BLOOD PERIIPIN A LEVELS IN APPARENTLY HEALTHY OBESE AND NON-OBESE ADULTS IN ACCRA.

By

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DECLARATION

I, Bright Selorm Letsu, do hereby declare that this thesis, which is being submitted in fulfilment of the requirements for an M.Phil. Degree in Chemical Pathology is the result of my own research conducted at Korle-Gonno, Mamprobi, Chorkor, Sempe and Agege (Accra), the School of Biomedical and Allied Health Sciences' Laboratory and the Central Laboratory unit of Korle Bu Teaching Hospital (KBTH) under the supervision of Dr. Seth Amanquah and Dr. Sylvester Yaw Oppong. References to other people's works have been duly acknowledged. This thesis presents results of original research undertaken by me and neither all nor part of this thesis has been presented for another degree in this institution or elsewhere.

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DEDICATION

This work is dedicated to my dear parents **Mawukoenya** *and* **Serwaa**.

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ABSTRACT

Perilipins are regulatory proteins that cover the surfaces of lipid droplets located in adipocytes. They protect against basal lipolytic activity of hormone sensitive lipase and promote its catalytic activity during stimulated lipolysis. Perilipin A is the commonest form found on the surface of lipid droplets in adipocytes and may be affected by obesity. With increasing adipocyte size in obesity, the expression of perilipin protein and its regulatory role in lipolysis may be lowered which may increase the release of free fatty acids leading to insulin insensitivity. The aim of this study was to assess blood Perilipin A levels in apparently healthy obese and non-obese subjects and relate it to percentage body fat, insulin resistance and blood lipid levels. A study population consisted of 46 apparently healthy obese and 40 non-obese subjects. The subjects recruited for the study were from a number of Ga communities located along the coast within the Ablekuma South Sub-metro who provided details on their health status through a questionnaire. Anthropometrics, fasting lipids and glucose were measured at baseline. Baseline fasting glucose values were used to confirm non-diabetic status of subjects. Serum perilipin A and insulin were measured on fasting samples using commercially available ELISA assays following strictly the manufacturer's protocol. The HOMA2 calculator was used to calculate insulin sensitivity. Blood perilipin A (ng/L) level was significantly higher in the non-obese compared with obese subjects (159.9±32.6 vs 130.8±23.9, $p<0.0001$). Blood perilipin A levels were higher in obese males than obese females (135.1±29.3 vs 126.4±19.5, $p=0.218$). Glucose and insulin levels were not significantly higher in obese than non-obese subjects (5.9±0.5 vs 4.9±0.5) and (11.8±3.8 vs 11.1±3.3) respectively. HOMA-IR level was higher in the obese compared with non-obese subjects (1.47± 0.5 vs 1.40±0.4, $p=0.480$). BMI (40.5±5.9 vs 22.9±2.6, $p<0.0001$), visceral fat (18.2±5.8 vs 5.9±2.6, $p<0.001$) and percentage body fat (44.1±10.1 vs 25.5±9.5, $p<0.001$)

were about two fold higher in the obese compared with non-obese subjects. Except for HDL all other lipid parameters were significantly higher in the obese than non-obese subjects ($p<0.05$ in all cases). There was no significant correlation between perilipin A levels and age ($p=0.87$), BMI ($p=0.91$), visceral fat ($p=0.10$) and % body fat ($p=0.162$). However, perilipin A generally showed a negative relationship with these parameters except for visceral fat. There was no significant correlation between perilipin A levels and HOMA-IR ($p=0.23$). Perilipin A levels were not significantly correlated with all lipid parameters ($p>0.05$ in all cases). Except for T.CHOL/ HDL ratio that showed a positive relationship, all other lipid parameters showed a negative relationship with perilipin A levels. HOMA-IR showed no significant correlation with age ($p=0.07$), BMI ($p=0.963$) and % body fat ($p=0.69$) but showed a significant correlation with visceral fat ($p=0.049$). There was significant positive correlation between HOMA-IR and insulin levels ($p<0.001$) but no significant correlation between HOMA-IR and all lipid parameters ($p>0.05$ in all cases). In conclusion, blood perilipin A levels were significantly higher in the non-obese subjects compared to the obese which may have accounted for the lower lipid levels but showed no significant correlations with HOMA-IR and serum lipid levels.

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LIST OF ABBREVIATIONS

ATGL……...……...Adipocyte Triglyceride Lipase

ACAT…….…….Acyl coA Cholesterol Acyltransferase

β-cell……...…….……beta cell

BIA……Bioelectric Impedance Analysis

BMI…….…….Body Mass Index

cAMP…….……cyclic Adenosine Monophosphate

cm……...…….centimeter

CMP-1……Monocyte chemoattractant protein-1

DGAT……...……Diacylglycerol Acyltransferaces

ELISA……Enzyme Linked Immunosorbent Assay

FBG……..…Fasting Blood Glucose

FFA……...Free fatty acid

Fig……..Figure

HOMA……..Homeostatic Model Assessment

HOMA-IR……Homeostatic Model Assessment – Insulin Resistance

HSL…….....Hormone Sensitive Lipase

g/L…….…… Gram per liter

GLU……Glucose

HDL……...……..High Density Lipoprotein

HRP……..……..Horseradish Peroxidase

IL-6……..…….…...Interleukin 6

IL-1B……...…….Interleukin 1 beta

INS…….……Insulin

kDa……..……kilodalton

Kg/m^2…….Kilogram per meter square

LD…….Lipid Droplets

LDL……...……...Low Density Lipoprotein

mL.....……...Milliliters

mmol/L……Millimole per liter

mRNA……...Messenger Ribonucleic Acid

ng/L……Nanogram per liter

^oC……..…Degree Celsius

%.....Percentage

OD……...Optical Density

PAT……....perilipin, adipocyte differentiation related protein, tail interacting protein

PBS……Phosphate Buffered Saline

PKA……...…Protein Kinase A

PLIN, PLIN A…….…Perilipin, Perilipin A

QUICKI…….Quantitative Insulin Sensitivity Check Index

Rpm.....….Revolution Per Minute

SD……. Standard Deviation

SNPs……Single Nucleotide Polymorphism

TBS…….…….Tris-Buffered Saline

T.Chol……..…...……Total Cholesterol

TG…….....…….…….…….Triglyceride

TMB……..3, 3', 5, 5', - tetramethyl benzidine

TNF-α…….…Tumour Necrosis Factor Alpha

VLDL-C……..Very Low Density Lipoprotein

Vs……...……..……Versus

WHO……..……...World Health Organization

μL……....Microliters

>…….……..Greater Than

<.....Less Than

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Worldwide, the prevalence of obesity continues to increase such that it has reached almost epidemic proportions in areas which have recently adopted westernized lifestyle (WHO, 2016). This has overburdened the already meagre healthcare budget by elevating the occurrence of diabetes, heart disease, hypertension, and cancer. In 2014, over 1.9 billion adult populations were overweight. Over 600 million of these adults were obese; representing close to 13% of adult population worldwide (WHO, 2016). Locally, the prevalence rate of obesity is 5.5% with about 23% of adults known to be overweight (Biritwum *et al*., 2005).

Some environmental factors, particularly overeating and sedentary lifestyle are major contributors to the occurrence of overweight and obesity (Hill & Peters, 1998). Nevertheless, genetic factors are regarded as major contributors (Bouchard, 1993) with some studies suggesting that as much as 50–70% of variation in body mass index is due to genetic difference (Comuzzie & Allison, 1998). In this regard, several obesity genes have been discovered which regulate human adipose tissue mass and function (Perusse *et al.,* 1999; Qi *et al.,* 2004). Also, some of these genetic components were linked to genes that were primarily expressed in adipocytes which regulate its metabolism such as the lipases and the perilipins proteins (Dahlman *et al.,* 2005).

The perilipins are a group of proteins that coats neutral lipid store surfaces in fat cells and steroidogenic cells. In adipocytes, perilipin A is the commonest form found coating surfaces of neutral lipids (lipid droplets) and functions to regulate the storage and release of these

lipids (Greenberg *et al.,* 1991). It is also the most well-characterized lipid droplets proteins whose genotypes and contents (Qi *et al.,* 2004; Kern *et al.,* 2004; Wang *et al*., 2003) have been linked to obesity and as a contributor to adipocyte metabolism. In view of this, many modern investigations in obesity have been skewed towards the lipid droplets biology.

There are generally about four isoforms of perilipin proteins with Perilipin A being the most abundant and well-characterized form (Greenberg *et al.,* 1991). Perilipin A is the only form whose genetic variability has so far been linked with human disease (Qi *et al.,* 2004). Perilipin B is the only other form that is also expressed in adipocytes but in small amounts (Richardson, *et al.,* 2011). Perilipin C and D forms are only known to be expressed in steroidogenic cells (Londos, *et al.,* 1999).

Perilipin A controls access to adipocytes lipid droplets that supplies most tissues with fuel, acting as an inhibitor of basal lipolysis and as a promoter of stimulated adipolysis by both hormone-sensitive lipase(HSL) and adipose triglyceride lipase(ATGL) (Zhang, *et al.,* 2003; Sztaryd *et al.,*2003). These lipases hydrolyze lipid stores to release free fatty acids (FFAs) and glycerol (Schweiger *et al*., 2006). The functions of Perilipin A were discovered in experiments using animal models where the perilipin genes were knocked out (Tansey *et al.,* 2001) and overexpressed (Miyoshi *et al.,* 2010). These functions were reproducible when human perilipin instead of mouse perilipin was overexpressed in mice (Miyoshi *et al.,* 2010).

In humans perilipin A genotypes have been shown to relate to obesity (Qi *et al.,* 2004). Studies investigating the association between perilipin A level and obesity however have not been consistent. Some studies have reported lower perilipin A expression on fat cells of obese individuals compared to non-obese (Wang *et al.,* 2003; Mottagui-Tabar, *et al.,* 2003). In another study by (Kern *et al*., 2004) in non-diabetic subjects, higher perilipin expression were

observed in the obese which was suggested to correspond to increasing adipocyte size in the obese.

In spite of the seemingly contradictory results, these studies support an association between obesity and perilipin A levels. This relationship has been suggested to be bidirectional one in which metabolic phenotypes regulates perilipin A levels, and vice versa. Possibly, investigation of blood perilipin A and its association with obesity which this study sought to determine may provide valuable information and further insight on existing literature obtained through investigations using tissue culture and animal models.

1.2 Problem statement

Worldwide, the increasing prevalence rate of obesity and its associated disorders put pressure on scarce health resources (WHO, 2016). Obesity is the cause of cardiovascular diseases (leading cause of death in 2016), stroke (second highest cause of death in the world), diabetes (the cause of about 15 million deaths in 2015), high blood pressure, musculoskeletal disorders and some cancers (WHO, 2016). There is lack of information on perilipin A levels in blood and its association with obesity and related problems such as dyslipidaemia and insulin resistance.

1.3. Justification

One major metabolic disorder associated with obesity is dyslipidaemia (Horowitz *et al.,* 1999) which is a major risk factor for atherosclerotic heart disease and insulin resistance (Krahmer *et al.,* 2013). Perilipin A proteins control the storage and release of free fatty acids by regulating activities of lipases. Studies linking perilipin A levels with obesity have only been carried out in animal models and Caucasians using adipose tissue which have proposed

contrasting linkage between perilipin A expression and obesity (Wang *et al,* 2003; Kern *et al*.,2004). It is possible blood Perilipin A levels could relate to obesity and its attendant health problems such as insulin resistance and dyslipidaemia. Findings may provide a new insight into the seemingly conflicting results from previous studies using adipose tissue and animal models. The study would provide information on blood perilipin A levels in apparently healthy individuals in Accra. Clearly, huge benefits would be derived if research could discover effective prevention and therapies for obesity and related health problems.

1.4. Hypothesis

Blood perilipin A is not related to obesity and has no relationship with insulin resistance and lipid levels in circulation.

1.5. Aim

To assess blood level of perilipin A and insulin resistance in apparently healthy obese and non-obese individuals.

1.6. Specific objectives

- To determine blood perilipin A levels in obese and non-obese subjects.
- To determine insulin resistance levels in obese and non-obese subjects
- To determine blood lipid levels in obese and non-obese subject

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Overview of Adipose tissue

The storage of lipids is a universal feature of cells and organisms to buffer energy fluctuations in order to survive. In humans, adipose tissue is specialized to carry out this function in addition to being an endocrine organ. It buffers lipid excesses such as sterols and fatty acids by esterifying them to form neutral lipids (triacylglycerols) stored in specific organelles called lipid droplets (LD) in adipocytes (Fujimoto *et al.,* 2008). In humans, the excess energy which is usually obtained from diet is mainly packaged in white adipose tissue (Rosen *et al.,* 2006) whilst brown adipose tissue is specific for thermogenesis which is an adaptation for non-shivering in many homeotherms (Smith &Horwitz, 1969). Brown adipose tissue is known to be present only in human infants but recent investigations suggest a dispersed form maybe present in adults as well (Nedergaard *et al.,* 2007). Lipid droplets are present in other cells such as adrenocortical cells, macrophages, enterocytes, hepatocytes and bone marrow or thymus. The adipose tissue in recent times has gained the recognition as an important endocrine organ which secretes hormones such as leptin and adiponectin that control inflammation and insulin sensitivity (Friedman, 2009).

2.2. Overview of Lipid Droplets

Lipid droplets have a special form made of a hydrophobic interior of neutral lipids which comprises mainly of triacylglycerol (in white adipose tissue) and sterol esters (in macrophages). The core is coated with a phospholipid monolayer and surrounded by specific proteins (Ohsaki *et al.,* 2014). In human cells, the phosphatidylcholine monolayer is the

predominant form with phosphatidylethanolamine, phosphatidylinositol, lyso-phosphatidylcholine and lyso-phosphatidylethanolamine occurring in lesser amounts. The phosphatidylcholine functions as a surfactant which prevent the lipid droplets from coalescence (Szymanski *et al.,* 2007). The proteins coats the lipid droplets surface and regulate its size and number. The lipid and protein content of these lipid droplets varies between different cell types. In white adipocyte cells a single lipid droplet fills up almost the entire cytoplasm which probably is the most extreme with regard to the way fat is stored in cells (Cushman, 1970).

Majority of neutral lipid structures are synthesised in the endoplasmic reticulum in the presence of enzymes such as Acyl coA cholesterol acyltransferases (ACATs) for sterol esters synthesis and Diacylglycerol acyltransferases (DGATs) for triacylglycerol synthesis in the endoplasmic reticulum (Farese *et al.,* 2000, 2008). As lipid droplets mature, neutral lipids formation and phospholipids synthesis are coordinated. Volume increases due to neutral lipids accumulation results in surface expansion which require a corresponding increase in phospholipids to cover the neutral lipid core and reduce the surface tension thereby preventing lipid droplets from combining (Krahmer *et al.,* 2011). Alterations in phospholipids compositions therefore may likely affect the morphology of lipid droplets seen in diseases which are characterized by altered lipid storage (Krahmer *et al.,* 2011). The surface of lipid droplets is covered by a group of proteins that includes the perilipins and adipophilins which are known to regulate lipid storage and mobilization (Wolins *et al.,* 2005). These proteins are recognized as regulators of lipid droplets formation and breakdown to provide free fatty acids and sterols for the cell's energy or membrane lipids needs (Wilfling *et al.,* 2014) by regulating the activities of lipid hydrolases, known as lipases (Schweiger *et al*., 2006).

2.2.1. Hydrolysis of Lipid Droplets

The breakdown and retrieval of stored lipids is a highly regulated procedure. Hormone sensitive lipase (HSL) and adipocyte triglyceride lipase (ATGL) are the two main enzymes which act in concert under catecholamine stimulation to breakdown the lipid core in adipose tissue. Under catecholamine stimulation triglycerides are hydrolysed into diacylglyceride, by ATGL which is further broken into FFAs and glycerol by HSL and monoacylglyceride lipase. Free fatty acids are then transported to muscle tissue for oxidation, to the liver for synthesis of triglycerides or oxidation, and adipose tissue for re-esterification. Free glycerol is transported to the liver and used for hepatic glucose production. Lipid cores are also degraded by lipases present in autophagosomes (Singh *et al.,* 2009).

Lipid storage diseases usually result from changes that affect the normal breakdown and retrieval of lipids. Changes in lipid droplet proteins and lipases contribute to this dysregulation of lipolysis seen in lipid storage diseases. This dysregulation is linked to the occurrence of metabolic disorders in humans such as dyslipidemia, type 2 diabetes and atherosclerosis (Krahmer *et al.,* 2013).

2.2.2. Lipid Droplets in Obesity and Associated Diseases

Lipid droplets have an impressive TG storage capacity due to their plasticity; however, when the fat storage capacity is exceeded it leads to diseases such obesity and its associated disorders. In obesity, adipose tissue become increasing dysfunctional resulting in higher circulating free fatty acids delivery to peripheral tissue and disordered adipokines production (van Herpen & Schrauwen-Hinderlin, 2008). Also, the generation of more bioactive lipids such FFAs and other derivatives could cause lipotoxicity and are also known to interfere with

insulin signaling pathway thereby promoting insulin resistance both in liver and skeletal muscle (Schaffer, 2003; Virtue & Vidal-Puig, 2010).

In obesity, with adipocyte hypertrophy, adipokines such as adiponectin and leptin secretion are suppressed (Wellen & Hostamisligil, 2003). Rather cytokines with pro- inflammatory activities such as macrophage chemo-attractant protein-1(MCP-1) and tumour necrosis factor-α (TNF-α) are secreted leading to inflammation (Hostamisligil *et al.,* 1993). Usually, microvasculature does not expand to match the increasing adipocytes volume which causes microhypoxia resulting in inflammation within the tissue (Pasarica *et al.,* 2009). In addition TNF-α increases lipolysis in adipocytes and suppress adipose tissue proteins which control the storage and breakdown TG (Guilherme *et al.,* 2008). Perilipin A synthesis is particularly known to be suppressed by TNF-α leading to reduced activity which could cause increased basal lipolytic rate (Souza *et al.,* 1998).

Lipid droplets associated proteins in adipose tissue such as fat-specific protein 27(FSP27) (Puri *et al.,* 2007) and perilipin A (Greenberg *et al,* 2011) are important for synthesis and function of lipid droplets by controlling abnormalities of excess triglycerides storage and release. The FSP27/CIDEC protein promotes the synthesis of lipid droplets with single locules (Gong *et al.,* 2011). Their depletion prevents LD formation and their expression results in bigger and fewer LDs (Jambunathan *et al.,* 2011). Polymorphisms in FSP27 proteins can influence obesity risk and disorders of metabolism (Dahlman *et al.,* 2005). Perilipin A is another LD associated protein that regulates triglyceride storage and release in adipose tissue (Greenberg *et al,* 2011). LDs may take part in other activities in the cell such as the breakdown and storage of proteins and viral replication (Murphy, 2012; Thiele & Spandl, 2008).

2.3. Overview of Perilipin

The lipid droplets surface is covered by proteins previously referred to as Perilipin, Adipocyte differentiation related protein and Tail-interacting protein (PAT) family of proteins (Muira, *et al.,* 2002). This name has since been revised and a new nomenclature, perilipin protein, adopted as a common nomenclature for these proteins. Perilipin 1 to perilipin 5 is currently used and these describe the location of the perilipin proteins on the lipid droplets periphery (Kimmel, *et al.,* 2010). The perilipin gene in mouse was the first to be studied which generated at least four isoforms (perilipin A to perilipin D).These isoforms have a similar amino terminal sequences in addition to a common protein kinase A (PKA) phosphorylation sites which range from two to six sites. Perilipin A and B (small amount) are distinctively expressed on adipocyte lipid droplets and have identical amino terminal (406 amino acids), after which they differ (Londos *et al.,* 1999). Perilipin A phosphorylation is up to a maximum of 6 different protein kinase A sites (81,223,277,434,492 and 517) whilst perilipin B is only phosphorylated up to three PKA sites (Londos *et al.,* 1999). Perilipin A is the most plentiful and well-characterized of the two isoforms (Greenberg *et al.,* 1991) and the only one with genetic variability linked to human obesity and related conditions (Qi *et al.,* 2004).

During stimulated lipolysis, catecholamines stimulate β-adrenergic receptors on adipocyte. This causes the activation of adenylyl cyclase which converts ATP to cAMP. PKA is then activated by cAMP and activated PKA then phosphorylates both hormone sensitive lipase (HSL) and perilipin (Londos *et al.,* 1999). Phosphorylated perilipin A then goes through a conformational change to accommodate the addition of HSL to the neutral lipid core.

Activated HSL moves from the cytosol to the lipid core to hydrolyze it and release FFAs and glycerol (Zhang *et al.*, 2003; Sztaryd *et al.*, 2003).

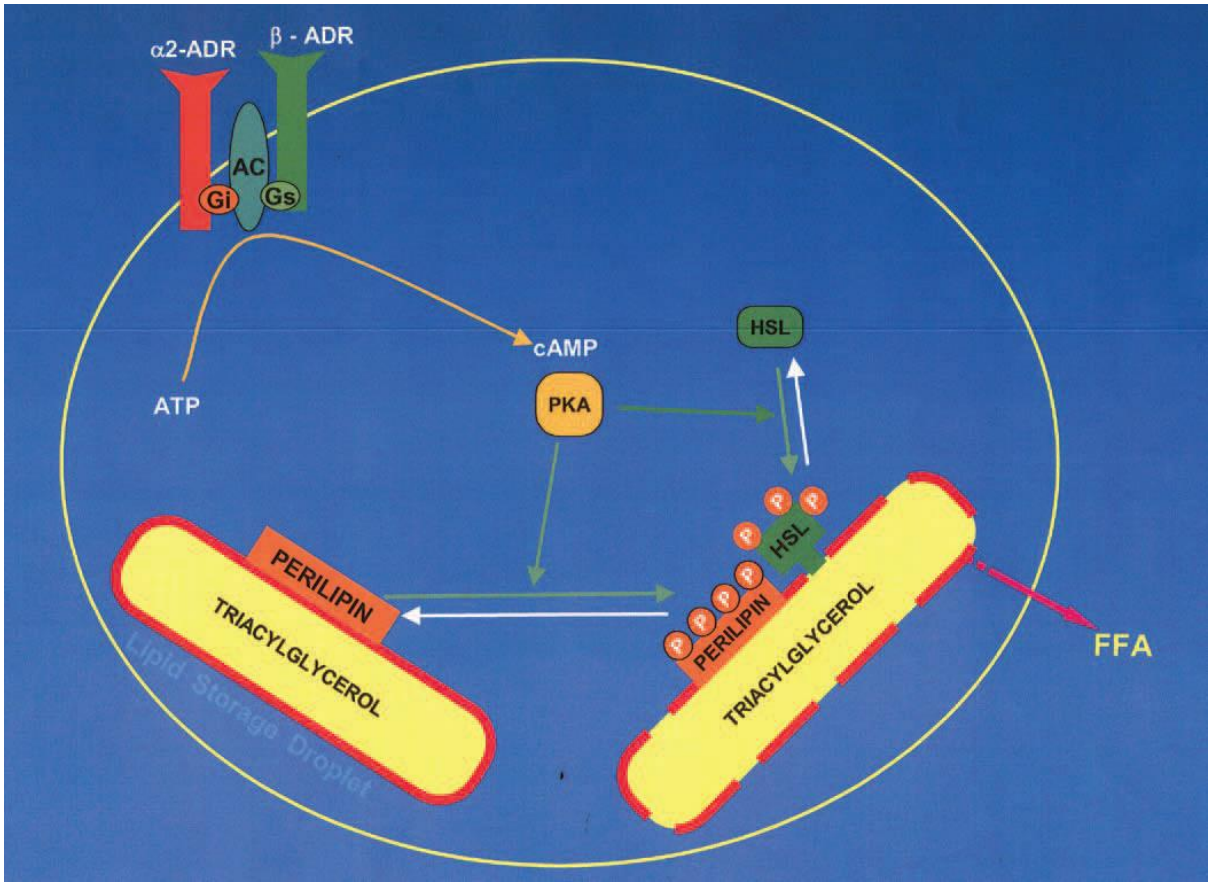

Figure 2.1: PKA-stimulated lipolysis in adipocytes (The USA National Library Medicine)

2.3.1. The Discovery of Role of Perilipin in Lipolysis

The functional role of perilipin A was first discovered using animal models when the perilipin A gene was knocked out. When Perilipin knocked out mice and the wild type were given a diet high in fat they both exhibited similar feeding pattern. Interestingly, perilipin knocked out mice were protected against obesity and their basal lipolysis increased. They visibly had a reduced adipose tissue and demonstrated a higher glucose intolerance and insulin resistance which was related to unregulated lipolysis (Zhai *et al.*, 2010; Tansey *et al.*, 2001). Perilipin A was then considered to be a lipogenic factor since it was required for the accumulation of normal amounts of adipose triacylglycerol. When lipolysis was stimulated in the same

experiment it was observed that perilipin knocked out mice had a blunt response. This showed perilipin was required during stimulated lipolysis, hence a lipolytic factor (Matinez *et al.,* 2000; Tansey *et al.,* 2001). This demonstration showed that perilipin A as much as HSL is required for maximum stimulated lipolytic activity (Mayoshi *et al.,* 2006).

It was also demonstrated that the deletion of perilipin showed reversal of obesity in an obese mice and obesity associated with feeding on a diet high in fat but did not reverse diabetes (Martinez *et al*., 2000; Tansey *et al*., 2001). Also, mutation of the three PKA sites (81,223,277) required for maximum lipolytic response may lead to unphosphorylation which result in inhibition of lipolysis even in cells that have been activated. This showed that HSL translocation will not occur if the 3 perilipin A PKA sites are mutated (Souza *et al.,* 2002).

When the perilipin A gene was overexpressed in mice it resulted in protection against obesity, increase in adipocyte size and volume and glucose intolerance when these mice were fed on high-fat diet. There was also decrease in basal and stimulated lipolysis. These benefits were observed when perilipin A gene was overexpressed in both human and mouse tissues (Miyoshi *et al.,* 2010).

2.3.2. Perilipin A Genotypes and Obesity

A set of six SNPs of the perilipin A gene were discovered (Qi *et al.,* 2004) which became the basis for many other genetic studies to detect association between perilipin gene and obesity in humans. Only two of these SNPs (rs2289487 and rs894160) which were gender-specific had associations with anthropometric and metabolic traits. The remaining four SNPs were not associated with any metabolic outcomes (Qi *et al.,* 2004). Two other SNPs (rs2304795, rs1052700) were discovered and were related to obesity traits in women, confirming the importance of the Perilipin (PLIN) locus (Qi *et al.,* 2004).

Despite these findings showing an association between the PLIN genes with obesity some other studies found no associations between PLIN A and obesity-related phenotypes (Bergman *et al.,* 2008; Dong-Shen *et al.,* 2009). Also, since the risk of obesity is strongly influenced by diet, investigations to determine dietary modulation of PLIN A became necessary. Here when dietary modulation of the PLIN A gene was assessed for the four SNPs that has been linked to baseline obesity, it confirmed reports that showed minor allele of rs894160 provided a protective effect against obesity (Corella *et al.,* 2005).

2.3.3. Perilipin A Content and Obesity

Studies in humans to investigate the association between adipocyte perilipin A level and obesity has not shown a consistent results. (Wang *et al.,* 2003) reported lower perilipin A expression per fat cell surface in obese individuals (BMI, 53 kg/m^2) than in non-obese individuals (BMI, 25kg/m^2). They found perilipin A protein content to be lower in obese subjects, but observed a similar perilipin A protein mass per fat cell in the obese and non-obese. Interestingly, in another study it was discovered that adipose perilipin A protein and mRNA contents were greater in non-diabetic obese individuals compared to non-obese individuals (Kern *et al.,* 2004). This they thought was to make up for the increasing adipocyte size. They noticed strong positive correlation when they compared perilipin A levels with percent body fat, but perilipin A levels showed no significant correlation with insulin resistance and cytokines (Kern *et al.,* 2004). In another study by (Mottagui- Tabar *et al*., 2003), they obtained a lower perilipin A protein content in the adipocytes of obese women (mean BMI, 40kg/m^2), which they related to greater rate of lipolysis in the obese. They also obtained noticeable inter individual differences in adipocyte perilipin A content in the population (Mottagui-Tabar *et al.,* 2003).

Results of these studies support an association between obesity and perilipin levels despite the contradicting results which indicate a possible bidirectional relationship. Thus, perilipin A levels may be influenced by factors such as obesity or rather perilipin A genotypes determine the level perilipin A in individuals.

2.4. Overweight and obesity

Excess accumulation of fat that negatively impact health is referred to as overweight and obesity. It usually results from a disparity between energy storage and its usage over a long duration which leads to shortened life expectancy and a number of health problems (Haslam *et al.,* 2005; Hubert *et al.,* 1983; WHO, 2016).Worldwide, the prevalence of obesity continues to increase such that even in poor countries, it is reaching epidemic proportion (WHO, 2016). Obesity is strongly implicated in dyslipidemia, insulin resistance, cardiovascular disorders, certain types of cancers and stroke (Krahmer *et al.,* 2013), contributing heavily to the weight of chronic health conditions. It is mainly classified based on anthropometric indices which include body mass index (BMI), waist hip ratio or waist circumference, skinfold thickness and other methods which provide direct estimates of body composition such as the percentage body fat by the bioelectric impedance method. Internationally BMI cut-off values of (25 to 29.5kg/$m^{2)}$ for overweight and (≥30kg/m^2) for obesity have been recommended and are commonly used classifications (WHO, 2016). In both adult males and females with an average weight, their percentage body fat is about 15-20% and 25-30% in males and females respectively (Seidel *et al.,* 1997).

2.4.1. Aetiology of Overweight and Obesity

Overweight and obesity result from a combine interaction between genes and the environment or both (Lau *et al*., 2006). This is traditionally attributed to the ingestion of large

quantities of food, genetic susceptibility and the lack of physical activity (Lau *et al*., 2006). Other causes which may contribute significantly but occur in limited numbers include medical reasons such as endocrine and psychiatric illness (Bleich *et al.,* 2008). Aside this, there are several medical and lifestyle causes which were identified in a 2006 review as contributors to the current rise in obesity (Keith *et al.,* 2006).

2.4.2. Medical Consequences of Obesity

Obesity usually leads to dysregulation of metabolic homeostasis which increases the risk for various chronic diseases, such insulin insensitivity and type 2 diabetes, heart diseases, high blood pressure and stroke, obstructive sleep apnea, dyslipidaemia, arthritis, asthma, and cancer (Bray, 2004). Childhood obesity is also implicated in an increased risk to obesity leading to disability and premature death later in adulthood (WHO, 2016).

Obesity presents with increased release and production of pathological products from adipose tissue. The pathologies of obesity fall under two main categories; those due to increased adipose tissue mass such as osteoarthritis which is as a result of wear and tear of joints due to the increased weight (Felson *et al.,* 1988), obstructive sleep apnea due to increased parapharyngeal fat deposits and social stigmatization from the oversized stature (Choban *et al.,* 1999). The second category is characterized by the metabolic effects of increased released of secreted products and it includes diseases such as diabetes mellitus, hypertension, cardiovascular complications, gallbladder diseases, and some forms of cancer (Bray, 2004).

Insulin resistance which is also a common feature in obesity results from a combination of factors present during obesity; increased released of fatty acids (Boden, 1997) from fat cells and overproduction of cytokines (Bouzakri *et al.,* 2007) that reduces insulin sensitivity. Obesity also induces a procoagulant state through chronic inflammation and impaired fibrinolysis both of which leads to increase risk of thrombosis. Chronic inflammation results

from transient hypoxia developing from a poorly vascularized adipose tissue leading to the recruitment of macrophages. In addition to these factors, changes in endothelial function and increased circulating free fatty acids put the obese individual at the increased risk of cardiovascular diseases, stroke and hypertension (Balistreri *et al.*, 2010). Obesity also comes with an increased risk for certain form of cancers (Manson *et al.*, 1995) with males facing increased risk of cancer of the colon, rectum and prostate and women having increased risk for cancers of the gallbladder and the reproductive system (due higher estrogens production by stromal cells of adipose tissue) (Schapira *et al.*, 1994). The combined effects of all these pathologies results in reduction in life expectancy (Haslam *et al.*, 2005).

2.4.3. Measurement of Overweight and Obesity

Numerous methods exist for the measurement of body fat composition. Each of these methods has its own benefits and limitations with only a small number suitable for epidemiological or clinical practice. Usually in clinical practice and epidemiological studies, methods which are technically simple, less time consuming and costs less to operate are favoured. Anthropometry measurements such as skinfold thickness (Deurenberg *et al.*, 1991) or weight and height index (Garrow & Webster, 1985), bioelectrical impedance method (Lukaski *et al.*, 1985; Segal *et al.*, 1988) and infra-red interactions (Conway *et al.*, 1984) are some of the simple methods preferred. The BMI is the simplest and commonest method using the weight and height index. Other indices such as the waist-hip circumference ratio have been developed to predict specifically intra-abdominal fat. This has been shown to be more predictive of adverse outcomes such dyslipidemia and insulin resistance than total fat (Pouliot *et al*., 1994).

2.5. Body Mass Index (BMI)

Body mass index is the ratio of body weight measured in kilograms to height in square meters (Quetelet, 1869). The World Health Organization (WHO) classifies individuals using BMI as overweight (BMI between 25.0 - 29.9 kg/m^2) and obese (BMI greater than 30.0 kg/m^2). It is the commonest measure of adiposity used in adults which provides a direct way of classifying individuals as underweight and overweight and also serves as a global nutritional status index. It also an extensively used method for determining obesity status due to its low cost and simplicity. It provides an approximation of fat mass and mortality risk with a total performance better than any other weight stature index (Cole *et al.,* 1995). Its standardization by age and sex in recent times makes it more sensitive, especially in children since BMI changes are greatest during childhood due to unrelated variations in height (Dietz *et al.,* 1999). However, it is considered a rough guide due to its failure to distinguish adipose tissue mass from lean mass. Also weight differences in people are not entirely due to body fat but are age, sex and population specific which may not be considered (Deurenberg *et al.,* 1991). Despite this obvious challenge, it has been proven that body fat estimation using BMI correlate very well with bioelectric impedance measurement (Ranasinghe *et al.,* 2013).

2.6. Bioelectric impedance analysis (BIA)

This method provides a safe and non-invasive approach to estimating body composition by measuring the resistance of the body when a small electric current is passed through it. The device provides measurement of how the current is impeded in different tissue types. Current flow is affected by the amount of water in the body with tissues containing large amount of fluid and electrolytes such as blood having a higher conductivity and other tissues such as fat slows the current. The method assumes the whole body to be a single

cylinder and takes bioelectric measurements from electrodes placed at the wrist and ankle. Data generated is adjusted for height and used for the estimation of total body water and fat free mass. Absolute or relative fat mass are then calculated from the estimated total body water or free fat mass values (Lukaski *et al.*, 1985; Chumlea *et al.*, 1994).

The method is suitable for population groups in epidemiologic studies where specific equations are generated based on age, sex and metabolic status of the group. Its accuracy for estimating body composition maybe limited because the equations used are mostly based on assumptions. Assumptions such as a homogeneous ion composition of the conductor, a constant cross-sectional area and a uniform distribution of current density do not necessarily relate to the body. Because the BIA incorporates these assumptions, they are considered to provide crude estimates of body composition unless corrected for sex, age and metabolic status of group used. Moreover, these equations mostly are population specific and were only suitable for populations that closely match the reference population in body and size (Deurenberg *et al.*, 1996).

However, there have been several comprehensive reviews of the principles and applications of the method which has led to a tremendous improvement on the accuracy, precision, and reliability of bioelectrical impedance in determining body composition in humans (Lukaski *et al.*, 1986; Heitmann *et al.*, 1994; Baumgartner *et al.*, 1990).

2.7. Insulin resistance

Insulin is a peptide hormone made by the islet cells of the pancreas beta cells that plays a major role in regulating glucose concentration in blood. It facilitate glucose uptake into body tissues such as skeletal muscle and adipose tissues and also prevents glucose generation in the liver. Insulin has other target tissues such as the pancreatic beta cells, the brain, heart and

vascular endothelium. Here it promotes the coordination and coupling of metabolic and vascular homeostasis in healthy conditions (Prodi & Obici *et al.,* 2006; Muniyappa *et al.,* 2007). Insulin actions are stimulated by glucose level, acting in concentration-dependent manner to decrease whole body glucose. Its maximal effects on tissues define its responsiveness whilst the insulin concentration required for half of the maximal effects refers to its sensitivity.

In insulin resistance there is a diminishing sensitivity of body cells to the metabolic activity of insulin. These include insulin mediated glucose uptake and inhibition of hepatic glucose production (Boden *et al.,* 2005). This eventually results in the need for higher levels of insulin for glucose disposal which beta cells compensate for this by increasing insulin secretion. Over time, due to increasing cells insensitivity and a higher level of insulin requirement which cannot be matched by beta cell production, prediabetes and type 2 diabetes develop.

Insulin resistance is well recognized for its major role in pathophysiology of type 2 diabetes with close association with metabolic syndrome (Petersen *et al.,* 2007). Individuals with excess body weight, physical inactivity and genetic predisposition usually stand a greater risk of developing insulin resistance.

2.7.1. Mechanism of Insulin Resistance in Obesity

Insulin resistance is a common condition present in obesity which may lead to occurrence of type 2 diabetes. Adipose tissues in the obese are enlarged and stressed which cause the release of fatty acids into circulation (Boden, 1997). These FFAs also tend to prevent the anti-lipolytic activity of insulin which further elevates the level of FFAs released into the circulation (Jensen *et al.,* 1989). These circulating FFAs have long been proposed to mediate the process of insulin resistance showing strong association with insulin resistance in

peripheral tissue (Savage *et al.,* 2007). To further demonstrate this, drastic reduction of FFAs with anti-lipolytic drug acipimox showed an improved insulin action on glucose delivery into peripheral tissue (Santomauro *et al.,* 1999).

In addition, adipose tissue secretes adipokines such as leptin and adiponectin which promotes insulin sensitivity of body tissues, serving as an important endocrine organ. In the absence of adipose tissue in humans, there is increased insulin resistance due to impaired secretion of adipokines (Oral *et al.,* 2002). Adipose tissue promotes whole-body metabolism in esterifying excess FFAs so that in its absence there is elevated concentration of FFAs leading to lipotoxicity and other complications in both humans and mice (Laustsen *et al.,* 2002). These observations re-emphasize the role of adipose tissue to body size in maintaining optimal insulin sensitivity and glucose balance when its mass is proportional to the body.

In obesity, due to the increased deposition of triglycerides and the ensuing inflammatory state of the adipose tissue the endocrine function is impaired. In addition, microvasculature is not increased in proportion to increased adipose tissue mass which result in microhypoxia. Adipose tissue secretes especially high amounts of monocyte chemoattractant protein-1(MCP-1) that induce the infiltration of adipose tissue by macrophages (Curat *et al.,* 2004). The continued production of MCP-1 and the subsequent macrophage infiltration of the hypertrophied cells lead to a pro-inflammatory state. The final development into an inflammatory state is characterized by adipocytes and macrophages secreting large amount of MCP-1 and other cytokines such as TNF-α, interleukins 6 and 1β (IL6 and ILβ) (Lagathu *et al.,* 2006). These cytokines directly increase lipolytic rates and decrease the formation of triglyceride in adipocytes resulting in increased amounts of circulating FFAs. These excess circulating FFAs form fatty acid derivatives such as long chain fatty acyl-CoA which

accumulate in peripheral tissues such as liver cells, muscle tissue and β-cells. This impairs the natural metabolic and secretory activities such as insulin sensitivity of these tissues (Unger, 1995, 2002). Aside this, TNF-α by direct action on insulin signalling in muscle can also cause insulin resistance (Bouzakri & Zierath, 2007).

2.7.2. Measurement of insulin resistance

Numerous techniques are available for measuring insulin sensitivity in diabetic management and research. The hyperinsulinemic euglycemic glucose clamp technique is regarded as the reference method for assessing insulin sensitivity in humans (DeFronzo *et al.,* 1979). However, due to the complicated nature of this procedure it is not suitable for large scale usage (Muniyappa *et al.,* 2008). There are other tests such as the insulin suppression test, minimal model analysis of frequently sampled intravenous glucose tolerance test and oral glucose tolerance test which are sensitive but also cumbersome. Alternative simple methods have been developed that employ fasting insulin and glucose concentrations to determine insulin sensitivity. These methods include; quantitative insulin sensitivity checks index (QUICKI) and the homeostasis model assessment (HOMA) methods.

2.8.Homeostasis Model Assessment (HOMA)

The HOMA was developed as a simple inexpensive alternative to the glucose clamp method. It provides an estimate for beta cell function and insulin sensitivity in a steady state using fasting insulin and glucose values in computer generated model. It is based on feedback mechanism between glucose levels and insulin secretion in the homeostatic state (Mathews *et al.,* 1985).

An individual's fasting plasma insulin level is mainly determined by β-cell response to glucose concentration whereas fasting glucose concentration is controlled by hepatic glucose

production (HGP) under the influence of insulin. This glucose insulin homeostasis is what is described by HOMA using a set of nonlinear equations that have been empirically derived. A computer generated model of this equation is now used to transform fasting glucose and insulin values from subjects to determine unique combinations of insulin sensitivity (HOMA%S) and β-cell function (HOMA β %) from steady-state conditions.

HOMA1-IR = [**fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5.**

HOMA1-%β = **(20×fasting insulin)/ (fasting glucose–3.5),** assuming 100% β-cell function and insulin resistance of 1(Mathew *et al.,* 1985).

IR= insulin resistance (inverse of %S) and %β= percentage β-cell function.

The current model, HOMA2 is an update on the first model, HOMA. It has been corrected to consider differences in hepatic and peripheral glucose resistance, increases in insulin secretion curve for plasma glucose concentration above 10 mmol/L and the contribution of circulating proinsulin (Levy *et al*, 1998). The model had a recalibration also to include specific insulin and c-peptide in determining %S and %β values.

HOMA is among the best and most extensively validated method for estimation of insulin resistance. Measurements by this method have shown strong correlation with measurement of insulin resistance and β-cell function by hyperglycaemic and euglycaemic clamp and intravenous glucose tolerance test (Anderson *et al.,*1995; Emoto *et al.,*1999; Bonora *et al.,*2000).

CHAPTER THREE

3.0. MATERIALS AND METHOD

3.1. Study design

This was a community based cross-sectional study with the purposive and simple random sampling technique adopted for recruiting obese and non-obese subjects respectively.

3.2. Study site

Subjects for the study were recruited from Korle-Gonno, Mamprobi, Chorkor, Sempe and Agege communities, all within the Ablekuma South Sub-metro located in the Accra Metropolis District of Greater Accra. These communities are located at the southern and central part of Accra along the shores of the Atlantic Ocean with the indigenous inhabitants (Gas) mainly fishermen, fishmongers and petty traders by occupation. Individual homes within these communities were visited and subjects recruited were assembled at a central site for the procedure. In Ghana, the prevalence of obesity is highest among the Gas (14.6%) with a national prevalence of about 5.5% (Biritwum *et al*., 2005).

3.3. Study population.

The study was carried out on apparently healthy obese and non-obese adults between the ages of 30-70 years. For the purpose of this study, apparently healthy adults were defined as men and women between the ages of 30 to 70 years who were without any known disease, who were not on admission or have been diagnosed for a particular disease for which they are on medication. The selection criteria for the subjects were based on response to a questionnaire, Appendix C, which obtained information on current health status, age, sex and

family history of obesity. Subjects selected were screened at baseline for fasting glucose to confirm non-diabetic status. A total of 140 subjects were recruited out of which 86 (both obese and non-obese subjects) were selected for the study. All subjects recruited provided an informed consent.

3.4. Inclusion and Exclusion criteria.

3.4.1. Inclusion criteria

Subjects recruited for the study were apparently healthy men and women between ages 30 to 70 years. These included both obese and non-obese subjects.

3.4.2. Exclusion criteria.

Subjects who did not fall within the age category of (30-70 years) were excluded from the study as well as heavy drinkers, drug abusers and smokers. Subjects whose baseline fasting glucose levels were above 6.1 mmol/L were excluded. Subjects who had any known disease conditions and subjects on medication for a disease condition or for other reasons were also excluded.

3.5. Sample size determination

The minimum sample size was determined by the formula;

Sample size (N) = $\frac{Z_{1-\alpha/2}^2 (SD^2)}{d^2}$ (Charan & Bisas, 2013)

$$N = \frac{(1.96)^2 (0.21)^2}{0.05^2}$$

N= 34

$Z_{1-\alpha/2}$ = standard normal variate (at 5% type 1 error =1.96 and $p<0.05$).

SD= standard deviation obtained from previous study or through pilot study (0.71±0.21, Kern *et al*, 2004).

d = the absolute error or minimum allowable error (5% or 0.05).

Based on the formula above, a sample size of 86 was used for the study, comprising 46 obese subjects and 40 non-obese subjects.

3.6. Ethical Approval

Ethical approval was obtained from the Ethics and Protocol Review Committee of the University of Ghana, College of Health Sciences.

3.7. Sampling and sample collection

3.7.1. Specimen collection, Transport and Storage

Sampling was done after 8 to 12 hours of overnight fast. About 4 mls of venous blood samples was drawn from the antecubital space of the forearm from each participant by a certified phlebotomist: About 3 mls of blood was pipetted into gel separator tube (contains a gel separator and clot activator) and 1 ml into a fluoride tube (contains fluoride oxalate). Blood samples were collected between the hours of 7:00 am and 9:00 am. Blood sample drawn was kept in a cool dry specimen container before transported to the laboratory for processing. The samples were then centrifuged at 4000 rpm for 3 minutes and the plasma analyzed for glucose immediately. Blood samples drawn into serum separator tubes were allowed to clot for 20 minutes minimum and centrifuged at 4000 rpm for 3 minutes. Serum was aliquoted into Eppendorf tubes and stored at -20 ^{o}C.

3.7.2. Anthropometric Measurements.

Participants' anthropometrics were determined using the Omron's HBF-514 Full Body Sensor Body Composition Monitor and Scale (OMRON Healthcare, Netherlands) following strictly standard operating procedures**.** To provide estimates of BMI, percentage body fat, and visceral fat the monitor required inputs of age, sex, and height (for BMI calculation). Height measurements were provided using a stadiometer after subjects had taken off their foot wears. Patients were required to stand upright and bare footed on the monitor with the two arms outstretched to hold the handles (electrodes) of the monitor. A small electric current, about 5mA passes through the sole of palm and feet after standing on the monitor scale which is captured and computed to provide estimates of visceral fat and percentage body fat on the monitor of the scale. The Omron scale and monitor provide estimates using the bioelectric impedance method. Body mass index (BMI) was calculated from body weight (in kilograms to 0.1 kg) divided by square of the height (in meters to 0.1cm) which was manually entered on the scale.

3.7.3. Data Collection

A structured questionnaire comprising mainly close-ended questions was used to obtain participants data on socio-demographic, health status and lifestyle behaviours after they have signed an informed consent form.

3.8. Biochemical Analysis

The enzyme-linked immunosorbent assay (ELISA), sandwich technique, was performed for the estimation of perilipin A and insulin content in serum. Fasting glucose and lipids were measured by an enzymatic colorimetric method using the VITROS 5, 1 FS Chemistry

Systems (J&J Ortho Clinicals Dagnostic, USA) at the Central Laboratory, Korle Bu Teaching Hospital (KBTH). The Homeostatic Model Assessment (HOMA2) (Diabetic Trials Unit, University of Oxford, UK) calculator was used to quantify insulin resistance.

3.8.1. Measurement of Perilipin A

Test principle

The kit uses the sandwich ELISA technique to assay Human Perilipin A (PLIN A) level in serum. A purified PLIN A antibody specific to Perilipin A is used to line the microtiter plate wells, making a solid-phase antibody. Samples are then added to the right microtiter strip plate wells to combine with the specific antibody. An antibody specific for PLIN A, labelled with horseradish peroxidase (HRP) enzyme is then added to the wells to form an antibody-antigen enzyme-antibody complex after washing thoroughly. A 3, 3', 5, 5', -tetramethylbenzidine (TMB) substrate solution is then added to be catalyzed by the enzyme HRP if an antibody-antigen enzyme-antibody complex is formed. TMB substrate becomes blue coloured when it is catalyzed by HRP enzyme. Sulphuric acid solution is then added to stop the reaction. The yellow colour change is then measured at 450 nm using the spectrophotometry plate reader. The OD value is proportional to the concentration of perilipin A. Concentration of Human Perilipin A is then determined by comparing the optical density of samples to the standard curve.

Test Procedure

Aliquoted serum samples were brought to room temperature to thaw and vortexed for 10 seconds. The Human Perilipin A kit (Kono Biotech Co., Ltd, China) which was previously stored in the refrigerator at (2- 8)^{o}C was brought to room temperature. Different concentrations of the standard were prepared as per the protocol (200 ng/ml, 100 ng/ml, 50

ng/ml, 25 ng/ml and 12.5 ng/ml) with the first well reserved for a blank. 50 µl of standard samples were added to the next five wells with the remaining wells filled with 50 µl of test samples (prepared by adding 10 µl of test samples to wells filled with 40 µl of sample diluent, dilution factor of 5) and gently mixed. The plate was then closed with a closure plate membrane and incubated at 37 ^{0}C (in an oven) for 30 minutes. A 30-fold wash solution was prepared with distilled water and was used to wash the plates. After 30 minutes of incubation, the closure plate membrane was uncovered, samples discarded and plate dried by swinging. Washing buffer was then added to each well for 30 seconds and drained. This was repeated four times and drying done by patting after each procedure. 50 µl of HRP-conjugate reagent was then added to each well, except the blank well and incubated for 30 minutes at 37 ^{0}C after closing plate with closure plate membrane. This was followed by washing and patting plate five times as described earlier. 50 µl of chromogen A and 50 µl of chromogen B was added to each plates followed by incubation at 37 ^{0}C for 15 minutes avoiding light. 50 µl of stop solution was then added to each well to stop the reaction with a colour change from blue to yellow. With the blank well taken as zero, the absorbance was read at 450 nm using Microtiter Plate Reader within 15 minutes of addition of stop solution. Four control samples were interspersed in test samples to validate the process.

3.8.2. Measurement of Insulin

Test Principle

The test uses the sandwich-ELISA technique to assay insulin in serum. The microtiter plate well pre-coated with an antibody specific to insulin is used. Samples or standards are then loaded to the appropriate microtiter plates well to combine specifically with these antibodies. An antibody specific to insulin which is conjugated with horseradish peroxidase (HRP) is then added to each microtiter well, incubated and followed by a thorough washing. A TMB

substrate solution is then added to each well to be catalyzed by the enzyme HRP. Only wells containing the insulin antibody antigen and HRP conjugated INS antibody complex will show a blue color appearance and turn yellow when the stop solution (sulphuric acid) is added. The optical density (OD) is then measured spectrophotometrically at a wavelength of 450 nm. The concentration of insulin is proportional to the OD value obtained. To calculate insulin concentration, the OD obtained is compared to the standard curve.

Test procedure

Different concentrations of the standard were prepared as per the protocol (18 mU/L, 12 mU/L, 6 mU/L, 3 mU/L and 1.5 mU/L) with the first well reserved for a blank. From each standard concentration prepared, 50 µl was added to two wells. Thus, five different concentrations, ten wells, with each well having 50 µl Standard Solution. The remaining wells were loaded with test samples in the order; 40 µl of sample dilution buffer plus 10 µl of sample (dilution factor of 5). The wells were then loaded with the samples; ensuring samples were delivered to the bottom of the well without touching the well wall and mixed well by gentle shaking. Plate was closed with a closure plate membrane and incubated at 37^0C (in an oven) for 30 minutes. A 30-fold wash solution was prepared from the stock with distilled water and was used to wash the plates. Carefully, the closure plate membrane was peeled off, test samples aspirated and plate filled again with the wash solution. This was followed by discarding the wash solution after a resting period of 30 seconds and repeating the washing procedure for 4 times. This was followed by 50 µl HRP-Conjugate reagents addition to each well except the blank control well and incubation at 37^0C for 30 minutes after the plate has been sealed with a closure plate membrane. Plate was then washed following the washing procedure as described earlier. 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mixed by gentle shaking and incubated at 37°C for 15 minutes.

Light was avoided during this incubation episode. 50 µl of stop solution was then added to each well to stop the reaction with a colour change from blue to yellow. Absorbance was read at 450 nm using a Microtiter Plate Reader within 15 minutes after adding stop solution. The OD value of the blank control well was set as zero.

3.8.3. Measurement of Glucose

Test Principles

This test method quantitatively measures glucose (GLU) concentration in plasma using the VITROS CHOL Slides and the VITROS Chemistry Products Calibrator Kit 1 on VITROS 5, 1 FS Chemistry Systems (Johnson & Johnson, New Jersey, USA). The VITROS CHOL Slide is a multilayered, analytical element coated on a polyester support. The method is based on an enzymatic colorimetric procedure described by (Curme *et al.*, 1978). About 10 microliters (10 µL) of sample was dropped on the slide and evenly spread by the spreading layer to the layers underneath. The sample glucose is then oxidized to form hydrogen peroxide and gluconate catalysed glucose oxidase. The reaction is then followed by an oxidative coupling catalysed by peroxidase in the presence of dye precursors to produce a dye. The intensity of dye formed corresponds to the concentration of glucose present in the sample which is measured at a wavelength of 540 nm by reflectance spectrophotometry. The procedure requires an incubation of reaction at 37^{o}C for about 5 minutes. Control samples were run to verify system performances before test samples were ran.

Reaction Scheme

B-D-glucose+O_2+H_2O $\xrightarrow{\text{glucose oxidase}}$ D-gluconic acid +H_2O_2

2H2O2 + 4 – aminoantipyrine + 1, 7 – dihydroxynaphthalene $\xrightarrow{\text{peroxidase''}}$ red dye

3.8.4. Measurement of Total Cholesterol

Test principle

The test method measures quantitatively total cholesterol (CHOL) levels blood(serum and plasma) using the VITROS CHOL Slides and the VITROS Chemistry Products Calibrator Kit 2 on VITROS 5, 1 FS Chemistry Systems. The VITROS CHOL Slide is a multilayered, analytical element coated on a polyester support. This enzymatic method is based on similar method proposed by (Allain *et al.*, 1974). A patient sample dropped on the slide is evenly spread using the spreading layer to the layers underneath. Cholesterol and cholesterol esters are then dissociated from lipoprotein complexes present in the sample by the Triton X-100 (TX100) surfactant which is present in the spreading layer. Cholesterol esters are then hydrolysed to cholesterol catalysed by cholesterol ester hydrolase. The available free cholesterol is then oxidized to form cholestenone and hydrogen peroxide in the presence of cholesterol oxidase. Hydrogen peroxide is used in the last step to oxidize a leuco dye in a reaction catalysed by peroxidase to generate a coloured dye. The intensity of colour (dye) developed correspond to the concentration of cholesterol in the sample and is measured at a wavelength of 540 nm by reflectance spectrophotometry. The procedure requires about 5µL of sample (serum) volume and an incubation time of 5 minutes at 37^0C. Control samples were run to verify system performance before test samples were ran.

Reaction scheme

Lipoprotein $\xrightarrow{\text{TX100}}$ cholesterol + cholesterol esters + proteins

Cholesterol esters + H2O $\xrightarrow[\text{Hydrolase}]{\text{cholesterol ester}}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow[\text{Oxidase}]{\text{cholesterol}}$ cholest-4-en-3-one + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{\text{peroxidase}}$ dye + 2H2O

3.8.5. Triglycerides measurement

Test principle

This test method measures quantitatively the concentration of triglyceride (TRIG) in blood (serum and plasma) using the VITROS TRIG Slides and the VITROS Chemistry Products Calibrator Kit 2 on VITROS 5, 1 FS Chemistry Systems. This is an enzymatic method based on similar method described by (Spayd *et al.*, 1978). About 6 µL of patient sample (plasma or serum) is dropped on the slide and spread evenly to the layers underneath by the spreading. Triglycerides are then dissociated from lipoprotein complexes present in the sample by Triton X-100 surfactant in the spreading layer. This is followed by the hydrolysis of triglyceride molecules by lipase to release fatty acids and glycerols. The glycerol is phosphorylated by glycerol kinase in the presence of adenosine triphosphate (ATP) after diffusing to the reagent layer. In the presence of L-α-glycerol- phosphate oxidase, L-α-glycerophosphate is then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. In the final step, the leuco dye is oxidized by hydrogen peroxide in a reaction which is catalyzed by peroxidase to produce a dye. The intensity of the dye formed is corresponds to the concentration triglyceride available in the sample and is measured at 540 nm. The procedure requires 5minutes of incubation time at 37^{o}C. Control samples were run to validate the testing process before test samples were ran.

Reaction Scheme

Lipoproteins $\xrightarrow{\text{surfactant}}$ triglycerides + proteins

Triglycerides + H2O $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ L-α-glycerophosphate + ADP

L-α-glycerophosphate + O2 $\xrightarrow[\text{Phosphate oxidase}]{\text{L-α-glycerol-}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{\text{peroxidase}}$ dye + 2H2O

3.8.6. Measurement of high density lipoprotein

Test principle

This test method measures quantitatively the concentration of HDL in blood (serum and plasma) using the VITROS dHDL Slides and the VITROS Chemistry Products Calibrator Kit 25 on VITROS 5, 1 FS Chemistry Systems. This method is based on similar non-HDL precipitation method described by (Burstein *et al.,* 1980) which is followed by an enzymatic method similar to that proposed by (Allain *et al.,* 1974). A drop of blood sample (10 µL serum) is dropped on the slide and is evenly spread to the layers underneath. This is followed by precipitation of non- High Density Lipoproteins (non-HDL) using phosphotungstic acid (PTA) and magnesium chloride ($MgCl_2$) to separate HDL in the spreading layer. HDL lipoprotein complex in the sample is then selectively dissociated from cholesterol and cholesterol esters by the Emulgen B-66 surfactant present in the spreading layer. HDL-derived cholesterol esters are hydrolysed to cholesterol in a reaction catalysed by a selective cholesterol ester hydrolase. The free cholesterol available is oxidized to form cholestenone and hydrogen peroxide in the presence of cholesterol oxidase. In the final step, the leuco dye is oxidized by hydrogen peroxide in a reaction which is catalyzed by peroxidase to produce a dye. The density of dye formed is proportional to the HDL cholesterol concentration present in the sample and is measured by reflectance spectrophotometry at 670 nm. The procedure requires 5 minutes of incubation time at 37^{o}C. Control samples were run to validate the testing process before test samples were ran.

Reaction Scheme

HDL + non-HDL $\xrightarrow{PTA/MgCl_2}$ high density lipoproteins + non-HDL (↓)

High density lipoproteins $\xrightarrow{\text{Emulgen B-66}}$ cholesterol + HDL cholesterol esters +proteins

HDL cholesterol esters + H_2O $\xrightarrow[\text{ester hydrolase}]{\text{cholesterol}}$ cholesterol and fatty acids

Cholesterol + O_2 $\xrightarrow[\text{Oxidase}]{\text{cholesterol}}$ cholest-4-en-3 + H_2O_2

H_2O_2 + leuco dye $\xrightarrow{\text{peroxidase}}$ dye + H_2O

3.8.7. Measurement of low density lipoprotein (LDL-C) Cholesterol

Test principle (Friedewald Equation)

The Friedewald equation is most common equation used for the indirect determination of the concentration of LDL- cholesterol. It is calculated from measurement of total cholesterol, triglycerides and HDL- cholesterol using the empirical equation of (Friedewald *et al*., 1972). LDL- cholesterol concentration of samples was estimated using the equation:

LDL-C (mmol/L) = [TC (mmol/L) – [TG](mmol/L)/2.2- HDL(mmol/L).

(Equation valid at TRIG concentration not greater than 4.4 mmol/l).

3.9. Data Handling

All data were entered into Microsoft excel spreadsheet for storage and subsequent analysis. Data was handled confidentially. Unique identifiers were used for computer based data entry. The supervisors and Investigator ensured that all study forms together with all identification code lists were kept safe and confidential.

3.10. Statistical analysis

Data gathered was analyzed in accordance with the requirements of the research objectives. Graph Pad Prism version 7.03 was used for the analysis of all data. For comparison of demographic, anthropometric and biochemical parameters, quantitative data was presented as mean ± S D. The statistical significance between means was estimated by student's t–test when appropriate. Pearson's and Spearman's correlation coefficient (r) were used to measure the strength of the association between variables where appropriate. Differences were considered statistically significant at $p<0.05$

CHAPTER FOUR

4.0. RESULTS

4.1. General baseline demographic and anthropometric characteristics of the study population.

The study involved 86 subjects comprising 46 obese and 40 non-obese. There were 23 males and 23 females in the obese group and 20 males and 20 females in the non-obese control group**.** About 90% of the obese group had obesity present in the family whilst 80% of the non-obese group reported of no family history of obesity. As expected the obese group had almost twofold increase in total body fat compared to the non-obese. Visceral fat was also significantly higher in the obese than the non-obese. The difference in mean age and BMI between the two groups was about 10years and 18 Kg/m^2 respectively as shown in Table 4.1.

TABLE 4.1: General baseline demographic and anthropometric characteristics in obese subjects compared with non-obese controls.

Parameters	**Obese (n=46)** Mean ± SD	**Non-obese (n=40)** Mean ± SD	**p-value**
Age (years)	50.0±10.5	40.4±10.5	<0.0001*
BMI (Kg/m^2)	40.5±5.9	22.9±2.6	<0.0001*
Visceral Fat	18.2±5.8	5.9±2.6	<0.0001*
% Body Fat	44.1±10.1	25.5±9.5	<0.0001*

*In table 4.1 data was presented as mean± SD. * mean difference is significant (p<0.05) at 95% confidence interval. BMI is body mass index, % body fat is percentage body fat, % body fat is percentage body fat.*

4.2. Mean demographic data in obese males and females

Within the obese group, when subjects were matched for age, there was significant difference in BMI, visceral fat and percentage body fat between male and female subjects. Female subjects had higher BMI, total body fat and a lower visceral fat compared to their male counterparts, Figure 4.1.

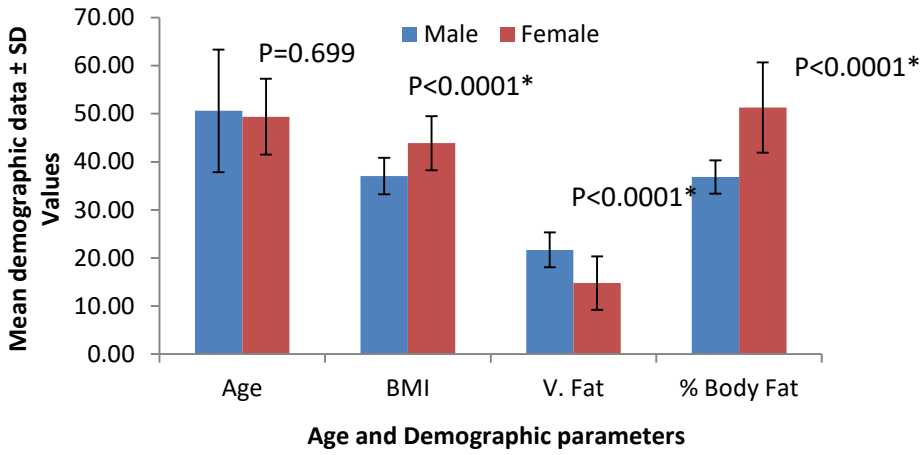

Figure 4.1: A bar graph of mean demographic data in obese male and females

** mean difference is significant (p<0.05) at 95% confidence interval. BMI is body mass index, V. fat is visceral fat, % body fat is percentage body fat.*

4.3. Mean demographic data in non-obese males and females

For the non-obese group when subjects were matched for age, there was no significant difference in BMI and visceral fat between males and females except for percentage total body fat which was significantly increased in women, Figure 4.2.

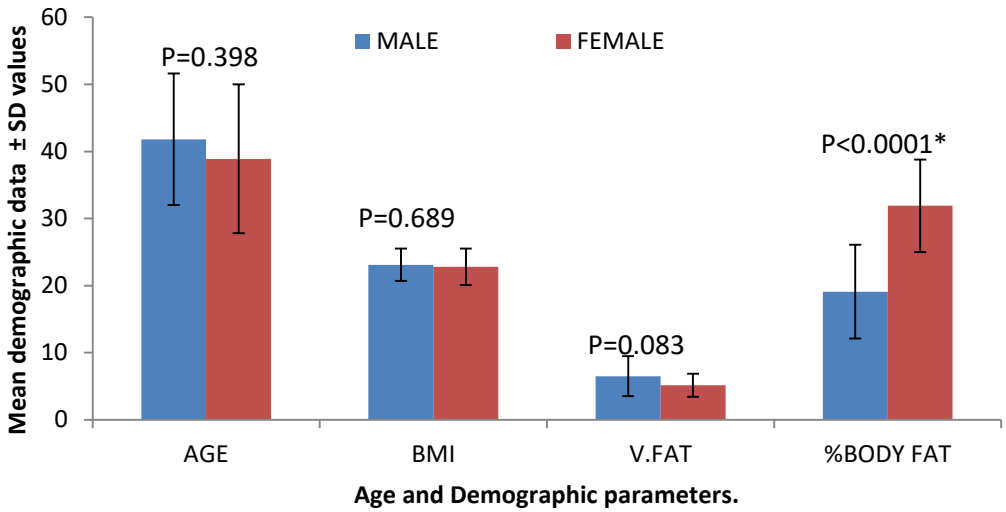

Figure 4.2: A bar graph of mean demographic data in non-obese males and females.

** mean difference is significant (p<0.05) at 95% confidence interval. BMI is body mass index, V. fat is visceral fat, % body fat is percentage body fat.*

4.4. Biochemical Parameters of the Study Population

Data on biochemical parameters of the study population have been presented as means ± standard deviation (Table 4.2). Mean fasting blood glucose levels were non-significantly higher in the obese than non-obese (p= 0.60). In the obese group, mean fasting insulin levels (p=0.67) and HOMA-IR (p=0.48) levels were not significantly different between obese and non-obese groups. Mean perilipin A levels in the obese were significantly lower compared to the non-obese groups (p=0.0001). The various lipid parameters (T.CHOL, TRIG, HDL, LDL, CHOL/HDL ratio) were significantly higher in the obese than the non-obese groups.

TABLE 4.2: Biochemical Characteristics for Study Population.

Parameters	**Obese (n=46)** Mean ± SD	**Non-obese (n=40)** Mean ± SD	**p-value**
FBG (mmol/L)	5.0±0.5	4.9±0.5	0.6000
INSULIN (mU/L)	11.8±3.8	11.1±3.3	0.6700
HOMA-IR	1.4±0.5	1.4±0.4	0.4800
PERILIPIN A (ng/ml)	130.8±23.9	159.9±32.6	0.0001*
T.CHOL (mmol/L)	4.9±0.9	4.5±0.7	0.0200*
TRIG. (mmol/L)	1.3±0.5	0.7±0.3	0.0001*
HDL (mmol/L)	1.3±0.3	1.6±0.4	0.0006*
LDL (mmol/L)	3.0±0.9	2.5±0.8	0.0060*
VLDL (mmol/L)	0.5±0.2	0.3±0.1	0.0001*
CHOL/HDL ratio	3.8±0.9	2.9±0.9	0.0001*

*In table 4.2 data on biochemical parameters of the study population was presented as means ± standard deviation. * mean difference is significant at 95% confidence interval (p<0.05). FBG is fasting blood glucose, HOMA-IR is insulin resistance index, T-CHOL is total cholesterol, TRIG is triglycerides, HDL is high density lipoprotein, LDL is low density lipoprotein and VLDL is very low density lipoprotein.*

4.5.Gender differences in mean perilipin A levels in obese and non-obese

When subjects were matched for age, mean perilipin A level was higher in men compared to women in the obese group (P=0.218). In the non-obese group mean perilipin A levels was not significantly different between men and women (P=0.792), Fig. 4.3.

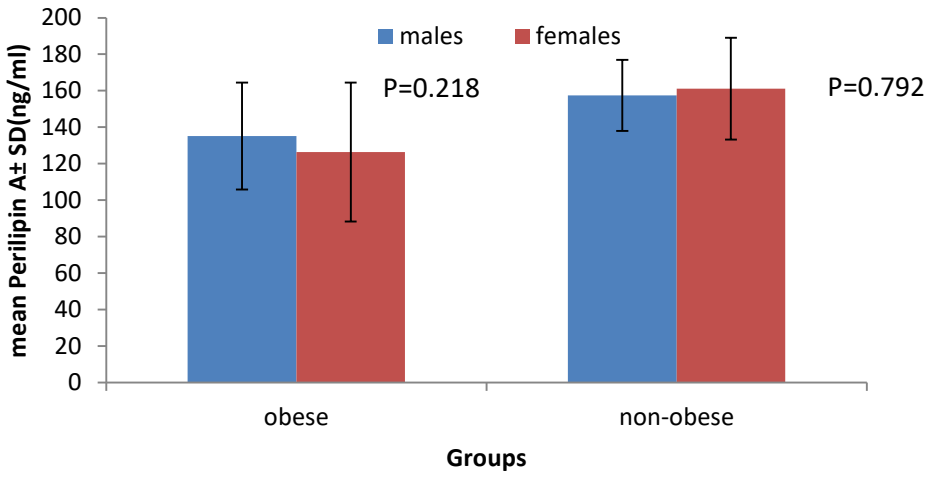

Figure 4.3: A bar graph of mean perilipin A levels in obese and non-obese males and females. ** mean difference is significant (p<0.05) at 95% confidence interval.*

4.6. Association of T.CHOL, TRIG and CHOL/HDL Ratio with age and anthropometric parameters in the obese.

Lipid indices showed insignificant positive correlation with anthropometric indices and age. T.CHOL (p=0.06) and TRIG (p=0.11) showed the highest insignificant positive correlation with BMI, Table 4.3.

Table 4.3: Correlation of T.CHOL, TRIG and CHOL/HDL Ratio with age and anthropometric parameters.

		T.CHOL	TRIG	CHOL/HDL
Age (years)	**R**	0.128	0.043	0.098
	P	0.420	0.772	0.514
BMI (Kg/m^2)	**R**	0.275	0.233	0.098
	P	0.063	0.118	0.514
Visceral fat	**R**	0.062	0.119	0.600
	P	0.681	0.430	0.689
% Body Fat	**R**	0.209	0.015	0.115
	P	0.161	0.161	0.443

*Data presented as Pearson's correlation coefficient, r. * Correlation was significant at 95% confidence interval ($p<0.05$).*

4.7. Association of Perilipin A with Age and Anthropometric parameters.

Perilipin A showed a general negative correlation with age, BMI and % body fat in the obese group but none of these correlations were significant. In the control group, Perilipin A showed a reversed positive correlation with BMI and % body fat except for age which still showed a negative correlation. With visceral fat, perilipin A showed a positive correlation in both obese and non-obese groups, Table 4.4.

TABLE 4.4: Correlation of Perilipin A with Age and Anthropometric parameters

		PERILIPIN A	
		OBESE	**NON-OBESE**
AGE (years)	**R**	-0.024	-0.160
	P	0.871	0.323
BMI (Kg/m^2)	**R**	-0.016	0.208
	P	0.913	0.196
VISCERAL FAT	**R**	0.244	0.027
	P	0.101	0.864
% BODY FAT	**R**	-0.209	0.201
	P	0.162	0.212

*Data presented as Spearman's correlation coefficient, r. * Correlation was significant at 95% confidence interval ($p<0.05$). BMI is body mass index, % body fat is percentage body fat.*

4.8. Association of Perilipin A with Biochemical parameters.

No observed significant correlation was obtained between Perilipin A levels and lipid parameters. However, all these relationships were negative except for CHOL/HDL ratio which showed a positive correlation in the obese and a negative correlation in non-obese. Perilipin A also showed a non-significant negative correlation with insulin and HOMA-IR, Table 4.5.

Table 4.5: Correlation of Perilipin A with Biochemical parameters.

		PERILIPIN A	
		OBESE	**NON-OBESE**
INSULIN (mU/L)	**R**	-0.075	-0.222
	P	0.616	0.167
HOMA-IR	**R**	-0.178	-0.238
	P	0.234	0.138
T.CHOL (mmol/L)	**R**	-0.178	- 0.130
	P	0.234	0.421
TRIG. (mmol/L)	**R**	-0.026	-0.060
	P	0.861	0.712
HDL (mmol/L)	**R**	-0.123	0.160
	P	0.415	0.321
LDL (mmol/L)	**R**	-0.088	-0.239
	P	0.558	0.137
VLDL (mmol/L)	**R**	-0.038	-0.062
	P	0.801	0.703
CHOL/HDL ratio	**R**	0.124	-0.234
	P	0.409	0.145

*Data presented as Spearman's correlation coefficient, r. * Correlation was significant at 95% confidence interval ($p<0.05$). FBG is fasting glucose, HOMA-IR is insulin resistance index, T-CHOL is total cholesterol, TRIG is triglycerides, HDL is high density lipoprotein, LDL is low density lipoprotein and VLDL is very low density lipoprotein.*

4.9. Relationship between HOMA-IR and age and anthropometric parameters

HOMA-IR showed a general non-significant positive correlation with age and anthropometric indices such as BMI and percentage body fat but a significant positive correlation with visceral fat (p=0.049). In the non-obese group HOMA-IR showed a significant positive correlation with age (p=0.020), Table 4.6.

Table 4.6: Correlation of HOMA-IR with age and anthropometric parameters

		HOMA-IR	
		OBESE	**NON-OBESE**
AGE (years)	**R**	0.264	0.364*****
	P	0.076	0.020
BMI (Kg/m^2)	**R**	0.006	0.069
	P	0.963	0.660
VISCERAL FAT	**R**	0.178 *	0.051
	P	0.049	0.754
% BODY FAT	**R**	0.059	0.099
	P	0.693	0.539

*In table 6 data was presented as Pearson's correlation coefficient, r. * Correlation was significant at 95% confidence interval (p<0.05). BMI is body mass index, % body fat is percentage body fat.*

4.10. Relationship between HOMA-IR and biochemical parameters in obese and non-obese.

HOMA-IR showed a strong positive correlation with insulin (p<0.001) and a negative non-significant correlation with Perilipin A. Correlations between HOMA and lipid parameters were positive and non-significant except for HDL which showed a weak negative correlation, Table 4.7.

TABLE 4.7: Correlating HOMA-IR with biochemical parameters

		HOMA-IR	
		OBESE	**NON-OBESE**
FBG (mmo/L)	**R**	0.016	0.091
	P	0.912	0.576
INSULIN (mU/ L)	**R**	0.996 *	0.998*
	P	<0.001	< 0.001
PERILIPIN A (ng/ml)	**R**	-0.178	0.059
	P	0.234	0.130
T.CHOLESTEROL	**R**	0.226	0.029
(mmol/L)	**P**	0.129	0.858
TRIG. (mmol/L)	**R**	0.038	0.089
	P	0.797	0.580
HDL (mmol/L)	**R**	-0.208	0.062
	P	0.496	0.703
LDL (mmol/L)	**R**	0.283	0.049
	P	0.056	0.762
VLDL (mmol/L)	**R**	0.029	0.086
	P	0.846	0.596
CHOL/ HDL RATIO	**R**	0.239	0.043
	P	0.109	0.790

*Data presented as Pearson's correlation coefficient, r. * Correlation was significant at 95% confidence interval (p<0.05). FBG is fasting glucose, HOMA-IR is insulin resistance index, T-CHOL is total cholesterol, TRIG is triglycerides, HDL is high density lipoprotein, LDL is low density lipoprotein and VLDL is very low density lipoprotein.*

CHAPTER FIVE

5.0. DISCUSSION

Perilipin A is a protein coat that surrounds neutral lipid stores in adipocytes. It regulates access to triglycerides stores by protecting against basal lipolytic activities of lipases and promotes stimulated lipolysis. Studies have shown that levels may be affected by obesity therefore reducing its protective function against basal lipolysis. This may contribute to the excess release of free fatty acids possibly leading to insulin insensitivity.

The effect of obesity on blood Perilipin A levels and the correlation between Perilipin A and insulin resistance in apparently healthy subjects were investigated in this study. Differences in the anthropometric measurements between obese and non-obese have been presented in Table 1. The obese group showed significantly higher levels of BMI, visceral fat and percentage body fat compared to the non-obese group with these parameters generally showing a positive correlation with insulin resistance. Increased BMI seen in obesity is a well-known contributing factor in the pathogenesis of insulin resistance leading to the occurrence of type 2 diabetes. This relationship even though is well recognized is fraught with some reservation since not all obese individuals have insulin resistance (Ferrannini *et al.,* 1996).

Visceral adipose tissue and abdominal subcutaneous adipose tissue mass have been proposed as a more sensitive indicator of insulin resistance in obese persons with visceral fat particularly strongly associated with insulin resistance (Banerji *et al.,* 1997; Pouliot *et al.,* 1992). Both visceral and subcutaneous adipose tissue are also well-recognized determinant of liver fat (Garg & Misra, 2002) and have been hypothesized to increase the delivery of FFAs to the liver through the portal vein leading to increase in hepatic lipids resulting in insulin resistance (Kabir *et al.,* 2005). This corroborates with findings in this study since visceral fat

showed a significant positive correlation with insulin resistance compared to BMI and percentage body fat which showed positive correlations with insulin resistance but not significant, Table 4.6. When obese subjects were matched for age, females had significantly higher BMIs and percentage body fat compared to men but had lower visceral fat mass, Figure 4.1. This is in line with other studies that showed visceral adiposity as a masculine pattern of fat deposition (Seidell *et al.,* 1988) whilst females generally tend to have greater percentage of total body fat which is stored in the gluteal-femoral region as a safe lipid reservoir for excess energy (Krotkiewski *et al.,* 1983; Jackson *et al.,* 2002). Contrastingly in the non-obese subjects, males and females subjects showed similar levels of visceral fat but body fat percentage was significantly increased in females than males, Figure 4.2. This further shows that as BMI or total body fat mass increases males turn to accumulate more visceral fat compared to females but in normal weight visceral fat mass may not differ significantly between male and females.

In this study except for high density lipoprotein cholesterol that was elevated significantly in the non-obese compared to obese, total cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol were significantly higher in the obese subjects. Coronary risk calculated from the ratio of total cholesterol to high density lipoprotein cholesterol was also significantly increased in the obese subjects, Table 4.2. The relationship between obesity and dyslipidaemia is one that is well-recognized. Obese individuals had increased lipids level compared with non-obese individuals because they have more stored fat and there is high turnover into circulation (Kissebah *et al*., 1982). The higher levels of lipids in obesity have been linked to increased risk to certain pathologies such as insulin resistance and atherosclerotic cardiac problems (Krahmer *et al.,* 2013).

There was a general insignificant positive correlation in both groups between insulin resistance calculated using HOMA-2 and the various lipid parameters except HDL-C which showed a negative relationship, Table 4.7. The positive relationship is in line with various studies that showed insulin resistance in obesity to be associated with hypertriglycedemia, increased LDL-C and VLDL-C and reduced levels of high density lipoproteins (Olefsky *et al.,* 1974; Steiner, 1994). Hypertriglyceridemia is a surrogate for increased circulating free fatty acids (Mostaza *et al.,* 1998) which causes insulin resistance in the obese. These relationships mostly have been observed in obese individuals who did not have an apparently healthy status. The relatively weak positive correlation seen in this study was probably due to the apparently healthy status of subjects used. Moreover, despite the higher mean lipid levels in the obese, the levels were well within the normal range.

Several clinical and genetic studies have pointed to the important role of perilipin in regulating basal and stimulated lipolysis in humans using adipose tissue. Mean perilipin A levels in blood of obese subjects were significantly lower than in non-obese, Table 4.2. This observation corroborated well with several other studies using adipose tissue (Mottagui-Tabar, *et al.,* 2003; Wang, *et al.,* 2003; Ray *et al.,* 2009). This also reflected in the negative relationship of perilipin A levels with BMI and percentage body fat in obese subjects, Table 4.4. Thus, when adipocytes increase in size and volume as seen in obesity, it is not followed by a corresponding increase in perilipin A level. Since perilipin A functions as a regulator of basal lipolysis, it was proposed that the high basal lipolysis seen in the obese subjects may be due to the relative decrease in its protective function (Wang *et al.,* 2003).

Obesity is characterized by the secretion of various inflammatory cytokines and TNF-α in particular has been linked with the down regulation of perilipin A in the adipose tissue of obese individual (Souza *et al.,* 1998). Aside this, the decrease in perilipin expression in the

obese individual was also proposed to be a peculiar one (Lithel & Boberg, 1978) since it contrast with increase in other proteins present in adipose tissue such as lipoprotein lipase showing its expression is probably downregulated in obesity. In contrast perilipin A showed a positive relationship with BMI and percentage body fat in non-obese, Table 4.4, suggesting that in the absence of cytokines (TNF-α) presumably in non-obese individuals, average increments in adipocyte size induce a corresponding increase in perilipin expression. Studies in non-diabetic obese individuals have shown higher perilipin A levels compared to non-obese subjects (Kern *et al.*, 2004).

Perilipin A levels showed a negative relationship with age both in the obese and non-obese subjects, Table 4.4. This could be explained in two ways, either age has no correlation with perilipin A levels or perilipin A levels may decrease with increasing age which may account for increased risk to age related dyslipidemia (Humayun *et al.,* 2009). Also since age did not show a significant correlation with Perilipin A, differences between perilipin A levels in the obese and non-obese may not be attributed to age in this study. The positive relationship between perilipin A and visceral fat mass in both non-obese and obese subjects cannot be directly explained since BMI and percentage body fats have shown negative correlations, Table 4.4. However, since perilipin A levels were determined in blood, levels cannot be attributed to a specific fat depot (e.g. visceral fat) but rather to a total body fat determined by both BMI or percentage body fat. Within the obese group, mean perilipin A levels were higher in men than women, Figure 4.3. This corroborated with previous studies where they found levels to be higher in adipose tissue of men than women (Mottagui-Tabar, *et al.,* 2003; Wang, *et al.,* 2003). Previous studies have also shown that females are more susceptible to the genetic effects on perilipin than men which may account for the lower levels (Qi *et al.,*

2004). Females also turn to have higher basal lipolysis per fat cell than men undergoing bariatric surgery (Löfgren *et al.*, 2002), which may indicate a lower perilipin content.

Perilipin A levels showed a weak negative correlation with triglycerides. The same relationship was observed for low and very low density lipoproteins, Table 4.5. Low levels of peripin A in the obese would mean increased basal lipolysis which may result in increased release of FFAs leading to increased hepatic synthesis of low density and very low density lipoprotein hence the negative correlation. The seemingly weak negative correlation may be due to the apparently healthy status of the obese subjects whose triglycerides level even though was higher still fell within the normal range. There was no relationship between perilipin A and insulin resistance probably due to the normal glucose, insulin and HOMA-IR status of the obese subjects which is line with a study by (Kern *et al.*, 2004) which found no relationship between perilipin A and insulin resistance in the obese.

5.1. Conclusion

- The findings of the study showed blood perilipin A to be significantly elevated in non-obese subjects than obese subjects.

- The lower perilipin A levels in obese subjects may be due to elevated production of cytokines (especially TNF-α) in the obese which probably suppresses perilipin A expression and function thereby increasing basal lipolysis.

- Perilipin A levels did not significantly correlate with insulin sensitivity and showed weak negative correlation with lipid levels probably due to the apparently healthy status of obese subject.

5.2. Recommendation

- Further research should be carried out in non-obese dyslipidemic patients to compare perilipin A levels with normal subjects in confirming or ruling out the influence of obesity.
- Further studies should also be conducted to determine the relationship between inflammatory cytokines such as TNF-α and Perilipin A levels in the obese.
- Further studies should be done in all age groups to properly determine the influence of age on perilipin A since age is a major contributor to dyslipidaemia.
- Further work should be done with a larger sample size to properly determine reference levels of perilipin A in blood.

5.3. Limitation

There was lack of funds to include other biochemical parameters such as hormone sensitive lipase and inflammatory cytokines and to recruit a larger sample size for the study.

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APPENDICES

APPENDIX A: ETHICAL CLERANCE

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.:.....

4th October, 2016.

Mr. Bright Selorm Letsu
Department of Chemical Pathology
School of Biomedical and Allied Health Sciences
Korle-Bu, Accra

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.1 – P 4.7/2016-2017**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 29th of September, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Blood Perilipin A levels in Apparently Healthy obese and non-obese Adults in Accra"**

PRINCIPAL INVESTIGATOR: **Mr. Bright Selorm Letsu**

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 30th September, 2017.

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

Signed: .....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
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APPENDIX B: INFORM CONSENT FORM

I, **Bright Selorm Letsu** of the Department of Chemical Pathology, School of Biological and Allied Health Sciences, University of Ghana, wish to embark on a study entitled; ***Blood Perilipin A Levels in Apparently Healthy Obese And Non Obese Adults in Accra*.**

Perilipin is a protein that coats the surface of lipid droplets (fat droplets) which regulates the release of free fatty acids into circulation. The purpose of this study is to find out if obesity has an effect on the level of this protein which may affect its function leading to increased release of fatty acids. Increased circulating free fatty acids may lead to insulin resistance resulting in type 2 diabetes and can also cause heart problems. Blood perilipin A levels may therefore serve as a good indicator of circulating free fatty acid level and may relate to insulin resistance.

The study will require you to provide some information on your personal details, lifestyle and health. As a participant, you would be required to undergo an overnight fast after which 5mls of blood would be drawn and your body weight and height would be measured. Both procedures may involve slight discomfort.

Participation is entirely voluntary. You may choose to withdraw from the study whenever you wish. You are assured of the strictest confidentiality of your personal information. The study will adhere to all applicable protocols and will maintain quality assurance in accordance with good laboratory practice. The blood samples collected will bear an identification code to ensure anonymity, confidentiality and ease of identification. There is the possibility that you might not benefit directly from participation. However, the information obtained and conclusions drawn will be applied in the adoption of relevant health policies as well as the appropriate care and management.

You will incur no costs and you will also not be paid for participating in this study. However, you will be entitled to know the outcome of the laboratory results and this will be well

explained to you. All data will be entered onto a lock/word-protected Microsoft Excel spreadsheet. Study questionnaires will be kept in a locked cabinet in a locked office.

If you have any questions, you may ask them now or later. My contact;

BRIGHT S. LETSU, Department of Chemical Pathology, SBAHS, University of Ghana.

(Principal investigator)

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OR

DR. S.D AMANQUAH, Department of Chemical Pathology, SBAHS, University of Ghana.

(Principal supervisor)

Telephone: 0244293987

Participant Consent Response

I, …… have been invited to take part in this research. I have been told of the purpose and procedure of this study. I have agreed to give personal information such as my education, health, physical activity etc. I understand I will not be reimbursed monetarily for participating in this study. I am aware of the nature, risk and benefits of the study and that I may withdraw at any time. I promise to comply with the requirement of study and I consent accordingly**.**

……. Date……...

Signature of participant

……. Date……...

Signature of Investigator

APPENDIX C: STUDY QUESTIONAIRE-DATA SHEET

Participant's ID …….

Contact Number…….

Date……

A. **SOCIO –DEMOGRAPHIC STATUS**

1. Age (yr) 30-39 [ ] 40-49[ ] 50 -60 [ ] above 60[ ]

2. Sex i. Male [ ] ii. Female [ ]

3. Marital Status i. single [ ] ii. Married [ ] iii. Divorced [ ] IV. Widowed [ ]

4. Educational Background I. No formal education [ ] ii. Basic Education (middle/JHS) [ ] iii. SHS/O –Level [ ] iv. HND/Diploma Certificate [ ]

v. Bachelor Degree [ ] vi. Post Degree [ ]

5. Employment Status: i Employed [ ] ii Unemployed [ ] iii Retired [ ] iv. Student [

B. MEDICAL HISTORY

6. Do you have a family history of **obesity?** YES [ ] NO [ ]

7. Do you have any **known** medical condition? YES [ ] NO [ ]

8. When was the last time you reported sick to any health facility…....

9. Are you on medication? YES [ ] NO [ ]

10. What type of medication? Conversional [ ] Herbal [ ]

11. How long have you been on this medication? ……

12. Do you use drugs? YES [ ] NO [ ]

12b. If yes, how long have you been using this (es) drug(s)? 0-5 [ ] 5-10 [ ] 10-15[ ] >15

C. LIFE STYLE BEHAVIOURS

10. Do you smoke? YES [ ] NO [ ]

10b. If yes how often? Daily [ ] Weekly [ ] Monthly [ ] Occasionally [ ]

11. Do you drink alcohol? YES [ ] NO [ ]

11b. If yes how often Daily [ ] Weekly [ ] Monthly [ ] occasionally [ ]