2

30

$$\text{H}_2\text{O}_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\textit{Peroxidase}} \text{4-(p-benzoquinone-monoimine)-phenazone} + 2\text{H}_2\text{O} + \text{HCl}$$
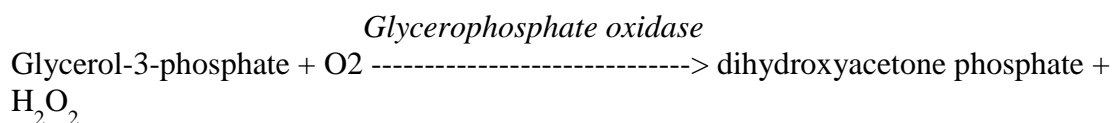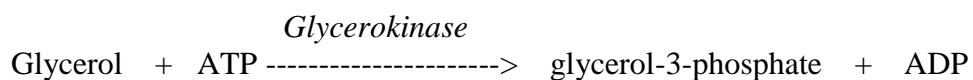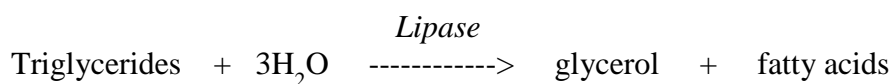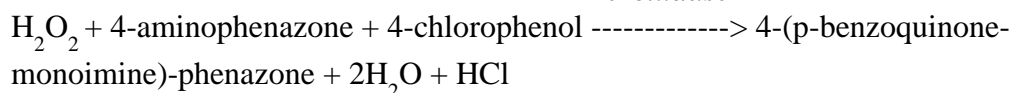
Assay Procedure: This is a modification of Trinder's (1969) method. Three hundred microliters (300µl) of triglyceride reagent (ELITech Clinical Systems, France) was pipetted into a cuvette and three microliters (3µl) of patient's serum was added. The content was well mixed and incubated for 15 minutes at room temperature. The intensity of the pink colour formed was measured at 500nm and the readings (absorbance) recorded.

3.8.8 MEASUREMENT OF HIGH DENSITY LIPOPROTEIN (HDL-CHOLESTEROL)

Test Principle: Apolipoprotein-B containing high density lipoproteins combine with sulphated alpha-cyclodextrin in the presence of magnesium ions to form complexes with ApoB containing high density lipoprotein. Polyethylene glycol-coupled cholesterol esterase (PEG-Cholesterol esterase) converts high density lipoprotein cholesterol esters (HDL-cholesteryl esters) to fatty acids and HDL-unesterified cholesterol which is acted on by PEG-cholesterol oxidase to chelestenone and hydrogen peroxide. The hydrogen peroxide react with 5-aminophenazone and N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine in the presence of peroxidase to produce a red dye (qunoneimine dye). The intensity of the colour was measured at 600nm.

ApoB containing high density lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 ---> HDL-cholesteryl esters (soluble non-reactive complexes with ApoB-containing lipoproteins)

31

PEG-cholesteryl esterase

HDL-cholesteryl esters ---------------------------> HDL-unesterified cholesterol + fatty acid

PEG-cholesterol oxidase

Unesterified cholesterol + O_2 ---------------------------> cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine + H2O + H^+ *peroxidase*--------- > qunoneimine dye + H_2O

Assay Procedure: Two hundred and forty microliters (240µl) of HDL direct reagent (R1) (ELITech Clinical Systems, France) was pipetted into a cuvette and 2.4µl of patient's serum was added. The content was well mixed and incubated for 4 minutes at room temperature. Absorbance A1 was measured. Eighty microliters (80 µl) of HDL direct reagent (R2) was then added and was well mixed and incubated for 4 minutes at room temperature and absorbance A2 was measured. The concentration of HDL was the difference in absorbance A1 and A2 measured at 600nm.

3.8.9   ESTIMATION OF LOW DENSITY LIPOPROTEIN (LDL-CHOLESTEROL)

Low-density lipoprotein Cholesterol (LDL-Cholesterol) levels were calculated using the Friedewald's formula (Friedewald *et al.,* 1972)

[LDL-Cholesterol] = [Total Cholesterol] – [HDL-Cholesterol] – [1/2.2 Triglycerides]

Results for LDL-Cholesterol were expressed in mmol/L. The limitation of this indirect method of estimating LDL-Cholesterol using Total Cholesterol, Triglycerides and HDL-Cholesterol was that, Triglycerides levels greater than 4.4mmol/L was not valid.

3.8.10 MEASUREMENT OF SERUM LEPTIN

Human serum leptin levels were detected by Enzyme Linked Immuno-sorbent Assay (ELISA) Kit (GenWay Biotech Inc., CA, USA). The Human Leptin ELISA kit is a solid phase sandwich enzyme-linked immune-sorbent assay in which a monoclonal antibody specific for human leptin has been coated onto the wells of the microtiter plates provided.

Principle: In the GenWay Human Leptin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human leptin antibody. After 60 minutes of incubation and washing, polyclonal anti-human leptin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (3,3',5,5'-tetramethylbenzidine). The reaction is stopped by addition of acidic solution (tetramethylbenzidine stop reagent) and absorbance of the yellow product is measured spectrophotometrically at 450nm. The absorbance values against concentrations of standards, and concentrations of unknown samples are determined using a standard curve (GenWay Biotech Inc., 2015).

Assay procedure: Hundred microliters (100μl) of diluted standards, quality controls, diluted buffer (blank) and patients' sera were pipetted into the appropriate wells. The microtitre plate were covered, placed on mechanical shaker (shaking at 300 revolution per minute) and incubated for 60 minutes at room temperature (25^0C – 27^0C). The wells were washed 3-times with diluted wash solution using a microtitre washer /aspirator and blotted onto a paper towel. Hundred microliters (100μl) of conjugate solution was added to each well, covered, placed on mechanical shaker (shaking at 300 revolution per minute) and incubated for 60 minutes at room temperature (25^0C – 27^0C). The

33

content of the plate were washed with diluted wash solution using a microtitre washer/aspirator and blotted onto paper towel. The amount of human leptin-Biotin bound was determined in the third incubation (incubation period of 10 minute with microtitre plate not shaking) step by addition of 100µl of substrate solution. The reaction was stopped by the addition of stop solution (100µl) which changed the colour to yellow immediately. The absorbance of each well was read within 5minutes at 450nm using a microplate reader with the reference wavelength set to 630nm.

Calculations: The standard curve was constructed by plotting the mean absorbance (y) of standards against the known concentration (x) of standard in logarithmic scale. Results of patients were interpolated from the standard curve of Human Leptin ELISA as concentrations of leptin (ng/ml) in samples (figure 3.1).
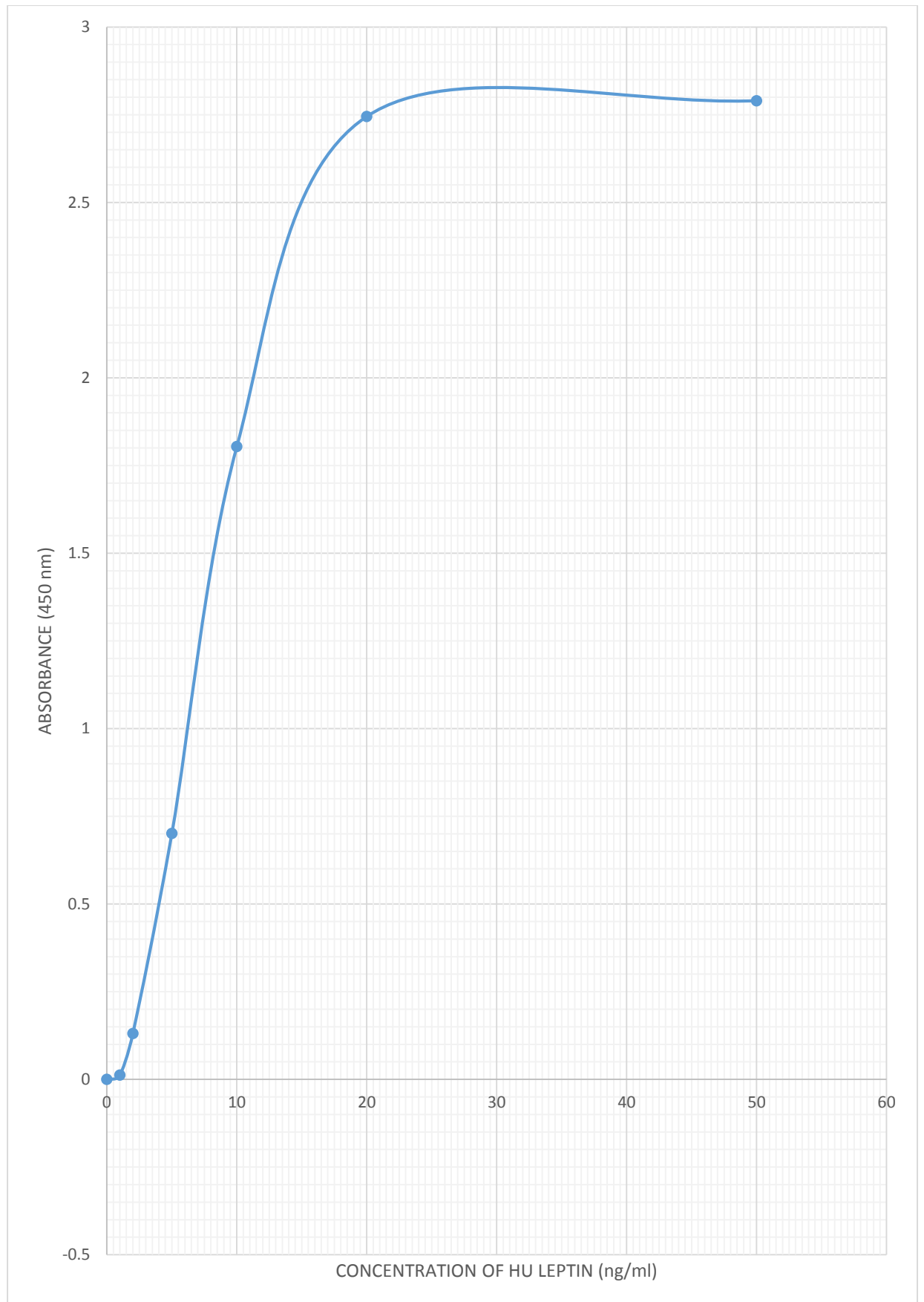
Figure 3.1: Typical standard curve for Human Leptin ELISA, Clinical Range

3.8.11 MEASUREMENT OF SERUM HIGH SENSITIVE C-REACTIVE PROTEIN

Human High Sensitive C-reactive protein (hs-CRP) levels were detected in serum by Enzyme Linked Immuno-sorbent Assay (ELISA) Kit (GenWay Biotech Inc., CA, USA). The principle of Human hs-CRP ELISA kit is a double antibody sandwich enzyme-linked immune-sorbent assay in which a monoclonal antibody specific for human hs-CRP has been coated onto the wells of the microtiter plates provided.

Principle: High sensitive C-reactive protein present in the samples reacts with the anti-CRP antibodies coated in polystyrene microtitre wells. After 3 series of washing to remove of unbound proteins, the enzyme bound to the immune-sorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of hs-CRP in the sample tested measure at 450 nm (GenWay Biotech Inc., 2015).

Assay procedure: All reagents were brought to room temperature (25^0C – 30^0C) and 100µL of standard, controls and human sera were pipetted into the pre-designated wells. The microtitre plate were covered and incubated at room temperature for 15minutes. The wells were washed 3 times with diluted wash solution using a microtitre washer /aspirator and blotted onto a paper towel. Hundred microliters (100µL) biotinylated enzyme-antibody conjugate were added to each well and incubated at room temperature (25^0C – 30^0C) for 15minutes in the dark. The wells were washed 3 times with diluted wash solution using a microtitre washer /aspirator and blotted onto a paper towel. Hundred microliters (100µl) of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution were added to each well and incubated in the dark at room temperature for 10 minutes. The reaction was stopped by the addition of stop solution (100µl) to each wells which changed the colour to yellow immediately. The absorbance of each well was read at 450nm using a microtitre plate reader and a standard curve was plotted.

Calculations: The standard curve was constructed by plotting the mean absorbance (y) of standards against the known concentration (x) of standard in logarithmic scale. Test samples values were interpolated from the standard curve (figure 3.2). These values were corrected for sera dilution factor to arrive at the hs-CRP concentration in original samples.
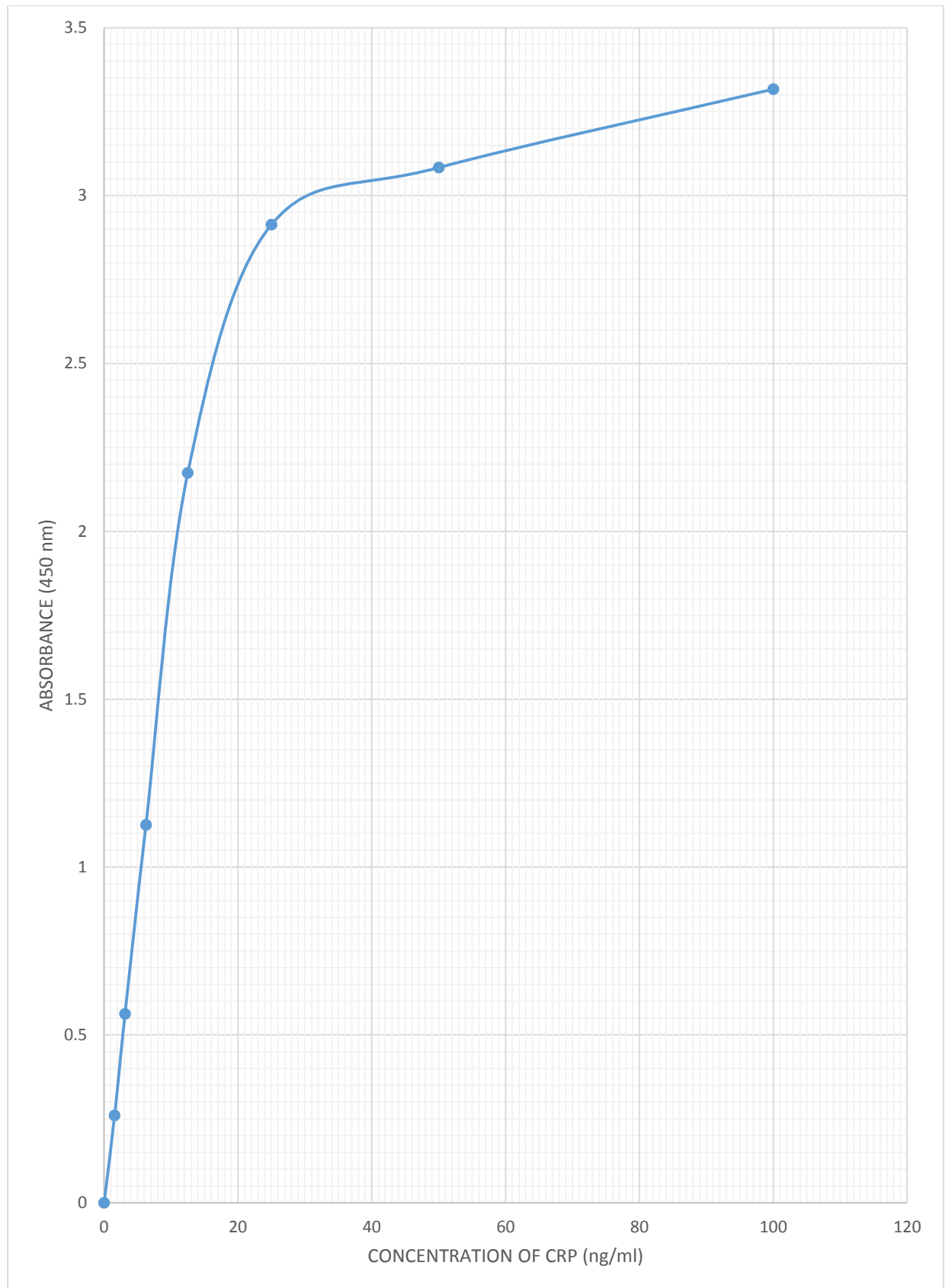
Figure 3.2: Typical standard curve for Human high sensitive C-reactive protein ELISA, Clinical Range

CHAPTER FOUR

4.0   RESULTS

4.1   SOCIO-DEMOGRAPHIC CHARACTERISTICS OF CASE AND CONTROL GROUPS

A total of 80 obese Ghanaians between the ages of $30 - 55$ years recruited for the study. The case group were obese type 2 diabetic subjects and the control group were apparently healthy obese individuals without type 2 diabetes mellitus. The case group comprised of 15 (33.3%) males and 25 (66.7%) females with an approximate ratio of 1:2; that is one male to two females. The control group also comprised of 15 (33.3%) males and 25 (66.7%) females with a ratio of 1:2 respectively (table 4.1).

Thirty-one (31) (77.5%) of the case group were married whereas single, divorced and the widowed constituted 3 (7.5%), 2 (5.0%) and 4 (10.0%) respectively. On the other hand, the control group who were married were 26 (65.0%), whiles single, divorced and widowed were 4 (10.0%), 6 (15.0%) and 4 (10.0%) as shown in table 4.1.

Obese diabetic subjects (case group) who had universal primary and secondary education were 24 constituting 60.0%, 12 (30.0%) had tertiary education, and 4 (10.0%) had no formal education. Twenty-six (26) of the obese non-diabetic subjects (control group) constituting 65.0% had universal primary and secondary education, 11 (27.5%) had tertiary education and 3 (7.5%) did not attend school as shown in table 4.1.

For occupation, 27 (67.5%) of the case group were self-employed, 12 (30.0%) government workers and 1 (2.5%) unemployed with about 90.0% of the work being sedentary. Twenty-three (57.5%) of the control group were self-employed, 5 (37.5%) government workers, and those who were unemployed were the least, 2 (5.0%) (table 4.1) also with 86.7% being sedentary workers.

39

Table 4.1: Siocio-demographic characteristic of case and controls

	VARIABLES	Case Group (40)		Control Group (40)	
		Number	Percent	Number	Percent
Sex	Male	15	33.3	15	33.3
	Female	25	66.7	25	66.7
Marital Status	Single	3	7.5	4	10.0
	Married	31	77.5	26	65.0
	Divorced	2	5.0	6	15.0
	Widowed	4	10.0	4	10.0
Educational Status	Never attended	4	10.0	3	7.5
	Primary	1	2.5	6	15.0
	Secondary	23	57.5	20	50.0
	Tertiary	12	30.0	11	27.5
Occupation	Unemployed	1	2.5	2	5.0
	Self employed	27	67.5	23	57.5
	Gov't worker	12	30.0	15	37.5
Ethnicity	Akan	15	37.5	11	27.5
	Ga	15	37.5	26	65.0
	Ewe	5	12.5	3	7.5
	Northerner	5	12.5	0	0.0
Family history of DM	Yes	23	57.5	8	20.0
	No	17	42.5	32	80.0
Herbal Remedy	Yes	8	20.0	11	27.5
	No	32	80.0	29	72.5

Data is presented as number and percentages.

40

For ethnicity, Ga and Akan tribe dominated among the obese diabetic subjects with each having 15 constituting 37.5%, Ewes were 5 (12.5%) and 5 (12.5%) Northerners. On the other hand, 26 of the obese non-diabetic group were from the Ga tribe constituting 65.0%, followed by Akan tribe, 11 (27.5%) and Ewes 3, (7.5%). Twenty-three (23) of obese diabetic subjects and 8 of the obese non-diabetic weight-matched controls had family history of diabetes constituting 57.5% and 20.0% respectively (table 4.1).

4.2   CLINICAL AND ANTHROPOMETRIC INDICES OF CASE AND CONTROL GROUP

The mean age of the obese diabetic and the obese non-diabetic group were 49.65 ± 5.73 and 47.28 ± 7.82 respectively. Difference in means for age in the two study group did not show statistical significance (P > 0.05) between both groups. The difference in means for body mass index (BMI), percentage body fat, percentage muscle mass, and visceral fat of both the obese diabetic and weight matched (obese non-diabetic group) did not also show significant difference (P > 0.05). There was however significant difference between the means of systolic blood pressure (SBP) of case and control groups (P<0.05) (table 4.2).

Table 4.2: Clinical and anthropometric indices of case and control group

Variables	Cases (40) Mean ± SD	Controls (40) Mean ± SD	P-value
Age	49.65 ± 5.73	47.28 ± 7.82	0.125
Height (cm)	164.77 ± 9.53	161.92 ± 6.81	0.132
Weight (Kg)	93.78 ± 16.03	89.82 ± 14.16	0.245
BMI (Kg/m^2)	35.55 ± 4.71	35.52 ± 5.13	0.975
% Body Fat	40.32 ± 9.62	41.71 ± 8.86	0.503
% Muscle Mass	27.24 ± 4.86	26.35 ± 4.72	0.409
Visceral Fat	13.78 ± 4.07	13.30 ± 3.96	0.598
SBP (mmHg)	137.53 ± 9.92	128.58 ± 7.18	**0.000***
DBP (mmHg)	89.30 ± 10.15	85.60 ± 6.57	0.057
Pulse (bpm)	82.83 ± 8.10	79.73 ± 6.27	0.059

*A comparison of age, body mass index (BMI), percentage body fat (% body fat), percentage muscle mass (% muscle mass), visceral fat, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse between the obese diabetic group (Obese DM) and obese non-diabetic group (Obese NDM) group. Data is presented as Mean ± Standard Deviation (SD). *P-value < 0.05 were considered as statistically significant.*

4.3   CLINICAL AND ANTHROPOMETRIC INDICES OF MALES IN THE STUDY

The differences in means for the anthropometric indices of males in the study showed no significant differences for age, height, weight, BMI, percentage body fat, percentage muscle mass, and visceral fat ($P > 0.05$). The mean systolic blood pressure (SBP) between males obese diabetic and obese non-diabetic group showed statistically significant ($P < 0.05$) (Table 4.3).

Table 2.3: Clinical and anthropometric indices of males in the study

Variables	Cases (15) Mean ± SD	Controls (15) Mean ± SD	P-value
Age	48.60 ± 6.03	48.40 ± 8.02	0.939
Height (cm)	172.23 ± 10.17	168.57 ± 4.71	0.216
Weight (Kg)	98.55 ± 18.86	91.30 ± 9.11	0.191
BMI (Kg/m²)	34.08 ± 4.04	33.13 ± 2.40	0.442
% Body Fat	30.18 ± 7.45	31.47 ± 4.04	0.561
% Muscle Mass	32.72 ± 3.06	31.64 ± 3.12	0.347
Visceral Fat	15.27 ± 3.86	16.00 ± 4.28	0.626
SBP (mmHg)	138.40 ± 9.19	126.87 ± 7.92	**0.001***
DBP (mmHg)	85.87 ± 8.44	83.23 ± 6.80	0.412
Pulse (bpm)	79.27 ± 6.11	80.80 ± 4.68	0.447

*A comparison of age, body mass index (BMI), percentage body fat (% body fat), percentage muscle mass (% muscle mass), visceral fat, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse between the Obese diabetic males and Obese non-diabetic males of the study participants. Data is presented as Mean ± Standard Deviation (SD). *P-value < 0.05 were considered as statistically significant.*

4.4 CLINICAL AND ANTHROPOMETRIC INDICES OF FEMALES IN THE STUDY

Systolic blood pressure and pulse showed statistical significance (P<0.05) between females in the case group and females in the control group. The mean age, height, weight, body mass index, percentage body fat, percentage muscle mass, and visceral fats did not show statistical difference (table 4.4).

Table 4.4: Clinical and anthropometric indices of females in the study

Variables	Cases (25) Mean ± SD	Controls (25) Mean ± SD	P-value
Age	50.28 ± 4.56	46.63 ± 7.78	0.060
Height (cm)	160.23 ± 5.55	157.92 ± 4.27	0.106
Weight (Kg)	90.92 ± 13.69	88.94 ± 16.58	0.647
BMI (Kg/m^2)	35.44 ± 4.93	35.96 ± 5.79	0.734
% Body Fat	46.40 ± 3.92	47.86 ± 3.62	0.178
% Muscle Mass	23.96 ± 1.69	23.18 ± 1.63	0.106
Visceral Fat	12.88 ± 3.99	11.68 ± 2.73	0.221
SBP (mmHg)	137.00 ± 10.49	129.60 ± 6.65	**0.005***
DBP (mmHg)	91.36 ± 10.68	86.84 ± 6.24	0.074
Pulse (bpm)	84.96 ± 8.49	79.08 ± 7.07	**0.011***

A comparison of age, body mass index (BMI), percentage body fat (% body fat), percentage muscle mass (% muscle mass), visceral fat, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse between the obese diabetic females and obese non-diabetic females of the study participants. Data is presented as Mean ± Standard Deviation (SD). *P-value < 0.05 were considered as statistically significant.

4.5 CORRELATION BETWEEN MARKERS (LEPTIN AND hs-CRP) AND ANTHROPOMETRIC INDICES IN THE STUDY

Association between several anthropometric indices correlates (height, body mass index, and percentage body fat) with leptin in the study population. Serum leptin

correlate strongly with percentage body fat (r = 0.561; P < 0.05) and weakly with body mass index (r = 0.300; P <0.005). The study also found negative correlation (r = -0.325; P < 0.05) between leptin and height. Although there were correlations between C-reactive protein and anthropometric indices, none of them showed statistical significance (P > 0.05) (table 4.5).

Table 4.5: Association between markers and anthropometric indices in the study

ANTHROPOMETRIC		LEPTIN (ng/ml)	hs-CRP (ng/ml)
HEIGHT (cm)	r	**-0.325**	-0.135
	p	**0.003***	0.231
WEIGHT (Kg)	r	0.030	0.059
	p	0.792	0.603
BMI (Kg/m^2)	r	**0.300**	0.196
	p	**0.007***	0.082
% BODY FAT	r	**0.561**	0.165
	p	**0.000***	0.144
% MUSCLE	r	-0.102	-0.108
	p	0.370	0.341
VISCERAL FAT	r	-0.220	0.027
	p	0.050	0.812

The association between markers (leptin and hs-CRP) and anthropometric indices. P-value < 0.05 is considered statistically significant. Pearsons' correlation coefficient r > 0.5 shows strong positive correlation and r < 0.5 shows weak positive correlation. Correlation coefficient r > -0.5 shows strong negative correlation and r < -0.5 shows weak negative correlation.

4.6 CORRELATION BETWEEN MARKERS (LEPTIN AND hs-CRP) AND ANTHROPOMETRIC INDICES IN MALES AND FEMALES IN THE STUDY

The study compared the relationship between markers and anthropometric indices in both obese males and obese females in the study. For obese males, there was a strong positive correlations between leptin and body mass index (BMI), and leptin and weight

in subject group but no such correlation existed in control group. A relationship existed between leptin and percentage body fat in control group but did not exist in subject group (table 4.6).

For obese females, leptin correlated (weak negative correlation) with percentage muscle for subject group and correlated with height for the control group. High sensitive C-reactive protein did not show correlation with any of the anthropometric indices in both males and females (table 4.6).

Table 4.6: Association between markers (leptin and hs-CRP) and anthropometric indices in males and female in the study

ANTRO.		MALES				FEMALES			
		Controls (15)		Cases (15)		Controls (25)		Cases (25)	
		Leptin	CRP	Leptin	CRP	Leptin	CRP	Leptin	CRP
Height (cm)	r	0.306	-0.011	-0.218	-0.117	0.056	0.015	**0.506**	-0.114
	p	0.267	0.970	0.435	0.677	0.790	0.944	**0.010***	0.588
Weight (Kg)	r	**0.587**	-0.388	0.192	0.079	0.155	0.206	0.142	0.126
	p	**0.021***	0.153	0.493	0.780	0.428	0.323	0.499	0.549
BMI (Kg/m^2)	r	**0.551**	-0.461	0.468	0.176	0.230	0.280	-0.096	0.192
	p	**0.033***	0.084	0.078	0.531	0.269	0.176	0.648	0.358
% Body Fat	r	0.189	-0.417	**0.609**	0.281	0.388	0.262	-0.147	0.174
	p	0.500	0.122	**0.016***	0.310	0.055	0.205	0.482	0.106
% Muscle Mass	r	0.417	0.219	-0.363	-0.024	**-0.404**	-0.055	0.100	-0.233
	p	0.071	0.434	0.184	0.933	**0.045***	0.795	0.634	0.261
Visceral Fat	r	0.445	-0.350	0.456	0.053	0.138	0.375	-0.246	0.174
	p	0.096	0.201	0.088	0.849	0.509	0.065	0.236	0.406

The association between markers (leptin and hs-CRP) and anthropometric indices. *P-value < 0.05 is considered statistically significant. Correlation coefficient r > 0.5 shows strong positive correlation and r < 0.5 shows weak positive correlation. Correlation coefficient r > -0.5 shows strong negative correlation and r < -0.5 shows weak negative correlation. CRP in the table is hs-CRP.

4.7   BIOCHEMICAL INDICES OF CASE AND CONTROL GROUP

Biochemical values for cases and control group of the study participants showed significant difference ($P < 0.05$) for glucose, total cholesterol, and LDL-cholesterol and serum leptin. No significant difference ($P > 0.05$) were observed for triglycerides, HDL-cholesterol, VLDL cholesterol, coronary risk, and high sensitive C-reactive protein (table 4.7).

Table 4.7: Biochemical indices of case and control group

Variables	Cases (40) Mean ± SD	Controls (40) Mean ± SD	P-value
Glucose (mmol/L)	6.85 ± 2.56	4.95 ± 0.68	**0.000***
Total Cholesterol (mmol/L)	4.33 ± 1.19	4.94 ± 1.16	**0.024***
Triglycerides (mmol/L)	1.20 ± 0.62	1.15 ± 0.85	0.745
HDL-cholesterol (mmol/L)	1.07 ± 0.26	1.07 ± 0.31	0.928
LDL-cholesterol (mmol/L)	2.71 ± 1.06	3.34 ± 0.95	**0.007***
VLDL-cholesterol (mmol/L)	0.55 ± 0.28	0.53 ± 0.39	0.749
Coronary Risk (T. Chol/HDL-c)	4.25 ± 1.40	4.85 ± 1.40	0.057
Leptin (ng/ml)	13.84 ± 4.76	17.92 ± 5.51	**0.001***
hs-CRP (ng/ml)	0.18 ± 0.09	0.23 ± 0.11	0.057

*A comparison of means of Glucose, Total Cholesterol, Triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Coronary Risk, Leptin, and C-reactive protein (CRP) between the obese diabetic group (Obese DM) and the obese non-diabetic (Obese NDM) group. Data is presented as Mean ± Standard Deviation (SD). *P-value < 0.05 were considered as statistically significant.*

4.8   BIOCHEMICAL INDICES OF MALES IN THE STUDY

From the biochemical parameters provided in table 4.8, the mean glucose of males for both case and control were 6.50 ± 3.01 and 4.61 ± 0.85 respectively and showed significant difference ($P < 0.05$) in the means. For total cholesterol, triglycerides, HDL, LDL, VLDL, and coronary risk of males in the study population, no significant differences ($P > 0.05$) were determined. The difference in means for serum leptin between males of obese diabetic group and obese non-diabetic group also showed significant difference ($P < 0.05$) (table 4.8).

Table 4.8: Biochemical indices of males in the study

Variables	Cases (15) Mean ± SD	Controls (15) Mean ± SD	P-value
Glucose (mmol/L)	6.50 ± 3.01	4.61 ± 0.85	**0.027***
Total Cholesterol (mmol/L)	4.37 ± 0.91	5.06 ± 1.20	0.088
Triglycerides (mmol/L)	1.37 ± 0.76	1.51 ± 1.23	0.693
HDL-cholesterol (mmol/L)	1.02 ± 0.21	1.02 ± 0.28	0.988
LDL-cholesterol (mmol/L)	2.72 ± 0.76	3.35 ± 0.98	0.062
VLDL-cholesterol (mmol/L)	0.63 ± 0.35	0.69 ± 0.56	0.696
Coronary Risk	4.44 ± 1.28	5.16 ± 1.42	0.156
Leptin (ng/ml)	10.12 ± 4.33	14.73 ± 4.93	**0.011***
hs-CRP (ng/ml)	0.18 ± 0.11	0.20 ± 0.09	0.672

*A comparison of means of Glucose, lipid profiles leptin and high sensitive C-reactive protein (hs-CRP) between the obese males for each group. Data is presented as Mean ± Standard Deviation (SD). *P-value <0.05 were considered as statistically significant.*

4.9   BIOCHEMICAL INDICES OF FEMALES IN THE STUDY

For biochemical indices of females in the study population, the mean difference for lipid profile (total cholesterol, triglycerides, high density lipoprotein, very high density lipoprotein, and coronary risk) did not show statistical significance (P>0.05) females of case and control group. However, there was significant difference (P<0.05) between mean of glucose, serum leptin and high sensitive C-reactive protein in females of the study population (table 4.9).

Table 4.9: Biochemical indices of females in the study

Variables	Cases (25) Mean ± SD	Controls (25) Mean ± SD	P-value
Glucose (mmol/L)	7.06 ± 2.28	5.15 ± 0.46	**0.000***
Total Cholesterol (mmol/L)	4.31 ± 1.34	7.06 ± 1.15	0.125
Triglycerides (mmol/L)	1.11 ± 0.51	0.92 ± 0.40	0.179
HDL-cholesterol (mmol/L)	1.09 ± 0.29	1.11 ± 0.32	0.923
LDL-cholesterol (mmol/L)	2.71 ± 1.22	3.33 ± 0.95	0.050
VLDL-cholesterol (mmol/L)	0.51 ± 0.23	0.43 ± 0.18	0.187
Coronary Risk	4.13 ± 1.49	4.67 ± 1.39	0.194
Leptin (ng/ml)	16.08 ± 3.46	19.83 ± 5.00	**0.003***
CRP (ng/ml)	0.18 ± 0.09	0.24 ± 0.12	**0.045***

*A comparison of means of Glucose, lipid profiles leptin and hs-CRP between the obese females for each group. Data is presented as Mean ± Standard Deviation (SD). *P-value < 0.05 were considered as statistically significant.*

4.10   RELATIONSHIP BETWEEN MARKERS AND LIPID PROFILES IN THE STUDY

Association between total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and coronary risk (CR) with markers (leptin and hs-CRP) were determined (table 4.10). There was no significant correlation between the markers (leptin and hs-CRP) and total cholesterol, high density lipoprotein (HDL) and coronary risk. Triglycerides and very low density lipoprotein (VLDL) showed an inverse correlation ($r < 0.5$; $p < 0.05$) with leptin but these association was weak (table 4.10).

Table 4.10: Relationship between markers and lipid profiles

LIPID PROFILE		LEPTIN (ng/ml)	hs-CRP (ng/ml)
T. Cholesterol	r	0.080	0.068
(nmol/l)	p	0.480	0.549
Triglycerides	r	**-0.251**	0.121
(nmol/l)	p	**0.025***	0.286
HDL	r	0.166	0.034
(nmol/l)	p	0.140	0.766
LDL	r	0.127	0.126
(nmol/l)	p	0.261	0.265
VLDL	r	**-0.253**	-0.123
(nmol/l)	p	**0.024***	0.275
Coronary Risk	r	-0.097	0.027
	p	0.393	0.214

*The association between markers (leptin and hs-CRP) and lipid profile. *P-value < 0.05 is considered statistically significant. Correlation coefficient r > 0.5 shows strong positive correlation and r < 0.5 shows weak positive correlation. Correlation coefficient r > -0.5 shows strong negative correlation and r < 0.5 shows weak negative correlation.*

4.11   RELATIONSHIP BETWEEN LIPID PROFILES AND ANTHROPOMETRIC INDICES IN THE STUDY

An association between anthropometric indices and biochemical parameters were determined (table 4.11). Significant inverse correlation ($r < 0.5$; $P < 0.05$) was found between percentage body fat and triglycerides as well as percentage body fat and very low density lipoprotein. Again, significant positive correlation ($r < 0.5$; $P < 0.05$) was found between percentage muscle mass and triglycerides as well as percentage muscle mass and very low density lipoprotein (VLDL). The correlation between height, weight, BMI and visceral and lipid profiles did not show significance (table 4.11).

Table 4.11: Relationship between lipid profiles and anthropometric indices in the study

		T CHOL (mmol/l)	TRIG. (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)	CR (mmol/l)
Height (cm)	r	-0.018	0.126	-0.079	-0.041	0.129	0.020
	p	0.874	0.264	0.485	0.716	0.253	0.863
Weight (Kg)	r	0.044	0.052	-0.136	0.070	0.054	0.084
	p	0.697	0.646	0.230	0.539	0.633	0.458
BMI (Kg/m²)	r	0.101	-0.049	-0.080	0.154	-0.049	0.092
	p	0.371	0.663	0.481	0.174	0.667	0.417
% Body Fat	r	-0.019	**-0.280**	0.138	0.031	**-0.283**	-0.116
	p	0.867	**0.012***	0.222	0.784	**0.011***	0.305
% Muscle	r	0.060	**0.302**	-0.100	-0.002	**0.305**	0.113
	p	0.599	**0.007***	0.379	0.986	**0.006***	0.319
Visceral Fat	r	0.033	0.181	-0.172	0.024	0.182	0.108
	p	0.774	0.107	0.128	0.832	0.106	0.342

The relationship between lipid profiles (Total Cholesterol, Triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), and Coronary Risk) and anthropometric indices. Correlation coefficient $r > 0.5$ shows strong positive correlation and $r < 0.5$ shows weak positive correlation. Correlation coefficient $r > -0.5$ shows strong negative correlation and $r < 0.5$ shows weak negative correlation. *P-value < 0.05 were considered as statistically significant.

4.12 CORRELATION BETWEEN BIOCHEMICAL INDICES AND ANTHROPOMETRIC INDICES OF CASE AND CONTROLS

The study compared biochemical indices and anthropometric indices of both the case and control group. Significant positive correlation (r<0.5; P<0.05) existed between glucose and percentage body fat, and negative correlation between glucose and percentage muscle mass of the control group. No correlation was however seen between glucose and anthropometric indices of the case (obese diabetic) group (table 4.12).

A positive correlation (r = 0.351; P < 0.05) existed between triglycerides and percentage muscle mass as well as triglycerides and visceral fats (r = 0.504; P < 0.05) of obese non-diabetic subjects but no significant correlation was found between triglycerides and anthropometric indices in the case group (obese diabetic subjects) as shown in table 4.12.

Significant positive correlation (r<0.5; P<0.05) between leptin and percentage body fat was found in both case (obese diabetic) and control (obese non-diabetic) group. Furthermore, significant negative association was found between leptin and percentage muscle mass in both groups. However, leptin correlated with BMI in obese non-diabetic group and no correlation was found between leptin and BMI of the case (obese diabetic) group (table 4.12).

Although, total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL) correlates with anthropometric indices of both case group and control, no significant correlation (P > 0.05) were found. But significant positive correlation (r = 0.355; P < 0.05) was found between very low density lipoprotein and percentage muscle mass as well as visceral fat (r = 0.515; P <0.05) in obese non-diabetic group as shown in table 4.12. No correlation was found between very low density lipoprotein (VLDL)

and anthropometric indices in the control group. A relationship exited between coronary risk and visceral fat in obese non-diabetic group but no relationship was found in obese diabetic group (table 4.12).

Table 4.12: Relationship between biochemical indices variables and anthropometric indices of study participants

Variables		CONTROL GROUP (OBESE NON-DIABETICS (40))				CASE GROUP (OBESE DIABETICS (40))			
		BMI	% Body fat	% muscle mass	Visceral fat	BMI	% Body fat	% muscle mass	Visceral fat
Glucose	r	0.258	**0.315**	**-0.378**	-0.091	0.077	0.104	-0.080	0.145
	p	0.107	**0.048***	**0.016***	0.579	0.636	0.525	0.624	0.373
T. Chol	r	-0.004	-0.138	0.195	0.300	0.078	0.004	0.022	-0.077
	p	0.981	0.397	0.229	0.060	0.633	0.979	0.895	0.638
Trig	r	0.025	-0.301	**0.351**	**0.504**	0.075	-0.178	0.197	0.222
	p	0.878	0.059	**0.026***	**0.001***	0.644	0.271	0.224	0.169
HDL	r	-0.227	0.165	-0.160	-0.179	-0.033	0.117	-0.101	-0.230
	p	0.159	0.310	0.325	0.357	0.841	0.473	0.535	0.153
LDL	r	0.131	-0.057	0.125	0.292	0.075	0.024	-0.004	-0.090
	p	0.421	0.727	0.443	0.067	0.645	0.884	0.978	0.579
VLDL	r	0.029	-0.303	**0.355**	**0.515**	0.076	-0.184	0.204	0.222
	p	0.858	0.058	**0.025***	**0.001***	0.641	0.255	0.207	0.169
CR	r	0.278	-0.166	0.242	**0.350**	0.044	-0.070	0.062	0.076
	p	0.082	0.306	0.133	**0.027***	0.786	0.668	0.705	0.639
Leptin	r	**0.444**	**0.572**	**-0.555**	-0.114	0.237	**0.635**	**-0.602**	-0.144
	p	**0.004***	**0.000***	**0.000***	0.483	0.141	**0.000***	**0.000***	0.377
CRP	r	0.033	0.211	-0.158	-0.005	0.172	0.113	-0.035	0.125
	p	0.840	0.191	0.329	0.973	0.289	0.489	0.830	0.443

*Table 4.12 shows the relationship between metabolic variables (Total Cholesterol, Triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Coronary Risk, leptin and C-reactive protein) and adiposity indices. Correlation coefficient r > 0.5 shows strong positive correlation and r < 0.5 shows weak positive correlation. Correlation coefficient r > -0.5 shows strong negative correlation and r < 0.5 shows weak negative correlation. *P-value < 0.05 were considered as statistically significant.*

4.13 RISK ASSESSMENT OF LEPTIN IN STUDY PARTICIPANTS

In this study, 19 of the cases (obese diabetic group) and 30 of the controls (obese non-diabetic group) had leptin greater than 15 ng/dl respectively with an odds ratio of 3.315 (table 4.13).

Table 4.13: Risk assessment of serum leptin in study participants

GROUPS	LEPTIN (ng/dl)		TOTAL	ODDS RATIO	95% CONFIDENCE IINTERVAL	
	5-15g/dl	>15ng/dl			Lower	Upper
Cases	21	19	40	3.315	1.17	7.78
Controls	10	30	40			

The table shows the odds ratio of obese diabetic (cases) and obese non-diabetic group

CHAPTER FIVE

5.0   DISCUSSION

The global increase in the prevalence of obesity in most human population is alarming (World Health Organization, 2012). Obesity is a major contributor to the burden of disabilities and several chronic diseases including type 2 diabetes mellitus (International Diabetes Federation, 2013; Doumatey *et al.,* 2010). Obese individuals produce large fat mass (about 7 folds) compared with lean mass (Seidell and Flegal, 1997), hence have larger adipose tissues.

Adipocytokines are biological active molecules secreted by adipose tissues (Schuster, 2010). Leptin, an adipocytokine is involve in the regulation of food intake, energy storage, and lipid metabolism (Attele *et al.,* 2002; Zimmet *et al.,* 1996). Even though leptin is reported to reduce food intake (Attele *et al.,* 2002), obesity may be the consequences of leptin resistance (Emanuelli *et al.,* 2001; Wei *et al.,* 1997).

The study evaluated serum leptin and high sensitive C-reactive protein (hs-CRP) levels in obese Ghanaians subjects with and without type 2 diabetes mellitus. These markers (leptin and hs-CRP) were found to vary among obese diabetic subjects and obese non-diabetic controls (table 4.7). Both leptin and high sensitive C-reactive protein were found to be lower in the case group compared with control group as shown in table 4.7. This is in agreement with other studies carried out in Caucasian patients with type 2 diabetes which reported that, reduced levels of both leptin and hs-CRP in diabetics may be due to insulin resistance (Mohammadzadeh and Zaghami, 2013; van Gaal *et al.,* 1999).

Leptin was found to correlate positively with BMI and percentage body fat in both case and control subjects (table 4.5) but was lower in case group compared with control group (table 4.7). A possible explanation for reduced levels of leptin was due to higher

55

visceral fat in obese diabetic group compared with obese non-diabetic controls (table 4.2). Diabetic subjects have been found to produce more visceral fats compared with non-diabetic subjects and visceral fat has been reported to produce less leptin (Susan *et al.,* 2000), This study found similarly higher levels of visceral fats (table 4.4) in obese diabetic group compared with obese non-diabetic controls which agrees with previous report by Susan *et al.,* (2000).

In this study, females had significantly higher serum leptin and high sensitive C-reactive protein concentrations than males in both groups (tables 4.6 and 4.8). This may be as a result of gender differences in body fat distribution. Higher percentage body fat was found in females compared to males even though this was not statistical significant in both group (table 4.4 and 4.3). Females were also found to be more obese (have higher body mass index) compared with males (table 4.4 and 4.3) and this may explain higher leptin in females. This is in line with previous studies which reported that, leptin increases with increases BMI and body fat (Mohammedzadeh and Zarghami, 2013; Mehmet *et al.,* 2004; Marguerite *et al.,* 1999; Wei *et al.,* 1997). Higher body mass index (BMI) and percentage body fat found in females may have also accounted for the significant increase of high sensitive C-reactive protein in females (table 4.4).

Obesity is considered as a pro-inflammatory condition (Chan-Hee *et al.,* 2012; Pickup, 2004) and C-reactive protein levels increase in systematic circulation during inflammation (Schuster, 2010). This study did not find significant difference between levels of high sensitive C-reactive protein in obese diabetic and obese non-diabetic group although the concentration of hs-CRP was lower in obese diabetic cases as against obese non-diabetic controls (table 4.7). This is because, obese diabetic subjects were on antidiabetic medications some of which included; metformin and rosiglitazone. Some studies have shown that, singly or combined oral administration of hypoglycemic

medication such as metformin and rosiglitazone in the management of type 2 diabetes mellitus decreases C-reactive protein levels in subjects (Andreas *et al.,* 2010; Farah *et al.,* 2008; Stewart *et al*., 2006). Angiotensin receptor blockers (ARB), angiotensin-converting enzymes (ACE) inhibitors and beta blockers in the management of hypertension and type 2 diabetes are also known to decrease C-reactive proteins on single therapy (Susan *et al.*, 2005). These medications may explain the reduced levels of high sensitive C-reactive proteins in obese diabetic subjects.

Studies have shown that, hyperlipidemia is a strong indicator for the association of diabetes with dyslipidemia (Jiao and Xu, 2008; Deepa *et al.,* 2007). The reason may be that, a decreased insulin effect in subjects with insulin resistance may result in excessive adipose tissue lipolysis and increased flux of free fatty acids to the liver, which may drive up hepatic triglyceride synthetic rates (Abhimanyu, 1996). Obese diabetic subjects were found to have higher concentration of triglycerides compared with obese non-diabetes controls even though not statistical significant (table 4.7). Again, total cholesterol and low density lipoprotein (LDL) showed significant difference (table 4.7) and were found to be lower in obese diabetic subjects compared with obese non-diabetic controls. A possible explanation was that, diabetic subjects were on hypertension and lipid lowering drugs to manage the hypertension and dyslipidemia, and perhaps may have accounted for the lower levels of total cholesterol and low density lipoprotein.
Manson *et al.,* (1990), found that, hypercholesteremia was significantly associated with BMI. Even though this study showed weak correlation between hypercholesteremia and BMI, there was no significant correlation found (table 4.11). Metabolically, visceral fats have been reported to produce more free fatty acids (FFAs) which may lead to increase levels of cholesterol, heart disease, and type 2 diabetes mellitus (Rebrin *et al.,* 1996). This study found higher visceral fats among obese diabetic cases compared with

obese non-diabetic controls (table 4.2). No significant difference were found between visceral fats and cholesterol (table 4.11). The significant difference between systolic blood pressures (SBP) of the case group and the control group (table 4.2) may be associated with hypertension.

Serum triglycerides are often high in obese individuals and hence some studies have link triglycerides with inhibited leptin transport (Banks *et al.,* 2004). In this study, serum leptin correlated negatively to triglycerides and very low density lipoprotein (VLDL) in the study participant (table 4.10). The explanations to these inverse relationship may be that, impaired leptin transport is believed to account for at least a portion of leptin resistance and may explain why there is an accumulation of leptin in obese individuals.

Serum leptin circulate normally in the blood at 5-15ng/dl and increased levels above 15 ng/dl indicates hyperleptinemia (Sinha *et al.,* 1999). According to Marguerite *et al.,* (1999), obese individuals with higher leptin levels above the upper reference limits (>15 ng/ml) increase risk of development of type 2 diabetes mellitus. This study recorded an odds ratio of 3.315 (table 4.13) implying that, obese non-diabetic subjects have 3 times the chance of having higher leptin levels compared with obese diabetic group.

Furthermore, body fats distribution may also differ between ethnic groups suggestion the link between obesity and type 2 diabetes mellitus (Cleland and Sattar, 2005). Obesity have been reported to be common among Ga and Akan tribes in Ghana (Benkeser *et al.,* 2007; Biritwum *et al.,* 2005; Amoah *et al.,* 2003). The prevalence of type 2 diabetes have also been found to be common among these tribes (Amoah *et al.,* 2002). Although incidence of obesity and type 2 diabetes mellitus were common among Ga and Akan tribes, this study found that, leptin and high sensitive C-reactive protein

concentrations were less in obese diabetic group as against obese non-obese subjects (table 4.1).

5.1   CONCLUSION

Obesity is known to be an important risk factor for development of type 2 diabetes mellitus. This study reported that, obese Ghanaians with type 2 diabetes mellitus have lower serum leptin and high sensitive C-reactive protein (hs-CRP) compared with obese non-diabetic controls. Higher levels of serum leptin in obese non-diabetic subjects may suggest a possible link between obesity, insulin resistance, and type 2 diabetes mellitus. High sensitive C-reactive protein (hs-CRP) was found to correlate positively only in diabetic females.

5.2   LIMMITATION

This study involve relative small group because subjects (both obese diabetics and obese non-diabetic) were not complying.

5.3   RECOMMENDATION

- Further studies in larger populations are necessary to clarify the role of leptin and C-reactive protein levels in obese Ghanaians

- Assessment of insulin and free fatty acids (FFAs) to establish the link between obesity, insulin resistance and type 2 diabetes mellitus is also recommended

- Different study sites should have been considered as this would have given a better comparison for persons from diverse walks of life.

REFERENCES

Abhimanyu, G., 1996. Insulin resistance in the pathogenesis of dyslipidemia. *Diabetes Care,* 19(4), pp. 387-389.

Abubakari, A. & Bhopal, R., 2008. Systematic review on the prevalence of diabetes , overweight / obesity and physical inactivity in Ghanaians and Nigerians. *Public Health,* Volume 122, pp. 173-182.

Al-Daghri, N., Al-Rubean, K., Bartlett, W. & Al-Attas, O., 2003. Serum leptin is elevated in Saudi Arabian patients with metabolic syndrome and coronary artery disease.. *Diabetic Medicine : A Journal of the British Diabetic Association,* 20(10), pp. 832-837.

Alexander, D. V., Irina, T. V. & Inna, M. V., 2013. Leptin levels in women with metabolic syndrome. *International Journal of BioMedicine,* 3(2), pp. 63-66.

Allain, C., Poon, L., Chan, C. & Richmond, W., 1974. Enzymatic determination of total serum cholesterol. *Clinical Chemistry,* Volume 20, pp. 470-475.

Amati, F., Dube, J., Coen, P. & Stefanovic-Racic, M., 2009. Physical inactivity and obesity underlie the insulin resistance of aging. *Diabetes Care,* Volume 32, pp. 1547-1549.

American Diabetes Association, 2012. Diagnosis and classification of diabetes mellitus. *Diabetes Care,* 35(1), pp. 64-71.

American Diabetes Association, 2014. Standards of medical care in diabetes. *Diabetes Care,* 37 (1), pp. 2-18.

Amoah, A. G., 2003. Sociodemographic variations in obesity among Ghanaian adults. *Public Health Nutrition,* 8(6), pp. 751-775.

Amoah, A., Owusu, K. & Adjei, S., 2002. Diabetes in Ghana: a community prevalence study in Greater Accra.. *Diabetes Research and Clinical Practice,* Volume 56, pp. 197-205.

Amy, F. & Erinn, T. R., 2009. Management of obesity , insulin resistance and type 2 diabetes in children : consensus and controversy. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* Volume 2, pp. 185-202.

60

Anand, S., Tarnopolsky, M., Rashid, S. & Schulze, K., 2011. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One,* Volume 67, pp. 22112-22118.

Andreas, P., Thomas, S., Markolf, H. & Thomas, F., 2010. High-sensitivity c-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with rosiglitazone.. *Journal of Diabetes Science and Technology,* Volume 5, pp. 706-716.

André, T. & Jean-Pierre, D., 2013. Pathophysiology of human visceral obesity: an update. *Physiology Review,* Volume 93, p. 359–404.

Attele, A., Shi, Z. & Yuan, C., 2002. Leptin, gut, and food intake.. *Biochemical Pharmacology,* Volume 63, pp. 1579-1583.

Banks, W. A., Alan, B. C., Sandra, R. M. & Asif, M., 2004. Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes,* Volume 5, pp. 1253-1260.

Benkeser, R., Biritwum, R. & Hill, A., 2012. Prevalence of overweight and obesity and perception of healthy and desirable body size in urban Ghanaian women. *Ghana Medical Journal,* 46(2), pp. 66-75.

Biritwum, R. B., Gyapong, J. & Mensah, G., 2005. The epidemiology of obesity in Ghana. *Ghana Medical Journal,* 39(3), pp. 82-85.

Bradley, R., Cleveland, K. & Cheatham, B., 2001. The adipocyte as a secretory organ: mechanisms of vesicle transport and secretory pathways. *Recent Prog Horm Res,* Volume 56, p. 329–358..

Bray, A. G., Kathleen, A. J., Wilfred, Y. F. & Elizabeth, B.-C., 2008. Relation of central adiposity and body mass index to the development of diabetes in the Diabetes Prevention Program. *The American Journal of Clinical Nutrition,* Volume 87, p. 1212–1218.

Bullo, M., Garcia-Lorda, P., Megias, I. & Salas-Salvado, J., 2003. Systemic inflammation, adipose tissue, tumour necrosis factor, and leptin expression.. *Obesity Research,* Volume 85, pp. 3338-3342.

Ceddia, R., William, W. J., Carpinelli, A. & Curi, R., 1999. Modulation of insulin secretion by leptin. *General Pharmacology,* Volume 32, pp. 233-237.

Ceriello, A., Mokdad, A. H., Bowman, B. A. & al., e., 2001. The continuing epidemics of obesity and diabetes in the United States.. *Journal of America Medical Association (JAMA),* Volume 286, pp. 1195-1200.

Chan-Hee, J., Bo-Yeon, K., Chul-Hee, K. & Sung-Koo, K., 2012. Association of serum adipocytokine levels with cardiac autonomic neuropathy in type 2 diabetic patients. *Cardiovascular Diabetology,* 11(1), pp. 1-9.

Chan, R. S. M. & Woo, J., 2010. Prevention of overweight and obesity : How effective is the current public health approach - review. *Internal Journal of Environmental Research and Public Health,* Volume 7, pp. 765-783.

Cleland, S. & Sattar, N., 2005. Impact of ethnicity on metabolic disturbance, vascular dysfunction and atherothrombotic cardiovascular disease. *Diabetes, Obesity and Metabolism,* Volume 5, pp. 463-470.

Coleman, R. A. & Herrmann, T. S., 1999. Nutritional regulation of leptin in humans. *Diabetologia,* Volume 42, pp. 639-646.

Collins, A. A., Matilda, S.-A. & Gloria, E. O., 2014. Predictors of overweight/obesity in urban Ghanaian women. *International Journal of Clinical Nutrition,* 2(3), pp. 60-68.

Deepa, R., Sandeep, S. & Mohan, V., 2007. *Abdominal obesity, visceral fat and type 2 diabetes-Asian Indian phenotype.* India-South Asian: s.n.

Dehghan, A., Kardys, I., de Maat, M. & Uitterlinden, A., 2007. Genetic variation, c-reactive protein levels, and incidence of diabetes.. *Diabetes Care,* Volume 56, pp. 872-878.

Dehghan, A., Mandy, V., Eric, J. S. & T., S., 2007. Risk of type 2 diabetes attributable to C-reactive protein and other risk factors. Diabetes Care, Volume 30, pp. 2695-2699.. *Diabetes Care,* Volume 30, pp. 2695-2699.

Despres, J., 1993. Abdominal obesity as important component of insulin-resistance syndrome.. *Nutrition,* Volume 9, p. 452–459.

Despres, J., Lemieux, I., Bergeron, J. & Pibarot, P., 2008. Abdominal obesity and the metabolic syndrome; contribution to global cardiometabolic risk.. *Arteriosclerosis Thromb Vasc Biology,* Volume 28, p. 1039–1049.

Després, J. et al., 1990. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis,* Volume 10, pp. 497-511.

Diabetes Prevention Program Research Group, 2002. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New English Journal of Medicine,* Volume 346, p. 393– 403..

Dixon, J. & O'Brien, P., 2002. Health outcomes of severely obese type 2 diabetic subjects 1 year after laparoscopic adjustable gastric banding.. *Diabetes Care,* Volume 25, pp. 358-363.

Doumatey, A., Lashley, K. & Huang, H., 2010. Relationships among Obesity, Inflammation, and Insulin Resistance in African Americans and West Africans. *Obesity,* 18(3), pp. 598-603.

Eckersal, P., 2000. Recent advances and future prospects for the use of acute phase proteins and markers of disease in animals.. *Revue Med. Vet.,* 151(7), pp. 577-584.

Emanuelli, B., Peraldi, P., Filloux, C. & Chavey, C., 2001. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *Journal of Biological Chemistry,* 276(51), pp. 47944-47949.

Farah, R., Shurtz-Swirski, R. & Lapin, O., 2008. Intensification of oxidative stress and inflammation in type 2 diabetes despite antihyperglycemic treatment. *Cardiovascular Diabetology,* Volume 22, pp. 7-20.

Festa, A., D'Agostino, R. J., Williams, K. & Karter, A., 2001. The relation of body fat mass and distribution to markers of chronic inflammation.. *International Journal of Obesitty Related Metabolic Disorder,* Volume 25, p. 1407–1415.

Festa, A., Ralph, D., Jr., G. H. P. & M., H., 2000. Chronic subclinical inflammation a part of the insulin resistance syndrome: the insulin resistance atherosclerosis study (IRAS).. *Circulation,* 102(1), pp. 42-47.

Freddy, C., Mary, L. & y Manuel, V., 2011. Relationship between leptin and hypertention. *Síndrome Cardiometabólico,* 1(3), pp. 60-66.

Freeman, D., Norrie, J., Caslake, M. & Gaur, A., 2002. C-reactive protein is an independent predictor of risk for development of diabetes - West of Scotland coronary study.. *Diabetes,* Volume 51, pp. 1596-1600.

Friedewald, W., Levy, R. & Fredrickson, D., 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. *Clinical Chemistry,* Volume 18, pp. 499-502.

Garber, A., Handelsman, Y., Einhorn, D. & Bergman, D., 2008. Diagnosis and management of Prediabetes in the continuum of hyperglycemia-When do the risks of Diabetes begin? A consensus statement from the American College of Endocrinology and the American Association of Clinical Endocrinologists.. *Endocrinology Practical,* 14(7), pp. 933 - 946..

GenWay Biotech Inc.,, 2015. *Protocol of Human C-rective Protein (CRP): Immunoperoxidase assay for determination of C-reactive protein in human samples.* CA, USA: GenWay Biotech Inc..

GenWay Biotech Inc., 2015. *Protocol for leptin ELISA Kit.* CA, USA: GenWay Biotech Inc..

GenWay Biotech, Inc, 2015. *Human Leptin ELISA Kit: Protocol for ELISA Kit.* San Diego, CA 92121: GenWay Biotech, Inc, www.genwaybio.com.

Ghana Demographic Health Survey, 2009. *Ghana demographic and health survey 2008, Accra, Ghana, Ghana Statistical Service (GSS), Ghana Health Services (GHS) and ICF Macro., Ghana.,* Accra: GSS, GHS and ICF Macro.

Haffner, S., Miettinen, H., Mykkanen, L. & Stern, M., 1998. Leptin concentrations are associated with higher proinsulin and insulin concentrations but a lower proinsulin/insulin ratio in nondiabetic subjects. *International Journal of Obesity Relation Metabolic Disorder,* Volume 22, pp. 899-905.

Hajer, G., van Haeften, T. & Visseren, F., 2008. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases.. *European Heart Journal,* Volume 29, pp. 2959-2971.

Han, T., Seidell, J., Currall, J. & Morrison, C., 1997. The influences of height and age on waist circumference as an index of adiposity in adults. *Internation Journal of Obesity Related Metabolic Disorders,* 21(1), pp. 83-89.

Hoffler, U., Hobbie, K., Wilson, R. & Bai R, R. A., 2009. Diet-induced obesity is associated with hyperleptinemia, hyperinsulinemia, hepatic steatosis, and glomerulopathy in C57Bl/6J mice.. *Endocrine,* Volume 36, pp. 311-325.

Huffman, F. G., Suzanne, W., Gustavo, G. Z. & Subrata, N., 2010. Waist circumference and BMI in relation to serum high sensitivity c-reactive protein (hs-CRP) in Cuban Americans with and without type 2 diabetes. *International Journal of Environmental Research and Public Health,* 7(3), pp. 842-852.

International Diabetes Federation, 2003. *Screening for Type 2 Diabetes: Report of a World Health Organization and Internation Diabetes Federation meeting,* Geneva: Internation Diabetic Federation (IDF).

International Diabetes Federation, 2013. *International Diabetes Federation Diabetes Atlas, sixth edition.,* USA: International Diabetes Federation, http://www.idf.org/membership/afr/ghana..

Jackson, A., Stanforth, P. & and Gagnon, J., 2002. The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *International Journal of Obesity Related to Metabolic Disorders,* Volume 26, pp. 789-794.

James, W. P. T., 2008. The epidemiology of obesity : the size of the problem (Review Article). *Journal of Internal Medicine,* Volume 263, pp. 336-352.

Javad, M. & Doudi, Q., 2005. Relation between leptin and insulin in patients with type 2 diabetes mellitus. *Turkish Journal of Endocrinology and Metabolism,* Volume 2, pp. 55-58.

Jiao, P. & Xu, H., 2008. Adipose inflammation : cause or consequence of obesity-related insulin resistance. *Diabetes, Metabolic Syndrome and Obesity,* Volume 1, pp. 25-31.

Kieffer, T., Heller, R. & Habener, J., 1996. Leptin receptors expressed on pancreatic beta cells. *Biochemistry Biophysiology Research Communication,* Volume 224, pp. 512-527.

Klein, S., Fontana, L., Young, V. & Coggan, A., 2004. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *New English Journal of Medicine,* Volume 350, p. 2549–2557.

Lemieux, I., Pascot, A., Prud'homme, D. & Almeras, N., 2001. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity.. *Arteriosclerosis Thromb Vasc Biology,* Volume 21, p. 961–967.

Liang, X., Changting, X., Robert, H. & Mark, N., 2011. C-reactive protein impairs hepatic insulin sensitivity and insulin signaling in rats: role of mitogen-activated protein kinases. *Hepatology (Baltimore,Md.),* 53(1), pp. 127-135.

Manson, J., Colditz, G. & Stampfer, M., 1990. A prospective study of obesity and risk of coronary heart disease in women. *New England Journal of Medicine,* Volume 322, pp. 882-889.

Mantzoros, C. & Moschos, S., 1998. Leptin: in search ofrole(s) in human physiology and pathophysiology. *Clinical Endocrinology (Oxf),* 49(5), pp. 551-567..

Marguerite, J. M., Edward, J., David, S. W. & J.B., S., 1999. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans.. *Diabetes Care,* 22(1), pp. 65-70.

Martin, S., Qasim, A. & Reilly, M., 2008. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease.. *Journal of american College of Cardiology,* Volume 52, pp. 1201-1210.

Matsuda, M. & Shimomura, I., 2013. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer.. *Obesity Research and Clinical Practice,* Volume 7, p. 330–341.

Matsuda, M., Shimomura, I., Sata, M. & Arita, Y., 2002. Role of adiponectin in preventing vascular stenosis. *The Journal of Biological Chemistry,* 277(40), pp. 37487-37491.

Mehmet, A. B., Ali, C., Ramazan, K. & Aytekin, G., 2004. Leptin levels in obese women with and without type 2 diabetes mellitus.. *Mediators of inflammation,* 13(5), pp. 321-325.

Meyre, D. & Froguel, P., 2006. ENPP1, the first example of common genetic link between childhood and adult obesity and type 2 diabetes.. *Medical Sciences (Paris),* 22(3), pp. 308-312.

Mittelman, S. & Bergman, R., 2000. Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in sulin. *American Journal of Physiology, Endocrinology and Metabolism,* Volume 279, p. 630–637.

Mohamed-Ali, V., Pinkney, J. & Coppack, S., 1999. Adipose tissue as an endocrine and paracrine organ.. *International Journal of Obesity Related Metabolic Disorders,* Volume 22, pp. 1145-1158.

Mohammedzadeh, G. & Zarghami, N., 2013. Serum leptin level is reduced in non-obese subjects with type 2 diabetes.. *International Journal of Endocrinology and Metabolism,* 11(1), pp. 3-10.

Moran, O. & Phillip, M., 2003. Leptin, obesity, diabetes and other peripheral effects - a review.. *Pediatric Diabetes,* 4(2), pp. 176-182.

Mukesh, S., Updesh, S. B., Param, P. S. & Sandeep, K., 2010. Leptin and the clinical cardiovascular risk (Revew). *International Journal of Cardiology,* Volume 140, pp. 266-271.

Nicklas, B., Penninx, B., Ryan, A. & Berman, D., 2003. Visceral adipose tissue cutoffs associated with metabolic risk factors for coronary heart disease in women. *Diabetes Care,* Volume 26, p. 1413–1420.

Ntuk, U., Mackay, D., Gill, J. M. & Sattar, N., 2014. Ethnic specific obesity cut-offs for diabetes risk: Cross-sectional study of 490,288 UK Biobank participants.. *Diabetes Care,* Issue In Press..

Oti, S., Van de Vijver, S., Agyemang, C. & Kyobutunqi, C., 2013. The magnitude of diabetes and its association with obesity in the slum of Nairobi, Kenya:results from a cross-sectional survey. *Tropical Medical and Internal Health 2013, 18:1520–1530.,* Volume 18, p. 1520–1530.

Parkinson, L., Gibson, R., Robinson, I. & Byles, J., 2010. Older women arthritis: Tracking impact over time.. *Australas Journal of Ageing,* Volume 29, pp. 155-160.

Petersen, K., Dufour, S., Feng, J. & Befroy, D., 2006. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Science, USA ,* Volume 103, p. 18273–18277..

Pickup, J., 2004. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care,* Volume 27, pp. 813-823.

Pouliot, M., Després, J., Nadeau, A. & Moorjani, S., 1992. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels.. *Diabetes,* Volume 41, pp. 826-834.

Pradhan, A., Manson, J., Rifai, N. & Ridker, P., 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of American Medical Association,* Volume 286, p. 327–334.

Rajesh, P., Narendra, K., Seema, P. & Ram, P., 2013. Impact of obesity and diabetes on arthritis: An update.. *Health,* Volume 5, pp. 143-156.

Rana, S. K., Helen, R. G., Plácido, N. & James, E. B., 2014. The Interaction between metabolic disease and ageing-review article. *Global Journal of Obesity, Diabetes and Metabolic Syndrome,* 1(1), pp. 102-107.

Rebrin, K., Steil, G., Mittelman, S. & Bergman, R., 1996. Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *Journal of Clinical Investigation,* Volume 98, p. 741–749.

Ren, J., 2004. Leptin and hyperleptinemia - from friend to foe for cardiovascular function. *Journal of Endocrinology,* Volume 181, pp. 1-10.

Ridker, P., Hennekens, C., Buring, J. & Rifai, N., 2000. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease. *Journal of Medicine,* Volume 342, pp. 836-843.

Roschlau, P., Bernt, E. & Gruber, W., 1974. Enzymatishe Bestimmung des Censamt-Cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.,* Volume 12, p. 226.

Rosemary, B. D., Rudolph, D., Joseph, S. & Richard, M. A., 2007. Prevalence of Obesity in Women of Accra, Ghana. *African Journal of Health Science,* Volume 14, pp. 154-159.

Ross, R., Freeman, J., Hudson, R. & Janssen, I., 2002. Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *Journal of Clinical Endocrinology Metabolism,* Volume 87, p. 5044–5051.

Ruth, E. G. & Lori, D. K., 2005. Adipose tissue as an active endocrine organ: recent advances.. *Current Opinion in Pharmacology,* Volume 5, pp. 122-128.

Santos, A., Lopes, C., Guimaraes, J. & Barros, H., 2005. Central obesity as a major determinant of increased high-sensitivity C-reactive protein in metabolic syndrome.. *International Journal of Obesity,* Volume 29, pp. 1452-1456.

Sattar, N. & Jason, M. G., 2014. Type 2 diabetes as a disease of ectopic fat? Obesity: exploring the causes, consequences and solutions. *BMC Medicine 2014,* Volume 12, pp. 123-129.

Schuster, D. P., 2010. Obesity and the development of type 2 diabetes : the effects of fatty tissue inflammation-review. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* Volume 3, pp. 253-262.

Seidell, J. C. & Flegal, K. M., 1997. Assessing obesity : classification and epidemiology. *British Medical Bulletin,* 53(2), pp. 238-252.

Shamsuzzaman, A., Winnicki, M. & Wolk, R., 2004. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation,* Volume 109, pp. 2181-2185.

Shemesh, T., Rowley, K., Jenkins, A. & Brimblecombe, J., 2007. Differential association of c-reactive protein with adiposity in men and women in an Aboriginal community in northeast Arnhem Land of Australia.. *International Journal of Obesity,* Volume 31, pp. 103-108.

Sinha, M. K., Opentanova, I., Ohannesian, J. P. & Kolaczynski, J. W., 1999. Evidence of Free and Bound Leptin in Human Circulation: Studies in Lean and Obese Subjects and During Short-Term Fasting. *Journal of Clinical Investigation,* 98(6), pp. 1277-1282.

Snijder, M. G., van Dam, R. M., Visser, M. & Seidell, J. C., 2006. What aspects of body fat are particularly hazardous and how do we measure them ?. *International Journal of Epidemiology,* 25(December, 2005), pp. 83-92.

Sun, Q., R.M., v. D., James, B. M. & Oscar, H. F., 1998. Leptin and levels and risk of type 2 diabetes in women: research design and methods ascertainment of type 2 diabetes laboratory procedures. *Diabetes,* Volume 61, pp. 1-10.

Susan, K. F., Matthew, R. R., Colleen, D. R. & Blandine, L. r., 2000. Symposium : Adipocyte function , differentiation and metabolism regulation of leptin production in humans. *Journal of Nutrition,* Volume 130, pp. 3127-3131.

Susan, S., Steven, H. & Michael, H. D. R. B. D., 2009. Hypertriglyceridemic waist phenotype predicts increased visceral fat in subjects with type 2 diabetes. *Diabetes Care,* Volume 32, p. 1916–1920.

Sweeney, G., 2002. Leptin signalling. *Cell Signal,* 14(8), pp. 655-63.

Tabak, A., Kivimaki, M., Brummer, E. & Lower, G., 2010. Changes in C-reactive protein levels before type 2 diabetes and cardiovascular death: the whitehall II study. *European Journal of Endocrinology,* Volume 163, pp. 89-95.

Tartaglia, L., 1997. The leptin receptor. *Journal of Biological Chemistry,* Volume 272, p. 6093–6096.

Tataranni, P. & Ortega, E., 2005. Does an adipokine-induced activation of the immune system mediate the effect of overnutrition on type 2 diabetes?. *Diabetes,* Volume 54, pp. 917-927.

Thorand, B., Baumert, J., Kolb, H. & Meisinger, C., 2007. Sex difference in prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Diabetes Care,* Volume 30, pp. 854-860.

Thorne, A., Lonnqvist, F., Apelman, J. & Hellers, G., 2002. A pilot study on longterm effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. *International Journal of Obesity Relatated Metabolic Disorders,* Volume 26, p. 193–199.

Tillett, W. & Francis, T., 1930. Serological reactions in pneumonia with non-protein somatic fraction of pneumococcus.. *J. Exp Med.,* Volume 52, pp. 561-571.

Trayhurn, P. & Beattie, J., 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proceedings of the Nutrition Society,,* 60(PubMed Abstract), pp. 329-339.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor.. *Annals of Clinical Biochemistry,* Volume 6, p. 24.

Tritos, N. & Mantzoros, C., 1997. Leptin: its role in obesity and beyond. *Diabetologia,* 40(12), pp. 1371-1379.

Van Gaal, L., Wauters, M., Mertens, I. & Considine, R., 1999. Clinical endocrinology of human leptin. *International Journal of Obesity Related Metabolic Disorder,* 23(1), pp. 29-36.

Wannamethee, S., Satter, N., Rumley, A. & P.H., W., 2008. Tissue plasminogen activator, von Willebrand factor and risk of type 2 diabetes in older men. *Diabetes Care,* Volume 31, pp. 995-1000.

Wei, M., Stem, H., Haffner & al, e., 1997. Serum leptin levels in Mexican American and non-hispanic whites: association with body mass index in cigarette smoking.. *Ann Epidemiology,* Volume 7, pp. 79-80.

World Health Organization, 2000. *Obesity: Preventing and managing the global epidemic,* Geneva: WHO Technical Reports Series No. 894..

World Health Organization, 2005. *Summary of Technical report and recommendation,* Geneva: WHO.

World Health Organization, 2007. *The challenge of obesity in the WHO European Region and the strategies for response,* Denmark: WHO Library Cataloguing in Publication Data.

Zeman, M., Jirak, R., Jachymova, M. & Vecka, M., 2009. Leptin, adiponectin, leptin to adiponectin ratio and insulin resistance in depressive women.. *Neuro Endocrinol Lett,* Volume 30, pp. 387-395.

Zhang, Y., Proenca, R., Maffei, M. & Barone, M., 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature,* Volume 372, pp. 425-432.

Zimmet, P. & et al., 1996. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study.. *British Medical Journal,* Volume 313, pp. 965-969.

APPENDIX

CONSENT FORMS

THESIS TOPIC: SERUM LEPTIN AND C-REACTIVE PROTEIN LEVELS IN OBESE GHANAIANS WITH TYPE 2 DIABETES MELLITUS AT KORLE-BU TEACHING HOSPITAL (KBTH), ACCRA

You have been invited to take part in the above titled research. The **purpose** of the study is to lookout for biochemical markers of type 2 diabetes mellitus in risk groups.

A brief **background:** The prevalence of obesity is increasing in Ghana. Obesity is associated with increased risk of many chronic diseases especially type 2 diabetes mellitus. Researching into biochemical markers of type 2 diabetes mellitus is very important especially among risk groups such obese individuals between the ages of 30-50 years. Leptin and C-reactive protein have been shown to be implicated in the pathogenesis of diabetes related disorders such as obesity.

You will be asked to fast for 10–12 hours and a small amount of blood (5mls) will be drawn by inserting a needle in your forearm for the study. Serum leptin, C-reactive protein, lipid profile and fasting glucose levels will be determined. The risk involved in this blood collection procedure is negligible and it will cause only minimal pain. A structured questionnaire will be administered to each participant to assess demographic characteristics. Anthropometric and blood pressure measurement will also be undertaken.

Your participation in the study is **voluntary**, and you can withdraw from the study at any time without any disadvantage concerning your medical care at the diabetic clinic or any other clinic in the hospital.

All information gathered will be treated confidentially and it will be made known only to the researcher. Your privacy and anonymity will be ensured in data collection, storage and publication of research material. Should the information be published in any scientific journal, you will not be identified by name.

Name of Researcher: **ADAMS YUSSIF (**Mphil. Part II student**)**

Signature of researcher: …………………… Date: ……………………….…….

Name of Institution: (Department of Chemical Pathology, University of Ghana)

CONSENT SHEET:

I understand my participation is totally **voluntary and free**, and that, I am not going to be subjected to any risk, danger or discomfort. I have been informed that, the confidentiality of the information will be safeguarded and that my privacy and anonymity will be ensured in the data collection, storage and publication of the research material. I have the right to refuse to participate at any time I wish to. I have read the information provided and all questions have been answered to my satisfaction. I therefore volunteer to take part in the study.

Signature/Thumb print of participant: ……………………… Date: …………………

QUESTIONNAIRE

To be completed by each subject participating in the study

Name: …………………………………………. Code: ……………………………..

Date: ………………………………… Patient ID: …………………………………….

Tel.: …………………………............. E-mail: ……………………………………......

Postal Address: ……………………………………………………………………………

Demographic and Anthropometric measurement

1. Age: ……………………….
2. Weight (kg): ………………
3. BP: ………………………..
4. % body fat: ……………….
5. % Muscle mass: …………..
6. Height (m): ………………………….
7. BMI (kg/m^2): ………………..………
8. Pulse: ……………………………..
9. Visceral fat: …………………………
10. Bone mass: …………………..………

Please tick [ ] the appropriate box where applicable

11. Sex   [ ] male
                 [ ] female

12. Marital status [ ] single   [ ] married
                    [ ] divorced   [ ] widowed

13. Education   [ ] none   [ ] primary
                   [ ] secondary [ ] tertiary

14. Occupation   [ ] unemployed   [ ] trader/self employed
                   [ ] government worker   [ ] others (please specify)……

15. Ethnicity   [ ] Akan   [ ] Ga   [ ] Ewe
                   [ ] Northerner   [ ] others (please specify)……

Lifestyle

16. How many times do you eat in a day?

                 [ ] two

74

[ ] three

[ ] four

[ ] five

17. What type of food do you mostly eat?

[ ] more carbohydrates and less meat or fish

[ ] less carbohydrates and more vegetables and fruits

[ ] less carbohydrates and more meat and fish

18. Do you exercise?

[ ] yes

[ ] no

If yes to question 13, how many times do you exercise?

[ ] once a week

[ ] twice a week

[ ] 3 – 5 times a week

[ ] every day of the week

Medical History

19. Do you smoke cigarette?

[ ] yes

[ ] no

If yes,   [ ] 1 pack/day   [ ] 2 pack/day   [ ] >2 pack/day

20. Family history of diabetes mellitus

[ ] yes

[ ] no

21. Do you have any of the following conditions?

[ ] none   [ ] hypertension   [ ] diabetes mellitus

[ ] chronic kidney disease   [ ] others (please specify)…

22. Are you taking any herbal remedy or preparation?   [ ] yes

[ ] no

Clinical data

To be completed by diabetic subjects only

23. Duration of diabetes since diagnosis

[ ] < 6months   [ ] 1 year   [ ] 2 years

[ ] 5 years   [ ] >5years

24. Do you experience any of the following complications

[ ] none   [ ] retinopathy   [ ] neuropathy

[ ] nephropathy [ ] skin lesion   [ ] recurrent infection

[ ] cardiovascular disease   [ ] oral cavity lesion

[ ] others (please specify)………………………

25. Type of drugs: [ ] none   [ ] metformin   [ ] rosiglitazone

[ ] insulin   [ ] Angiotensin receptor blockers (ARB)

[ ] Angiotensin-converting enzymes (ACE) inhibitors

[ ] beta blockers   [ ] others (please specify)…….………

26. Are you taking any herbal remedy or preparation?

[ ] yes

[ ] no

End of questionnaire.

Thank you for participating in this study. God richly bless you.

76

UNIVERSITY OF GHANA
SCHOOL OF MEDICINE AND DENTISTRY

Ref. No.: .... MS-AA/C.2/Vol.18^A

11th March, 2015

Mr. Adams Yussif (M.Phil)
Department of Chemical Pathology
SBAHS
Korle-Bu

ETHICAL CLEARANCE

<u>Protocol Identification Number</u>: **MS-Et/M.6 – P 3.2/2014-2015**

The Ethical and Protocol Review Committee of the School of Medicine and Dentistry on 10th March, 2015 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Assessment of Serum Leptin and C-Reactive Protein Levels in Obese Ghanaian Subjects with Type II Diabetes Mellitus at Korle-Bu"**

PRINCIPAL INVESTIGATOR: **Mr. Adams Yussif**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till November, 2015.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: ....

PROFESSOR ANDREW A. ADJEI
(FOR: CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE)

cc: Dean, SMD
        Head of Department
        Research Office

COLLEGE OF HEALTH SCIENCES

• P. O. Box GP4236, Accra, Ghana.   • Telephone: +233 (0) 302 666 987/8   • Fax: +233 (0) 302 663 062
            • Email: smd@chs.ug.edu.gh   • Website: www.chs.ug.edu.gh

BUDGET

Item	Quantity	Unit cost (GHS)	Total cost (GHS)	Unit cost (USD)
Cotton	1 roll	8.00	8.00	0.00
Gloves	2 boxes	14.50	29.00	0.00
Phlebotomist plaster	2 boxes	10.00	20.00	0.00
Tourniquets	1 set	13.00	13.00	0.00
Methylated spirit	1 litres	12.00	12.00	0.00
Alcohol pads	2 boxes	13.00	26.00	0.00
Eppendorf tubes	400 pieces	0.35	135.00	0.00
Cryotubes	400 pieces	0.25	95.00	0.00
100µl pipette tips	1000 pieces	30.00	30.00	0.00
1000µl pipette tips	1000 pieces	34.00	34.00	0.00
Syringe/Needles	200 pieces	28.00	28.00	0.00
Gel separator tubes	100 pieces	-	32.00	0.00
Fluoride oxalate tubes	100 pieces	0.25	25.00	0.00
Plain tube	100 pieces	0.35	35.00	0.00
EDTA Tubes	200 pieces	-	58.00	0.00
Sub-total			**580.00**	**0.00**
Glucose ELITech reagent	1 box	111.80	111.80	0.00
Triglyceride ELITech reagent	1 box	344.40	344.40	0.00
Total ELITech Cholesterol reagent	1 box	189.80	189.80	0.00
HDL ELITech reagent	1 box	880.13	880.13	0.00
Human Leptin ELISA Kit	1 box	0.00	0.00	302.40
Hurman C-reactive protein ELISA Kit	1 box	0.00	0.00	270.00
Shipping	-	0.00	0.00	165.00
Reagent Clearance	-	0.00	910.00	0.00
Sub-total			**2436.13**	**737.40**

Stationery	4 rim	15.00	60.00	0.00
Printing and Binding	-	0.50	500.00	0.00
Statistical analysis	-	-	250.00	0.00
Transportation	-	-	500.00	0.00
Miscellaneous	-	-	800.00	0.00
Sub-total			**2110.00**	**0.00**
Grand Total			**GHS 5126.13, USD 737.40**	