

**LEVELS OF ADIPOKINES IN GHANAIAAN WOMEN IN JAMESTOWN,
ACCRA.**

AFUA BONTU ADJEI

INDEX NUMBER: 10362437



**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF A
MASTER OF PHILOSOPHY (M. PHIL) DEGREE IN CHEMICAL
PATHOLOGY**

JULY, 2013

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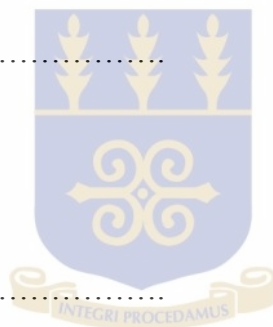
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DECLARATION

I AFUA BONTU ADJEI do hereby declare that with the exception of references to other people's work, which have been duly acknowledged, this thesis is the outcome of my own research conducted at the Department of Chemical Pathology, University of Ghana Medical School, College of Health Sciences under the supervision of Prof. Francis Agyemang-Yeboah and Dr. Henry Asare-Anane. Neither all nor parts of this project have been presented for another degree elsewhere.

.....
AFUA BONTU ADJEI
(Student)



Date:

.....
PROF. FRANCIS AGYEMANG - YEBOAH
(Supervisor)

Date:

.....
DR. HENRY ASARE – ANANE
(Supervisor)

Date:

DEDICATION

I dedicate this work to my family, most particularly to my mum, who has always been my motivation and has never ceased to encourage me to see beyond the skies as my limit in whatever I do. My insightful appreciation goes to my lovely husband for his invaluable support and encouragement, support and understanding during my time in school.



ACKNOWLEDGEMENT

The promises of the Lord are yea and amen. I am much grateful to the Almighty God, who has honoured His promise for his care and guidance over my life throughout my period of this research.

I am extremely grateful to my supervisors, Prof. Francis Agyemang Yeboah, Dr. Henry Asare – Anane, and my Head of Department, Dr S.Y. Oppong for urging me to undertake this research project in the first place. I genuinely appreciate their criticisms, suggestions and corrections which have helped me produce this work. May God reward their invaluable efforts. My thanks also go to my co-supervisor, Dr. Seth Amanquah for his support and sense of direction. Also to all the noble staff in the department Chemical Pathology, I am very grateful for their inputs and their various supports.

My sincere thanks to the staff of Clinical Virology Unit (Microbiology Department, UGMS) for their support and technical assistance particularly during the bench phase of the project.

Finally, to my colleagues especially Kwame Yeboah for his timeless support, encouragement and assistance from the very beginning of the project to the very end.

ABSTRACT

Adipokines are bioactive mediators that communicate between adipose tissue and other biological systems. Adipokines have strongly been linked to adiposity and obesity. The body's balance of adipokines is fundamental to prevent obesity and other non-communicable disease. However, their levels in the aetiology of obesity or otherwise have not been studied in Ghana.. The aim of the study was to investigate the levels of plasma Leptin, Adiponectin, Resistin (major adipokines) among women between the ages of 20 to 80 years in James Town, Accra. One hundred and eleven women were used for this study using invitational sampling. A structured questionnaire covering subjects' lifestyle and anthropometrics was administered to each consented subject. The subjects' anthropometrics (weight, height, BMI, percentage body fat, visceral fat, skeletal muscle and resting metabolism) and blood pressure (BP) were taken. Total cholesterol (TC), high density lipoproteins cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), triglycerides (TRIG), fasting blood glucose (FBG), Leptin, Adiponectin and Resistin were assessed.

The prevalence of obesity in this study was 48% and body composition correlated with blood pressures of study subjects. Adiponectin levels correlated positively with LDL-c ($r = 0.219$, $p = 0.030$), TCHOL/HDL-c ($r = 0.281$, $p = 0.005$) and LDL/HDL ($r = 0.269$, $p = 0.007$). On the other hand, adiponectin correlated inversely with HDL-c ($r = -0.226$, $p = 0.024$). Adiponectin was high in normal subjects with normal SBP which supports other studies and therefore has the potential in protecting one from cardiovascular damage.

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ACRP30.....	Adipocyte complement related protein 30
AdipoR.....	Adiponectin receptor
AMPK.....	AMP activated protein kinase
APM1.....	Adipose most abundant gene transcript 1
Apo A1.....	Apolipoprotein A1
Apo B.....	Apolipoprotein B
BIA.....	Bioelectrical Impedance Analysis
BMI.....	Body mass index
CCL2.....	Chemokine (c-c-motif) ligand 2
CETP.....	Cholesteryl ester transfer protein
CHD.....	Coronary heart disease
CNS.....	Central nervous system
CRP.....	C- reactive protein
CT.....	Computed Tomography
CVD.....	Cardiovascular disease

DBP.....	Diastolic blood pressure
DXA.....	Dual energy x-ray absorptiometry
ELISA.....	Enzyme-linked Immunosorbent Assay
eNOS.....	endothelial Nitric oxide synthase
FBG.....	Fasting blood glucose
FFA.....	Free fatty acid
GBP 28.....	Gelatin –binding protein 28
GK.....	Glycerol kinase
GM-CSF.....	Granulocyte-macrophage colony stimulating factor
GPO.....	Glycerolphosphate oxidase
HDL.....	High density lipoprotein cholesterol
HIV.....	Human immunodeficiency virus
HL.....	Hepatic lipase
HMW.....	High molecular weight
IFN- γ	Interferon gamma
IL- 6.....	Interleukin-6
IL-12.....	Interleukin- 12

IL-1RA.....	Interleukin-1 receptor- antagonist
LDL.....	Low density lipoprotein cholesterol
LDLR.....	Low density lipoprotein cholesterol receptor
LMW.....	Low molecular weight
LPL.....	Lipoprotein Lipase
LPS.....	Lipopolysaccharide
MAP.....	Mean arterial pressure
MCP-1.....	Monocyte chemo attractant protein-1
MRI.....	Magnetic Resonance Imaging
mRNA.....	messenger Ribonucleic acid
MSR.....	Macrophage scavenger receptor
NADPH.....	Nicotinamide adenine dinucleotide phosphate
NEFAs.....	Non-esterified fatty acids
NKkB.....	Nuclear factor kappa light chains enhancer of activated β cells
NOS.....	Nitric oxide synthase
OBRb.....	Long isoform of leptin
PAI-1.....	Plasminogen activator inhibitor-1

PP.....	Pulse Pressure
PPAR- γ	Peroxisome proliferator-activated receptor γ
RAAS.....	Renin-angiotensin-aldosterone system
SAT.....	Subcutaneous adipose tissue
SBP.....	Systolic blood pressure
T2DM.....	Type 2 Diabetes Mellitus
TBHA.....	Tribromo-3-hydroxy-benzoic acid
TNF- α	Tumour Necrosis factor alpha
VAT.....	Visceral adipose tissue
VEGF.....	Vascular endothelial growth factors
VLDL.....	Very low density lipoprotein cholesterol
WAT.....	White adipose tissue
WHO.....	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Obesity is a non-communicable chronic disease which affects over a billion adults world-wide. It is predicted that, its prevalence will be doubled by the year 2030 and that obesity epidemic would become the biggest health problem of the century (Pudal & Ellrott, 2004). With increasing number of patients suffering from obesity, the prevalence of obesity-associated complications resulting from excess fat of adipose tissue would be also on the increase. Ghana is among seven Sub-Saharan African countries (Congo Brazzaville, Liberia, Nigeria, Niger, Senegal and Sierra Leone) with the largest number of overweight and obese people, with over 3 million out of an estimated population of 25 million (Ghana News Agency, 2007). As the prevalence of obesity increases in modern society, there seems to be a concomitant rise in research and investigations geared towards the probable causes of the dysfunction of the adipose tissue.

Obesity is associated with increased occurrence of metabolic syndrome which includes numerous diseases like hypertension, dyslipidaemia, atherosclerosis, insulin resistance and diabetes (Weiss et al., 2004). The mechanisms by which obesity leads to vascular dysfunction/ disorders like hypertension are unknown. It has been suggested that alterations of immune function could probably link obesity to vascular disorders as well as to the risk factors of atherosclerosis (Channon & Guzik, 2002). Furthermore, obesity predisposes individuals to several other independent diseases like asthma, breast cancer or non-alcoholic liver steatosis (Yanovski & Yanovski, 2002). Although these

associations have been unquestionably proven in large clinical trials, their mechanisms and therefore prospects for therapeutic interventions remain unexplained (Yanovski & Yanovski, 2002). Obesity is accompanied by generalized inflammation, characterized by increased plasma C-reactive protein levels as well as dys-regulated cytokine production by monocytes, lymphocytes and other immune cells (Ouchi, Kihara, Funahashi, Matsuzawa, & Walsh, 2003). The occurrence of obesity has also long been associated with the presence of endothelial and vascular dysfunction, this perhaps, provides partial explanation of how obesity, may lead to cardiovascular diseases (Matsuzawa, 2006).

In sub-Saharan Africa, non-communicable diseases are quite prevalent among urban and peri-urban dwellers. For example, obesity has been shown to be linked to Type 2 diabetes mellitus (T2DM) and atherosclerosis. This observation is likely to be due to compromised life-style viz; lack of exercises, adoption of westernized life-habits, and consumption of processed foods among others. Physiologically, the adipocytes as well as other cells present within fat tissues are capable of releasing numerous vasoactive factors like IL-6 and TNF- α which can lead to cardiovascular morbidity in obese individuals. These adipocyte-derived substances called adipokines can exert significant effects on the immune system (Lago, Dieguez, Gómez-Reino, & Gualillo, 2007).

The most widely known adipokine is leptin which is involved in regulation of appetite, resting metabolism and fertility (Myers, Cowley, & Munzberg, 2008). In addition, adipose tissue produces substances involved in glucose and lipid metabolism (e.g. adiponectin and cholesteryl ester transfer protein), inflammation (e.g. tumour necrosis

factor- α [TNF- α] and interleukin-6 [IL-6]) and coagulation (e.g. plasminogen activator inhibitor-1) (Hajer, van Haeften, & Visseren, 2008).

Adipokines play a significant role in the regulation of metabolic processes and also regulates systemic processes, displaying typical endocrine properties. Therefore, adipose tissue is an important part of the endocrine system (Trayhurn & Beattie, 2001). The abundantly present macrophages have also been found to exhibit endocrine properties. The adipose tissue as an active endocrine organ affects the function of other organs and it is an important source of several proinflammatory cytokines, chemokines, growth factors and complement proteins (Gnacinska, Malgorzewicz, Stojek, Lysiak-Szydlowska, & Sworczak, 2009). Mature adipocytes are the main source of leptin, adiponectin, resistin and visfatin, while prostaglandin E₂, interleukins, vascular endothelial growth factor, hepatocyte growth factor are synthesized by stromal and vascular cells. Adipokines synthesized by the adipose tissues interacts between the adipose tissue, muscular tissue, adrenal cortex and the central and sympathetic nervous system (Romijn & Fliers, 2005) resulting in a positive or negative effect of the individual tissues or glands.

Adipose tissue dysfunction in obesity eventually leads to endothelial dysfunction, vascular hypertrophy and impaired pressure natriuresis. Important mechanisms involved include systemic inflammation, oxidative stress, activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic overdrive (Dorresteijn, Visseren, & Spiering, 2012).

Dysfunctional adipose tissue in obesity is microscopically characterized by hypertrophied adipocytes and increased infiltration by macrophages and functionally by marked changes in secretion of adipokines and saturated free fatty acids (Weisberg et al., 2003); (Hajer et al., 2008). The release of most adipokines, including leptin, TNF- α and IL-6 is elevated, while production of adiponectin, exclusively secreted by adipose tissue, is suppressed (Z. V. Wang & Scherer, 2008). This has important metabolic consequences. Decreased levels of adiponectin for example, contribute to insulin resistance, since adiponectin receptors in liver and muscle cells mediate β -oxidation of fatty acids, glucose uptake, gluconeogenesis and peroxisome proliferator activated receptor- γ activation (Z. V. Wang & Scherer, 2008).

Notably, decreased production of adiponectin by dysfunctional adipose tissue may also provide a direct link between obesity and hypertension. Adiponectin is an important stimulant of endothelial nitric oxide synthase (eNOS), which plays critical roles in controlling vascular tone, inflammation and smooth muscle cell proliferation (Z. V. Wang & Scherer, 2008).

Studies have clearly indicated that obesity is associated with a low-grade inflammation processes in adipose tissue. However, the pathophysiological mechanisms underlying this observation are poorly understood. Another physiological and pathological aspect that has generated a considerable scientific interest during the last decade is the observation that adipocytes have the capacity to synthesize and secrete several bioactive mediators some of which appear to play an important role in obesity associated insulin resistance and cardiovascular complications (Das, 2002).

It is well established that obesity is an independent risk factor for type 2 diabetes, dyslipidemia, and cardiovascular diseases (CVD) (DeFronzo & Abdul-Ghani, 2011). There is also strong evidence that, for a given adiposity, there is a large heterogeneity in the metabolic and cardiovascular risk. (Bastard et al., 2006). Visceral adipose tissue accumulation has been observed as an important predictive factor of lipid, glucose or atherogenic disturbances. Ever since the description of the metabolic syndrome (Reaven, 1998) the awareness of its lethal consequences and the dramatic rise in the prevalence of obesity had led physicians and public health services to consider it as a major health problem which is linked to considerable morbidities.

The combination of obesity and hypertension are also often associated with metabolic abnormalities, such as high triglycerides, low high-density lipoprotein cholesterol and high fasting glucose which predispose individuals to cardiovascular disease and diabetes mellitus (Kotchen, 2010). The causal association between obesity and elevated blood pressure has been demonstrated by large population based studies (Timpson et al., 2009). The contribution of adiposity to the burden of hypertensive disease was estimated to be 78% in men and 64% in women according to the Framingham Offspring Study (Garrison, Kannel, Stokes, & Castelli, 1987). The relation between adiposity and hypertension is particularly high among individuals who are under 60 years of age. This remains consistent even in adolescents and children irrespective of their ethnicity (Weiss et al., 2004).

1.2 Assessment of Body Composition

Although several methods are available to measure anthropometric indices (e.g. muscle mass, skin fold thickness) among obese subjects, these methods are impractical for routine use due to the fact that they are either inaccessible or their use is not cost-effective (Lukaski, 2003). Furthermore, problems with accuracy have generally limited the use of anthropometry in longitudinal studies, particularly when small changes are anticipated (Lukaski, 2003). An alternative approach is the use of electronic devices which employ a four-electrode bioelectrical impedance analysis (BIA) to estimate body composition (Brown, Karatzas, Nakielnny, & Clarke, 1988). However, this tetra polar BIA method has only received limited use to date.

In vivo methods for studying human body composition continued to develop, along with more advanced reference methods that use the information obtained with new technologies. Consequently, several methods for investigating human body composition at different levels are currently used. Densitometry, isotopic dilution, bioelectrical impedance, whole body potassium-40 counting, neutron activation, X-ray absorptiometry, computed tomography (CT), magnetic resonance imaging (MRI), and spectroscopy are the techniques most widely employed (A Battezzati, S Bertoli, C Testolin, & G Testolin, 2003)

1.3 Problem Statement

Obesity is a fast health growing problem that is reaching epidemic proportions worldwide and is often associated with an increased risk of premature death (Adams et al., 2006).

Furthermore, individuals with a central deposition of adipose tissue can experience elevated cardiovascular morbidity and mortality, including stroke, congestive heart failure, myocardial infarction and cardiovascular death (Kenchiah et al., 2002).

The incidence of obesity among women is increasing tremendously in Ghana and generally among women in the coastal regions. (Biritwum, Gyapong, & Mensah, 2005). The high prevalence of obesity among women leads to high morbidity, low socio-economic productivity and enormous health cost burden.

Adipokines have been shown to be strongly linked to adiposity and obesity; however their levels in Ghanaian women have not been investigated. This study sought therefore to evaluate the levels of adipokines in a section of Ghanaian women.

1.4 Justification

The incidence of obesity in the Ga traditional area is perhaps a high health burden to the nation in terms of healthcare management. (Amoah, 2003), reported that the overall crude prevalence of obesity in Ghana was 14.1%. However, (Biritwum et al., 2005) reported 5.5%. (Amoah, 2003) and (Biritwum et al., 2005) reported that the prevalence of obesity were 20.2% and 16.1% respectively in Greater Accra. Knowing the levels of adipokines will aid clinicians to manage conditions associated with metabolic syndrome which will help improve the health of women in our local communities and the nation as a whole.

1.5 Hypothesis

Adipokines are potential diagnostic biomarkers for obesity among women in the James Town catchment area in Accra, Ghana.

1.6 Aim

To investigate the levels of adipokines in relation to body composition among women in James Town, a suburb- dwelling in Accra.

1.7 Specific Objectives

These were:

- To investigate the levels of obesity among women in the James Town suburb of the Greater Accra Region and compare data with previous studies in other parts of the Greater Accra Region of Ghana.
- To investigate the association between the levels of adipokines with the anthropometric characteristics of subjects.
- To investigate the association between the adipokines and plasma lipids as risk indices of obesity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition and classification of obesity

Obesity can be described as a chronic inflammatory response, characterized by abnormal adipokine production, and the activation of some pro-inflammatory signalling pathways which, results in the induction of several biological markers of inflammation (Sartipy & Loskutoff, 2003). Conversely, a reduction in body weight is accompanied by a decrease or even a normalization of these biological parameters (Esposito et al., 2003). Several animal models suggest that these inflammatory processes have a causal relationship with obesity and its co-morbidities such as insulin resistance, type 2 diabetes and CV (Bastard et al., 2006).

The role of fat cells as repository for fats in metabolic dysfunctions has long been considered, but their potential role in an inflammatory process is still an ongoing concept. However, several findings have indicated that adipocytes share with immune cells, certain properties such as complement activation (Rosen et al., 1989) and pro-inflammatory cytokine production (Hotamisligil, Shargill, & Spiegelman, 1993). Fat cell precursors also share features with macrophages. Preadipocytes have the capacity for phagocytosis in response to stimuli (Charrière et al., 2003). Fat cells are also able to synthesize and secrete a chemokine, monocyte chemo attractant protein-1 (MCP-1), a recruiting factor for circulating monocytes that is over-expressed in obesity (Christiansen, Richelsen, & Bruun, 2005). Its main known function is related to guiding monocytes to leave the circulation and become tissue macrophages, the first step in the initiation of

inflammation. (Rull, Camps, Alonso-Villaverde, & Joven, 2010). Genes that code for transcription factors, cytokines, inflammatory signalling molecules, and fatty acid transporters are essential for adiposity.

Available scientific evidence suggests the presence of an overall, low-grade inflammation in obesity, with altered levels of circulating factors such as increases in the plasma levels of C-reactive protein (CRP), tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), and other biological markers of inflammation (Das, 2002); (Engstrom et al., 2003). Other adipose-specific molecules that are involved in the control of energy metabolism also regulate immune responses. For example, leptin in addition to its key role in food intake and energy expenditure also regulates immune processes. Leptin-deficient mice or humans display an altered immune status (Farooqi et al., 2002). The reduction in leptin levels could be responsible for fat-associated immuno-suppression (Lord et al., 1998). Reduction in body weight is accompanied, not only by an improvement in the inflammatory process and the co-morbidities, but also by a decrease in the expression of genes coding for inflammatory proteins (Clement, Schmidt, Bernaix, Covington, & Carr, 2004). This phenomenon is detectable with a small decrease in body weight. In a related study, it was observed that, three months following a by-pass surgery, resulted in a significant decrease in both macrophage infiltration and in the steady state levels for mRNA involved in the inflammatory response (Cancello et al., 2005). Adipokine production is altered in obesity, type 2 diabetes and metabolic syndrome. This is observed for leptin, TNF- α , IL-6, adiponectin and resistin. Other adipokines such as angiotensinogen, Plasminogen activator inhibitor-1 (PAI-1) or visfatin are also important players in vessel and metabolism regulation (Curat et al., 2004).

The worldwide incidence of obesity continues to increase, despite increased awareness and global efforts to understand and confront its origins. In essence, dysregulated energy homeostasis stems from a societal reduction in physical activity, an increase in the accessibility of energy-dense foods and overindulgence in the energy-dense foods, combined with a myriad of genetic, social and economic complicating factors (Mitchell, Armstrong, Robker, & Norman, 2005). Overweight and obesity that were considered problems only in high-income countries are considerably on the increase in low and middle income countries particularly in urban settings. Consumption of junk foods and western lifestyles that causes abdominal obesity especially among women needs to be controlled. (Biritwum et al., 2005) reported that the prevalence of obesity in Ghanaians of 18 years and above was 5.5% and that its incidence was more in the Southern part as compared to the Northern sector of the country. Obesity was highest in Accra (16.1%) and virtually absent in Upper East and West. By ethnicity, obesity was highest among Ga-Dangme (14.6%), Ewes (6.6%), and Akans (6.0%). Obesity was also more common in married females than unmarried ones and highest in the employed compared to self-employed (Biritwum et al., 2005).

Obesity is associated with diverse set of metabolic disorders, and has reproductive consequences that are not well understood. A significant proportion of the infertile or sub-fertile population are obese or overweight (Hamilton-Fairley, Kiddy, Watson, Paterson, & Franks, 1992); (Zaadstra et al., 1993); (Pettigrew & Hamilton-Fairley, 1997); (Norman & Clark, 1998); (Crosignani, Vegetti, Colombo, & Ragni, 2002), with a plethora of reproductive complications including menstrual dysfunction and anovulation

(Hartz, Barboriak, Wong, Katayama, & Rimm, 1979), (Lake, Power, & Cole, 1997) and miscarriage (J. X. Wang, Davies, & Norman, 2002).

Obesity is an important risk factor for future incidence of hypertension (Cassano, Segal, Vokonas, & Weiss, 1990) and for progression from prehypertension to hypertension (De Marco et al., 2009), (Tomiyama et al., 2009). Studies done by (Dorresteijn et al., 2012), showed that with normal weight individuals as the reference, the odds ratio for the presence of hypertension was 1.7 for overweight individuals (body mass index [BMI] 25.0–29.9), 2.6 for class I obesity (BMI 30.0–34.9), 3.7 for severe or class II obesity (BMI 35.0–39.9) and 4.8 for morbid or class III obesity (BMI > 40.0) (Nguyen, Magno, Lane, Hinojosa, & Lane, 2008). Mendelian randomization studies estimated that each 10% increase in BMI is associated with elevation of systolic blood pressure by 3.9 mmHg (Timpson et al., 2009). Yet, waist circumference and waist-hip ratio, both measures of centralized obesity, are even more strongly related to hypertension than BMI (C. M. Lee, Huxley, Wildman, & Woodward, 2008). Each 5% weight gain is associated with 20–30% increased odds of hypertension (Droyvold, Midthjell, Nilsen, & Holmen, 2005), while weight reduction results in blood pressure decrease (Dall'Asta et al., 2009).

Obesity has been also been shown to be linked to insulin-resistance which plays an important role in the pathogenesis of several metabolic disorders including hypertension (Esteghamati et al., 2008), type 2 diabetes (Meigs et al., 2007), dyslipidemia (Semenkovich, 2006); (Chapman & Sposito, 2008), and cardiovascular disease (Bonora et al., 2002; Bonora et al., 2007).

2.2 Lipid disorders in Obesity

Dyslipidaemia in obesity is characterized by increased levels of very low-density lipoprotein (VLDL) cholesterol, triacylglycerols and total cholesterol, an increased in small dense LDL particles, and lower high density lipoprotein (HDL) cholesterol levels (Howard, Ruotolo, & Robbins, 2003). There is little evidence that LDL cholesterol known to be a major risk factor for coronary heart disease (CHD) is enhanced in obesity, among patients with visceral obesity in particular (Luc et al., 2006). It has also been shown that apolipoprotein (Apo) B/A1 ratio, small dense LDL particles and low HDL cholesterol, are predictive factors for cardiovascular disease in people with visceral adiposity (Yusuf et al., 2004). Insulin-resistant state of abdominal obesity adds substantially to the CHD risk of patients with familial hypercholesterolemia (Gaudet et al., 1998). Obesity also limits the beneficial effects of lipid-lowering strategies (Nicholls et al., 2006). However, aggressive reduction of lipids with the use of statins has been shown to offer beneficial effects on coronary plaque development in obese individuals (Nicholls et al., 2006). Regular exercise is also known to decrease the risk of cardiovascular disease (Leon & Sanchez, 2001). Comprehensive reviews, suggest that exercise has little effect on total cholesterol or low-density lipoprotein (LDL) cholesterol concentrations and only a minimal and inconsistent beneficial effect on high-density lipoprotein (HDL) cholesterol concentrations (Leon & Sanchez, 2001). It is clear that the concentrations of , small LDL particles, large HDL particles, and large very-low-density lipoprotein (VLDL) particles are better indicators of cardiovascular risk than are the elements of the traditional lipid profile (Pascot et al., 2001). In insulin-resistant states, the dyslipidaemia as seen in obesity is characterized by a different composition and

distribution of LDL particles, resulting in an increased concentration of small, dense LDL. LDL particles are enriched in triacylglycerols, which are rapidly lipolysed by hepatic lipase (HL) leaving smaller, denser LDL particles (Luc et al., 2006). The activities of both cholesteryl ester transfer protein (CETP) and HL seem to be increased in metabolic syndrome, leading to oxidation and glycation of such particles. Small dense LDL particles can move through endothelial fenestrations, entering the sub- endothelial space where inflammation and transformation into plaque can occur (Kwiterovich, 2002). The modified LDL is mostly taken up by macrophage scavenger receptors, rather than the normal LDL receptor (LDLR) pathway, thus inducing atherosclerosis.

Hepatic overproduction of VLDL seems to be the primary and crucial defect in obesity that is a consequence of hepatic steatosis. Insulin resistance in the liver, muscles and adipose tissue leads to an inability to suppress hepatic glucose production, impaired glucose uptake and oxidation, and inability to suppress release of non-esterified fatty acids (NEFAs) from adipose tissue. Another key factor in the regulation of VLDL secretion is the rate of ApoB-100 degradation, which is decreased in insulin resistance (Luc et al., 2006).

2.3 Body composition as an investigating tool in metabolic disorders

Indeed, weight, height, and body mass index are not sufficient for the assessment of nutritional status in health and disease. Body compartments and their relationships with changes at the onset of disease, during treatment and in the long-term follow-up are far more important in the management of diseases. The methods for body composition

assessment are critical for investigating physiology and pathophysiology of non-communicable diseases. Modern research increasingly uses this information for better describing clinical phenotypes and for understanding the pathogenesis of many diseases. The diseases most studied using body composition assessment methods are; chronic obstructive pulmonary diseases, cardiovascular diseases, obesity, HIV, sarcopenia, and metabolic diseases, in particular diabetes, osteoporosis, and glycogenosis. (A Battezzati et al., 2003). Body composition is affected virtually by all pathologic conditions like cardiac disorders, pneumonia, kidney problems, endocrine disorders and gastrointestinal diseases. Very often, diseases affect body composition at both the molecular level (fat mass, fat-free mass including body water, protein and glycogen content, bone mineral content) and the cellular or tissue level (cellular mass, adipose tissue, muscular tissue, body water distribution) (A Battezzati et al., 2003).

Pathogenic events that are related to changes in fat mass are the basis of obesity. The absolute amount of fat is important, and its distribution is also critical. Evidence on insulin resistance and cardiovascular risk suggests a pathogenic role for “ectopic” fat (Yki-Jarvinen, 2002), i.e., the fat stored in visceral adipocytes or in myocytes and hepatocytes. It has long been known that endocrine disease, such as glucocorticoid (cortisol) excess, causes an altered trunk fat distribution. Beyond the metabolic effects of stored and circulating fat, adipose tissue emerges as an innervated endocrine organ capable of secreting cytokines proportionally to its amount (Trayhurn & Beattie, 2001). More than any other body composition field, the study of fat and adipose tissue mass has developed rapidly together with new knowledge on energy metabolism regulation at the neuroendocrine, genetic/genomic, and molecular biology level.

It is not only possible to measure the amount of fat by various methods (plethysmography, dual energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), but also to localize the adipocytes by magnetic resonance imaging (MRI) or computed tomography (CT) imaging and to quantify the fat amount in muscle and hepatocytes (MRI clinical spectroscopy).

Changes in fat-free mass result from the endocrine milieu including anabolic and catabolic hormones and cytokines. Innervation is also important in maintaining muscular mass and metabolic activities, thus contributing to fat free mass. Since most fat-free mass is water, this compartment is also affected by all modulators of electrolyte fluxes, including hormones and drugs (A Battezzati et al., 2003).

Sarcopenia which is the wasting of the quality or quantity of muscular proteins is the last common pathway of most diseases. Specifically, it is a widespread and important contributor to the fragility and disability of the elderly. Sarcopenia could be an early lesion signalling energy or metabolic imbalance. For this reason, it is of special value to couple measurements related to metabolically active mass with those of energy expenditure (Toth & Poehlman, 2000). On the other hand, changes in body water per se are the subject of intensive investigation in endocrinology, nephrology, and cardiology, with body fluid retention being a pathogenic hallmark (A Battezzati et al., 2003).

2.4 Body composition as a clinical tool in the investigation of metabolic disorders.

A correct diagnosis of fat content and localization is very important in the assessment of overweight, obesity, lipodystrophy, eating disorders, and risk factors for cardiovascular and metabolic diseases. It is also important to monitor the therapeutic programs, for example to target the interventions on body fat excluding relevant changes on fat-free mass. A correct diagnosis of sarcopenia requires the measurement of fat-free mass, and possibly of cellular mass. The measurement of energy expenditure normalized to metabolically active mass should provide invaluable tool to define a hyper- or hypo-metabolic state at early stages. The assessment of body water compartments is widely used by nephrologists and increasingly used by cardiologists in order to monitor specific changes in body hydration so as to provide diagnoses and to monitor therapeutic interventions and patient outcome (A Battezzati et al., 2003).

Simply measuring weight or anthropometry is not sufficient for detecting changes in fat, lean mass, bone or water content and distribution. The advantages of the anthropometric data for diagnosis, prevention, therapy, and follow-up do not need any comments. Instruments such as DXA, BIA and, more recently, air plethysmography are widely available at a limited cost, with widespread know-how, a large range of applications, and present fully codified operative procedures.

Beyond that, it must be recognized that reference values are often incomplete for most geographic regions and ethnicities. Secondly, a consensus remains to be reached on: (a) the physiological meaning of measures (for example, intracellular water or fat-free mass as indicators of “lean tissue mass” or “cellular mass” or “metabolically active mass”); (A.

Battezzati, S. Bertoli, C. Testolin, & G. Testolin, 2003) (b) interpretation of the deviation from normal values in the various disease processes; and (c) selection of the application limits of the instruments and methods (for example to identify the cut-off values for accuracy of DXA in morbid obesity) (A. Battezzati et al., 2003). Only after the above requirements are met, will it be possible to adopt specific guidelines from scientific societies, which form the most reasonable basis for acceptance and accreditation by care providers. Finally, some body composition techniques are already being used in several settings, including the rapidly developing area of body fitness. Establishing guidelines for the clinical application of body composition study methods is important for physicians and their patients.

2.5 Adiposity, Adipokines and Obesity

Increasingly, it is becoming apparent that adipose tissue functions as highly specialized active endocrine and paracrine organ that releases several bioactive mediators that influence not only body weight homeostasis but produces an array of adipokines, as well as eliciting cell mediated effects via inflammatory and anti-inflammatory cells, producing various cytokines and chemokines. Adipokines have local and systemic biological effects, which influence insulin sensitivity and the development of obesity, and also coagulation, fibrinolysis, insulin resistance, diabetes, and atherosclerosis (Kopelman, 2000); (O'Rourke, Gronning, Yeaman, & Shepherd, 2002). Paradoxically, the cellular mechanisms linking obesity to atherosclerosis are complex and have not been fully explained or dealt with (David et al., 2005).

The emergent of knowledge in the understanding of the biological functions of adipokines and their resultant role coupled with their probable interactions with other hormones will throw a better scientific and evidenced-based light on the contribution of adipokines to obesity.

2.5.1 Leptin

Leptin was one of the first adipokines identified (Pelleymounter et al., 1995) and (G. H. Lee et al., 1996) and has since drawn substantial research attention. Leptin is a 167 amino-acid protein, encoded by the *ob* gene, belonging to a cytokine family, located within 7q31.3 locus (Geffroy et al., 1995). Adipocytes are the primary sites of leptin expression, although it has also been shown to be expressed in gastric wall, vascular cells, placenta, ovary, skeletal muscle and liver (Koerner, Kratzsch, & Kiess, 2005), (Brzozowski, Konturek, Konturek, Brzozowska, & Pawlik, 2005), (P. C. Konturek et al., 2004). Leptin's role in appetite control within so called brain-gut axis provides a satiety signal through its action on CNS receptors within the hypothalamus (P. C. Konturek et al., 2004) (S. J. Konturek et al., 2003), and (S. J. Konturek et al., 2003) and energy expenditure. Mice with mutated *ob* gene (*ob/ob* mice) develop severe obesity in relation to the lack of satiety signalling within their brain gut axis (Pelleymounter et al., 1995).

Similarly, adults with leptin deficiency (extremely rare genetic disorder) showed increased appetite and obesity which can be treated by leptin administration. The phenotype of these subjects includes T - cell hypo-responsiveness, hyperinsulinemia, insulin resistance, hyperlipidemia, immune dysfunction and neuroendocrine

abnormalities (Farooqi et al., 2002), (Montague et al., 1997). A link of leptin with insulin resistance and fertility was reported by (F. F. Chehab, Lim, & Lu, 1996), with leptin deficient mice having increased adiposity, severe insulin resistance and diminished fertility, of which were restored with leptin administration, but not by calorie restriction or weight loss (F. F. Chehab et al., 1996), (Mounzih, Lu, & Chehab, 1997).

Leptin levels are closely correlated with the fat mass, and decrease with weight reduction (Considine et al., 1996). Like majority of neurohormones, leptin levels exhibit important circadian rhythms with peak during night between midnight and morning hours (Sinha et al., 1996). Several agonists have been shown to increase leptin release from adipocytes. These include TNF- α and other pro-inflammatory cytokines, insulin, glucose, estrogens. Other vasoactive factors like angiotensin II or endothelin may also lead to leptin release (S. Kim, Whelan, Claycombe, Reath, & Moustaid-Moussa, 2002), although this is still under investigation, as this phenomenon may occur locally and does not seem to affect plasma levels of leptin during angiotensin II infusion (Ran et al., 2006).

Leptin receptors (a family of splice variants OB-R, differing with the size of cytoplasmic C terminus) are expressed in number of different tissues, (Kaminski et al., 2006); (Brzozowski et al., 2005); (Nawrot-Porabka et al., 2004); (Jaworek et al., 2003); (Accorsi et al., 2005), particularly within the cardiovascular and immune system (Stallmeyer, Pfeilschifter, & Frank, 2001); (Kougias et al., 2005). Different splice variants of the receptors may differ in relation to signalling pathways and sites of expression, with OB-Rb (long isoforms as the major signalling one). It is important to note that receptor splicing differs between mouse and human. Such ever-present expression of the receptors

in humans and widespread binding of leptin in various organs indicates its role in a constellation of vital processes including growth, metabolic control, immune regulation, insulin sensitivity regulation and reproduction. These aspects of leptin actions have been extensively reviewed (Koerner et al., 2005);(Tilg & Moschen, 2006); (Kaur & Zhang, 2005).

Leptin is involved in the regulation of energy homeostasis (Farid F Chehab, Qiu, & Ogus, 2004) and is almost exclusively expressed and produced by white adipose tissues (WAT) and more particularly by differentiated mature adipocytes (Ahima & Flier, 2000). Circulating levels and adipose tissue mRNA expression of leptin (Vidal et al., 1996) are strongly associated with BMI and fat mass in obesity. Thus, leptin appears as a real marker of adipose tissue mass in lean humans where the subcutaneous fraction of adipose tissue represents about 80 % of total fat. Leptin mRNA expression is higher in subcutaneous adipose tissue (SAT) than in visceral adipose tissue (VAT) in human (Lefebvre et al., 1998). Although leptin acts mainly at the level of the central nervous system to regulate food intake and energy expenditure, there is a relationship between leptin and the low grade inflammatory state in obesity, suggesting that leptin could exert peripheral biological effects as a function of its cytokine-like structure (Ahima & Flier, 2000). Leptin receptors belong to the cytokine class I receptor family, and several published works have reported that there is an increased inflammatory response associated with the presence of hyperleptinemia without obesity (Loffreda et al., 1998), (van Dielen et al., 2001) and that leptin is able to control TNF- α production and activation by macrophages (Loffreda et al., 1998). However, the underlying mechanisms have not been clearly identified. In peripheral tissues, leptin generally has a fat

metabolizing role with limited direct effect on glucose metabolism. However, leptin antagonizes insulin action and decreases its production by pancreatic β -cells (Seufert, 2004). Leptin indirectly affects glucose metabolism, for example glucose transport in skeletal muscle via the hypothalamus and central nervous system (Kamohara, Burcelin, Halaas, Friedman, & Charron, 1997); (Minokoshi, Haque, & Shimazu, 1999). Evidence suggests that leptin increases lipolysis in adipose tissue and cells, and in skeletal muscle, but it appears less critical to liver function (Cohen et al., 2001).

2. 5.2 Adiponectin

Adiponectin, the most intensely studied adipokine, has insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. Since the discovery of adiponectin (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995), several articles on this molecule, example of such are (Tamang et al., 2013) and (Stepien et al., 2013) have been published. The emergent evidence implicates adiponectin as a major insulin-sensitizing adipokine and an important biomarker and therapeutic target for obesity-associated metabolic diseases (Kadowaki et al., 2006). Adiponectin was also first described in cultured murine adipocytes (Scherer et al., 1995) and is abundantly synthesized by adipocytes. The adiponectin gene is located on chromosome 3q27 (Saito et al., 1999), in a region mapped as a susceptibility locus for type 2 diabetes and adiposity (Kissebah et al., 2000) ; (Vionnet et al., 2000), and is thought to potentially link obesity to insulin resistance.

Adiponectin, having been discovered by several groups, has been attributed several different names: adipocyte complement-related protein of 30 kDa (ACRP30) or adipoQ

in mouse and gelatin-binding protein 28 (GBP28) or adipose most abundant gene transcript 1 (APM1) in human (Kadowaki & Yamauchi, 2005). The expression of adiponectin mRNA is dependent on the adipose tissue localisation. It is lower in visceral adipose tissue (VAT) than in subcutaneous adipose tissue (SAT) (Lihn et al., 2004). Plasma levels of adiponectin, which constitutes 0.01% of circulating proteins, are between 5 to 30 mg/L in lean control subjects while those of leptin are between 2 to 8 µg/L (Bastard et al., 2006). Adiponectin has several peculiarities which distinguishes it from other adipokines: 1) circulating adiponectin levels are decreased in obese and/or type 2 diabetic patients and in patients with coronary heart diseases, 2) there is a strong positive correlation between adiponectinemia and insulin sensitivity, 3) there is an inverse correlation between adiponectinemia and obesity and more particularly with abdominal obesity and 4) adiponectin may play a protective role against atherosclerosis and insulin resistance. The insulin-sensitive action of adiponectin may involve the activation of AMP activated protein kinase (AMPK) (Bastard et al., 2006), which is known to regulate cellular malonyl CoA concentrations by inhibiting acetyl CoA carboxylase (Toshimasa Yamauchi et al., 2002). This inhibition results in a decreased level of intracellular malonyl CoA and a subsequent decreased lipogenesis associated with increased mitochondrial fatty acid beta-oxidation. Adiponectin is also able to regulate liver glucose production by lowering mRNA expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, two key enzymes of gluconeogenesis (Kadowaki & Yamauchi, 2005). Adiponectin enhances insulin sensitivity in muscle and liver and increases free fatty acid (FFA) oxidation in several tissues, including muscle fibres (Fruebis et al., 2001), (Toshimasa Yamauchi et al., 2001). It also decreases serum

FFA, glucose, and triacylglycerol concentrations in normal concentrations. Lean mice given injections of adiponectin in conjunction with a meal high in fat and sugar, the normal postprandial increases in plasma glucose, FFA, and triacylglycerol concentrations are smaller as the result of an increased rate of clearance from the blood rather than a reduced rate of absorption from the gut (Fruebis et al., 2001). Insulin-resistant mice treated with physiologic concentrations of adiponectin, had their glucose tolerance improved and insulin resistance is reduced (Toshimasa Yamauchi et al., 2001).

In addition to its insulin-sensitising effects, adiponectin may have a protective effect on the vascular wall by acting early at several steps in the atherogenesis process modulation of endothelial adhesion molecules (Ouchi et al., 1999), transformation of macrophages into foam cells (Ouchi et al., 2001) and modulation of vascular smooth muscle cells proliferation (Y. Arita et al., 2002). Moreover, adiponectin may modulate the TNF- α induced inflammatory response, since it has been shown that adiponectin reduces TNF- α secretion of macrophages (Ouchi et al., 2000). This anti-TNF- α effect of adiponectin may partly explain the anti-inflammatory and anti-atherogenic effect of adiponectin. In contrast, both TNF- α and IL-6 reduce human adipocyte mRNA expression of adiponectin (Bruun et al., 2003), which is an additional mechanism by which these two cytokines induce insulin resistance.

In humans, plasma adiponectin concentrations fall with increasing obesity and this effect is greater in men than in women (Yukio Arita et al., 1999). Reduced adiponectin concentrations correlate with insulin resistance and hyperinsulinemia (Hotta et al., 2001). In addition, several polymorphisms of the adiponectin gene (*APM1*, mapped to

chromosome 3q27) have been identified that are associated with reduced plasma adiponectin concentration (Kondo et al., 2002), (Hara et al., 2002) and that increase the risk of type 2 diabetes, insulin resistance, or the metabolic syndrome (Kissebah et al., 2000). Adiponectin is implicated in the development of atherosclerosis, in that their concentrations are reduced in patients with coronary artery disease (Hotta et al., 2000). and adiponectin inhibits tumour necrosis factor α (TNF- α), induced expression of adhesion molecules and the transformation of macrophages to foam cells, both of which are key components of atherogenesis (Ouchi et al., 1999);(Ouchi et al., 2001). Finally, in mice deficient in apolipoprotein E (and thus susceptible to atherosclerosis), treatment with human adiponectin significantly inhibits lesion formation in the aortic sinus by 30% compared with that in untreated control animals (Okamoto et al., 2002).

Two adiponectin receptors, adipoR1 and adipoR2, localized on chromosomes 1q32 and 12p13 respectively, have been cloned (T. Yamauchi et al., 2003). AdipoR1 is predominantly expressed in skeletal muscle while adipoR2 is mainly expressed in the liver. However, the physiological relevance and the transduction signal pathways of these two receptors remain to be determined.

2.5.3 Resistin

Resistin is a peptide hormone produced by mature adipocytes and regulates whole-body insulin sensitivity and first discovered by (Steppan et al., 2001). It is more highly expressed in visceral white fat than in subcutaneous fat (Steppan et al., 2001). The expression of resistin mRNA is suppressed in response to rosiglitazone, a peroxisome

proliferator-activated receptor- γ (PPAR- γ) agonist that enhances insulin sensitivity (Steppan et al., 2001). It has been shown that circulating and adipose tissue resistin levels were increased in obese rodents but decreased under treatment with thiazolidinediones (Steppan et al., 2001). Moreover, infusion of recombinant resistin into lean control animals induced insulin resistance, while its immuno-neutralisation improved insulin sensitivity in insulin-resistant obese animals. However, additional, contradictory studies have indicated a decreased mRNA gene expression in adipose tissue from various insulin-resistant rodent models. In cultured adipocytes, resistin reduced insulin-stimulated glucose transport, an effect which was reversed using an anti-resistin antibody. In addition, resistin inhibited adipocyte differentiation (K. H. Kim, Lee, Moon, & Sul, 2001). These studies suggest that resistin could be a link between adipose tissue, obesity and insulin resistance, however, recombinant resistin caused major liver insulin resistance (Jackson, Osei, & Ahima, 2005). It was recently shown that resistin-knockout mice have lower fasting glycaemia and increased glucose tolerance and insulin sensitivity associated with a reduced liver glucose production (Jackson et al., 2005). The lack of resistin could lead to the activation of AMPK and consequently to a decreased expression of genes involved in liver neoglucogenesis, suggesting that resistin could exert effects opposite to those of adiponectin. Finally, resistin-knockout mice under a high fat diet regimen became obese and insulin resistant as their wild type counterparts. However, fasting glycaemia was lower in resistin-knockout mice, suggesting the implication of resistin in the hyperglycaemia and insulin resistance observed in obesity.

With regards to resistin in humans, several discrepancies have been observed. Whilst some studies have shown that adipose tissue expresses resistin (Steppan et al., 2001)

others did not find its presence or detected only very low mRNA expression in this tissue. It is believed that the adipocyte is not the major cell type producing resistin in humans, which rather is produced by circulating monocytes and macrophages (Jackson et al., 2005). Finally, most of the studies found no correlation between plasma resistin levels, BMI and insulin resistance in human. Nonetheless, the macrophage localization of resistin and its inter-relationship with adipocyte metabolism and function are currently under investigation.

2.6 Effects of Adipokines on the Immune System

The excessive amounts of adipose tissue are related to increased systemic inflammation; it has also been proven in both clinical and experimental settings (Tilg & Moschen, 2006). This is related to the ability of adipocytes to produce cytokines as well as the inflammatory infiltration of fat by monocytes, macrophages and possibly other inflammatory cells like lymphocytes. The relative importance of those compartments of fat in obese individuals remains disputed (Fain, 2006). Although leptin is not a classical cytokine several immune cells (including polymorphonuclear leukocytes, monocytes, macrophages and lymphocytes) bear leptin receptors and their activity can be modulated by leptin (Zhao, Sun, You, Gao, & Tian, 2003). Most of leptin pro-inflammatory activities appear to be mediated by a long OBRb receptor. Leptin has certain structural similarities to classical cytokines like IL-6, GM-CSF or IL-12 (Tilg & Moschen, 2006). Interesting data regarding the role of leptin in mediating immunity have been obtained in two models giving insight into leptin biology (i.e. ob/ob mice lacking leptin and db/db

mice showing leptin resistance). Mice lacking leptin show numerous pathologies of the immune system. Leptin has been postulated to play an important role in linking nutritional status to immune system (Matarese, 2000). The most evident effects of leptin seem to occur at the level of adaptive immunity. Leptin-deficient (*ob/ob*) mice have severe thymic atrophy and this finding suggests that this hormone is required for normal thymopoiesis, although the role of leptin may be more complex in the setting of inflammation stimuli such as lipopolysaccharide (LPS). Leptin administration induced weight loss and stimulated thymopoiesis in *ob/ob* mice, but did not stimulate thymopoiesis in wild-type C57BL/6 nor BALB/c mice (Hick, Gruver, Ventevogel, Haynes, & Sempowski, 2006). Surprisingly, in endotoxin-stressed mice, leptin prevented LPS-induced thymus weight loss and stimulated TCR- α gene rearrangement. (Hick et al., 2006) demonstrated that leptin had a selective thymo-stimulatory role in settings of leptin deficiency and endotoxin administration, and may be useful for protecting the thymus from damage and augmenting T cell reconstitution in these clinical states (Hick et al., 2006). Interestingly, leptin appears to also protect from TNF- α induced toxicity. Acting on monocytes, leptin induces release of cytokines such as TNF- α or IL-6 as well as chemokine (c-c motif) ligand 2 (CCL2) and vascular endothelial growth factors (VEGF) (Tilg & Moschen, 2006). Moreover, leptin leads to increased proliferation and differentiation of monocytes. Leptin effects on the immune system appear to depend not only on the leptin concentrations, but also on the status of the immune system.

Leptin may lead to enhancement of auto-immune reactions, in part by reducing T regulatory cells. Leptin levels are also increased in patients with autoimmune diseases (Matarese, Moschos, & Mantzoros, 2005).

Adiponectin is considered to be a beneficial adipokine. Certain inflammatory mediators, such as TNF- α or IL-6 which have been shown to increase leptin expression in adipocyte lead to a decrease of adiponectin expression and release. Adiponectin can lead to numerous changes of immune cell functions that are NFkB dependent as well as vascular adhesion molecular expression further reducing inflammation. Acting on adaptive immunity, adiponectin inhibits T cell activation and proliferation, although data regarding adiponectin effects on adaptive immune responses are relatively bare. Adiponectin also inhibits B lymphopoiesis, but only when stromal cells were present and only when cultures were initiated with the earliest category of lymphocyte precursors (Yokota et al., 2003). Adiponectin induces the production of the anti-inflammatory mediators IL-10 and interleukin-1 receptor-antagonist (IL-1RA) in human monocytes, monocyte-derived macrophages, and dendritic cells (Wolf, Wolf, Rumpold, Enrich, & Tilg, 2004). In addition, adiponectin significantly impairs the production of the proinflammatory cytokine IFN- γ , and adiponectin-treated macrophages exhibit reduced phagocytotic capacity (Wolf et al., 2004). Different forms of adiponectin may exhibit differential actions on the immune system. Low molecular weight (LMW) - adiponectin as well as high molecular weight form (HMW) both induce apoptosis in non-differentiated monocytic THP-1 cells, reduce macrophage scavenger receptor (MSR) an mRNA expression, and stimulate phosphorylation of adenosine monophosphate-activated protein kinase (Neumeier et al., 2006). However, HMW form induces IL-6 in human monocytes, while LMW form reduces LPS mediated IL-6 release and stimulates immunosuppressive IL-10 secretion, most likely by reducing the abundance of inhibitor of nuclear factor (NF)-kappa B kinase b, leading to a diminished nuclear translocation of N Adiponectin,

acting on NK cells, a key component of innate immune system, suppresses the IL-2-enhanced cytotoxic activity of NK cells without affecting basal NK cell cytotoxicity (K. Y. Kim et al., 2006). This effect appears to be also mediated via the AMP-activated protein kinase-mediated inhibition of NF-kappa B activation (K. Y. Kim et al., 2006). IFN-gamma enhances NK cell cytotoxicity by causing an increase in the levels of expression of TRAIL and Fas ligand. The production of IFN-gamma, one of the NF-kappa B target genes in NK cells, was also found to be suppressed by adiponectin, accompanied by the subsequent down-regulation of IFN-gamma inducible TRAIL and Fas ligand expression (K. Y. Kim et al., 2006). The interaction of adiponectin with the immune system is also related to the fact that the elastase derived from macrophages is critical for the generation of active globular form of adiponectin (Waki et al., 2005).

2.7 Effects of Adipokines on Cardiovascular Functions.

Obesity is a common risk factor for diseases of the vascular system; these include atherosclerosis and hypertension. Elucidating additional potential links and pathways from obesity to these disease processes is vital for understanding the complicated role of adipose tissue on the cardiovascular system (Kougias et al., 2005), which can be very useful in designing future therapeutic approaches. Central adiposity is a key feature of obesity, reflecting the fact that the prevalence is driven by the strong relationship between waist circumference and increasing adiposity, and how these new insights may provide innovative therapeutic strategies to improve cardiovascular health.

In analogous to their effects in the immune system, adipokines exhibit extensive effects in the vascular system. While both of these aspects of actions of adipokines are likely to be related to each other as inflammation is critical for virtually all cardiovascular diseases (Lucas et al., 2003), some actions occur independently, as they can be observed in studies of direct vascular effects of adipocytokines *in vitro* and as adipokine receptors have been identified on endothelial cells and vascular smooth muscle cells.

As part of the adipokines effects on cardiovascular function, adiponectin induces the production of anti-inflammatory mediators in human inflammatory cells and impairs interferon- γ production, another target gene of NF- κ B (K. Y. Kim et al., 2006). Adiponectin directly stimulates the production of nitric oxide by endothelial cells, via PI3-K dependent pathways, which enhances endothelial nitric oxide synthase (eNOS) activity by triggering its AMPK- induce phosphorylation (Chen, Montagnani, Funahashi, Shimomura, & Quon, 2003). Other studies have also shown adiponectin's ability to decrease TNF- α - induced production of asymmetric dimethylarginine (Eid, Lyberg, Arnesen, & Seljeflot, 2007), an L- arginine analogue that inhibits NO formation and thereby impairing vascular function. The activation and up-regulation of eNOS could explain some of the observed vaso- protective properties of adiponectin. Adiponectin also improves endothelium redox state by suppressing NADPH oxidase derived superoxide generation (Motoshima, Wu, Mahadev, & Goldstein, 2004). It is also interesting to know that high molecular weight adiponectin suppresses endothelial cell apoptosis (Kobayashi et al., 2004) and promotes vascular healing and angiogenesis (Yang et al., 2006).

2.7.1 The Anti-atherogenic effect of Adiponectin

The principal hypothesis of the anti-atherogenic effect was confirmed by the finding that adiponectin prevents atherosclerosis in apolipoprotein-E-deficient mice (Okamoto et al., 2002). In addition, *in vitro* studies have also revealed some specific anti-atherogenic actions of adiponectin. Studies on human aortic endothelial cells have proved that adiponectin suppresses TNF- α -induced expression of adhesion molecules (Ouchi et al., 1999). Furthermore, adiponectin prevents macrophage's transformation into foam cells, a crucial step in atherogenesis (Ouchi et al., 2001). (Y. Arita et al., 2002) also showed that adiponectin suppresses proliferation of human aortic smooth muscle cells, suggesting a possible interference in vascular remodelling. Besides, as mentioned earlier, it has been shown that hypoadiponectinaemia is associated with coronary artery disease (Kumada et al., 2003). Moreover, adiponectin plasma levels correlate with various atherosclerotic risk factors, such as low-density lipoproteins, particle size and triglycerides (Kazumi, Kawaguchi, Hirano, & Yoshino, 2004). Therefore, it is generally believed that by acting as an anti-inflammatory, antioxidant and vasodilator agent, adiponectin prevents endothelial dysfunction and the progression of atherosclerosis.

2.7.2 Effects of Adiponectin on myocardium

Adiponectin is synthesized and secreted by human cardiomyocytes, while AdipoRs exist in cardiac muscle. As in other energy-consuming tissues, adiponectin also regulates energy homeostasis in cardiac muscle, increasing cardiac oxidation of fatty-acids (Frayn, Karpe, Fielding, Macdonald, & Coppack, 2003). Adiponectin also exerts a direct cardio-

protective action. Two recently published studies demonstrated that adiponectin accumulates in myocardial tissue that has been damaged by ischemia or reperfusion injury (Shibata et al., 2007) and protects myocardium by inhibiting inducible NOS and NADPH-oxidase expression and resultant oxidative stress (Tao et al., 2007), while it may protect against myocardial ischemia or reperfusion injury through AMPK and COX-2-dependent mechanisms (Shibata et al., 2005). Even more importantly, adiponectin seems to be involved in coronary plaque vulnerability as that is characterized by angiographic lesion complexity (Otsuka et al., 2006). In view of this evidence, it seems reasonable enough to claim that adiponectin at least takes part in the pathophysiology of ischemic heart disease if not altering its course and outcome. Generally, adiponectin seems to be a key player in cardiovascular homeostasis. Nonetheless, most of the mechanistic data currently available are based on observations from cell culture and animal models, and extrapolations to humans should be made with caution.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Sampling Population

Jamestown a suburb of Accra, the capital of Ghana is located east of the Korle lagoon. It is one of the oldest districts in the city of Accra and emerged as a community around the 17th century British James Fort on the Gulf of Guinea coast. The district was developed by the 19th century and followed the rapid growth of the city in the 20th century. It became an area of a dense mixture of commercial and residential use. Jamestown remains a fishing community inhabited primarily by the indigenes of the Ga tribe. Although in a state of decay following years of neglect by subsequent governments, the district is a popular tourist destination for those seeking to see the remnants of Accra's colonial past.

A total of 111 subjects were recruited into the study. Baseline surveys were conducted by regular home visits. During the interactive interview and examination, a standard structured questionnaire covering subjects' lifestyle, family disease history, dietary habits, and anthropometrics was administered to each consented subject.

Data on demographic variables (age, sex), smoking status, alcohol use and educational level was also obtained from the subjects. Measurements of height and weight were taken using a standardized protocol. Height was measured with subjects wearing no shoes using a stadiometer and weight measured using a weighing scale with subjects in light clothing and without shoes. The Body Mass Index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (Kg/m^2). Study participants were

individually counselled for participation in the study and informed consent was obtained by signature or thumbprint.

According to WHO criteria, BMI is staged into 3 groupings, normal ($\text{BMI} < 25\text{kg/m}^2$), overweight ($\text{BMI } 25\text{kg/m}^2 \leq \text{BMI} < 30\text{kg/m}^2$) and obese ($\text{BMI} \geq 30\text{kg/m}^2$). Alcohol consumption was defined as the weekly consumption of beer, wine and hard liquor of approximately 75g/l. Smokers were classified as people who smoked at least one cigarette per day and continued for at least a year. Lipid profile of the individuals was analyzed enzymatic method using a standardized auto analyzer and the levels of adipokines namely, Leptin, Adiponectin and Resistin in serum were also analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) Techniques.

3.2 Study Design

This was a community based cross-sectional survey that adopted a least vigorous technique involving the selection of the most accessible subjects of 111 consented participants from the James Town catchment area within a month of sampling period.

3.3 Minimum Sample Size Calculation

For the mean levels of the selected Adipokines,

	Control subjects	obese subjects
Resistin	$21.5 \pm 3.2 \mu\text{g/l}$	$28.8 \pm 5.8 \mu\text{l}$
Leptin	$5.9 \pm 0.7 \mu\text{g/l}$	$26.9 \pm 3.9 \mu\text{g/l}$

Adiponectin	13.3±1.8 mg/l	8.6±0.8mg/l
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$$N = \frac{2 * \sigma^2 (Z\beta + Z\alpha / 2)}{(\text{Difference means})^2}$$

Where:

N = sample size in the case group

Z β = desired power (typically 0.84 for 80% power)

σ = standard deviation of the outcome variable

Z $\alpha/2$ = level of statistical significance (typically 1.96)

$\frac{r+r}{r}$ = ratio of controls to cases

r

By this calculation, the minimum sample size for this study for cases = 23.8 ~ 24

For the purpose of the study, the chosen sample size was 50 subjects for cases and 50 subjects for controls.

3.4 Inclusion and Exclusion Criteria

Inclusion Criteria

- Women between the ages of 20-70 years were included.

- Women with BMI $\geq 30\text{kg/m}^2$ served as the cases.
- Women with BMI $\leq 25\text{kg/m}^2$ served as the controls.

Exclusion Criteria

- Pregnant women
- Women ordinarily on steroid products.
- Women with Type 2 diabetes mellitus.

3.5 Data Collection and Analysis

After 10-12 hours of overnight fast, 10ml of venous samples were obtained from the subjects according to the Helsinki protocol declaration (Puri, Suresh, Gogtay, & Thatte, 2009). The blood was centrifuged while the serum obtained was analyzed for lipid profile and adipokines concentrations. The blood samples were collected after consent had been sought from subjects. Two millilitres (2mls) of whole blood was transferred into a sodium fluoride containing tubes and the plasma separated for the estimation of glucose. The remaining eight millilitres (8mls) of whole blood was put into serum separator tubes for processing. The tubes were then placed in a centrifuge and spun at 3000g for 10 minutes to obtain the plasma and sera. Plasma glucose was measured enzymatically and serum samples were aliquoted in 1ml portions into sterile eppendorf tubes and stored at -20° C until required for use.

3.6 Anthropometric Parameters

Relevant anthropometric measurements were taken. Weight was taken to the nearest 0.1 kilogram using a weighing scale with the subjects barefooted and in light clothing. While for measurement of height, a stadiometer was used. Body mass index (BMI) was then calculated as a ratio of weight (in kilograms) per height squared and expressed as Kg/m^2 . Percentage body fat, visceral fat, resting metabolism and skeletal muscle were measured using the Bioelectrical Impedance (BI) method. The body fat composition was measured using an electronic device OMRON BF508 which measures the body fat percentage base on electrical impulses and transmission.

3.7 Blood Pressure Measurements of subjects

Blood pressure was measured by qualified nurses using an OMRON BLOOD PRESSURE CUFF from Omron Healthcare Inc, Illinois, USA. This device uses the oscillometric technique which shows the oscillations of pressure in a sphygmomanometer cuff gradual deflation, the point of maximal oscillation corresponds to the mean intra-arterial pressure. One advantage of the method is that no transducer has to be placed over the brachial artery, so the placement of the cuff is not critical. Other potential advantages of the oscillometric method for ambulatory monitoring are that it is less susceptible to external noise and the cuff can be removed and replaced by the patient. The measurements were taken from the left upper arm after at least 5 minutes rest in a sitting position. Duplicate measurements were taken with a 5 min rest interval between measurements and the mean value was recorded in mmHg.

3.8 Data Analysis

Data was entered into a spread sheet and analyzed using Microsoft Office Excel 2007 and the values were expressed as mean plus or minus standard deviations (mean $\pm$ SD). SPSS version 20.0 was used for independent sample *t*-test (student *t*-test) with a level of statistical significance set at $p < 0.05$ for all tests and at 95% confidence interval (CI). Student *t*-test was used for comparison of means of variables between the obese and non obese subjects. One way analysis of variance (ANOVA) was used to assess mean adipokine levels across various age groups for significant differences. Variables with significant associations were assessed through stepwise linear and multiple regression to determine their independent contributions to the variance of total adipokines.

3.9 Descriptive Statistics

Appropriate measures of centrality (mean, median and dispersion or standard deviation range) were calculated. Graphical displays were created where appropriate.

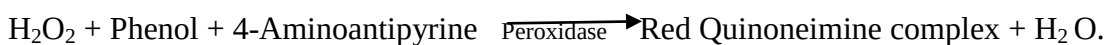
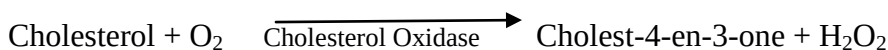
3.10 Biochemical Analysis

Serum total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) triglycerides (TG), fasting blood glucose (FBG) were determined using a manual spectrophotometer. Serum leptin (LEP), adiponectin (ADP) and resistin (RST) were also determined using a MULTISKAN MS PRIMARY EIA V. 1.5 -0.

3.11 Measurement of Total Cholesterol

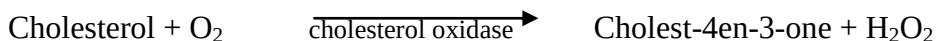
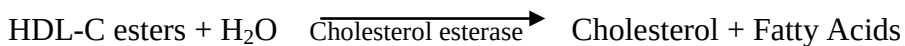
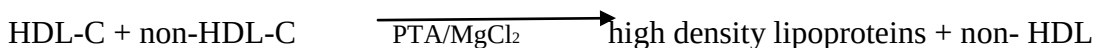
The method used in this study was based on an enzymatic method proposed by (Allain, Poon, Chan, Richmond, & Fu, 1974). A 10 microlitre (10 μ l) serum sample was pipetted into a plain tube containing 1 millilitre (1.0 ml) of the cholesterol reagent. Hydrolysis of the Cholesterol esters to cholesterol is catalysed by cholesterol esterase. Free cholesterol is then oxidised in the presence of cholesterol oxidase to form cholestenone (chole 4-en-3-one) and hydrogen peroxide. The hydrogen peroxide is then hydrolyzed by a peroxidase to form or generate a red dye (quinoneimine) in the presence of phenol and aminoantipyrine. The density of the dye formed is proportional to the cholesterol concentration present in the sample and the absorbance was measured by a spectrophotometer at a wavelength of 505 nm (500-540) nm against a standard and a blank. The incubation period for the entire reaction was 10 minutes at room temperature.

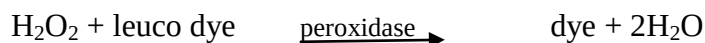
Reaction Sequence



3.12 Measurement of High Density Lipoproteins (HDL)

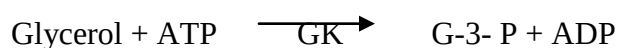
The method is based on phosphotungstate, a precipitating method described by (Allain et al., 1974). Two hundred microlitres (200 μ l) of the serum was dispensed into three hundred (300 μ l) of the precipitating reagent. The mixture was well mixed and allowed to stand at room temperature for five minutes. It was then spun at 3000 g for ten minutes. High density lipoproteins cholesterol (HDL-C) was separated by the precipitation of non HDL, LDL, VLDL and chylomicrons react with phosphotungstic acid and Magnesium Chloride. Thousand microlitres (1000 μ l) of the cholesterol reagent was pipetted into a plain tube. Hundred microlitres of the supernatant was dispensed into the working reagent, well mixed and incubated for ten minutes at room temperature. There was dissociation of the cholesterol and cholesterol esters from the HDL-C complexes present in the sample. Hydrolysis of HDL-derived cholesterol ester to cholesterol was catalysed by cholesterol ester hydrolase. Free cholesterol was then oxidised in the presence of cholesterol oxidase to form cholestenone and hydrogen peroxide. The hydrogen peroxide oxidised a leuco dye in the presence of peroxidase and generated a coloured dye. The optical density of the dye formed was proportional to the HDL-C concentration present in the sample and was measured at a wavelength of 505nm or a with green filter (500-540) using a spectrophotometer.





3.13 Measurement of Triglycerides

The method for the analysis is a modification of that of (Trinder, 1969). Thousand microlitres of the triglycerides reagent was dispensed into a plain tube and ten microlitres of the sera were pipetted into the plain tubes and were well mixed. The test was incubated for 15 minutes at room temperature. Triglycerides in the sample were catabolised by Lipoprotein Lipase (LPL) to glycerol and fatty acids. The glycerol was then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G-3-P) and adenosine-5 diphosphate in a reaction catalyzed by glycerol kinase (GK). Glycerol-3-phosphate was then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacted with 4-aminoantipyrine (4-AAP) and 2, 4, 6- Tribromo-3-hydroxy-benzoic acid (TBHA) in a reaction catalyzed by peroxidase (POD) to yield a red coloured quinoneimine dye in the sample. The intensity of the colour produced was measured at 505 nm and was directly proportional to the concentration of Triglycerides in the sample.





3.14 Measurement of Low density lipoprotein (LDL) cholesterol

In the most widely used indirect method, total cholesterol, triglycerides and HDL cholesterol are measured and LDL cholesterol is calculated from the primary measurements using the empirical equation of (Friedewald, Levy, & Fredrickson, 1972). In this study LDL concentration of the samples were estimated by the *Friedewald equation* as given below.

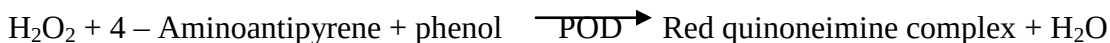
Results were expressed in mmol/l.

$$[\text{LDL cholesterol}] = [\text{Total cholesterol}] - [\text{HDL cholesterol}] - [\text{Triglycerides}]/2.2 \text{ (mmol/l)}$$

3.15 Measurement of Fasting Blood Glucose (FBG)

Serum glucose concentrations were measured using the glucose oxidase method (enzymatic colorimetric) described by (Trinder, 1969). Thousand microlitres of the glucose reagent was pipetted into a plain tube, and ten microlitres of the plasma was added. It was well mixed and incubated for fifteen minutes at room temperature. Glucose was oxidized to gluconate by atmospheric oxygen in the presence of glucose oxidase (GOD). The indicator 4-aminophenazone and phenol were oxidized by the hydrogen peroxide formed in the presence of peroxidase (POD). The intensity of the colour produced was proportional to the glucose concentration in the sample. Finally, colour

intensity was measured at 505nm using a spectrophotometer. The glucose reagent kit was supplied by the LIQUIZONE. Results were expressed as mmol/l.



3.16 Determination of Resistin in Human Serum

The Human Resistin ELISA Kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human resistin in samples. Standard dilution (32, 16, 8, 4, and 2) mg/L was prepared using an original standard reagent of 64 mg/L and standard diluents. Adiponectin antibody labelled with biotin, Streptavidin-HRP was not added to blank. 50 µl of Streptavidin-HRP were added to the standards since the standard already had combine biotin antibody, it was not necessary to add the antibody. Forty microlitres (40 µl) of the serum was pipetted into the wells and ten microlitres (10 µl) of resistin antibody and fifty microlitres (50 µl) of the Streptavidin-HRP added. It was then sealed using a sealing membrane and shaken gently and incubated for sixty minutes at 37°C to form immune complex. The sample was washed 5 times to remove the uncombined enzyme. 50 µl each of chromogen solution A and B were added to each well, gently mixed, and incubated for ten minutes at 37°C away from light. The colour of the liquid changed into blue. 50 µl of stop solution was added to each well to stop the reaction and the blue changed to yellow immediately at the effect of the acid. The blank well was taken as zero, and the optical density (OD) measured at 450nm wavelength

carried out within 15 minutes after adding the stop solution. According to the standards' concentration and the corresponding optical density values, the standard curve linear regression equation was calculated and then the concentrations of the samples were calculated using the linear regression equation with their corresponding OD values of the samples. The assay range was 20 ng/L to 6000 ng/L and the sensitivity was 10.21 ng/L.

The same procedure was used for Human Leptin and Adiponectin concentrations in serum following the same test principle. The assay range for Leptin was 2 ng/ml to 1500 ng/ml and its sensitivity was 1.02 ng/ml. For that of Adiponectin the assay range was 0.2 mg/L to 60 mg/L and its sensitivity was 0.11 mg/L.

3.17 Standardization of Method

Various standards were prepared to standardise the procedure before the samples were run for the concentrations of the adipokines. An original standard reagent and diluents were provided by the manufacturer for leptin, adiponectin and resistin. For leptin, 1: 2 serial dilution of the stock concentration (1600 ng/ml) was done down to 50 ng/ml. The standards were used to plot a curve against their optical densities of the standards. The same procedure was followed for adiponectin (64 mg/l) and resistin (6400 ng/l).

CHAPTER FOUR

4.0 RESULTS

4.1 General characteristics and clinical parameters of the study population

The total number of volunteers who participated in the study was one hundred and eleven (111) with age ranging from 20-80 years. The various parameters were; mean age (48 ± 13.1 years), mean BMI ($30.3 \pm 6.3\text{kg/m}^2$), mean Visceral Fat (8.9 ± 2.9) and mean Resting Metabolism (1467.5 ± 189.8). The mean Systolic Blood Pressure was ($126.9 \pm 23\text{mmHg}$), Diastolic Blood Pressure ($82.4 \pm 13.9\text{mmHg}$), pulse rate ($76.8 \pm 10/\text{min}$), pulse pressure ($44.5 \pm 12 \text{ mmHg}$), and the mean arterial pressure ($97.2 \pm 16.5\text{mmHg}$). The mean percentage (%) body fat and skeletal muscle mass were ($40.96 \pm 8.2\%$) and ($25.6 \pm 3.1\%$) respectively (Table 1).

Table 1: General characteristics and Investigated parameters of the study population

Parameter	N	$\bar{x} \pm SD$
Age/years	111	48.6 $\pm$ 13.1
BMI/ kgm ⁻²	109	30.3 $\pm$ 6.3
Percentage body fat (%)	108	40.9 $\pm$ 8.2
Visceral fat level	108	8.9 $\pm$ 2.9
Percentage skeletal muscle mean (%)	108	25.6 $\pm$ 3.1
Resting metabolism	108	1467.5 $\pm$ 189.8
Systolic blood pressure (mmHg)	110	126.9 $\pm$ 23.0
Diastolic blood pressure (mmHg)	110	82.4 $\pm$ 13.9
Pulse rate (min)	110	76.8 $\pm$ 10.1
Pulse pressure	110	44.5 $\pm$ 12.2
Mean arterial pressure	110	97.2 $\pm$ 16.5

4.2 Age distribution of Studied Subjects

The subjects were grouped according to age decades. 22.5% of the total subjects were below 40 years, 23.4% were between 40 – 49 years, 30.6% were between 50 – 59 years and 23.4% were above 60 years.

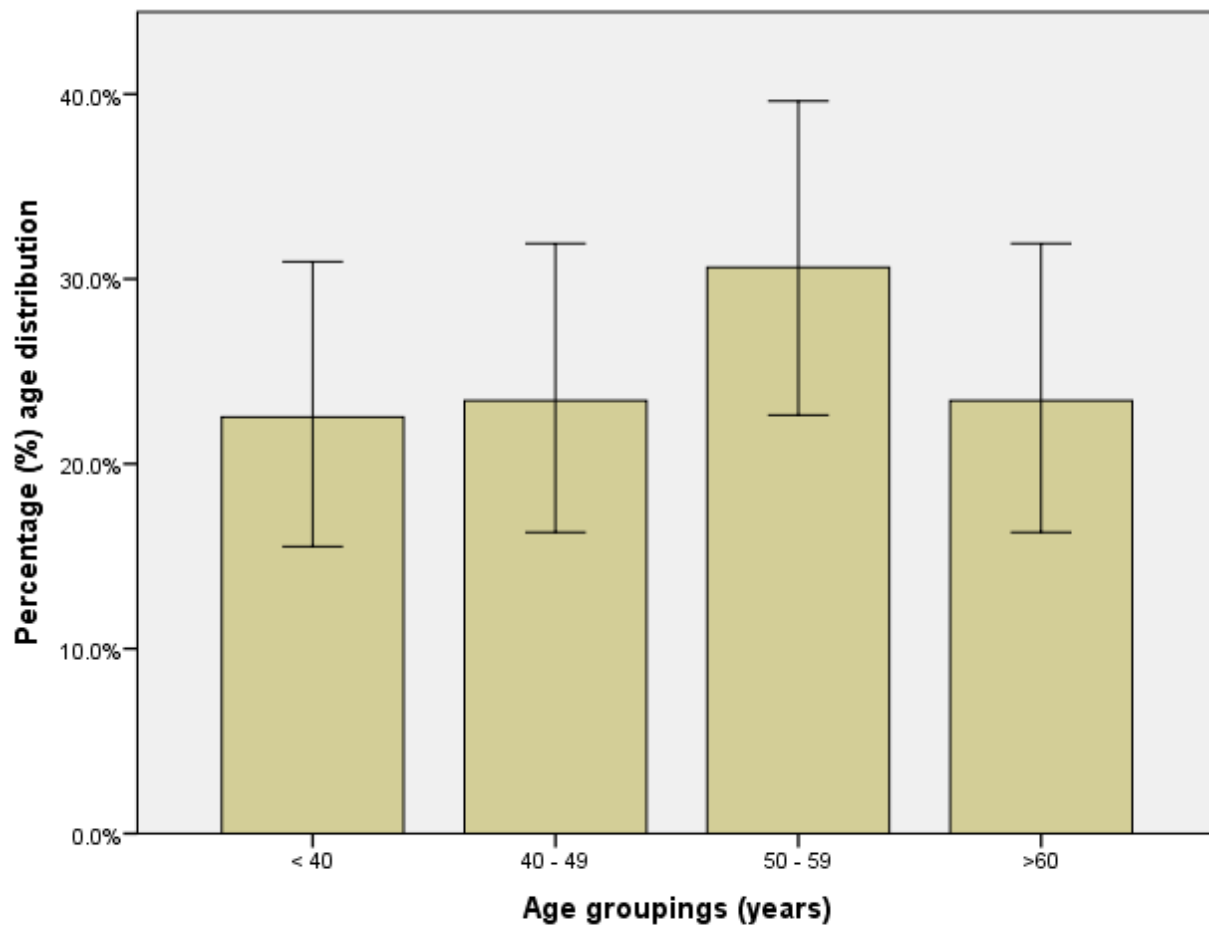


Figure 1: The age distribution among the subjects expressed as percentage of the studied population. Bars represent Error Bars at 95% CI

4.3 Body mass index (BMI) Categories of Studied Subjects

The subjects were staged into BMI categories according to the WHO criteria (2008) viz;

Normal ($18.5 \leq \text{BMI} < 25\text{kg/m}^2$),

Overweight ($25\text{kg/m}^2 \leq \text{BMI} < 30\text{kg/m}^2$)

Obese ($\text{BMI} \geq 30\text{kg/m}^2$)

Accordingly, 24 (21.6%) of the studied subjects were normal weight, 33 (29.7%) were overweight and 53 (48.7%) were obese.

4.4 Blood Pressures of various BMI categories of Subjects.

From the studied data, Systolic blood pressures were not statistically significant ($p = 0.231$) for the various BMI categories (normal weight, overweight and obese respectively). Also the pulse, pulse pressure and mean arterial pressure of subjects were not statistically significant ($p = 0.336$, 0.448 and 0.083 respectively) (Table 2). However, diastolic blood pressure for the various BMI categories was statistically significant ($p = 0.030$).

Table 2: Blood Pressures of various BMI categories of Subjects

Blood Pressure (mmHg)	Normal N= 24	Overweight N= 33	Obese N= 53	p- value (ANOVA)
Systolic	123.9 $\pm$ 29.0	127.4 $\pm$ 24.0	129.0 $\pm$ 19.0	0.231
Diastolic	80.7 $\pm$ 16.0	85.2 $\pm$ 12.5	82.4 $\pm$ 13.9	0.030*
Pulse	80.3 $\pm$ 12.7	75.9 $\pm$ 9.9	75.8 $\pm$ 8.9	0.336
Pulse Pressure	43.2 $\pm$ 16.0	47.1 $\pm$ 14.1	43.8 $\pm$ 8.9	0.448
Mean arterial pressure	95.1 $\pm$ 20.0	95.9 $\pm$ 16.3	99.8 $\pm$ 14.4	0.083

Table 2 shows the blood pressures: systolic, diastolic, pulse, pulse pressure, mean arterial pressure of the various BMI categories (normal weight, overweight and obese) of the subjects. N = number of subjects, * p -values ≤ 0.05 were considered significant. Data are presented as mean $\pm$ S.D.

4.5 Association between Body composition and Blood pressure.

Table 3 shows the correlation between the blood pressures of the studied subjects and their body composition. There were no significant differences between the BMI and the blood pressures. But body fat, visceral fat and skeletal muscle had some level of association. SBP, DBP and mean arterial pressure correlated positively with body fat. Visceral fat also correlated positively with SBP, DBP and mean arterial pressure but inversely correlated with pulse. Skeletal muscle inversely correlated with DBP and Mean arterial pressure.

Table 3: Association between Body composition and Blood pressure.

Body composition		Blood pressure (mmHg)				
		Systolic	Diastolic	Pulse	Pulse pressure	Mean arterial pressure
BMI/kgm-2	r	0.169	0.174	-0.087	0.041	0.171
	p	0.080	0.072	0.373	0.671	0.076
Body Fat %	r	0.196	0.241	-0.111	0.018	0.220
	p	0.043	0.012	0.254	0.853	0.023
Visceral Fat	r	0.263	0.211	-0.210	0.185	0.234
	p	0.006	0.029	0.030	0.057	0.015
Skeletal Muscle %	r	-0.168	-0.234	0.076	-0.008	-0.209
	p	0.084	0.015	0.435	0.937	0.031
Resting Metabolism	r	0.066	0.106	-0.154	-0.073	0.090
	p	0.498	0.279	0.112	0.457	0.355

Table 3: shows the association of blood pressures of subjects with their body composition. p- values < 0.05 were considered significant.

4.5 Blood Pressures of various age decades of Subjects

The blood pressures between the different age groups were investigated (Table 4). The mean systolic blood pressure, pulse, pulse pressure and mean arterial pressure were statistically significant ($p = 0.002, 0.021, < 0.001$ and 0.030 respectively) between the various age groups (<40years, 40-49 years, 50-59 years and > 60 years). However, the

mean diastolic blood pressure was not statistically significant ($p = 0.137$) for the various age groups.

Table 4: Blood Pressures of various age decades of Subjects

Blood Pressure	< 40 yrs N =25	40-49 yrs N =26	50 -59 yrs N =33	60 and above N = 26	p –value (ANOVA)
Systolic	117.7 ± 15.7	119.5 ± 14.3	130.8 ± 22.2	138 ± 30.8	0.002
Diastolic	77.9 ± 12	80.5 ± 10.7	85.5 ± 13.5	84.7 ±17.6	0.137
Pulse	81.7 ±10.4	77.1 ± 8.9	73.5 ± 9.6	75.9 ±10.3	0.021*
Pulse Pressure	39.8 ± 8.3	39.0 ± 6.9	45.3 ± 10.6	53.5 ± 16	< 0.001*
Mean arterial pressure	91.2 ± 12.8	93.5 ± 11.5	100.6 ±16.2	102.5 ± 21.6	0.030*

*Table 4 shows the blood pressures: systolic, diastolic, pulse, pulse pressure, mean arterial pressure of the various age decades (<40years, 40-49 years, 50-59 years and > 60 years) of the subjects. N = number of subjects, * p –values ≤ 0.05 were considered to be significant. Data are presented as mean ± S.D.*

4.6 Biochemical parameters for various BMI categories of Subjects

Measurement of biochemical indices among the BMI categories showed no significant differences as shown in the Table 5 below. The mean ratio of total cholesterol to high density lipoprotein Cholesterol (TC/HDL), and the ratio of low density lipoprotein

Cholesterol to high density lipoprotein Cholesterol (LDLC/HDL) were statistically not significant ($p = 0.461$ and 0.493 respectively) for the various BMI categories of subjects (Table 5).

Table 5: Biochemical parameters for various BMI categories of Subjects.

Biochemical parameter (mmol/l)	Normal N = 24	Overweight N = 33	Obese N = 54	P – value (ANOVA)
Fasting blood glucose	6.1 ± 1.4	6.0 ± 1.1	6.0 ± 1.1	0.818
Total cholesterol	6.8 ± 1.7	6.7 ± 1.7	6.7 ± 1.7	0.599
High density lipoprotein	2.1 ± 0.5	2.1 ± 0.5	2.1 ± 0.6	0.848
Low density lipoprotein	3.9 ± 1.6	3.9 ± 1.5	3.9 ± 1.8	0.561
Triglycerides	1.7 ± 0.2	1.8 ± 0.6	1.7 ± 0.4	0.321
TC/HDL	3.3 ± 0.9	3.4 ± 1.3	3.5 ± 1.3	0.461
LDL/HDL	1.9 ± 0.9	2.0 ± 1.0	2.1 ± 1.2	0.493

Table 5 shows the biochemical parameters for the various BMI categories. p- values for all categories were not significant. Data are presented as mean $\pm$ S.D.

4.7 Adipokine levels in the various age decades of subjects were investigated.

Measurement of adipokine levels among the various age decades showed no significant differences ($p = 0.317, 0.706$ and 0.484 respectively) for leptin, adiponectin and resistin from Table 6.

Table 6: Levels of Adipokines between the various age decades.

Adipokines Levels	< 40 yrs N = 25	40 – 49 yrs N = 26	50 – 59 yrs N = 34	60 and above N = 26	p – value (ANOVA)
Leptin (ng/ml)	469.4 ± 309.2	376 ±269.8	354.2 ± 213.5	453.3 ± 318.9	0.317
Adiponectin (mg/L)	14.7 ±7.0	13.8 ±7.1	14.0 ± 5.1	15.8 ± 8.2	0.706
Resistin (ng/ml)	241.3 ±117.8	206.1 ± 123.5	200.5 ± 87.5	231.9 ± 134.8	0.484

Table 6 shows the adipokine levels for the various age decades. p- values for all age groups were not significant. Data are presented as mean ± S.D.

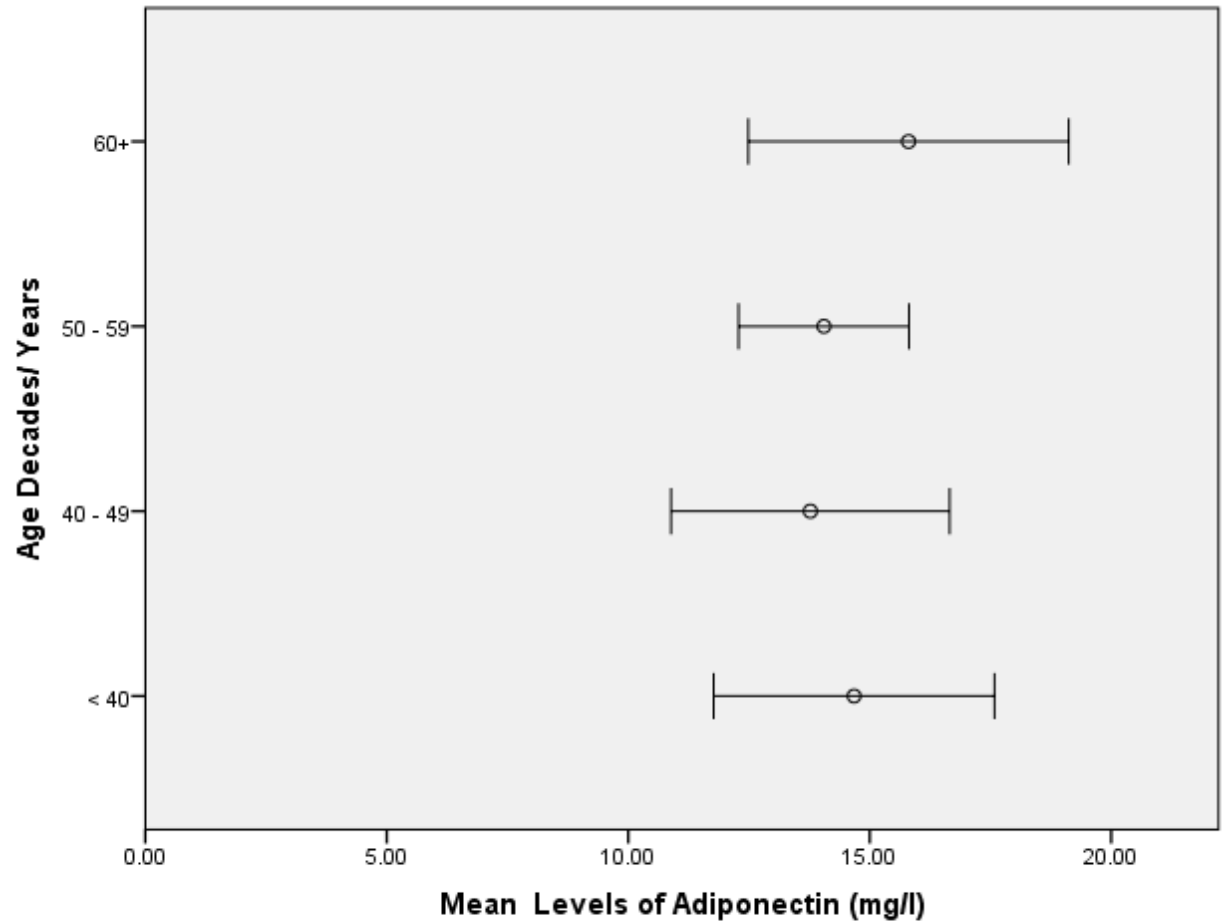


Figure 2: Shows the levels of adiponectin among the various age decades of study subjects expressed in mg/l. Bars represent Error Bars at 95% CI

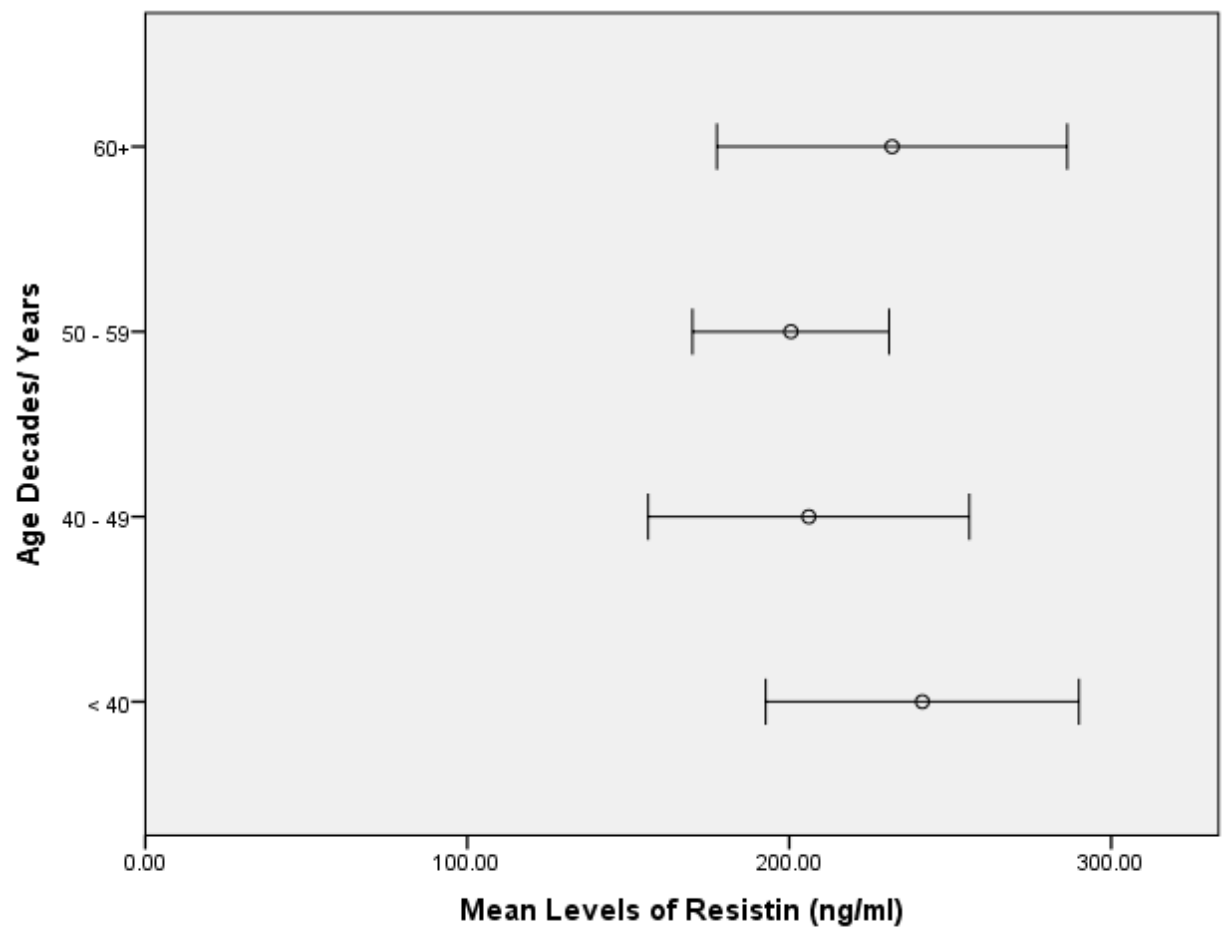


Figure 3: Shows the levels of resistin among the various age decades of study subjects expressed in ng/ml. Bars represent Error Bars at 95% CI

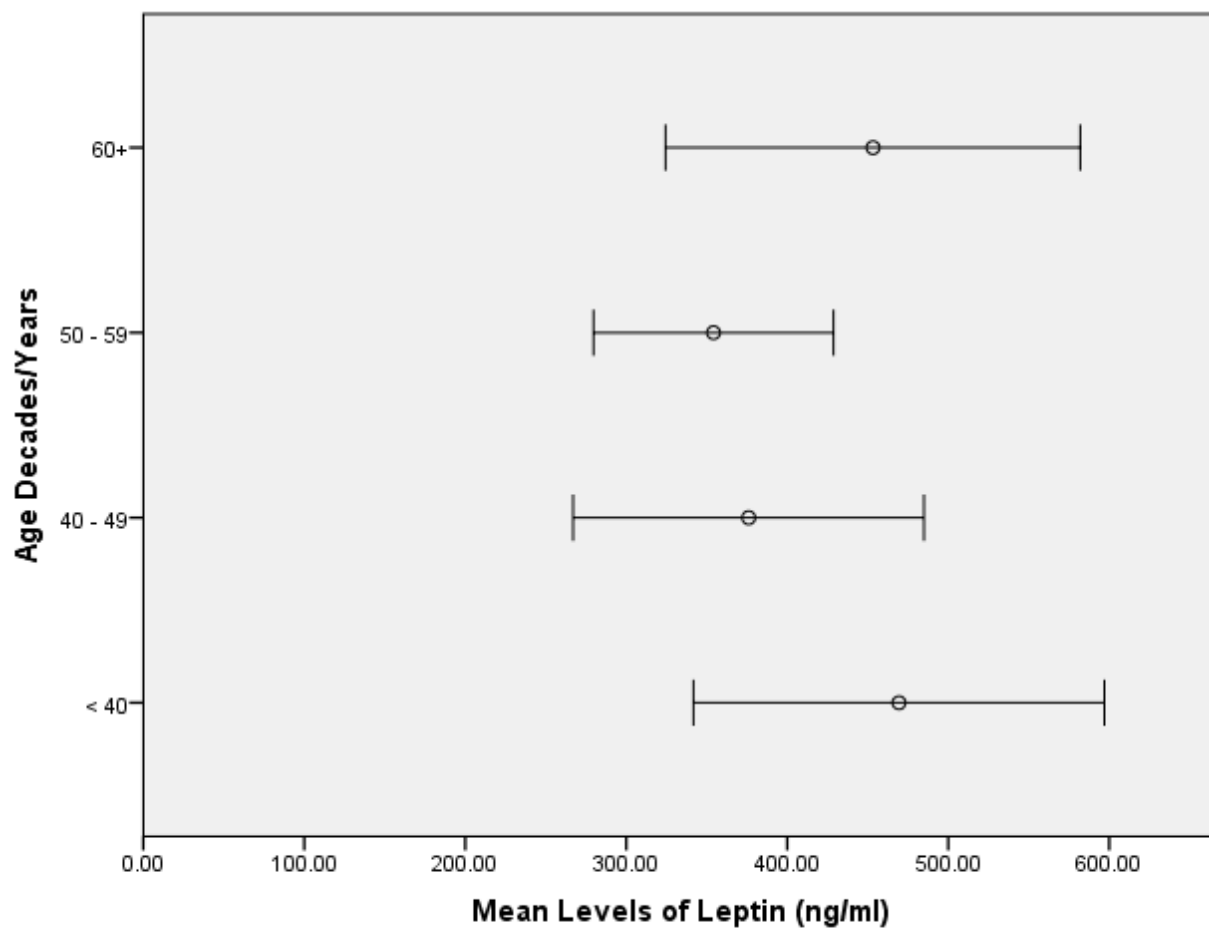


Figure 4: Shows the levels of leptin among the various age decades of study subjects expressed in ng/ml. Bars represent Error Bars at 95% CI

4.8 Levels of Adipokines with the various BMI categories of Subjects.

In this study, the levels of Adipokines among the various BMI categories were measured (Table 7). The mean levels of leptin for normal, overweight and obese subjects were

(362.1 ± 205.2 , 395.9 ± 271.6 and 437.5 ± 308.8) respectively and showed no significant difference ($p = 0.710$). Likewise adiponectin showed no significant levels among the BMI categories as shown in Table 6. Resistin also showed no levels of significance among the BMI groups.

Table 7: Levels of Adipokines with the various BMI categories

Adipokines Levels	Normal N =24	Overweight N = 33	Obese N = 54	p – value (ANOVA)
Leptin (ng/ml)	362.1 ± 205.2	395.9 ± 271.6	437.5 ± 308.8	0.710
Adiponectin (mg/L)	15.1 ± 6.1	14.7 ± 7.0	14.4 ± 7.1	0.864
Resistin (ng/ml)	224.6 ± 102.6	205.9 ± 120.1	225.6 ± 119.3	0.756

Table 7 shows the adipokine levels for the various BMI categories. p- values for all age groups were not significant. Data are presented as mean $\pm$ S.D.

4.9 Association between adipokines and body composition after adjustment of age decades.

BMI showed no correlations between Leptin, adiponectin and resistin. The differences were not statistically significant from Table 8.

Table 8: Association between adipokines and body composition after adjustments of age decades

Variables		Leptin N= 111	Adiponectin N = 111	Resistin N = 111
BMI/kgm ⁻²	r	0.129	0.043	0.0870
	p	0.202	0.673	0.390
Body fat (%)	r	0.092	0.019	0.041
	p	0.367	0.850	0.688
Visceral fat	r	0.044	- 0.006	0.023
	p	0.664	0.950	0.821
Resting metabolism	r	0 .014	-0.056	-0.039
	p	0.891	0.585	0.699
Skeletal muscle (%)	r	- 0.060	- 0.017	-0.039
	p	0.552	0.869	0.717

Table 8 shows the association of adipokine levels of subjects with their body composition. p- values were not significant.

4.10 Association between Adipokines and the biochemical parameters of subjects

From table 9, there were no associations between the Adipokines, FBG, triglycerides and the total cholesterol. However, high density lipoprotein Cholesterol, low density lipoprotein cholesterol, (TC/HDL) and low density lipoprotein: high density lipoprotein Cholesterol (LDL/HDL) ratios had some level of association with adiponectin, which

were statistically significant with HDL ($P = 0.024$), LDL ($P = 0.030$), TC/HDL ratio ($P = 0.005$) and LDL/HDL ratio ($p = 0.007$).

Table 9: Association between Adipokines and the biochemical parameters of subjects

Variables (mmol/l)		Leptin N = 111	Adiponectin N = 111	Resistin N = 111
FBG	r	0.062	0.016	- 0.017
	p	0.540	0.873	0.868
Total cholesterol	r	0.171	0.161	0.116
	p	0.091	0.112	0.252
High density lipoprotein	r	0.090	0.226	0.039
	p	0.376	0.024*	0.699
Low density lipoprotein	r	0.134	0.219	0.112
	p	0.188	0.030*	0.269
Triglycerides	r	0.066	0.147	0.150
	p	0.516	0.147	0.140
T. CHOL/HDL	r	0.066	0.281	0.126
	p	0.518	0.005*	0.213
LDL/HDL	r	0.069	0.269	0.116
	p	0.500	0.007*	0.254

Table 9 shows the association of adipokine levels of subjects with their biochemical parameters. p-values were not significant. $r > 0.5$ shows strongly positive correlation

*< 0.5 shows weakly positive correlation. $r > -0.5$ shows an inversely strong correlation and $r < -0.5$ shows an inversely weak association. $*p < 0.05$ are considered significant.*

CHAPTER FIVE

5.0 Discussion, Limitation, Recommendation and Conclusion

5.1 Discussion

The prevalence of obesity among women in this particular study as shown in Table 2 was 49 % which is contrary to reports by (Amoah, 2003) and (Biritwum et al., 2005) as discussed under literature review of this thesis. Studies in Ghana by (Amoah, 2003) and (Biritwum et al., 2005) reported the prevalence of obesity to be 20.2% and 16.1% of which 7.9% of Biritwum's were females in Greater Accra. The observed discrepancies between this current study and the previous studies could be due to various factors including sample size, their method of sampling and time frame when those studies were carried out. The method of sampling (Amoah, 2003) used was random cluster sampling, with a sample size of 2874 females from various study sites targeting different categories of women while (Biritwum et al., 2005) used random sampling of 334 females from various study sites and different categories of subject. However, this study adopted a convenient sampling approach at a particular market community targeting one hundred and eleven market women. Also, their studies were conducted a decade and 8 years ago respectively, within which a lot of lifestyle modification such as in urban settings with increased consumption of junk foods, western lifestyles and lack of physical activities due to easy access to transportation from their various homes to the market place.

Furthermore, studies conducted by (Agyemang & Owusu-Dabo, 2008) which also used random sampling approach after 5 and 3 years respectively of previous studies done in Ghana, investigated the differences in overweight and obesity between first generation

Dutch Ghanaian migrants in Netherlands and their rural and urban counterparts in Ghana. Dutch Ghanaian women had a significantly high prevalence of 79.5% overweight and obesity; urban Ghanaian women also had a significantly higher prevalence of 50.0% and rural Ghanaian women 19.0%. This gave credence to earlier reports of increase in prevalence of overweight and obesity with urbanisation within Africa and migration to industrialised countries (Agyemang & Owusu-Dabo, 2008). (Biritwum et al., 2005) also supported that overweight and obesity were becoming the biggest health burden in low and middle income countries particularly in urban settings.

This study reports for the first time the levels of leptin, adiponectin, and resistin and how they relate to obesity and other biochemical indices of metabolic syndrome in women in Accra.

Figures 2, 3 and 4 showed the mean levels of the various adipokines (adiponectin, resistin and leptin) respectively. There were no significant levels of adipokines between the various age groups. Also, no statistical significant correlation was observed between adipokine levels and BMI categories.

From Table 2 of this study, the obese subjects had the highest systolic blood pressure while the normal subjects had the least. Again, the association of body composition and the various blood pressures (Table 3) showed some correlations between percentage body fat with systolic blood pressure, diastolic blood pressure and mean arterial pressure. Visceral fat showed association with systolic blood pressure, diastolic blood pressure, mean arterial pressure, but inversely correlated with pulse. Abdominal fat accumulation has been shown to play a crucial role in the development of metabolic syndrome

(Matsuzawa, 2006). Abdominal fat accumulation particularly is closely correlated to the development of cardiovascular diseases and obesity-related disorders such as hypertension and hyperlipidemia.

This observation agrees with the outcome of a study by (Timpson et al., 2009) which reported that BMI was associated with elevated Systolic blood pressure of which (Droyvold et al., 2005) had previously reported that weight gain was associated with increased odds of hypertension, while reduction in weight resulted in blood pressure decrease (Dall'Asta et al., 2009). Waist circumference and waist-hip ratio, both of which measures centralized obesity, were more strongly related to hypertension than BMI according to (C. M. Lee et al., 2008).

Again, with normal BMI as the reference, (Dorresteyn et al., 2012) the odds ratio for the presence of hypertension for overweight individuals increased increasing BMI. This was also supported by (Nguyen et al., 2008). While in 1990, (Cassano et al., 1990) suggested that obesity was an important risk factor for future incidence of hypertension and for progression from prehypertension to hypertension it was supported by (De Marco et al., 2009) and (Tomiyama et al., 2009).

Systolic blood pressure, pulse pressure, mean arterial pressure and pulse showed significant differences among the age decades. From Table 4, the mentioned parameters above, increased with age. However, there was no significant difference between the diastolic blood pressures and the age groups. Cardiovascular risk also increased with age (Mancia & Grassi, 2013). They reported that the relationship between blood pressure values and cardiovascular, renal morbid and fatal events has been addressed in a large

number of observational studies. (Vishram et al., 2012) reported that systolic blood pressure appeared to be a better predictor of events than diastolic blood pressure after age of 50 years and in elderly individuals, pulse pressure had been reported to have a possible additional prognostic role (Benetos et al., 1997). The results of these earlier studies were in line with what was observed in this study supporting the contribution of blood pressure in the subjects.

Measurements of biochemical indices among the BMI categories were also investigated (Table 5). Levels of fasting blood glucose, total cholesterol, high density lipoproteins cholesterol, low density lipoproteins cholesterol and triglycerides were not significant among the BMI categories of the subjects. The mean ratio of total cholesterol to high density lipoproteins (TC/HDL-C), and the ratio of low density lipoproteins to high density lipoproteins (LDL-C/HDL-C) were also statistically not significant for the various BMI categories. However, the concentrations of LDL particles, small LDL particles, large HDL particles, and large very-low-density lipoprotein (VLDL) particles have been shown to be better indicators of cardiovascular risk than the elements of the traditional lipid profile (Pascot et al., 2001). In insulin-resistant states, dyslipidaemia as seen in obesity is characterized by different composition and distribution of LDL particles, resulting in an increased concentration of small, dense LDL-C. LDL-C particles are enriched in triacylglycerols, which is rapidly lipolysed by hepatic lipase (HL) leaving smaller, denser LDL-C particles (Luc et al., 2006). The activities of both cholesteryl ester transfer protein (CETP) and HL seem to be increased in metabolic syndrome, leading to such particles, which are more prone to oxidation and glycation. Small dense LDL-C particles can move through endothelial fenestrations, entering the sub-endothelial space

where inflammation and transformation into plaque can occur (Kwiterovich, 2002). The modified LDL-C is mostly taken up by macrophage scavenger receptors, rather than the normal LDL receptor (LDLR) pathway, thus inducing atherosclerosis.

The outcome of this study showed no statistical significant differences between the levels of adipokines within the various BMI categories of the subjects, even though the levels of leptin were shown to be highest in the obese than the normal subjects (Table 7). Also the association between adipokines and body composition observed (Table 8) showed no correlations between the various components of body composition.

However, (Azuma et al., 2003) carried out a study which indicated that adipokine levels correlated closely with adiposity with increasing levels in subjects with higher body mass index. Furthermore, (Oh, Ciaraldi, & Henry, 2007) reported that leptin and adiponectin are individually known to be involved in the pathogenesis of obesity and metabolic syndrome. Under obese condition, the leptin levels are higher and adiponectin levels are lower and thus, leptin: adiponectin ratio which is reported to be a surrogate new marker for atherosclerosis in subjects with obesity and type 2 diabetes can be relatively higher (Kotani, Sakane, Saiga, & Kurozawa, 2005).

Also as shown in Table 7, adiponectin showed no significant differences between the BMI categories of the subjects; though the levels of adiponectin in the normal subjects were insignificantly higher than the obese. This is supported by (Hajer et al., 2008) and (Weisberg et al., 2003), who reported that dysfunctional adipose tissue in obesity was microscopically characterized by hypertrophied adipocytes and increased infiltration of macrophages and functionally by marked changes in secretion of adipokines and

saturated free fatty acids (Weisberg et al., 2003); (Hajer et al., 2008). The release of most adipokines, including leptin, is elevated, while production of adiponectin, exclusively secreted by adipose tissue, was suppressed (Z. V. Wang & Scherer, 2008).

It was observed that there were statistical significant correlations between HDL, LDL, TC/HDL, LDL/HDL and adiponectin (Table 9). This has important metabolic consequences. It has been shown that obesity was an independent risk factor for type 2 diabetes, dyslipidemia, and cardiovascular diseases (CVD) (Chavez & Tripathy, 2011). Studies conducted by (Yu et al., 2011) concluded that adiponectin exhibited strong inverse associations with metabolic syndrome independent of body composition, inflammation and leptin, while the association of leptin was largely explained by fat mass. Decreased levels of adiponectin for example, contributed to insulin resistance, since adiponectin receptors in liver and muscle cells mediate β -oxidation of fatty acids, glucose uptake, gluconeogenesis and peroxisome proliferator activated receptor- γ activation (Z. V. Wang & Scherer, 2008). The findings of this study were similar to those of (Matsubara, Maruoka, & Katayose, 2002) who reported that low levels of adiponectin in obese subjects could be due to the fact that only diabetic subjects were considered as exclusion criteria for the subjects of their study, while hypertensive and dyslipidemia subjects were not. Adiponectin levels were reduced in hypertensives, diabetics and patients with coronary artery diseases (Hotta et al., 2000) and dyslipidemia was also associated with decreased adiponectin concentrations.

Particularly, decreased production of adiponectin by dysfunctional adipose tissue may also provide a direct link between obesity and hypertension.

5.2 Limitations of Study

This study clearly spelt out some interesting findings yet there were some few limitations.

- First, various study sites should have been considered with subjects randomly sampled. The variety of the study subjects would have given this study an opportunity to compare different women from different working environments, and their nutrition status with their levels of adipokines.
- Secondly a large sample size would have minimized error and given us a better representation of the prevalence of obesity within this study.
- Thirdly, using adipokines as a biomarker, one may have to consider a lot of factors that were considered worldwide since different study population from different geographical backgrounds genetically may also have some variations. There were no reference ranges that could be used to compare results though various studies reported their own ranges from their study and protocols only gave their assay ranges.

5.3 Recommendation

- The study indicates a need to further assess other environmental factors and lifestyle that lead to these increases in obesity and their impact on related

metabolic disorders, leading to cardiovascular risk factors among the study subjects.

- Large sample size should be considered in order to minimise error.
- There is a need to establish a reference range in Ghana.
- Further studies should be carried out to compare levels of adipokines in both genders in different conditions in metabolic syndrome.

5.4 Conclusion

The findings of this study demonstrate that the prevalence of obesity is overly high among the subjects of this research in Accra. Body composition which correlated with blood pressure of the study subjects indicating increases in body fat mass will be seriously detrimental to the already overburdened health care resources in Ghana. As the levels of leptin among the obese and overweight subjects were higher than the normal subjects, there were no significant differences. Other studies supported it could be attributed to poor nutrition and living conditions that might have caused some form of oxidative stress in these women hence the increase levels of leptin. Adiponectin on the other hand was higher in normal subjects compared with obese subjects. This anti atherogenic protein might have given the normal subjects some form of protection against vascular damage and normal blood pressures. Adiponectin correlated inversely with HDL-c but positively with low density lipoprotein cholesterol, total cholesterol: high density lipoprotein cholesterol ratio (TC/HDL-c), and low density lipoprotein cholesterol:

high density lipoprotein cholesterol ratio (LDL-c/HDL-c) which are predictors of cardiovascular diseases hence reducing their risk of cardiovascular diseases. This study having given us a general idea about the levels of adipokines in women in Ghana, could be documented and at least serve as a baseline for future research.

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