INSULIN RESISTANCE, ARTERIAL STIFFNESS AND CIRCULATING ATRIAL NATRIURETIC PEPTIDE LEVELS IN YOUNG GHANAIAN ADULTS

BY

AFFRIM KORMLA PATRICK
(10162114)

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JULY, 2017
DECLARATION

I, Affrim Kormla Patrick declare that this project was carried out by me at the Department of Chemical Pathology, University of Ghana under the supervision of Dr. Kwame Yeboah and Prof. Henry Asare-Anane.

AFFRIM KORMLA PATRICK
(STUDENT)

Date

………………………………..  …………………………………..

DR. KWAME YEBOAH       PROF. HENRY ASARE-ANANE.

(SUPERVISOR)                ( SUPERVISOR)

Date……………………………  Date……………………………. 
DEDICATION

This project work is dedicated to the Almighty God, to my lovely wife, Rita Dodzi Affrim and to my Mum, Miss Aculey Bernice.
ACKNOWLEDGEMENTS

I desire to express my sincere gratitude and indebtedness to Dr. Kwame Yeboah and Prof. Henry Asare-Anane for their pieces of advice, guidance, motivation and supervisory role in helping me to conduct this study.

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Finally, my gratitude to all the participants who volunteered and consented to take part in this study.
ABSTRACT

Insulin resistance is an independent predictor of a wide range of major illnesses, including stroke, type 2 diabetes, cardiovascular diseases (CVDs), hypertension and cancer. Insulin resistance commonly coexist with obesity and arterial stiffness resulting in hypertension and CVDs. Obese people with insulin resistance have lower plasma levels of natriuretic peptides such as atrial- and brain- natriuretic peptides (ANP and BNP), and these play an important role in the development of arterial stiffness and essential hypertension. Biomarkers such as plasma ANP levels and arterial stiffness measurements are improved tools that can help to identify individuals who are at high-risk of developing CVDs and for management. This study investigated the burden of insulin resistance, arterial stiffness and plasma levels of atrial natriuretic peptide, which are known markers and indicators of cardiovascular diseases (CVDs). This study was conducted in Accra, Ghana, a questionnaire was administered to assess information on the lifestyle of the participants and anthropometric indices such as body fat, weight, height, waist and hip circumferences were also measured. Tensionmed Arteriograph was used to measure blood pressure and aortic stiffness (pulse wave velocity). Fasting venous blood samples were obtained from each participant for fasting blood glucose, plasma lipids were measured, insulin and ANP levels were also determined by Sandwich ELISA. BMI, WHR, IR were determined and statistical analyses were done using SPSS to test for associations and significant differences. A total of 300 young adults within the age range of 20-30 years were studied, 36.1% males and 63.9% females. The mean age for the study participants was 23.9yrs, significant differences were observed in the mean weights (75.84 vs 72.66, p = 0.0103), Hips circumference (121.93 vs 105.31, p < 0.0001) and
BMI (26.95 vs 25.65, p = 0.0054) comparing the females to the males. The overall prevalence of obesity and overweight among the study participants were 20.61% and 48.48% respectively and these were significantly higher among the females as compared to the males (26.07 vs 10.92% and 48.82% vs 47.90%, p = 0.0003). The overall mean PWV, IR and ANP among the study participants were 7.22 ± 2.29, 0.92 ± 0.20 and 4.94 ± 1.88 respectively, however there was no significant difference in the mean PWV and IR with regards to gender (p > 0.05), however there were significant associations of PWV with WHR and BMI. Deductions from this study suggest that the prevalence of obesity and overweight were high among the young adults, therefore lifestyle modifications such as improved diets and increased physical activity are highly recommended for young adults in order for IR and arterial stiffness prevention.
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<td>Advanced Glycation End Products</td>
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<tr>
<td>ANP</td>
<td>Atrial Natriuretic Peptide</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>CVA</td>
<td>Cerebro Vascular Accident</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<td>LDL</td>
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<td>NAFLD</td>
<td>Non-Alcoholic Fatty Liver Disease</td>
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<td>NCDs</td>
<td>Non-Communicable Diseases</td>
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<td>NHANES</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<td>PWV</td>
<td>Pulse Wave Velocity</td>
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<td>RAAS</td>
<td>Renin Angiotensin Aldosterone System</td>
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<tr>
<td>TNF-α</td>
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<td>VSMC</td>
<td>Vascular Smooth Muscle Cell</td>
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<td>WHR</td>
<td>Waist-to-Hip Ratio</td>
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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND

Overweight and obesity during young adulthood and childhood have now and future impact on health. Obesity is mostly linked to the diseases of cardiovascular and metabolic origin (Mattah et al., 2013; Vercoza et al., 2009). Obesity was previously uncommon among the Ghanaian population, but this has changed with time as a result of increase sedentary lifestyles of Ghanaians, increased consumption of energy dense foods and increase alcohol consumption especially among the youth (Mattah et al., 2013). Recent findings indicate that about 400 million adults worldwide are obese of which 115 million (29%) are found in developing countries. In 2008 there was an increase from 25.5% to 30.5% in the rate of obesity in Ghana (Ofori-Asenso and Daireen, 2016). In insulin resistance, including hyperinsulinaemia, diabetes, hyperlipidaemia and high blood pressure, obesity is implicated as been at the center of these (Vercoza et al., 2009).

There were about 17.5 million deaths from cardiovascular diseases (CVDs) in the year 2012, accounting for 31% of deaths worldwide. It has been projected that there will be 20million CVD-related deaths by the WHO in 2015; however deaths in poor and developing countries such as Ghana accounts for about 80% of these deaths (Ofori-Asenso and Daireen, 2016). Cardiovascular diseases were the most major cause of mortality in Ghana compared to other non-communicable diseases (NCDs) and also the cause of most institutional deaths of 14.5% of total deaths reported in 2008 in Ghana (Ofori-Asenso and Daireen, 2016).
In Ghana, CVD is an important cause of mortality after diarrhoeal diseases (WHO, 2010; Agyemang et al., 2012). Evidence of CVD in childhood has been demonstrated in postmortem studies, showing the incidence plaques and streaks of fats in coronary arteries and the aorta of children and adolescents (Shanthi et al., 2011). Body mass index (BMI), smoking, hypertension, type 2 diabetes, alcoholism, sedentary lifestyle, and hypercholesterolemia are strongly associated with the development of CVDs (Shanthi et al., 2011). Arterial stiffness is proven to be vital for the assessment of Cardiovascular (CV) risk among markers of arterial disease. It defines the reduced ability of arteries to contract and expand in response to changes in pressure (Ferreira et al., 2012). Elevated stiffness of arteries is an important marker of CV risk and is linked to higher risk of CV disorders (Ferreira et al., 2012). Many degenerative alterations happen with age in big elastic artery walls. These are known to be the cause of elevated stiffening overtime, such as mechanical lamellar elastin fraying as a result of mechanical stress cycles; alterations in the type and elevations in the quantity of collagen proteins as a compensation for the loss of elastin and due to fibrosis; and cross-linking of collagen fibers adjacently positioned by advanced products of glycation (Dietz, 2007).

Measurement of aortic Pulse Wave Velocity (PWV) gives much of the evidence related to the significance of large artery stiffening. Increases in aortic PWV predict CV diseases, the prognoses of disease among subjects with hypertension, end stage renal failure, diabetes mellitus and in the normal populace (Alejandro et al., 2014; Hansen et al., 2006). Pulse wave velocity measurement is considered as much easy, reproducible, accurate and non-invasive, technique in the determination of arterial stiffness. The most acclaimed propagative model of the vascular system is the direct measurement of the
Carotid-femoral PWV, which is determined along the aortic-iliac and aortic pathway, and this is the most important clinically as the aorta and its first branches are what are seen by the left ventricle which is important for the effects of arterial stiffness pathophysiology (Alejandro et al., 2014; Stephane et al., 2006).

Insulin resistance (IR) is a condition characterized by reduced sensitivity of cells and tissues to the stimuli of insulin. Insulin Resistance has the following as risk factors: stress, genetics, being 40-45 years of age or older, obesity (abdominal obesity), sedentary lifestyle, hypertension, hypertriglyceridaemia, low level of high density lipoprotein (Milner et al., 2010). Excess body fat, large waist circumference and abdominal visceral fat have been implicated as risk factors for increase arterial stiffening in both middle-aged and elderly people.

The mechanism behind these associations could be the connection between obesity and insulin resistance (Milner et al., 2010). Regarding visceral adiposity, there are two links with IR. 1) Visceral adipocytes release large quantities of proinflammatory cytokines (e.g. IL-8, TNF-alpha, IL-6, IL-1) (Gyllenhammer et al., 2016). Interleukin-6 is known to modulate hepatic synthesis of C-reactive protein (CRP) which is a chronic inflammatory marker and can cause acute coronary syndrome (Kelly, 2014). 2) Hepatic accumulation of fat is linked to visceral adiposity which results in non-alcoholic fatty liver disease (NAFLD). NAFLD causes increased release of FFA into circulation, elevation in breakdown of glycogen and glucose synthesis. These worsen peripheral insulin sensitivity and increases the incidence of diabetes type 2 development (Muniyappa et al., 2008). Arterial stiffness is often present among all age groups of IR syndrome subjects.
Elevated activity of angiotensin type I in vascular tissue receptor and the renin angiotensin-aldosterone system increases fibrosis and wall hypertrophy development in conditions of long-term high blood glucose and insulin. Elevated LDL, endothelium-1 FFA, impaired vasodilatation by insulin and reduced levels of adiponectin further increases stiffness through endothelial dysfunction (Mitchell, 2014; Hall, 2003).

Atrial natriuretic peptide is hormone that modulates the pressure of blood, insulin release from the pancreas and prevention of arterial stiffness (Tauscher et al., 2015). Low levels of ANP predict future development of diabetes, arterial stiffness and other CVDs (Tauscher et al., 2015). Natriuretic peptides (NPs) are very important markers of myocardial ischaemia, due to left ventricular dysfunction. Additionally, NPs contributes in the development of atherosclerotic disease. Natriuretic peptides modulate remodeling of blood vessels through their effects on the growth, vitality and death of smooth muscles and endothelial cells (Noman et al., 2010).

In addition, anti-fibrotic properties ANP have been seen in fibroblasts, preventing proliferation of cells and synthesis of collagen caused by the effect of TGF-β, an important cardiac fibrosis mediator (Calvieri et al., 2012). New physiological properties of ANP have been found such as oxidation and breakdown of lipids and respiration by mitochondria, these protect against IR and obesity due to diet. Atrial Natriuretic Peptide also prevents the synthesis of pro-inflammatory cytokines from adipose tissues and macrophages which inhibits obesity-linked IR due to chronic inflammation (Nina et al., 2014; Tauscher et al., 2015).
1.2 Problem Statement

Insulin resistance is the cause of many diseases such as CVDs, diabetes type 2 and tumors. Insulin resistance always coexists with obesity, and drastic increase in childhood obesity and in young adults is a risk for CVD development, in under developed nations having health care system incapable of handling secondary prevention strategies (Shanik et al., 2008). The trends of obesity and overweight are increasing in Ghana; it increased from 13% to 30% in 1993 to 2008 among women in Ghana (15-49yrs) (Ofori-Asenso and Daireen, 2016). In 2014, 30% (17million) deaths were due to CVDs, deaths due to CVDs are found more in developing nations including Ghana, where majority of CVDs related deaths occurred and this is expected to grow to 23.6million by 2030 (Nathan, 2014). Coronary artery disease and stroke accounted for 6.9% and 7.3% of mortalities in 2011 in Ghana respectively (Ofori-Asenso, 2016).

Most studies in sub-Saharan Africa investigating into vascular diseases only report on risk factors associated with lipid deposition (atherosis), however information on the gradual stiffening of the walls of arteries (sclerosis), had not been sufficiently investigated (Yeboah, 2013). The modifiable risks linked to pathological processes in the occurrence of atherosclerotic CVDs begin in childhood and early adulthood many years before clinical manifestation (Katja et al., 2013; Sevith, 2015). Moreover, CVDs are initiated gradually during the non-clinically stage of hyperinsulinaemia and impaired glucose metabolism (Katja et al., 2013).
1.3 Justification

Obesity is linked to low ANP levels and elevated IR in young adults. Therefore, studies investigating into the relationship between ANP and IR can enhance the mechanisms underlining arterial stiffness. This knowledge will help to institute better therapeutic and non-therapeutic management of obesity (Yeboah, 2013). The ability to characterize the arterial system and derive related biomarkers that can predict the occurrence of future CVDs, discriminate high risk subject for management would be of immense importance to public health and chronic disease prevention. Assessment of arterial stiffness and central haemodynamic indices have shown to predict CVD events in healthy and diseased Caucasian and Asian population; few studies had been reported of the utility of arterial stiffness assessment in sub-Saharan African subjects (Yeboah, 2013).

There is a great importance given to the development of CVDs due to arterial stiffness and arterial stiffness assessment is used in the clinical diagnosis and management of patients in developed nations. The carotid to femoral pulse wave velocity is the standard technique for arterial stiffness measurement due it been easy to use, reproducibility and by its independent prediction and diagnoses of CVD in different populations (Yeboah, 2013). Most of the risk factors of CVDs initiate their debilitating effects via the vascular system several years in early 2nd to 3rd decades of life before the overt clinical manifestation after 5th decade. The above argument makes it imperative to institute vascular assessment and underlining mechanism in Ghanaian population where obesity is on the rise and risk factors of CVDs are also on the increase among young adults. This gives further justification for the evaluation of cardiovascular risk in young people and provides a rationale for prevalence, mechanistic and intervention studies (Dietz, 2007).
1.4 Hypothesis

H₀: There is no association between Arterial stiffness, Insulin resistance and ANP levels in young adults in Ghana.

1.5 Aim

This study was to investigate the association of arterial stiffness, insulin resistance and atrial natriuretic peptide levels in obese and non-obese young adults.

1.6 Specific Objectives

- To compare the levels of arterial stiffness, insulin resistance and plasma atrial natriuretic peptide levels between obese and non-obese young adults.

- To investigate the association between arterial stiffness, insulin resistance and plasma ANP levels.

- To determine the factors associated with insulin resistance and arterial stiffness in young adults.
CHAPTER TWO
LITERATURE REVIEW

2.1 Cardiovascular Diseases

Cardiovascular disease (CVD) refers to diseases of the heart or blood vessels. Some types of CVDs are coronary artery diseases (CAD) such as angina; others are stroke, hypertension, cardiomyopathy, endocarditis, thrombosis, cardiac arrhythmia and congenital heart disorder (Kohn et al., 2015). Cardiovascular diseases accounted for 17.3 million deaths (31.5%) in the year 2013 up from 12.3 million (25.8%) in 1990. Mortality at any age as a result of CVD is very frequent and is rising in most part of the underdeveloped countries; however it’s declining in the developed countries since the 1970s (Naghavi, 2015).

2.2 Risk factors for CVDs

Numerous factors are known to be risks for CVDs: age, excessive alcohol intake, unhealthy diet, smoking, sedentary lifestyle, obesity, social factors, poverty, history of cardiovascular disease in one’s family, hypertension and hyperlipidaemia (Naghavi, 2015).

2.3 Obesity

Obesity is the accumulation of excessive total body fat as a result of an imbalance between food intake and energy expenditure, an individual is classified as been obese when his/her body mass index (BMI) higher than 30kg/m² (Jung and Myung-Sook, 2014). There are many factors that results in the incidence obesity, such as socio-cultural, environmental, genetic, behavioral, economic and metabolic.
Obesity is now an epidemic in adults and children as well and is often linked to many disorders including CVDs, diabetes, tumors and sleeping disorders (Mattah et al., 2013). In addition to abnormal metabolism, other changes with function and structure of the heart take place with over accumulation of adipose tissues (Jung and Myung-Sook, 2014). Adipocytes synthesize and release different cytokines that regulates CV homeostasis (Jung and Myung-Sook, 2014). They are an important depot of TNF-α, IL-6, lipoprotein lipase, plasminogen activator inhibitor-1, leptin, adiponectin, IGF-I (Jung and Myung-Sook, 2014).

### 2.4 Insulin Resistance

Insulin is a hormone produced by the beta cells of the pancreas (Wright et al., 2014). Insulin modulates carbohydrates and fats metabolism through stimulating uptake of glucose from blood into the liver and skeletal muscles and also enhances lipids storage instead of them been expended. Moreover, insulin prevents the liver from releasing glucose and it also has other growth functions in the body (Wright et al., 2014). Insulin Resistance (IR) refers to disorder where cells are unable to respond to the stimulus of insulin resulting in hyperglycaemia. This then results in an elevation in insulin production from the pancreatic beta cells causing hyperinsulinaemia. This can be undetected and can results into diabetes type 2 (Chiu et al., 2007). Signs and symptoms of IR include; brain fogginess, hyperglycaemia, difficulty losing weight, elevated blood triglycerides levels, hypertension, increased pro-inflammatory cytokines associated with CVD, intestinal bloating, sleepiness (especially after meals), weight gain,, depression, acanthosis nigricans and increased hunger (Milner et al., 2010; Chiu et al., 2007).
Risk factors related with IR are the following: Genetic, stress, aged 40 years or being older, obesity (central obesity), inactive lifestyle, prediabetes, gestation diabetes, high blood pressure, hypertriglyceridaemia, low level of high density lipoprotein and medications (glucosamine, rifampicin, isoniazid and antiretrovirals) (Milner et al., 2010).

2.5 Obesity and Insulin resistance
Insulin Resistance is usually seen in people with central abdominal obesity, high blood pressure, hyperglycaemia and dyslipidaemia including hypertriglyceridaemia, LDL, and low HDL cholesterol (Emmanuella et al., 2015). This are known as been risks for dyslipidaemia, diabetes, high blood pressure, blood clotting disorders and increase arterial stiffening in both middle-aged and elderly people, the mechanism behind these associations could be the connection between obesity and insulin resistance (Preis et al., 2010). Regarding visceral adiposity, there are two mechanism linking it with IR. 1. Visceral adipocytes synthesize a lot of proinflammatory cytokines (IL-8, TNF-alpha, IL-6, and IL-1) (Preis et al., 2010; Emmanuella et al., 2015). A high IL-6 level in plasma is a risk for T2DM development and injection of IL-6 cause elevated blood lipid levels, high blood glucose and IR in humans and humans and animals. Interleukin-6 impairs signaling of insulin through down-regulating Insulin receptor substrate (IRS) and up-regulation of Suppressor of cytokine signaling-3 (SOCS-3) (Preis et al., 2010). Tumor necrosis factor-α was the cytokine to be identified as been associated with IR and obesity. Specific deletion of TNF-α gene and receptors improves insulin sensitivity in rodent obesity. Mechanisms for TNF-α’s effects includes activation of JNK and p38 mitogen-activated protein kinase (MAPK) which elevates the phosphorylation of IRS-1 and IRS-2, resulting
in them been weak substrates for insulin receptor-activating kinases and elevating their degradation (Emmanuella et al., 2015; Miyazaki et al., 2003).

2. Secondly, visceral obesity is associated with the hepatic fat accumulation, resulting in Non-alcoholic fatty liver disease (NAFLD). The consequences of NAFLD are elevated release of fats into the circulation (as a result of elevated lipids breakdown), elevated hepatic breakdown of glycogen and synthesis of glucose. These worsen peripheral IR and increase the rate of development of diabetes mellitus (Muniyappa et al., 2008).

2.6.0 Arterial stiffness

Arterial stiffness defines the incapability of an artery to contract and expand as a result of pressure changes (Kohn et al., 2015). Arterial stiffness increases as a result of aging and arteriosclerosis (Ferreira et al., 2012). Elevated stiffening is linked to increased rate of cardiovascular disorders e.g. myocardial infarction and CVA, the leading causes of mortality in the developed world (Ferreira et al., 2012). There are many degenerative alterations which take place in the walls of large elastic arteries that contribute to elevation in stiffening overtime, such as mechanical damage of lamellar elastin in the wall as a result of continuous cycles of mechanical stress; alterations in the type and quantity of arterial collagen proteins, partly in response against the loss of arterial elastin and due to fibrosis; and cross-linking of collagen fibers by Advanced glycation end-products (AGEs) (Dietz, 2007). The measurement of aortic PWV gives an indication about some of the prognostic significance of large artery stiffening. Increases in PWV predict CVDs; mortality in renal failure, hypertension, and diabetes mellitus subjects (Alejandro et al., 2014; Hansen et al., 2006). Elevations in arterial stiffness increase the workload on the heart, because the heart has to do more in order to maintain the stroke
volume. This elevated workload results in left ventricular hypertrophy and remodeling that can culminate in the failure of the heart (Cheng and Vasan, 2011).

2.6.1 Mechanisms of Vascular Stiffness

Vascular stiffening is developed from an interaction of stable and dynamic changes which involves cellular and structure of the vessel wall. These changes are modulated by hemodynamic factors and extrinsic factors e.g. salts, hormones and glucose regulation (Jane, 2013; Galis and Khatri, 2002).

2.6.2 Structural Components of Arterial Stiffening

The relative content of collagen and elastin determines the compliance, resilience and stability of vascular wall (Wagenseil et al., 2012; Lakatta, 2003), an imbalance of this regulation, mostly by stimulation of an inflammatory milieu, results in increased synthesis of abnormal collagen and reduced amounts of normal elastin contributing to arterial stiffness. Elevated luminal pressure also enhances excess collagen synthesis. (Luft, 2012; Lakatta, 2003). Collagen, elastin, glycoproteins and proteoglycans comprises the extracellular matrix (ECM) of the vessel wall, as a result of their collagenolytic and elastinolytic effects, Matrix metalloproteinases (MMPs) degrade the ECM by creating uncoiled, less effective collagen and broken and frayed elastin molecules, respectively (Luft, 2012).

Collagens produce tensile strength and are enzymatically cross-linked immediately after their synthesis which makes them non-soluble to hydrolytic enzymes. Breakages in the structure of these intermolecular bonds results in unraveling of the collagen matrix (Jane, 2013). Collagen is very prone to non-enzymatic glycation cross-linking, due to collagen’s
low hydrolytic turnover rate; as a result there is an elevated collagen content, mostly with a more disorganized and non-functional fiber distribution (Beenakker et al., 2012). Desmosine and isodesmosine are formed by Elastin molecules and stabilized through cross-linking by LOX; destruction of these cross-links causes the weakening of the elastin array and mineralization by calcium and phosphorous, increasing arterial stiffness. (Beenakker et al., 2012).

Arterial stiffness is also due to AGEs, this is due to non-enzymatic protein glycation which results in non-reversible cross-links of proteins e.g. collagen. Collagen that is AGE-linked is stiffer and non-susceptible to hydrolytic turnover (Varun et al., 2014). Elastin is similarly prone to AGE cross-linking causing a reduction of the elastic matrix of the wall. Endothelial cell function is also affected by AGE through reduction in nitric oxide synthesis and elevating the production of oxidative species e.g. peroxinitrite (Varun et al., 2014).

2.6.3 Cellular Role in Vascular Stiffening

Vascular stiffening is highly influenced by endothelial cell signaling and the muscle cell tone of vascular smooth muscle cell (VSMC). Vascular smooth muscle cell tone can be altered by mechanical stimulation partly because of cell stretch and alteration in calcium signaling, and through modulators e.g. nitric oxide, angiotensin II, endothelin and oxidative stress (Fleenor et al., 2010; D’ Alessio, 2004). This is partly due to an imbalance of nitric oxide, endothelial-derived hyperpolarizing factor, constricting hormones, and oxygenases (eg, cyclooxygenase and NADPH). Nitric oxide levels are diminished by activation of reactive oxygen species due to hormones and stress (Fleenor et al., 2010).
2.6.4 Vascular Stiffening Pathobiology

With regards to the heart, arterial stiffening increases the workload on the ventricles and how the heart is also perfused. Higher end-systolic pressures are needed for the heart to eject into stiff arteries in order to maintain the optimal net stroke volume, this results in an increase in the energy required for a given ejected flow. Long-term ejection into such stiff arteries causes hypertrophy of the ventricles (Cecelja and Chowienczyk, 2012). Coronary flow is normally during diastolic, this mean alterations during systolic have little influence on average perfusion. However, when cardiac ejects into a stiff arteries, the increased systolic pressure is associated with the coronary perfusion (Kohn *et al.*, 2015).

2.6.5 Assessment of pulse wave velocity

Arterial stiffness can be assessed at local, regional and systemic levels with diverse techniques (*Quinn et al.*, 2012). The use of PWV for arterial stiffness assessment gives an indication of arterial changes as a result of the activities of much different pathology. Thorough analyses of studies indicated an association of carotid-femoral PWV and CVD risk factors and between arterial stiffness and PWV (*Åstrand et al.*, 2011).

2.6.6 Arteriograph

The Arteriograph (TensioMed) is a novel equipment which uses an arm cuff to simultaneously measure all parameters, under strict stop-flow condition (*Quinn et al.*, 2012). The cuff is inflated to supra-systolic pressure, to totally occlude brachial artery (*Budoff*, 2015). The measurements of aortic PWV were compared with three non-invasive devices: Complior, SphygmoCor and Arteriograph. They observed that aortic
pulse wave velocity determined by Complior was significantly higher than that of SphygmoCor and Arteriograph. This difference was due to travelled distance measured (Budoff, 2015)

2.7 Insulin Resistance and Arterial stiffness (CVDs)

The mechanism that links body fat and arterial stiffness are not well understood, even though many mechanisms have been proposed (Chun-Lin and Hui, 2014; Lorenzo et al., 2010). Short-term increase in weight can even affect arterial stiffness and this could be due changes in sensitivity of insulin, increase stimulation the Renin-Angiotensin-Aldosterone system (RAAS). Insulin Resistance and long-term high blood glucose also cause vascular stiffening as a result of an elevated production of AGEs which is an independent predictor of PWV (Chun-Lin and Hui, 2014; Semba et al., 2009). Stiffening can also be due to endothelial dysfunction as a result of free fatty acids, elevated LDLs and impaired vasodilatation by insulin (Figure 2.1) (Chun-Lin and Hui, 2014; www.bioclinicnaturals.com). Arterial stiffness is present across all age groups in diabetics and Metabolic syndrome (MetS) and the level of metabolic alterations is prognostic of arterial stiffness (Semba et al., 2009). Long-term high blood glucose and elevated blood insulin increases the activity of the RAAS and angiotensin type I receptor expression in vasculature, resulting in wall fibrosis and hypertrophy (Chun-Lin and Hui, 2014). Visceral obesity is linked to elevated quantities of plasminogen activator inhibitor-1 (PAI-1), PAI-1 inhibits fibrinolytic activity and this is a known to promote arterial stiffening. High levels of PAI-1 are especially seen in insulin resistance syndrome (Chun-Lin and Hui, 2014).
2.8 Atrial Natriuretic Peptide

Natriuretic peptides (NPs) such as A-type, B-type, and C-type, refer to a family of hormones with haemodynamic and anti-remodeling functions in the cardiovascular system (Tauscher et al., 2015). Atrial natriuretic peptide (ANP) is potent vasodilator released by cells of the heart’s atria and are released in response to elevated circulatory volume. Atrial natriuretic peptide mediates homeostasis of salts (sodium and potassium), water and fat (Tauscher et al., 2015). Blood pressure is reduced by ANP through the natriuresis of water, sodium and adipose loads on the circulatory system; therefore ANP counters the actions of the RASS which increases blood pressure (Potter et al., 2009). Atrial Natriuretic peptide also have several other functions such as inhibition of Reactive Oxygen Species release, TNF-α and expression of adhesion molecules (Paolo, 2014).

2.9 Arterial stiffness (CVDs) and Plasma levels of Atrial Natriuretic Peptide

Atrial natriuretic peptide is a very important marker of myocardial ischaemia due to left ventricular (LV) dysfunction. NPs are also known to influence the pathogenesis of atherosclerosis, they modulates the remodeling of blood vessels through controlling the vitality, growth, deaths of both endothelial and muscle cells (Noman et al., 2010). Increased levels of atherosclerosis are associated with increases in NT-proANP and NT-proBNP levels (Noman et al., 2010), ANP also causes increment in endothelial permeability which results in hypovolemia and movement of proteins and fluid from plasma to the extra vascular space (Curry, 2005). Natriuretic peptides play an important role in inhibiting remodeling such as hypertrophy (induced by angiotensin II or ET-1), fibrosis and inflammation (Calvieri et al., 2012). Natriuretic peptides level in blood is a marker for the diagnosis and prognosis of heart failure; they also give an indication on
important knowledge on high blood pressure and diseases of arteries. Disorders of ANP gene (*NPPA*) and alterations in plasma levels of NPs are associated with CVDs and are markers of CV risk (Kuhn 2012).

### 2.10 Insulin Resistance and Plasma levels of Atrial Natriuretic Peptide

Many findings suggest a role for ANP and its receptor in the endocrine pancreas e.g ANP injections increases plasma levels of insulin in subjects with good health. Moreover, low plasma ANP levels can result in type 2 diabetes development, ANP can stimulate the function and the growth of cultured pancreatic β cells (Tauscher *et al.*, 2015; Magnusson *et al.*, 2012). Findings from many studies indicate that, low plasma NP level was common among individuals with obesity compared to non-obese individuals (Tauscher *et al.*, 2015). Moreover, reduced levels of ANP were significantly associated negatively with waist circumference; this could be due to elevations in the removal by receptors or a reduction in the release of ANP (Tekes and Cikim, 2007). ANP also stimulates the synthesis and release of adiponectin (adipokine), which enhances insulin sensitivity (Birkenfeld *et al.*, 2012). It has been found out that prolonged up regulation of Natriuretic peptide receptor-C (NPR-C) could result in obesity and associated CV and other metabolic disorders e.g. diabetes (Cannone *et al.*, 2011). Furthermore NPs modulates carbohydrate and lipid metabolism through NPR-A sensitization and activation of cGMP to promote breakdown of lipids and FFA mobilization in adipose tissues. ANP/cGMP signaling also enhances pancreatic β-cell growth, the synthesis and release of insulin (Cannone *et al.*, 2011).
Glucose intolerance, chronic inflammation and dyslipidaemia are associated with obesity; these together can result in insulin resistance which then causes CVDs through endothelial dysfunction, impaired thrombolysis and hypertension (Fig. 2.1).
CHAPTER THREE
METHODOLOGY

3.1 Study Design
A cross-sectional study design was used for this study and sampling was done by convenient sampling technique.

3.2 Study Site
The study was conducted at Martin Luther Health Training School at Odorkor in Accra, which is representative of the age group (inclusion criteria), conveniently selected as study site after approval from the head of the institution. The city of Accra is the largest and the capital city of Ghana, with about 3.27 million inhabitants as of 2012 (Mills et al., 2012) and is representative of the different tribes and ethnic groups in Ghana.

3.3 Subjects/ Study Population
The study subjects were apparently healthy male and female young adults aged between 20 – 30 years without any medical history for CVDs, renal or diabetes diseases, this was determined by interviewing the subjects about their medical history.

3.4 Inclusion Criteria

- Young adults within the age group of 20-30 years.

- Subjects should be of sound mind to voluntary informed consent.
3.5 Exclusion Criteria

- Subjects who fall outside the required age range of 20-30 years.

- Participants with history of diabetes, hypertension, kidney failure and liver failure were excluded.

3.6 Sample size determination

330 subjects (sample size) were selected by using a 95% Confidence level and the equation (Jaykaran and Tamoghna, 2013);

\[ n = \frac{z^2 \times p \times (1-p)}{E^2} \]

\( p = \) proportion estimate (50%), \( n = \) sample size, \( z = z\)-score (1.96), \( E = \) margin of error (5.4%)

\[ n = 1.96^2 \times 0.5 \times (1-0.5) \]

\[ 0.054^2 \]

\[ n = 330 \]

Therefore 330 subjects were selected for the study.

3.7 Recruitment of study subjects

A convenient sampling technique was used to recruit the study participants. The participants were given scheduled dates on which they would take part in the study. Prior
to the data collection, participants were educated on the fasting guidelines; overnight fasting between 8–12 hours before blood samples were taken.

### 3.8 Ethical Considerations

Ethical clearance was sought from the Ethics and Protocol Review Committee of the College of Health Sciences, University of Ghana. A letter was submitted to the authorities of the Tertiary Schools to seek institutional approval. Informed consent (Appendix I) was sought from all subjects before they were included in the study and the nature of the study, the constraints and implications of the procedures, the risks and side effects of the study were detailed. Participants were given specific codes for confidentiality and their data was handled with maximum confidentiality and it extended after the course of the study.

### 3.9 Questionnaire administration

The data from this study was collected by the aid of a structured questionnaire (Appendix III) used to collect the data from the study participants, this contained questions which assessed: socio-demographic and lifestyle information and this helped to assess the respondents’ CVDs risk. Information on the questionnaire was explained to the study participants.

### 3.10 Anthropometry

Participants’ body weights were measured by them wearing very light clothing and were barefooted using Omron digital scale (HN-288). Seca Stadiometer was used to determine the height (Seca, Germany) with participants barefooted. The BMI (kg/m²) was
determined by dividing an individual’s body weight by the square of the height. There was a categorization of the participants based on their BMI, where BMI < 18.50 kg/m² was for underweight, BMI 18.50 – 24.99 kg/m² for normal weight, BMI 25.00 – 29.99 kg/m² for overweight and BMI ≥ 30 kg/m² for obesity (WHO, 2003). Waist and hip circumference were measured by participants standing with feet close together and arms positioned at the sides. Measurement for the circumference of the waist was made after a normal duration of expiration, with a non-elastic tape measure, approximately the mid-section of the upper part of the iliac crest and the lower part of the last rib. The circumference of participants’ hips was determined at the greater trochanters. The Waist-hip ratio was then calculated as the ratio of the circumferences of the waist and the hips.

3.11 Body Fat composition

Participants’ fat body composition was measured using the bioelectrical impedance technique (Omron body composition monitor), measurements were made with participants’ standing upright and the 4 electrodes terminals touching both the heels and soles of each feet. The participant’s age, gender and height were entered into the equipment. The participants then grabbed the grip of the electrodes of the monitor by placing the palm around the terminals and with their thumbs upright and stretch their arms forward to approximately 90° to the axis of the body. The body fat percentage (%), visceral fat level and BMI in kg m⁻² were computed for each patient. This device releases a low current of 500mA and 50 kHz electricity into participants’ body and the quantity and proportion of fat tissue is then determined using the variables: sex, age, weight, height and electrical resistance.
3.12 Blood pressure measurements of participants

The blood pressures were determined by an Omron digital blood pressure monitor, before blood pressure (BP) measurement, the participants were asked to empty their urinary bladder if they have not passed out urine within the last four (4) hours. The blood pressure was measured by placing a cuff on the left arm around the brachial artery, the participants lying in a supine position and they were allowed to rest for at least 5 minutes. The blood pressure was measured three times; each measurement was spaced with at least 60 seconds interval with the preceding measurement. The first results were not used; however the mean of the last two measurements was used as the true blood pressure.

3.13 Measurement of arterial stiffness levels (Tensiomed Arteriograph)

The Arteriograph was used to measure indices of arterial stiffness such as brachial BPs, aortic systolic BP, aortic PWV, as well as brachial and aortic augmentation indices. The participants were informed about the procedures involved in the assessment and thereafter, asked to lie calmly on an examination couch in a supine position in a quiet, temperature-controlled room (20 ± 2°C) for at least 10 minutes. The length of each participant’s descending aorta was estimated as the jugulum-symphysis distance, which was measured using a specialized. The participant’s data was entered into the software (TensioWin 2.4.10) and through bluetooth connection, the arteriograph was programmed. The appropriate BP cuff size, as recommended by the software based on the arm circumference, was applied to the participant’s right arm. The participant was told to remain calm before and during the cuff inflation. The systolic and diastolic BPs are first determined by the Arteriograph, the cuff is inflated again, initially to measured diastolic
pressure and later to the supra-systolic pressure, the signals are then recorded for 8 seconds. These signals recorded are transmitted wirelessly to a computer. The behaviour of the first systolic pulse wave and the wave reflected from the bifurcation of the aorta as well as the time interval (return time) between the first and late systolic wave is used to determine aortic PWV. The aortic PWV determined by using this equation (Kohn et al., 2015):

\[
\text{Aortic PWV (m/s)} = 2 \left( \text{jugulum-symphysis distance} \right) / \text{return time}
\]

A schematic illustration of the direct and reflected waves generated by the pulse in the aorta is shown in the figure below.

**Figure 3.1**: Schematic illustration of the working principle of the Arteriograph

(www.bioclinicnaturals.com)
3.14 Biochemistry and clinical analysis

3.14.1 Blood sample collection

Blood samples (venous) were collected after 8-12 hours of overnight fast (10mls). These 10 mls was divided into 2 mls for fluoride (ash top) tubes for fasting plasma glucose (FPG) measurement, 3 mls into EDTA (violet top) tubes and 5 mls into gel-separator tubes (yellow top). Clear plasma and serum were obtained by centrifuging tubes for 10mins at 3000 rpm. Plasma glucose was measured enzymatically within 15 minutes after sample collection. Plasma and serum samples were aliquoted into sterile eppendorf tubes and stored at -20 until further analysis.

3.14.2 Chemical principle – glucose oxidase test for fasting plasma glucose

The level of glucose in the fasting plasma samples and the controls (standards) were measured with a Selectra Junior chemical auto analyzer from Namarka, using ELITech glucose PAP SL reagent from ELITech clinical systems, France, following the manufacturer’s instructions. The analysis involves enzymatic oxidation of glucose to form equimolar amount of gluconic acid and hydrogen peroxide.

\[
\text{GOD} \\
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{gluconic acid} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \\
2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{phenol} \rightarrow \text{quinoneimine} + 4 \text{H}_2\text{O}
\]

Glucose concentrations were calculated by the equipment after reading the absorbance of the quinoneimine at a wave length of 500 nm.
3.14.3 Lipid profile assay

Lipid profile of was analyzed using Selectra Junior chemical auto analyzer from Namarka, using ELITech cholesterol SL, ELITech cholesterol HDL SL 2G and ELITech triglycerides Mono SL New reagents from ELITech clinical systems, France, following the manufacturer’s instructions.

The total amount of cholesterol (TChol) in the plasma was assayed after enzymatic hydrolysis and oxidation.

\[ \text{cholesterol esterase} \]
\[
\text{Cholesterol ester + } H_2O \quad \rightarrow \quad \text{cholesterol + fatty acids}
\]

\[ \text{cholesterol oxidase} \]
\[
\text{Cholesterol + } O_2 \quad \rightarrow \quad \text{cholesten-3-one + } H_2O_2
\]

\[ \text{lipases} \]
\[
\text{Triglycerides + } H_2O \quad \rightarrow \quad \text{glycerol + fatty acids}
\]

\[ \text{GK} \]
\[
\text{Glycerol + } ATP \quad \rightarrow \quad \text{glycerol-3-phosphate +ADP}
\]

\[ \text{GPO} \]
\[
\text{Glycerol-3-phosphate + } O_2 \quad \rightarrow \quad \text{dihydroacetonephosphate + } H_2O_2
\]

\[ \text{POD} \]
\[
2H_2O_2 + 4\text{-aminophenazone +phenol} \quad \rightarrow \quad \text{quinoneimine + 4 } H_2O
\]

Cholesterol concentration was determined by the equipment after reading the absorbance of the indicator at a wavelength of 500 nm.

HDL cholesterol was assayed by the precipitation method. 500 μL of diluted precipitant solution, containing phosphotungstic acid in the presence of magnesium, was added to
200 μL of the plasma sample. The HDL cholesterol was assayed from the supernatant solution at an absorbance of 500nm.

The levels of LDL cholesterol were calculated from Friedwald’s equation, \( \text{LDL} = \text{TChol} - (\text{HDL} + \text{TG}/2.2) \).

The study subjects were further classified based on the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) using their lipid profile, FPG, BMI, WC and BP measurement into various CVD risk groups (Pinhas-Hamiel et al., 2015).

- High FPG \( \geq 6.1\text{mmol/L} \).
- High blood pressure (BP) \( \geq 130/85\text{mmHg} \).
- Low HDL-C (women \( \leq 1.01\text{mmol/L} \) and men \( \leq 0.9\text{mmol/L} \)).
- Raised Triglycerides (TGL) \( \geq 1.7\text{mmol/L} \).
- Abdominal/central obesity (waist circumference): \( \geq 88\text{cm} \) in women and \( \geq 102\text{cm} \) in men.

3.14.4 Insulin and ANP enzyme-linked immunoassay

Serum insulin and ANP were assayed using enzyme linked immunoassay (ELISA) method with duo set ELISA kits from R&D systems (United Kingdom).

Sample Dilution

For insulin, an amount of 1 μL of the serum samples was added to 499 μL of reagent diluent to achieve 1:500 dilutions; and also for ANP, 1 μL of the serum was added to 499 of reagent diluents to achieve 1: 500 dilutions. The samples were then stored in a refrigerator at a temperature of 2°C and used for the assay within one week.
Plate Preparation

- Plate Coating with Capture Antibody: Before the assay of insulin and ANP, capture antibody was used to coat the wells in ELISA microplate overnight. For insulin, anti-human insulin was reconstituted with 1.0 ml of PBS to obtain a concentration of 720 μg/mL, which was further diluted in PBS to achieve a working concentration of 4μg/ml. For ANP, 1.0 ml of PBS was added to reconstitute it and achieve a 180 μg/ml concentration, which was further diluted to a concentration of 1.0 μg/ml. 100 μl of diluted capture antibody solution was used to coat ten 96-well microplates (five for each hormone) overnight at room temperature.

- Washing: After overnight incubation, each well was aspirated and washing was done by filing each well with 400 μl of wash buffer twice, for a total of three times, using an auto washer. Blotting the microplate with paper towels was done to remove any leftover buffer.

- Microplates were then blocked by adding 300 μl of diluent to every well and followed by 1 hour incubation.

- Washing: washing process was done as above in ‘b’.

Assay procedure

- Sample and standard addition: In each well, diluted sample or standard was added (100μl each) and incubated for 2 hours at room temperature after covering with adhesive strip.

- Washing: repeated as in step ‘b’.
• Detection antibody addition: a volume of 100 μl of diluted detection antibody (diluents with biotinylated mouse anti-human insulin detection antibody for insulin and also biotinylated mouse anti-human ANP detection antibody for ANP) was added every well and incubated at room temperature for 2 hours.

• Washing: repeated as in step ‘b’.

• To each well a volume of 100 μl of Streptavidin-HRP (diluted) was added, covered and incubated for 20mins at room temperature, avoiding exposure of plates from direct light.

• The washing process was repeated as in step ‘b’.

• To each well a volume of 100 μl of substrate solution was added, incubated in darkness at room temperature for 20 mins.

• To each well, 50 μL of stop solution was added.

• The absorbances were read at a wavelength of 450 nm using a microplate reader.

The ADAMSEL software was used in converting the absorbances into concentrations.

3.15 Diagnosis of Insulin Resistance

The Homeostatic Model Assessment (HOMA) method was used (Hui-Qi et al., 2011).

\[
\text{HOMA –IR} = \frac{\text{Glucose x Insulin}}{22.5} \quad \text{or} \quad \text{HOMA-\(\beta\)} = \frac{20 \times \text{Insulin\%}}{\text{Glucose – 3.5}}
\]
3.16 Statistical analysis

Data were analyzed by statistical package for social sciences (SPSS) software, 20\textsuperscript{th} version. The parameters with normal distribution were presented as mean± standard deviation, median (inter quartile range) was used to present non-normal distribution variables. Independent student’s t-test was used to analyze means of data with two predictors. Categorical data were presented as frequency and analyzed by chi-square ($\chi^2$) test when necessary. Association between variables was analyzed using Pearson’s correlation for normally distributed data and Spearman’s correlation for non-normally distributed data. A 95\% confidence interval was used and considered a value of $p <0.05$ as statistically significant.
CHAPTER FOUR
RESULTS

4.1 Participant’s General Characteristics
A total of 330 young adults within the age range of 20-30 years were included in the study, made up of 119 (36.1%) males and 211 (63.9%) females. The mean age for the study participants was 23.9 years, with males being older than females (p = 0.0338) (Table 4.1).

4.2 Anthropometric characteristics of study participants
The females had significantly higher mean values of weight, hips circumference and BMI as compared to their male counterparts (p < 0.05). There were no significant differences in PWV and SBP between males and females participant (p > 0.05) (Table 4.1).

4.3 Gender distribution of the Prevalence of obesity and Overweight
The overall prevalence of obesity and overweight among the study participants were 20.3% and 48.8% respectively. Obesity and overweight were significantly higher among the females as compared to the males (p < 0.05) (Figure 4.1).
Table 4.1: Anthropometric characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Males, n (%)</th>
<th>Females, n(%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>119 (36.1)</td>
<td>211 (63.9)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>24.34 ± 2.73</td>
<td>23.69 ± 2.62</td>
<td>0.0338*</td>
</tr>
<tr>
<td>BMI, kgm²</td>
<td>25.65 ± 3.58</td>
<td>26.95 ± 4.29</td>
<td>0.0054*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>84.53 ± 19.75</td>
<td>87.22 ± 20.19</td>
<td>0.2423</td>
</tr>
<tr>
<td>Hips circumference, cm</td>
<td>105.31 ± 18.67</td>
<td>121.93 ± 18.32</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Waist-to-Hip ratio</td>
<td>0.81 ± 0.15</td>
<td>0.84 ± 0.17</td>
<td>0.1096</td>
</tr>
<tr>
<td>Visceral fat, cm</td>
<td>7.29 ± 3.69</td>
<td>7.95 ± 4.15</td>
<td>0.1501</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>117.59 ± 15.93</td>
<td>119.39 ± 15.89</td>
<td>0.3243</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>75.88 ± 7.82</td>
<td>76.48 ± 7.6</td>
<td>0.5017</td>
</tr>
<tr>
<td>Aortic PWV, m/s</td>
<td>7.37 ± 2.24</td>
<td>7.14 ± 2.32</td>
<td>0.3819</td>
</tr>
</tbody>
</table>

Table 4.1 shows the anthropometric and haemodynamic measures of the participants. Data are presented as mean ± standard deviation; p values were determined using independent t-test; *P < 0.05 is considered significant; n = total number in participants in category; BMI = Body Mass Index; BP = Blood Pressure; PWV = Pulse Wave Velocity
4.4 Biochemical measurements of the Participants

The mean IR and ANP among the study participants were 0.92 ± 0.20 and 4.94 ± 1.88 respectively and the males had a significantly higher mean value of ANP as compared to the females (p = 0.06), however there was no significant difference in the mean IR between the males and the females (p > 0.05) (Table 4.2). There were no significant differences in the means of the other biochemical measurements with regards to gender (p > 0.05) (Table 4.2).
Table 4.2: Biochemical characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Males (n=119)</th>
<th>Females (n=211)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG, mmol/l</td>
<td>4.88 ± 0.91</td>
<td>5.05 ± 1.13</td>
<td>0.1612</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.79 ± 0.29</td>
<td>0.73 ± 0.29</td>
<td>0.0720</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.27 ± 0.16</td>
<td>1.29 ± 0.15</td>
<td>0.2571</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>3.67 ± 1.39</td>
<td>3.51 ± 1.15</td>
<td>0.2618</td>
</tr>
<tr>
<td>IR</td>
<td>0.95 ± 0.18</td>
<td>0.91 ± 0.21</td>
<td>0.3891</td>
</tr>
<tr>
<td>ANP</td>
<td>5.58 ± 2.38</td>
<td>4.75 ± 1.65</td>
<td>0.0600</td>
</tr>
</tbody>
</table>

*Table 4.2 shows the results of the biochemical measurements, the IR and ANP. There was no significant difference in the mean values with regards to gender; n = number of participants; FBG = Fasting Blood Glucose; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; IR = Insulin Resistance, ANP = Atrial Natriuretic Peptide. Data are presented as mean ± standard deviation; p values were determined using independent t-tests.*

4.5 Association of BMI with CVD risk factors

Mean values of all the CVD risk factors were highest in the obese group and lowest among participants with normal BMI, significant associations of BMI with CVD risk factors were found in FBG and DBP (p < 0.05), and however, there were no significant associations found between BMI and the other CVD risk factors (p > 0.05) (Table 4.3).
4.6 Gender distribution of CVD risk factors

The females had significantly higher prevalence of high waist circumference, high BMI (obesity) and high WHR ratio (p < 0.05). However, a significantly higher prevalence of low HDL was seen among the males (p = 0.0054) (Figure 4.2). There were no significant differences in the prevalence of high FBG and high LDL with regards to gender (p > 0.05) (Table 4.2)

Figure 4.2: Gender distribution of the Prevalence of CVDs risk factors

*Figure 4.2 shows the distribution of CVD risk factors with regards to gender. The females had significantly higher prevalence of high WHR and High BMI (obese), however there was a significantly higher prevalence of low HDL in the males. WHR = Waist-to-Hip ratio; BP = Blood Pressure; TRIG = Triglycerides; LDL = Low Density Lipoprotein.*
Table 4.3: Association of BMI with risk factors

<table>
<thead>
<tr>
<th></th>
<th>Normal BMI</th>
<th>Overweight</th>
<th>Obese</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>97</td>
<td>161</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>24.43 ± 2.85</td>
<td>23.86 ± 2.71</td>
<td>23.40 ± 2.24</td>
<td>0.0460*</td>
</tr>
<tr>
<td>FBG</td>
<td>4.99 ± 0.96</td>
<td>4.87 ± 0.71</td>
<td>5.27 ± 1.69</td>
<td>0.0340*</td>
</tr>
<tr>
<td>HDL</td>
<td>1.29 ± 0.16</td>
<td>1.27 ± 0.16</td>
<td>1.26 ± 0.13</td>
<td>0.4260</td>
</tr>
<tr>
<td>LDL</td>
<td>3.61 ± 1.34</td>
<td>3.62 ± 1.18</td>
<td>3.44 ± 1.26</td>
<td>0.5840</td>
</tr>
<tr>
<td>TRIG</td>
<td>0.74 ± 0.26</td>
<td>0.75 ± 0.26</td>
<td>0.81 ± 0.37</td>
<td>0.2780</td>
</tr>
<tr>
<td>SPB</td>
<td>119.25 ± 17.93</td>
<td>117.53 ± 14.15</td>
<td>121.12 ± 16.77</td>
<td>0.2820</td>
</tr>
<tr>
<td>DBP</td>
<td>77.00 ± 9.18</td>
<td>74.86 ± 2.16</td>
<td>78.60 ± 2.39</td>
<td>0.002*</td>
</tr>
<tr>
<td>PWV</td>
<td>7.07 ± 2.45</td>
<td>7.18 ± 2.16</td>
<td>7.60 ± 2.39</td>
<td>0.3170</td>
</tr>
</tbody>
</table>

Table 4.3 shows the association of BMI with CVD risk factors. There were significant associations of BMI with FBG and DBP; FBG = Fasting Blood Glucose; TRIG = Triglycerides; SPB = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; HDL= High Density Lipoprotein; Data are presented as mean ± standard deviation, p values were determined using ANOVA tests; * P < 0.05 is considered significant;
4.7 Association between PWV and CVD risk factors

There were significant associations between BMI and WHR with PWV (p = 0.0274 and 0.0224), however, there were no significant associations between PWV and the other CVD risk factors. Moreover, there were weak positive correlations between FBG, SBP and TRIG with PWV. There was an inverse (negative) correlation between HDL and PWV (r = -0.0611) (Table 4.4).

Table 4.4: Association of PWV with CVD risk factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG</td>
<td>0.0068</td>
<td>0.9021</td>
</tr>
<tr>
<td>WHR</td>
<td>0.1257</td>
<td>0.0224*</td>
</tr>
<tr>
<td>SBP</td>
<td>0.0836</td>
<td>0.1296</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.0611</td>
<td>0.2692</td>
</tr>
<tr>
<td>BMI</td>
<td>0.1214</td>
<td>0.0274*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.0132</td>
<td>0.8112</td>
</tr>
</tbody>
</table>

Table 4.4 shows the association of PWV with the CVD risk factors. There was a significant association between WHR and BMI, however, PWV was inversely associated with HDL; Pearson Correlation was used to determine r and p-values; *P < 0.05 is considered significant; PWV = Pulse wave velocity; WHR = Waist-to Hip Ratio.
4.8 Association (correlation) between IR and ANP with PWV and CVD risk factors

There was a significant association of IR with PWV (p = 0.0155), however there were no significant associations of IR with ANP and the other CVD risk factors (p > 0.05). There were negative associations of ANP with IR, BMI, WHR and SBP, moreover ANP was not significantly associated with any of the risk factors (p > 0.05) (Table 4.5).

Table 4.5: Association of IR and ANP with CVD risk factors

<table>
<thead>
<tr>
<th></th>
<th>IR</th>
<th>ANP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>PWV</td>
<td>0.2368</td>
<td>0.0155*</td>
</tr>
<tr>
<td>ANP</td>
<td>-0.1230</td>
<td>0.9014</td>
</tr>
<tr>
<td>BMI</td>
<td>0.1174</td>
<td>0.2353</td>
</tr>
<tr>
<td>SBP</td>
<td>0.1470</td>
<td>0.1364</td>
</tr>
<tr>
<td>WHR</td>
<td>0.08489</td>
<td>0.3916</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>0.1239</td>
<td>0.2102</td>
</tr>
<tr>
<td>TRIG</td>
<td>0.0565</td>
<td>0.5689</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.1404</td>
<td>0.1531</td>
</tr>
</tbody>
</table>

Table 4.5 shows the association of IR and ANP with CVD risk factors. There was a significant association between IR and PWV, there were also negative associations of IR with ANP and HDL, there were negative associations of ANP with BMI, SBP and WHR. Pearson Correlation was used to calculate r and p-values; * P < 0.05 is considered significant; r = Correlation Coefficient; ANP = Atrial Natriuretic Peptide; IR = Insulin Resistance.
CHAPTER FIVE

5.1 DISCUSSION

Obesity is a major health concern in both children and adults worldwide, it is most often linked with a lot of diseases including CVDs, high blood pressure, diabetes type 2, sleep disorders and tumors. Therefore, studies that seek to characterize the metabolic and vascular systems and identify biomarkers such as IR, PWV and ANP which can help to diagnose and prognose the incidence and the development of CVDs in future and can also identify individuals at risk for management which could be very important to public health and the prevention of long-term diseases. The findings of this study showed that there was a high prevalence of obesity and overweight among the study participants (Figure 4.1). Moreover, obesity and overweight were significantly higher in the females as compared with their male counterparts (Table 4.1 and Figure 4.1). These findings were in agreement with a study conducted in young adults in Accra, Ghana by Afrifa-Anane et al., (2015).

CVD risk factors are a group of metabolic and cardiac risk factors such as high blood pressure, high blood glucose, dyslipidaemia and central obesity (Seerat and Saroj, 2012). The results of this study showed that the incidence of CV risks among the study participants was high (Figure 4.2). The results of this study were not in agreement with Owiredu et al., (2008) who had a lower incidence of CV risks in Ghana, this difference could be due to an increase in the incidence of the components of CV risks with time as a result of an increment in sedentary lifestyle and general unhealthy lifestyles of Ghanaians especially among the youth. Further analyses of the results indicated that most of the CVD risk factors were prevalent in the females, high WHR, elevated BMI, and
hypertension were the significant risk factors in the females, while in males these were elevated Triglycerides and hyperglycaemia (Figure 4.2, Table 4.2). Moreover, there were statistically significant differences in the prevalence of high WHR, BMI and low HDL in females compared with males. These findings were similar to that of Ashtari et al., (2012) and also in agreement with a study conducted by Ling-Ling et al., (2014). This higher prevalence found in the young female adults could be as a result of female’s social, biological, cultural and behavioral changes during puberty puts them at higher risk of inactivity and makes them prone to weight-associated chronic disorders including diabetes type 2, MetS and CVDs. Furthermore findings from NHANES assessing levels of physical activity shows that men spent a significantly 20mins more on moderate and vigorous exercise and 21mins less in sedentary lifestyle than women (Yi-Ju et al, 2014).

The relatively new technique developed for assessing vascular compliance, which is determined by PWV has enhanced research on markers of CVDs. The determination of carotid to femoral PWV is accepted as standard technique for the assessment of arterial stiffness in everyday practice due to its affordability, non-complicated to use, non-invasiveness, precision, accuracy and its predictive value of diagnosing and prognosing CVDs. The results of this study showed that mean aortic PWV was relatively high but was less than the threshold of 10m/s (Table 4.1). This mean PWV value was higher than findings from a similar study conducted by Alejandro et al., (2014) in the same age group. However, the findings in this study were similar to a work done by Oswaldo et al., (2011) The results as shown in Table 4.1 indicated that the female participants had significantly higher values of systolic BP, diastolic BP and pulse as compared to their
male counterparts. However, the females had lower values of aortic PWV as compared to their male counterparts, thus analyses of the results indicated that males had stiffer arteries as compared to their female counterparts, but there was no statistically significant difference in the aortic PWV mean values. Generally, CV risk is similar for adult women and men, but lower in women during the course of their fertility period. This gender difference is as a result of the influence of estrogen, estrogen is a protective factor against CVDs (Susana et al., 2012: Eliza and Wei, 2009). Findings from studies attribute this protection by estrogen to its regulatory effects on the endothelium of vascular system. Estrogen is known to enhance nitric oxide synthesis by the endothelium by elevations in the effects and expression of nitric oxide synthase and enhances thromboxane A2 and prostacyclin synthesis (Susana et al., 2012: Eliza and Wei, 2009). These pathways are important vascular tone regulators in females. Furthermore Estrogen is known to also regulate the RAAS, which controls the volume and pressure of blood and also the growth of smooth muscle cell. Estrogen inhibits angiotensin-converting enzyme expression hence prevents the release of angiotensin II (a vasoconstrictor) (Susana et al., 2012: Eliza and Wei, 2009).

Glucose-insulin metabolism in youth is related to atherosclerosis and CV risk in adulthood, therefore an increase in childhood and early adulthood obesity and insulin resistance represents a risk for CVDs development in under-developed countries where the health care system and second prevention strategies are poor. The assessment of insulin resistance by the Homeostasis model assessment (HOMA-IR) is widely accepted as the standard method for research purposes due to its convenience, accuracy and predictive value of IR and metabolic disorders. Analyses of results from this study show
that the mean IR among the participants was normal for this category of participants (apparently healthy young adults), however IR was non-significantly higher in males as compared to the females, this finding is similar to that of Eliza and Wei, (2009), who also had higher HOMA-IR in males. Estrogen effects are the major reason for the lower prevalence of IR in females, estrogen directs the deposition of fats into the gluteal and subcutaneous areas in women instead of in the viscera found in men. Estrogen also enhances the sensitivity of insulin through effects on glucose and insulin homeostasis such as reducing the synthesis and release of glucose by the liver and promoting the transport of glucose in muscles. Furthermore, estrogen enhances the release of adiponectin which has insulin sensitization and anti-atherogenic effects whiles testosterone is known to reduce the levels of adiponectin (Susana et al., 2012: Eliza and Wei, 2009). There were also non-significant negative correlation between IR with ANP and HDL. ANP was not significantly associated with any of the factors; however there were negative correlations of ANP with WHR, BMI and SBP, it is known that ANP levels are low among obese individuals (Yeboah, 2013) and this is in agreement with the negative correlation found in this study between ANP and the indices of obesity (WHR and BMI).

5.2 CONCLUSION

The metabolic and cardiovascular risk profile in overweight and obese young adults are associated with markers of atherosclerosis and adult CVD risk including lipid disorders, diabetes, hypertension and inflammation. The results of this study support the use of PWV, HOMA-IR and ANP levels as markers to diagnose CVDs and also to help improve
strategies on glucose-insulin metabolism in childhood and young adulthood in order to reduce CVD risk in adult life.

5.3 LIMITATIONS

The sample size of this study was not large enough (due to financial constraints) to generalize and make inferences on the total population of young adults in Ghana.

5.4 RECOMMENDATIONS

Longitudinal and experimental studies should be designed to compare with the findings of this study. Furthermore, a larger sample size should be used in order for inferences to be made on the findings. Lifestyle modifications such as improved diets and increased physical activity are highly recommended for young adults in order for IR and arterial stiffness prevention.
REFERENCES


APPENDIX I

INFORMED CONSENT FORM

Participant ID Number: ____________ Participant Name: _________________________

Study Title: The association between arterial stiffness, insulin resistance and ANP levels in young Ghanaian adults.

Purpose of study

Obesity (high body fat) is an important cause of diseases of the heart and blood vessels. People who are obese develop problems with the functioning of special blood vessels which carry bright red blood, called arteries. The arteries are supposed to be elastic (able to stretch and recoil) as blood passes through. However, in obese people, the arteries may become less elastic (arterial stiffness) and this may lead to diseases. The occurrence of arterial stiffness in young adults in Ghana is unknown. In this study, we want to learn the relationship between arterial stiffness and obesity in young adults. Also, we will take your blood sample to measure some factors that regulate your blood sugar (insulin) and blood pressure (atrial natriuretic peptide).

Your role in this study

You are likely to spend the best part of the morning going through this study. For you to qualify to be part of this research, you should be between the ages of 20 to 30 years. If you want to take part in the research, you would be asked to fast overnight for 10-12 hours. We will ask you to provide information about yourself and your health status. You may feel uncomfortable providing such information. Also, your blood pressure, height, weight and amount of fat in your body will be measured. In addition, some special
medical equipment that measure blood pressure and stiffness of the blood vessel will be applied to your arm.

**Possible Risks**

These procedures are painless and might give slight tingling sensations for few seconds when the cuffs inflate. Also, some amount of blood will be drawn to measure substances in the bloodstream. You are assured that this amount will not affect your health. All the tests we will do for you in connection with this research will be free of charge. You may experience a minor bruise and/or temporary discomfort at the site of taking the blood and this risk is not more than what you will normally be exposed to for having a blood drawn routinely at the hospital. We will reduce this discomfort happening by asking experienced staff to take the blood.

**Confidentiality**

Information we collect on you in this study will be kept confidential and secure. The information will only be available to the scientists conducting this study. You are further assured that if a report of this study is prepared for the scientific and medical community you will not be identified by name.

**Withdrawal**

You are to understand that taking part in the research is entirely voluntary. You are further to note that you may refuse to take part or withdraw from the study at any time without anyone objecting.
Possible Benefits

You may not have any direct benefit for taking part in the study. The study will, help us understand arterial stiffness and how this is associated with plasma factors like insulin and atrial natriuretic peptide in young Ghanaian adults. All your test results will be explained to you. You may through this study discover that you have bad fat in the blood, stiff arteries, hypertension or diabetes. You will be advised professionally and/or referred appropriately if you should have any of these conditions on testing.

Contacts

If there is something that you do not understand or you have any questions or concerns about this Research or should you later wish to have any matter or question relating to this study clarified, do not hesitate to contact Mr. Affrim K Patrick, Department of Chemical Pathology, University of Ghana, Korle-Bu, Accra. (Tel number, 0240977531), the principal investigator for this study, to answer any questions you may have.

I have fully explained to ____________________________ the nature and purpose of the above described research, its procedures, risks and benefits. I have allowed the subject to ask questions and have answered and will answer to the best of my ability, all questions relating to the study at any time.

_________________ ____________________________ ____________
Signature Full Name of Investigator Date
APPENDIX II

PARTICIPANT CONSENT RESPONSE

I ________________________, have read (or have had read to me in a language that I fully understand) the proposed study and that I have understood what is going to be done. Also, any concerns I have, have fully been addressed. My signature or thumbprint below indicates that I have understood what is going to be done and that I agree to take part in the study.

___________________________________    Date:__________________
(Signature/thumbprint of Subject)

___________________________________    Date:__________________
(Signature: Witness)
APPENDIX III

QUESTIONNAIRE FORM

The association between arterial stiffness indices, insulin resistance and ANP levels in young Ghanaian adults.

Date of Evaluation:____________________  Questionnaire #:____________

SECTION A: DEMOGRAPHIC DATA

1. Age …………………years
2. Gender…………….. [ ] Male [ ] Female (1 =M; 2=F)
3. Residential Status. [ ] boarding student [ ] day student
4. Marital status [ ] Married [ ] Single [ ] Cohabitation [ ] Divorce

SECTION B: RISK FACTOR DATA

{For questions 5-10 Use 1 =Yes, and 2=No}

5. Do you currently smoke cigarettes regularly? ______
   a) If yes, how many sticks on average do you smoke a day? ______
   b) How old were you when you first started to smoke? ______
6. Have you ever smoked cigarettes? ______
   a) if yes, how long did you smoke for?______yrs ________ months
   b) if yes, how many sticks did you smoke a day? ______
7. Do you have a close relation who is a smoker? ______
   a) if yes, who? ______________ (eg. wife, sister, father, son, grandfather, etc)
   (b) Did you live with that person(s)? ______ (Yes=1; No=2)
8. Why do you smoke? [ ] Mark occasions [ ] Feel good [ ] Peer pressure [ ] Relieve stress [ ] I don’t smoke [ ] To induce sleep [ ] Promote appetite
Other........................................................................

9. Do you currently drink alcohol? ________

(a) if yes, how many drinks on average do you have in a week? ________

(b) if not that frequent, how many drinks in a month? ________

(Please note that 1 unit of alcohol = half a pint of beer, a tot of gin / whisky, a glass of wine)

10. Why do you take the alcohol? [ ] Mark occasions [ ] Feel good [ ] Peer pressure [ ] Relieve stress [ ] I don’t take alcohol [ ] To induce sleep [ ] Promote appetite Other.................................................................

SECTION C: PAST HISTORY

{For question 11 use 1=yes, 2=no and 3=don’t know}

11) Have you ever been told by a healthcare person that you have any of the following?

(a) hypertension________

(b) heart attack________

(c) stroke________

(d) heart disease________

(e) high blood cholesterol or lipids________
SECTION D: FAMILY HISTORY

{For questions 12 & 13 Use 1 = Yes, 2 = No and 3 = Don’t know}

(12) Do you have anyone in your family (not a spouse) with diabetes? ________

if yes, who has ________________________________ (list all)

(13) Do you have anyone in your family with any of these conditions?

(a) Hypertension ________

(b) Stroke ________

(c) High blood cholesterol or lipids ________

(d) Limb amputation that is not through an accident ________

(e) Coronary artery bypass surgery ________

(f) Angina ________

(g) Vascular surgery - Varicose veins? ________

(h) Heart attack ________

(i) Heart disease ________

(j) Gout ________
### SECTION E: ANTHROPOMETRY/PHYSICAL MEASUREMENTS

<table>
<thead>
<tr>
<th>Physical measurements</th>
<th>Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height in (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist girth (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure 1</td>
<td>SBP1</td>
<td>HR1</td>
</tr>
<tr>
<td></td>
<td>DBP1</td>
<td></td>
</tr>
<tr>
<td>Blood pressure 2</td>
<td>SBP2</td>
<td>HR2</td>
</tr>
<tr>
<td></td>
<td>DBP2</td>
<td></td>
</tr>
<tr>
<td>Blood pressure 3</td>
<td>SBP2</td>
<td>HR3</td>
</tr>
<tr>
<td></td>
<td>DBP2</td>
<td></td>
</tr>
<tr>
<td>Hip circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm circumference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jugulum symphysis distance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you
APPENDIX IV

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: ..................................................

3rd October, 2016.

Mr. Affrim Kormla Patrick
Department of Chemical Pathology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu, Accra

ETHICAL CLEARANCE


The Ethical and Protocol Review Committee of the College of Health Sciences on the 27th of September, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: “Association between Arterial Stiffness, Insulin Resistance and Circulating Atrial Natriuretic Peptide Levels in Young Ghanaian Adults”

PRINCIPAL INVESTIGATOR: Affrim Kormla Patrick

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

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

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

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

This ethical clearance is valid till 31st August, 2017.

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

Signed: .............................................

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

cc: Provost, CHS
Dean, SBAHS
Head of Department