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SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES.

SERUM LEPTIN LEVELS AMONG CHRONIC KIDNEY DISEASED SUBJECTS WITH HYPERTENSIVE HEART DISEASE ATTENDING KORLE-BU TEACHING HOSPITAL

BY

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DECLARATION

I, Christian Nsormi Adekena, certify hereby; that this work is original and mine under the supervision of Dr. Seth Amanquah and Dr. Vincent Boima. Full recognition has been made wherever references were cited. No part nor all of this thesis has been submitted for any other degree.

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DEDICATION

God Almighty is worthy of all the praises for His grace, blessings, favour and mercies. This work is dedicated to my parents, Mr. and Mrs. Adekena for their continuous affection, advice, and encouragement.

This thesis is also dedicated to my brothers and sisters, especially Samuel, Acheampong, Derek Prince and Eva for their financial help and encouragements.

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LIST OF ABBREVIATIONS

ACE	-	Angiotensin Converting Enzyme
AHA	-	American Heart Association
BMI	-	Body Mass Index
BP	-	Blood Pressure
CKD	-	Chronic kidney disease
GFR	-	Glomerular Filtration Rate
HHD	-	Hypertensive Heart Disease
ESRD	-	End stage renal disease
LVH	-	Left Ventricular Hypertrophy
HFNEF	-	Heart Failure with Normal Ejection Fraction
TGF	-	Transcription Growth Factor
FDA	-	Food and Drugs Administration
CVD	-	Cardiovascular Disease
KBTH	-	Korle Bu Teaching Hospital
ELISA	-	Enzyme-Linked Immunosorbent Assay
NPY	-	Neuropeptide Y
THC	-	Tetrahydrocannabinol

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MSH	-	Melanocyte Stimulating Hormone
CCK	-	Cholecystokinin
PYY	-	Peptide tyrosine-tyrosine
JAK/STAT	-	Janus Kinase/Signal Transducers and Activators of Transcription
MAPK	-	Mitogen-activated Protein Kinase
PTHrP	-	Parathyroid Hormone related Protein
HPA	-	Hypothalamic Pituitary Adrenal
KDIGO	-	Kidney Disease Improving Global Outcomes
TNF	-	Tumour Necrosis Factor
mRNA	-	messenger Ribinucleic Acid
Th	-	T-helper
NO	-	Nitric Oxide
GBD	-	Global Burden of Diseases
WHO	-	World Health Organisation
NCD	-	Non-Communicable Diseases
ROS	-	Reactive Oxygen Species
RAAS	-	Renin Angiotensin Aldosterone System
ECG	-	Electrocardiography

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ACC	-	American college of Cardiology
CHF	-	Congestive Heart Failure
LDL	-	Low Density Lipoprotein
HDL	-	High Density Lipoprotein
HRP	-	Horseradish Peroxidase
TMB	-	Tetramethylbenzidine
SPSS	-	Statistical Package for Social Sciences
IHD	-	Ishchemic Heart Disease

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ABSTRACT

Background: Chronic kidney disease (CKD) is a serious public health issue. Leptin, a peptide hormone produced by the adipocytes is very important in the regulation of food and energy. Increased Leptin concentrations are seen in CKD, and have been observed to trigger further complications such as cardiovascular diseases with significant mortality. Despite the interrelationship between leptin and CKD, and their associated adverse health outcomes, the precise role of leptin in hypertensive heart disease and CKD is not fully known, and the few studies in this area have been inconsistent. .

General aim: This study aimed at evaluating serum leptin levels among CKD patients with hypertensive heart disease (HHD) attending the Korle Bu Teaching Hospital

Methodology: This is a cross-sectional study involving one hundred and eight (108) participants – seventy-two (72) CKD subjects and thirty-six (36) apparently healthy controls. Fasting venous blood samples were collected from the study participants and resulting sera, evaluated for leptin and other biochemical parameters. An independent-samples t-test was used to determine difference in clinical and biochemical parameters between study groups. Multiple regression analysis was conducted to identify predictors of serum leptin in the CKD and control groups.

Results: Results show significantly higher serum leptin levels among participants with CKD compared with the control group ($p < 0.0001$). In the CKD group, being at stage 5 made the largest unique contribution (beta = 0.37, $p < 0.0001$) to the variance in serum leptin levels, followed by HDL (beta = 0.269, $p < 0.0001$), FBG (beta = 0.267, $p = 0.001$), HHD diagnosis of more than 6 years (beta = -0.217, $p = 0.020$), systolic BP (beta = 0.201, $p = 0.030$), female gender (beta = 0.191, $p = 0.006$), Body Mass Index (BMI) (beta = 0.18, $p = 0.017$), and LDL (beta = 0.177, $p = 0.037$). In the control group, female gender made the largest unique contribution (beta = 0.709, $p < 0.0001$)

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followed by BMI (beta = 0.341, p < 0.0001), and eGFR (beta = -0.222, p = 0.011). **Conclusions**: Serum leptin levels were significantly higher among CKD subjects co-burdened with HHD in Accra. Stage 5 CKD was the most significant predictor of serum leptin. These findings underscore the role of leptin in the biochemical complexities observed in CKD subjects looking at the physiological functions of leptin.

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CHAPTER ONE

1.0 BACKGROUND

1.1 Introduction

Chronic kidney disease (CKD) is one of several medical conditions that is affecting millions of people worldwide (Di Angelantonio *et al.*, 2007). This disease condition represents a depreciation in the filtration rate of the glomerulus (GFR) that is irreversible and progressive (Webster *et al.*, 2017). The lack of specific treatment and the relentless progress to end stage kidney disease (ESKD) in most chronic nephropathies has resulted in the increasing prevalence of CKD worldwide (Webster, *et al*., 2017). Predisposing factors to CKD include diabetes, hypertension and cardiovascular diseases, among others (Iribarren *et al*., 2007; Parhami *et al*., 2001).

The limited data on CKD emanating from countries including Ghana, Senegal, Tanzania, and the Democratic Republic of Congo, though being of varied quality, have indicated prevalence's ranging from 5% to 17%. These prevalences are also synchronous with low levels of awareness (Stanifer *et al*., 2015). In addition to the ill-preparedness of most of these countries to managing the cardiovascular consequences of CKD, they also fail at providing access to renal replacement remedies (Katende *et al*., 2015).

Hypertensive heart disease (HHD), which encompasses heart failure, ischemic heart disease, and left ventricular hypertrophy, is the leading culprit in high blood pressure-associated mortality (Sharp *et al.*, 2008). HHD, a consequence of chronic hypertension, has the following as its hallmark: anatomical and physiological alterations in the myocardium without the presence of other primary anomalies in the cardiovascular system depicting a collection of responses from target organs, ranging from systolic and diastolic dysfunctions, left ventricular hypertrophy

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(LVH), as well as their resultant clinical presentations (arrhythmias and heart failure) (Drazner 2011). The risks of developing heart failure is increased by two folds if hypertension occurs in men, and by three folds, if it occurs in women (Levy *et al.,* 1990). In women, the occurrence of hypertension could additionally result in more than two folds increased risks for developing acute myocardial infarction, as well as stroke (Levy *et al.,* 1990). Besides its probable effects on the heart, chronic hypertension could in addition, potentially damage peripheral arteries, the eyes, and even the kidneys (Berk *et al*., 2007). Despite the fact that elevated blood pressure starts the chain reaction, neuro-hormonal factors, predominantly, the renin-angiotensin system, are mainly implicated in the remodeling of the geometry of the cardiac chamber and walls (Susic *et al*., 2007).

Leptin, a peptide hormone of energy expenditure, is essentially produced by adipocytes (mainly, white adipocytes, and less frequently, brown adipocytes) and also by gastric chief cells, bone marrow, ovaries, mammary epithelial cells, the lower part of the stomach's fundic glands, skeletal muscle, and placenta (Pan *et al*., 2014). Leptin inhibits hunger by regulating the balance of energy (Brennan *et al*., 2006; Halaas *et al*., 1995). It operates through hypothalamic arcuate nuclear receptors (Brennan *et al*., 2006). Obesity can result from a reduced sensitiveness to leptin (Pan *et al*., 2014). Primarily, leptin regulates fat stores, but owing to the fact that it is produced by non-fat cells too, and that many other types of cells besides cells of the hypothalamus express receptors for leptin, it is evident that leptin is involved in other physiological processes also, but these secondary functions are yet to be precisely elucidated. In the blood, leptin could either be complexed to proteins or be freely circulating (Considine *et al.,* 1996).

Not many studies have investigated serum leptin levels with CKD. Few reported studies have the leptin-cardiovascular parameter (Singhal *et al.,* 2002) or the leptin-cardiovascular disease associations to be positive (Wallace *et al*., 2001; Reilly *et al*., 2004). Others have demonstrated

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contrary findings (Iribarren *et al*., 2007). Okpechi *et al* from South Africa established an inverse association between the levels of leptin in plasma and that of eGFR (Okpechi *et al., 2007).* Furthermore, experimental studies in animals suggest that increased levels of leptin may cause hyperglycemia, blood pressure elevations, and loss of renal function (Carlyle *et al*., 2002). In rat models, natriuresis has been shown to be induced by increased leptin levels with the goal of maintaining the sodium-water balance, which as a consequence increases pressure in the arteries (Jackson and Li, 1997). Leptin has also been shown to be involved in the activation of Transforming Growth factor (TGF)-beta cofactor, which promotes the proliferation of endothelial cells of the kidney, and could potentially be involved in renal glomerulosclerosis (Wolf & Ziyadeh, 2006; Wolf *et al*., 2002). This study aimed to investigate serum leptin levels and their relationship with CKD in patients at the Korle-Bu Teaching Hospital.

1.2 Problem statement

Uremic factors, including, leptin, could negatively interact with biological functions (Vanholder *et al.*, 2003). Reported to be higher in concentration among CKD sufferers (Nordfors *et al*., 1998; Merabet *et al*., 1997; Heimbürger *et al*., 1997), leptin is significantly associated with cardiovascular problems (Iribarren *et al*., 2007), a leader in CKD mortality (Tesar, 2003). Advances in technological and medical innovations in the past two decades for treating CKD has not translated into an improvement in survival rates of CKD sufferers (Shankar *et al*., 2010). Classical CKD risk factors provide only a partial explanation for the high mortality rates observed among CKD sufferers. Mortality due to hypertensive heart disease remains very high among CKD patients, and this needs to be minimized (Vanholder *et al*., 2003). This context is really serious, as CKD could exclusively cause hypertension (Vanholder *et al*., 2003). Nonetheless, the concept of leptin and CKD has not been explored much, hence limiting pharmacological interventions that

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could reduce blood leptin levels. Also, diet control and physical exercise are recommended for those with leptin resistance, but CKD sufferers are usually too weak to exercise, so they are normally placed on strict diets. The recommended approach to helping clear such a high molecular weight protein as leptin is to use high-flux permeable membrane for the dialysis. In spite of the emphasis on the employment of high-flux permeable membranes and the crucial importance of using such membranes, many health facilities utilize low-flux permeable membranes (Tattersall *et al*., 2007) which cannot clear leptins.

If leptin levels are positively correlated with eGFR, then synthetic leptin analogs can be used to correct the low levels (FDA, 2014). If leptin levels are negatively correlated with eGFR, then high flux haemodialysis would be recommended to help clear a high molecular weight protein like leptin (Vanholder *et al.*, 2003). If the efficiency of hemodialysis is not adequate, the level of blood toxins and the clinical symptoms of the patient are not controlled and can lead to either an increase in the duration of each dialysis session or the frequency of necessary dialysis per week. This will consequently increase the mortality and morbidity of the patients and the cost of dialysis (Tramadon *et al*., 2014).

Blood leptin levels in CKD patients with or without hypertension has not been established in the Ghanaian population and therefore specific treatment options as stated above cannot be recommended. There is therefore the need to get baseline data to help drive treatment options.

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1.3 Justification

Given the adverse health outcomes for leptin and CKD (Heimbürger *et al*., 1997; Merabet *et al*., 1997; Nordfors *et al*., 1998), and the implication of both leptin and CKD in CVD, mortality, and the high economic burden on countries (Parhami *et al*., 2001; Iribarren *et al*., 2007), it is necessary to further explore the role that leptin plays among subjects with CKD and/or hypertensive heart disease. Few studies that have evaluated the relationship between leptin and CKD have reported inconsistent outcomes (Merabet *et al*., 1997; Wahba & Mak, 2007; Nordfors *et al*., 1998; Heimbürger *et al*., 1997). In Ghana, despite the high burden of the disease, there is a paucity of information on its epidemiology. Moreover, classical CKD risk factors provide only a partial explanation for the high mortality rates observed among CKD sufferers. These limitations have made it difficult to improve pharmacological regimens and enhance prognosis in CKD sufferers. It is therefore necessary to explore other factors that could play a role in prognosing the CKD state of patients. The study therefore purported to provide baseline data on the the interaction between CKD and leptin. It is anticipated that findings from this study to fill knowledge gaps, improve insights, and contribute to better outcomes among CKD sufferers in Ghana and beyond.

1.4 General aim

The study aimed to evaluate serum leptin levels among CKD subjects with hypertensive heart disease (HHD) attending the Korle Bu Teaching Hospital (KBTH).

1.5 Specific Objectives

i. To determine serum leptin levels among CKD and apparently healthy individuals using ELISA.

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ii. To compare clinical and biochemical parameters among CKD patients with HHD and apparently healthy individuals using statistical tools.

iii. To determine factors that predispose to increasing serum leptin levels among CKD subjects with HHD and apparently healthy subjects.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Leptin

2.1.1 Leptin structure

Kiess (1997) identified leptin to be a part of the most important hormones derived from adipose tissue. It is a 16 kDa molecular weight hormone and contains one hundred and sixty seven amino acids, and is a key element in intake and expenditure of energy, including appetite regulation and metabolism (Ahima & Flier, 2000; Friedman & Halaas, 1998).

In humans, the locus of the gene that encodes the leptin hormone is found on chromosome 7. The leptin hormone is elongated, bearing the following approximate dimensions: $20 \times 25 \times 45$ Å and is made up of four alpha helices – A, B, C, and D – to which two long cross over links (AB and CD) as well as a short BC loop are connected, arranged in a left-hand twisted helical bundle, which takes an up-up-down-down fold that forms a two-layer packing of antiparallel helix pairs A and D against B and C (Zhang *et al*., 1997). As noted earlier, in the blood, leptin could either be freely circulating or complexed to proteins; a soluble form of the receptor for leptin could be a part of such plasma binding proteins. It is theorized that lean individuals have a large proportion of their leptin circulating in the leptin-protein complex form, whereas in obese individuals, it is the free form that dominates (Considine *et al.*, 1996).

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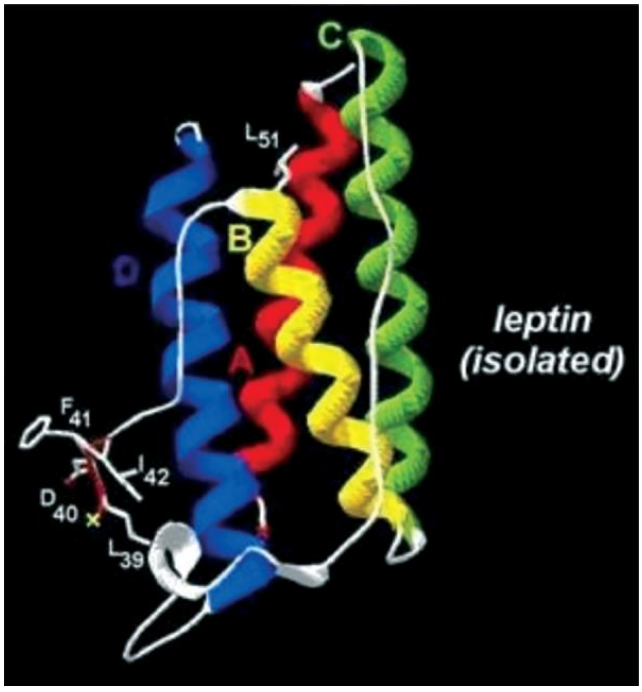

Figure 1: Tertiary leptin structure (Zhang *et al*., 1997)

2.1.2 Leptin, its action and function

Leptin elicits its primary responses in the brain, specifically, the hypothalamus; it also has targets other than the hypothalamus, called peripheral targets. The interactions in each of these targets vary in their importance (Margetic *et al*., 2002). Their responses are also dependent on the physiologic state and the species involved (Margetic *et al*., 2002). These responses are facilitated by the binding of leptin to its receptor, a single-transmembrane-domain type I cytokine receptor that is present on different cell types, particularly of the human body. It indirectly modulates the effects of such ligands as growth hormone, insulin-like growth factor, insulin, glucocorticoids, glucagon, metabolites, and cytokines, through its interaction with other hormones and energy regulators (Margetic *et al*., 2002).

Leptin plays its cardinal role in the modulation of the use of food energy, energy balance, physical exercise, and hunger, to regulate adipose tissue mass via its effects on the hypothalamus. In its

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peripheral targets, leptin plays a role in the activation of immune cells, energy expenditure regulation, regulation of permissive factor in puberty, growth factor activation, modulation between maternal and fetal metabolism, and the activation of beta islet cells (Doherty *et al;* 2013). In humans, cognitive changes associated with Alzheimer's disease, depression, and anorexia have all been linked to the occurrence of low levels of circulating blood leptin (Lieb *et al*., 2009). There are evidences from studies that use transgenic mouse models to provide insights on Alzheimer's disease that suggest that it is possible to improve brain pathology and cognitive performance through long-term leptin administration, which decreases two defining characteristics of Alzheimer's Disease (beta amyloid and hyper-phosphorylated Tau) (Doherty *et al.,* 2013).

The choroid plexus is believed to be the route through which leptin enters the brain; this is as a result of the high level expression of a receptor that closely resembles the receptor for leptin, which is thought to act as a transport mechanism (Lynn *et al*., 1996). Increased melatonin levels downregulate leptin, but seems to do the reverse in the presence of insulin, thus decreasing appetite during sleep. Another phenomenon linked with reduced leptin levels is partial deprivation of sleep (Copinschi *et al*., 2005).

2.1.3 Leptin and the hypothalamus

When bound to its receptors present on the lateral part of the hypothalamus, leptin activates hunger inhibition, but activates satiety when bound to receptors in the medial part of the hypothalamus and achieves hunger inhibition through the lateral hypothalamic receptors by virtue of its antagonistic effects on the potent hunger stimulant called neuropeptide Y (NPY), secreted by gut and hypothalamic cells (Elias *et al.,* 1999).

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Additionally, leptin antagonizes anandamide, whose activity is similar to neuropeptide Y and targets receptors which are also targets of tetrahydrocannabinol (THC). To achieve hunger inhibition in the medial part of the hypothalamus, leptin facilitates production of the hunger suppressant identified as alpha-MSH (Fekete *et al*., 2000).

Thus anorexia is caused by a lesion in the lateral part of the hypothalamus, whereas excessive hunger is the result of a lesion in the medial part; this type of appetite inhibition has long term effects, differentiating it from both rapid hunger inhibition that is modulated via cholecystokinin (CCK), as well as a PYY3-36-mediated slower hunger inhibition between meals (Mars *et al*., 2006). Hence when leptin or its receptor is absent, or if there is a defect in the receptor, uncontrolled hunger, and consequently, obesity could result. The levels of leptin are lowered when an individual takes in diets that are highly deficient in calories, or fasts, but are less contingent on increasing food intake than they are on decreasing food intake. Notably, leptin dynamics in response to acute energy balance changes could have a connection with appetite and subsequently, food intake, but not fat stores (Williams *et al*., 2009). In the arcuate nucleus, leptin interacts with NPY neurons to downregulate their activity. It signals the hypothalamus to generate a feeling of satiety. In addition, its signals could ease the desire of taking in high-calorie diets (Baicy *et al*., 2007).

Leptin recognizes six receptor types (LepRa-LepRf or Ob-Ra–Ob-Rf), products of the same gene, LEPR (Wang *et al*., 1996). Ob-Rb, the only receptor isoform capable of intracellular signaling through the Jak-Stat and MAPK pathways, is present in hypothalamic nuclei (Malendowicz *et al*., 2006). Upon binding the Ob-Rb receptor, leptin activates stat3 through downstream phosphorylation, which elicits alteration in gene expression (such as repressing the

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expression of endocannabinoids, whose effect is seen in increment in hunger), following its migration to the nucleus (Di Marzo *et al.*, 2008).

2.1.4 Leptin and circulatory system

The role of leptin and its receptors in regulating the activities of T lymphocytes has been demonstrated in experiments involving mice: they mediate the immune response to atherosclerosis, a condition which is significantly linked with obesity (Knight *et al.*, 2009).

Exogenous leptin has the capacity of promoting angiogenesis through causing an increase in the titre of vascular endothelial growth factor. Hyperleptinemia resulting from infusion or adenoviral gene transfer is known to reduce rat blood pressure (Taleb *et al*., 2007; Zhang *et al*., 2010).

Microinjections of leptin into the solitary tract nucleus potentiate the cardiovascular response to activation of the chemoreflex, as well as effect sympatho-excitatory responses (Ciriello *et al.*, 2012).

2.1.5 Leptin and fetal lung

In the lungs of fetuses, the formative alveolar epithelium of the alveolar interstitial fibroblasts secretes PTHrP during conditions of moderate stretch to induce leptin production, which subsequently gets bound to the leptin receptors expressed on the epithelia of the alveolar type II pneumocytes; this leads to an induction of surfactant expression (Torday & Rehan, 2006).

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2.1.6 Leptin and the ovulatory cycle

Leptin is significant for fertility in mice, and by extension, in humans. In females, the ovulatory cycle is more connected with energy balance and flux than it is with energy status and thus negative energy balance and high energy flux causes the ovarian cycle to stop, and menstruation ceases and the energy status only affects menstruation if the body fat percentage of the female is very low (Anifandis *et al*., 2005). It is reported that if leptin levels fall outside a certain acceptable range, the quality of eggs and outcomes during *in vitro* fertilization are negatively affected (Anifandis *et al*., 2005). A key way through which leptin exerts its influence on reproduction is through the stimulation of gonadotropin-releasing hormone from the hypothalamus (Comninos *et al*., 2014).

2.1.7 Leptin and the immune system

Factors that acutely influence the levels of leptin also have bearings on inflammation markers; these include emotional stress, sleep, testosterone, levels of body fat, and caloric restrictions. It has been hypothesized that leptin regulates inflammatory responses by responding to inflammatory cytokines derived from adipose (Caldefie-Chezet *et al*., 2001). Leptin is considered to be a part of the superfamily of cytokines, as it resembles interleukin-6 in structure, as well as function (Fantuzzi *et al*., 2000). Owing to its seeming effect on the HPA axis, leptin is additionally thought to be involved in stress responses and in both males and females, high concentrations of leptin have been linked with increases correspondingly, in white blood cell counts (Mabuchi *et al*., 2005). Just as is the case during chronic inflammation, consistently high leptin levels are connected to overeating and obesity, and also such inflammation-linked diseases as CVDs, metabolic syndrome, and hypertension. Interestingly, despite the connection between leptin and body fat mass, it is not affected by exercise, hence the speculation that leptin reacts particularly to inflammation that is

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adipose-derived. It is a pro-inflammatory, pro-angiogenic, and also mitogenic factor, whose actions are strengthened via crosstalk with IL-1 family cytokines in cancer (Perrier *et al*., 2009). Intake of calories results in rises in the levels of leptin, which in this instance, serves as an acute inflammatory response mechanism that forestalls extreme cellular stress caused by overeating. When high intake of calories exceeds the ability of fat cells to swell in size and number proportionally to the amount of calorie taken, cellular inflammation and ectopic fat storage results. In response to the excess calories, insulin concentration increases, effecting a proportional increase in leptin levels; this response is potentiated by elevated levels of cortisol (LaPensee *et al*., 2008). It has been observed too that administration of acipimox, in a bid to prevent lipolysis, results in a marginal rise in leptin levels, regardless of synchronous intake of low-calorie diets and loss of weight (Worm *et al*., 2000).

A small proportion of the human population are homozygous for mutants of the gene that encodes leptin, and hence constantly desire food, so consequently become obese. In a study involving such individuals, leptin half-life was found to be 9.4 ± 3.0 minutes, and its rate of production was 3.6 ± 1.2ng/100 g fat/min (Margetic *et al*., 2002). The kidney of humans takes up and degrades leptin, thus playing a key role in removing leptin from plasma; this uptake is estimated to explain ~80% of the leptin withdrawn from blood (Meyer *et al.*, 1997). Quite logically then, kidney pathologies would be expected to translate to high titres of leptin in the plasma.

2.1.8 Role of leptin in CKD

CKD is a set of abnormalities of renal structure and/or function, present for ≥3 months, with association to health (National Kidney Foundation. KDIGO, 2012). In Sub-Saharan Africa, CKD burden has been estimated to be 13.9%, a prevalence that is higher than the global prevalence of

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10% (Stanifer *et al.*, 2015). The CKD prevalence in many Sub-Saharan African countries could be comparable to, or be higher than, those of countries with higher income (Stanifer *et al.*, 2015). In one study in Ghana, 5% of patients admitted to hospitals had renal disease, and of these, 27% died from renal failure (Collins, 2012). In Ghana, most CKD sufferers fall within the age group of 20 to 50 year olds, which represents the most economically productive group (Osafo *et al.*, 2011).

Leptin, a part of interleukin-6 family of pro-inflammatory cytokines, is eliminated from circulation by the renal system through glomerular filtration and metabolic break down in the kidney tubules (Mak *et al*., 2006). As what could be a mechanism to compensate for the decreased capability of the aberrant kidney in clearing leptin from circulation, in the adipose tissues of CKD sufferers, the expression of the gene that encodes leptin is lower (Wahba & Mak, 2007). In CKD sufferers, the leptin concentration in the blood is positively correlated with that of C-reactive proteins, suggesting that inflammation could be complicit in hyperleptinemia and this is corroborated by a Kalantar-Zadeh *et al*. work in 2004 which reported inflammatory cytokines to be linked with anorexia in patients on dialysis. Furthermore, in CKD patients, amendment of metabolic acidaemia, a possible stimulant of ubiquitin-proteasome induced cachexia, is associated with increased serum leptin levels (Zheng *et al*., 2001; Kalantar-Zadeh *et al*., 2004). Nonetheless, many researchers have contradicted this with reports that suggest that higher concentrations of serum leptin is inversely related to improved nutritional status and outcome in CKD (Don *et al*., 2001; Pecoits-Filho *et al*., 2003). Leptin, just as serum albumin, is also a negative acute phase reactant in CKD patients (Don *et al.*, 2001). Scholze *et al* in 2007, after spending close to seven years studying 71 patients undergoing hemodialysis, demonstrated that the minimum blood levels of leptin were lower in dead patients compared with that of survivors. There was a higher risk of

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mortality among patients with leptin concentrations below the median value. This shows leptin could be yet another so-called "counterintuitive" predictor of death in CKD.

Serum levels of leptin are increased in CKD sufferers primarily as a result of decreased clearance by kidneys. Leptin is not cleared by dialysis using conventional dialysers because it is a 16 Kda protein. However elevated serum leptin is not universally present in severe renal failure (Sanjay *et al.,* 2002). Increased leptin levels are reversed following successful kidney transplantation (Briley & Szczech, 2006). Leptin, insulin levels and body weight are interrelated; concentration of insulin is noted to correlate positively with leptin concentration, regardless of uremic status of patients and long term insulin infusion acts as a stimulant of leptin secretion in humans (Mantzoros *et al*., 2011; Leury *et al*., 2003). It has also been reported that such cytokines as interleukin-1 (IL-1) and tumor necrosis factor (TNF) induce increases in the concentration of leptin messenger RNA (mRNA) and anorexia in animals (Yuen *et al*., 2002). It can thus be inferred that increases in the concentration of leptin could account for one of the mechanisms chronic inflammation induces anorexia.Th1-mediated immune response is promoted by leptin and unregulates the synthesis of inflammatory cytokines in human monocytes (Lord *et al*., 1998). Continuous inflammation is a frequently encountered phenomenon in CKD sufferers, and is a predictor of untoward outcome (Stenvinkel *et al*., 2005). It must be mentioned though that there is few information on the relationship between leptin and metabolic syndrome, its link with clinical outcomes in CKD sufferers undergoing dialysis, as well as its involvement in the early stage of CKD. Yet, it is evident that an relationship between leptin levels and CKD is plausible, as its roles in the activation of the sympathetic nervous system and chronic blood pressure elevations, as well as in renal dysfunction have been elucidated (Carlyle *et al*., 2002).

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El Meligi *et al* in 2003, evaluated the association between fasting serum leptin levels and the stage of diabetic nephropathy among patients with type 2 diabetes.

Scholze *et al*. (2007) reported that in haemodialysis patients, low levels of leptin significantly predicted mortality; by inference, in patients undergoing haemodialysis, high levels of leptin could predict favourable outcomes. In an earlier study among haemodialysis patients, being obese was associated with higher survival rates (Kalantar-Zadeh *et al*., 2006). This is a classic example of reverse epidemiology in which factors that could otherwise predispose to unfavourable health outcomes contrastingly predict better outcomes in individuals suffering from chronic diseases. In a study conducted in Netherlands, however, no such survival advantage was observed among obese haemodialysis patients in relation to leptin levels (de Mutsert *et al*., 2007). It is noteworthy though, that in both studies (Kalantar-Zadeh *et al*., 2006 and de Mutsert *et al*., 2007), the leptin levels of patients at earlier CKD stages were not factored in the equation.

2.1.9 Leptin and hypertension

In conditions of good health, administration of leptin on a short term basis exerts no net effect on blood pressure. To illustrate, it has been reported that among lean animals, although leptin activates the sympathetic nervous system, the response is balanced by NO-dependent vasorelaxation and natriuresis, hence effecting no changes in blood pressure (Wallace *et al*., 2001). Contrastingly, chronic hyperleptinemia causes an increase in BP as acute depressor effects are weakened and/or further sympathetic nervous system-independent depressor effects appear, such as oxidative stress, nitric oxide deficiency, increased renal Na^+, K^+-ATPase activity, and Na^+ reabsorption and increased production of endothelin (Bełtowski *et al*., 2006). Blood leptin levels were higher in male subjects who subsequently had first-ever myocardial infarction than that of control subjects

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(Söderberg *et al*., 1999). High levels of leptin in the plasma was shown to predispose to coronary activities in males during a five-year follow-up period (Wallace *et al*., 2001).

2.2 Cardiovascular conditions

In Ghana, CVDs were the most implicated NCDs in deaths (institutional deaths inclusive), accounting for 14.5% of the total number of deaths recorded in the year 2008 (Bosu, 2012). It is estimated that among those aged between 30 and 70 years in Ghana, 20% are likely to die of chronic respiratory disease, diabetes, cancer, or CVD (WHO 2015).

Factors that predispose to CVDs are usually interrelated, and encompass history of heart disease in the family, lack of exercise, overweight or obesity, diabetes, elevated blood cholesterol, smoking, elevated BP, and age (Yusuf *et al*., 2014). Worldwide, approximately 13% of CVD-related deaths resulted from hypertension, 9% from diabetes, 6% from physical inactivity, and 5% from obesity and overweight (Bosu, 2012).

2.2.1 Hypertensive Heart Disease

In Ghana, in the year 2013 alone, hypertensive heart disease (HHD) accounted for 1.07 million deaths, thus increasing by almost two folds in comparison with the figure recorded in the year 1990 – 630,000 (GBD, 2013). In one systematic review, the prevalence of hypertension was reported to be 19.3% and 54.6% respectively in rural and urban areas in Ghana (Addo *et al*., 2012). It is noteworthy though that a considerable proportion of hypertension cases go undiagnosed in the country. To illustrate, in a WHO study involving participants from urban communities in Ghana, just 7.4% of hypertensive participants were aware of their condition (WHO, 2015). In fact, among those with hypertension in Ghana, less than 5% control their blood pressures (Awuah *et al*., 2014).

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HHD is caused by high blood pressure. The WHO has identified CVDs as one of the top two (WHO 2014) causes of death in Ghana after diarrheal illnesses (WHO 2013).

The pathogenesis of HHD is known to involve all of the heart's components, namely, coronary vessels, fibrillary collagen, proteins of the extracellular matrix, and such myocytic and non-myocytic cells as endothelial cells and fibroblasts (Susic *et al*., 2007). The widely accepted theory of HHD is that LVH is a response that compensates for increased stress on the walls of the heart, and is succeeded by a dilation of the left ventricles, plus a progressive marginal reduction in ejection fraction, and eventually culminating in dilated heart failure (Drazner, 2011). Nonetheless, the adaptive response, such as LVH pattern (which is believed to be associated with such interrelated factors as differences in neurohormonal responses, genetic influences, and pressure loading) significantly varies from person to person (Drazner 2011). Development of diastolic dysfunction and/or heart failure with normal ejection fraction (HFNEF), which is related to extracellular matrix remodeling and an elevation in left ventricular filling pressures, has increasingly been reported in individuals suffering from HHD (Berk *et al*., 2007).

Remodeling of HHD structurally, is defined by enlarged cardiac myocytes with changed energy metabolism, fibroblast-myofibroblast transformation, fibroblast multiplication and activation, and excessive collagen deposition, all of which result in a more rigid myocardium (Lip *et al*., 2000; Levick *et al*., 2006). Coronary resistance vessels as well, are affected, perivascular fibrosis of intramyocardial heart arteries and arterioles yield intimal-medial thickening (Schwartzkopff *et al*., 1992). One of these essential processes, owing to its contribution to the development of hypertension as well as acceleration of hypertension-associated organ damage, through vascular and cardiac remodeling, vascular inflammation, reactive oxygen species (ROS) generation, and vasoconstriction is the activation of renin-angiotensin-aldosterone system (RAAS) (Sun *et al*.,

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1993; Ronald *et al*., 2012). Moreover, the angiotensin converting enzyme (ACE) produces angiotensin II (Ang II), which correlates to left ventricle hypertrophy (LVH). The ACE gene is highly polymorphic – some individuals have deletions in the gene, and others, insertions – and this translates to a variation in plasma ACE levels, and consequently risks for left ventricle hypertrophy among various individuals (Lip *et al*., 2000).

2.2.2 Left ventricular hypertrophy (LVH)

LVH is a major response to maladaptive chronic pressure overload and also a significant risk factor in hypertensive subjects. LVH development is highly linked with hypertension (systolic). From the Framingham Heart Study, small isolated increase in systolic hypertension at an elderly age was associated with higher thickness of walls of the left ventricles and impaired diastolic filling (Sagie *et al*., 1993). It is now recognized that LVH is modulated by the mechanical stress of pressure overload and also various neurohormonal substances that independently put trophic effects on myocytes and nonmyocytes in the heart (Post *et al*., 1994). Hypertensive patients of Mexican-Hispanic, Caribbean, or African American descents are each associated with a higher incidence of LVH, and, hence it becomes necessary for clinicians to suspect and screen for LVH in such individuals (Sharp *et al.,* 2008). Various studies have also noted that these categories of individuals additionally have greater left ventricular mass than white patients with comparable blood pressures. The most difficult patients to identify are those with "masked" hypertension, as they react in an opposite way to individuals with "white coat" hypertension – such individuals are observed to have overwhelmingly high systolic blood pressure when engaging in their daily routines, but appear to have normal blood pressures when they sit in the consulting rooms of doctors (López *et al*., 2007). Hence during performance of their daily routines, the afterloads of their hearts are increased, and this stimulates the development of hypertrophy.

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Markers for "masked hypertension" include abnormal EKG, S4 gallop, as well as arteriolar thickening on funduscopic examination; exercise testing could also provide a presumptive diagnosis; all these could be confirmed through the monitoring of ambulatory blood pressure (Grandi *et al.*, 2001). ECG is a highly specific means for LVH diagnosis. Hypertrophied myocardia provide larger myocardial mass for passage of electrical activation, i.e., the amplitude of the QRS complex, representing ventricular depolarization, becomes higher. Similarly, abnormally thickened myocardia increases the duration with which electrical activity traverse the heart in its entirety, and therefore could widen the duration of the QRS complex – identified as "LVH with QRS widening". Repolarization could also be strained under conditions of myocardial hypertrophy, through parallel mechanisms, which could result in aberrant ST segments or T waves – identified as "LVH with strain" or "LVH with repolarization abnormality" (Sun *et al.*, 2015). These anomalies in repolarization could occasionally resemble changes in ischemic ST, significantly complicating their distinction from those observed in conditions of myocardial infarction (Kligfield *et al.*, 2007). The regular pattern observed in LVH includes a departure of the ST segment from the QRS complex (discordance), and a typical T wave inversion pattern is present (Kligfield *et al.*, 2007).

2.2.3 Congestive heart failure

A frequently encountered complication of chronically elevated BP is heart failure. From the American College of Cardiology (ACC)/American Heart Association (AHA), patients with hypertension fall into either of the following groups: asymptomatic, but at a higher chance of developing heart failure – Stage A or B, and contingent on the patients' development of structural heart disease resulting from hypertension and suffering from Stage C or D symptomatic heart

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failure. Hypertension usually goes unrecognized as the etiology of CHF, partly due to the fact that at the time of the development of heart failure, the defunct left ventricle fails at generating the elevated blood pressure. Up to 33% of asymptomatic diastolic dysfunction in hypertensive patients without LVH could exist. Chronically increased afterload and the resultant LVH could adversely influence the active early relaxation phase and the late compliance phase of ventricular diastole (Sun *et al*., 2015).

2.2.4 Diastolic dysfunction

Diastolic dysfunction is a ubiquitous phenomenon in hypertensive individuals. It is frequently, but not consistently, concurrent with LVH. Besides elevated afterload, probable contributory factors to diastolic dysfunction development include structural anomalies like fibrosis and LVH, systolic dysfunction, aging, and synchronous coronary artery disease. Diastolic dysfunction, when it occurs, precedes asymptomatic systolic dysfunction.

Early left ventricular (LV) diastolic disharmony could be associated with remodeling of the left ventricles, and could contribute to LV diastolic dysfunction in hypertensive patients (Sun *et al*., 2015). The degree of diastolic dysfunction seemingly correlates with accretionary severity of hypertension; peak rate of myocardial systolic strain could independently determine the degree of LV remodeling and diastolic function (Sun *et al.*, 2015).

2.2.5 Systolic dysfunction

Later in the course of disease, the LVH is unable to requite the increased blood pressure with an increased cardiac output, and the left ventricular cavity initiates dilation, so cardiac output could be sustained. There is a further decrease in the systolic function of the left ventricle as the disease

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progresses to the end stage. This results in further increased activation of the neurohormonal and renin-angiotensin systems, increasing peripheral vasoconstriction and salt and water retention as a consequence. Subsequently, the already endangered left ventricle becomes overwhelmed; the end result is that the patient progresses to the symptomatic systolic dysfunction stage.

2.2.6 Decompensation

Apoptosis (also referred to as programmed cell death), triggered by myocyte hypertrophy and its stimulant-inhibitor imbalance, is assumed to play a key role in the switch from compensated to decompensated stage. As a result of alterations in afterload conditions or the presence of other myocardial challenges (e.g., ischemia, infarction), the patient could develop symptoms in the course of the asymptomatic stages of the LV systolic or diastolic dysfunction. Any abrupt elevations in blood pressure could result in acute pulmonary edema which does not inevitably alter the LV ejection fraction (Gandhi *et al*., 2001).

Normally, the occurrence of asymptomatic or symptomatic LV dilatation or dysfunction promotes rapid deterioration in clinical status and a markedly elevated death risk. Besides LV dysfunction, the right ventricles also thicken, and diastolic dysfunction develops due to septal thickening and LV dysfunction.

2.2.7 Ischemic heart disease

Hypertension increases the risks of coronary artery disease (ischemic heart disease) development by almost two folds, and this could be attributed to several factors. A key factor is that angina could occur in hypertensive patients, regardless of the non-existence of epicardial coronary artery disease. Two reasons account for this. Enhanced afterload secondary to hypertension causes an increase in LV wall tension and transmural pressure, compromising coronary blood flow during

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diastole. Also, the microvasculature beyond the epicardial coronary arteries is dysfunctional in hypertensive patients, and could not be able to make up for increased metabolic and oxygen demand (Sun *et al*., 2015).

2.3 Leptin and obesity

There is a well-established relationship between mass of fat in the body and the amount of produced and secreted leptin (Liuzzi *et al*., 1999; Ostlund *et al*., 1996). Leptin is found to be an anti-obesity hormone. The first and major function of leptin to be described is the control of body weight and fat deposition by its effects on receptors in the hypothalamus, which causes appetite inhibition, and also its effects on metabolic rate stimulation and thermogenesis (Lönnqvist *et al*., 1996; Misra *et al*., 1996).

Increased levels of leptin are not enough of an interference to halt disturbances in energy balance in obesity, suggesting that obese people could be leptin-resistant (Seufert *et al*., 2004). Possibly, also, mechanisms not related to leptin play a more significant part than leptin under the physiological alterations in obesity. Obesity is related with enhanced sympathetic nerve activity, and leptin, partially by increasing sympathetic nerve activity in the kidneys, has been shown to take part in autonomic nervous system control. Therefore, the role of leptin to sympathetic activation in the leptin-resistant state, is contradictory. This leads to the novel concept of selective leptin resistance, in which resistance appears to be basically limited to the metabolic (weight losing and satiety) functions of leptin, leaving the other functions in obese individuals (Montani *et al*., 2002).

It has been postulated that leptin could be a significant link between obesity and the emergence of CVDs (Fekete *et al*., 2007). This may be mediated through several effects of leptin, including effects on blood pressure(Cooke *et al*., 2001), platelet aggregation (Chaldakov *et al*.,

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2001), formation of arterial thrombosis (Bełtowski *et al*., 2002), and inflammatory vascular response (Bodary *et al*., 2002). Increased levels of leptin is believed to be linked with decreased arterial distensibility, an index of cardiac and circulatory function, and leptin plays a role in the pathogenesis of the atherosclerotic process through processes other than vascular relaxation (Chu *et al*., 2000). Leptin has also been demonstrated to promote angiogenesis, regulate osteoblastic differentiation, increase the calcification of vascular cells, and potentiate prothrombotic platelet aggregation through a novel leptin receptor process (Chaldakov *et al*., 2001). Numerous studies have demonstrated leptin as a predictor of myocardial infarction, coronary events, and cardiovascular accident, independent of body mass index (BMI) (Ren *et al*., 2004).

2.4 Leptin levels and association with diabetes

Three hundred million people are estimated to be afflicted with Type 2 diabetes by 2020 (Muoio *et al*., 2008). Minimal studies have investigated a direct link between concentration of leptin and diabetes mellitus (Bandaru *et al*., 2011). Research has demonstated that blood leptin levels are mostly higher in subjects with diabetes mellitus except cases when the data is regulated for body mass index (Bandaru *et al*., 2011). Leptin can improve or conversely cause induction insulin resistance (Ceddia *et al*., 2002). It is also documented to play a role in the induction of pancreatic β cells to release insulin (Ceddia *et al*., 2002). Studies have shown that leptin has the ability to be useful in the treatment and management of diabetes that is lipoatrophic (Ebihara *et al*., 2001). Very interesting of note is the fact that obesity directly is linked with resistance to insulin and the occurance of diabetes mellitus in humans (Ceddia *et al*., 2002). Since obesity could be a result of leptin resistance, it means resistance to leptin is likely to play a role in the pathophysiology of the obesity. Leptin resistance, just as leptin deficiency, is very vital in the pathophysiology of severe

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resistance to insulin in uncontrolled diabetes type 1 (German *et al.*, 2010). Leptin signaling can, however, be restored in neurons by overexpression of anorexigenic peptides and or repression of orexigenic peptides (Aragonès *et al.*, 2016). Compounds in food such as, reservatrol, teasaponins, caffeine, celastrol and taurine are able to put back in place the neuronal leptin signaling by the use of repression or expression of these peptides (Aragonès *et al.*, 2016). It has also been reported that vitamins D and A helps in the transportation of leptin through the blood brain barrier (Aragonès *et al.*, 2016).

2.5 Leptin and lipids

Higher lipid stores in non-adipose tissues, such as muscle is linked to functional problems, called "lipotoxicity," which lead to insulin resistance and impaired insulin secretion (Unger *et al*., 1999). Although the lipid factor that leads to "lipotoxicity" is undentified, involvement of fatty acyl-CoA or diacylglycerol, acting via a type of protein kinase C, is suspected (Savage *et al.*, 2007). It has been shown that treatment with leptin reduces lipid pile up in non-adipose tissues and improves serious diabetes in lipodystrophy subjects and also rodents (Oral *et al*., 2002). Serum leptin levels are increased in obesity, being strongly associated with fat mass and BMI (Wauters *et al*., 2000). Animals genetically lacking in leptin, plus being hyperphagic, hypometabolic and obese, have a significant range of abnormalities in macronutrient breakdwon including disordered handling of carbohydrates, lipids and proteins. These include elevation of circulating free fatty acids, raised triglycerides, an increase in serum cholesterols and increased hepatic steatosis (Wiegman *et al.*, 2003; Silver *et al.*, 2000).

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2.6 GFR estimation – its value and concerns

In subjects living with chronic kidney disease, it is important that the GFR (measured as clearance of an ideal filtration marker from urine or plasma) is determined, as it is a marker of prognosis (Go *et al.*, 2004). It has been reported that individuals whose GFR falls below 60 ml per minute per 1.73 m^2 are at a higher risk of developing new and reoccuring cardiovascular disease, even in those of the general population, and are more likely to die from CVD than progression to kidney failure, hence the need to evaluate and treat such individuals for CVD risk factors (Sarnak *et al.*, 2003; Go *et al.*, 2004; Weiner *et al*., 2004). However, the use of exogenous markers in the measurement of GFR cannot be practiced as a routine in clinical settings, owing to its complexity and extremely high costs (Mohanram & Toto, 2005). Hence to ensure improvements in how CKD iis detected, evaluated, and managed, it is highly recommended that GFR be estimated through standardized equations (National Kidney Foundation, 2002; Levey *et al*., 2003; Levey *et al*., 2005; Sarnak *et al*., 2003). Two of such mostly applied equations are those of Cockcroft-Gault and the MDRD study, both of which are based on the marker creatinine (Sokoll *et al.*, 1994; Levey *et al*., 1999; Levey *et al.*, 2000). The Cockcroft-Gault equation is given as Creatinine clearance = [(140 – age) × ((weight) ÷ (72 × serum creatinine) × 0.85 (if the subject is female)]; the creatinine clearance is expressed in milliliters per minute; age is expressed in years; weight is expressed in kilograms; serum creatinine is expressed in milligrams per deciliter (Sokoll *et al.*, 1994). That of the MDRD study is as follows: GFR = 186 × $(\text{Serum creatinine})^{-1.154}$ × $(\text{age})^{-0.203}$ × 0.742 (if the subject is female) or × 1.212 (if the subject is black) (Levey *et al.*, 2000; Levey *et al*., 1999).

The challenge with the use of these equations is that in individuals without CKD, such as potential kidney donors and young type 1 diabetics with no microalbuminuria, these equations have been shown to have lower accuracy, probably as a result of determinants of serum creatinine that are

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not GFR related (Lin *et al.*, 2003; Rule *et al*., 2004; Froissart *et al.*, 2005; Ibrahim *et al*., 2005; Poggio *et al*., 2005; Stevens *et al.*, 2006). Some studies have asserted that the two equations produce similar outcomes (Gonwa *et al*., 2004; Ibrahim *et al*., 2005; Verhave *et al*., 2005). However, others have favoured the MDRD equation over the Cockcroft-Gault equation, reporting that the former is better in predicting GFR than the latter (Rule *et al*., 2004; Froissart *et al.*, 2005; Poggio *et al.*, 2005). In fact, the superiority of the MDRD study equation to the Cockcroft-Gault equation has been reported in older and obese individuals (Froissart *et al.*, 2005; Verhave *et al.*, 2005). Another marker, cystatin C, is considered as a plausible alternative to creatinine in estimating GFR, and is being investigated for such purposes (Rule *et al*., 2006; Grubb *et al*., 2010; Inker *et al*., 2012). That too however is distraught with its concerns. For instance, its serum levels are affected by coricosteroid use, age, gender, weight, smoking status, and C-reactive proteins levels, even when creatinine clearance is factored out (Knight *et al*., 2004). Still some studies have demonstrated that it is cleared extrarenally if its titers reach high levels (Grubb, 2000; Sjostrom *et al.*, 2005). Regardless of these controversies, GFR remains the gold standard for evaluating kidney function (Smith, 1951), and hence improvements in its determination are centainly of utmost importance.

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study site

This study was conducted at the Renal Dialysis Center of the Department of Medicine of the Korle-Bu Teaching Hospital (KBTH) in Accra. KBTH is a premier tertiary and referral hospital in Accra with a two thousand bed capacity and about three thousand members of staff. It serves the Accra Metropolis and the whole of the southern sector of Ghana. KBTH provides both inpatient and outpatient services to clients. The Renal Dialysis Unit is involved in the management of acute and chronic renal failures (including follow ups on transplant patients, performing epidemiological research, carrying out hemodialysis of patients, donor screening for kidney transplantation, kidney transplant, training facility for various health professionals, postgraduate and undergraduate students). About 3,000 patients with kidney related problems were seen at the renal clinic in 2013 and approximately 10% of all deaths on the medical wards of KBTH are due to CKD (2013 annual report, KBTH).

3.2 Study design and participants

This was a cross-sectional study, involving 72 subjects with CKD and 36 apparently healthy persons without CKD who were randomly recruited from the renal dialysis unit of KBTH during the period spanning from August, to October, 2018.

3.2.1 Inclusion criteria

Confirmed CKD subjects (eGFR < 60ml/min for > 3 months and/or having ultrasound scan showing small kidneys < 9cm on right and left kidney) with documented diagnosis of hypertensive heart disease (HHD) by a qualified specialist, and/or on chronic hemodialysis were recruited for this study.

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The control group (n=36) were apparently healthy participants without CKD or HHD.

3.2.2 Exclusion criteria

CKD patients on transplant, and with a history of mycobacterium tuberculosis, autoimmune disease, gout, allergies, malignancy, urinary tract infection and acute cardiovascular events were excluded from the study.

3.2.3 Minimum sample size determination

The minimum sample size was calculated using the Cochran's (1977) sample size formula shown below:

$$n = \frac{z^2 \times p\,(1-p)}{d^2}$$

Where *n* represents minimum sample size, z represents confidence level at 95% (standard value of 1.96), d represents acceptable error margin (5%), and *p*, the estimated prevalence of CKD in Ghana = 5% (Collins, 2012)

$$n = \frac{1.96^2 \times 0.05\,(1-0.05)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.05\,(0.95)}{0.05^2}$$

$$n = 72.9904 \approx 73$$

The minimum sample size was determined to be 73, but 72 subjects were recruited falling short of the minimum sample size by one participant. This was because the CKD sufferers co-burdened with HHD group were scarce. Also, the time frame for recruitment and limited resources did not allow us to engage a larger cohort.

3.4 Clinical and Laboratory Assessment

For all the participants recruited into the study, body height and weight were measured using a standard scale and a wall-mounted meter rule, to the nearest 1.0 kg and 0.005m respectively. These

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measurements were taken when the participants were without footwear and wearing light outfits. Body mass indices (BMIs) were calculated as weight/(height)^2 with the unit kg/m^2. Using an automatic cuff blood pressure equipment, the participants' blood pressures were measured after they had been in sitting position and rested for at least, 5-10 minutes.

Fasting venous blood samples (5ml) were obtained from all study participants. These samples were taken after an overnight fast (between 10-12hrs), from the antecubital vein of the forearm of participants, by a qualified phlebotomist, into serum separator tubes (4 ml). The remaining blood (1ml) was dispensed into a sodium fluoride tube for glucose estimation. Blood samples were collected between 7:00am and 9:00am each day.

A standard questionnaire (Appendix III) was used to collect data on socio-demographics and patient profile, such as age, gender, duration of condition, coexistence of other infectious and metabolic diseases, and family history of common metabolic diseases, and drug history.

3.5 Sample processing and Laboratory tests

Blood samples were processed quickly in the Laboratory by centrifuging at 4000rpm for 5minutes. Sera and plasma were checked for hemolysis. Sample that had undergone hemolysis were not processed further. The sera were put into Eppendorf tubes and stored at -20^{o}C for subsequent estimation of creatinine, HDL Cholesterol, total cholesterol, triglycerides, and total Leptin. The plasma obtained were used to estimate blood glucose concentration on the same day of blood collection.

3.5.1 Reagents and equipment

The reagents and equipment used in the biochemical analysis were:

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3.5.1.1 Reagents:

Creatinine, Direct HDL Cholesterol Triglycerides, Glucose and total Cholesterol slides (VITROS Chemistry Products, USA), and total Human Leptin by ELISA Kit (ALPCO® kit; USA).

3.5.1.2 Equipment:

VITROS 350 Chemistry Analyzer (VITROS Chemistry systems, USA),

URIT 660 microplate Reader (China).

3.5.3 Laboratory analysis

Fasting blood glucose (FBG), serum creatinine, high density lipoprotein (HDL) cholesterol, triglycerides (TG), and total cholesterol (TC) were assayed using standard auto-analyzer dry slides techniques (VITROS 350 Chemistry Systems, U.S.A). The Friedewald equation was used to determine serum low density lipoprotein (LDL) cholesterol (Friedewald *et al.*, 1972). The Friedewald equation is as follows:

LDL cholesterol = total cholesterol - HDL - $\frac{\text{triglycerides}}{5}$

The Modification of Diet in Renal Disease (MDRD) formula for creatinine-based GFR served as the basis for GFR estimation. The equation is as follows:

$$\text{GFR} = 186 \times \left(\frac{\text{Creatinine}^{-1.154}}{88.4}\right) \times \text{Age}^{-0.203} \times \left(0.742\ (\text{if female})\right) \times \left(1.210\ (\text{if black})\right)$$

For the purposes of description, the CKD participants were categorized into CKD stages 1, 2, 3A, 3B, 4, and 5 following the guidelines of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) (National Kidney Foundation, 2002).

3.5.4 Biochemical analysis

3.5.4.1 Serum creatinine (Cr)

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Serum creatinine was analyzed with VITROS 350 automated chemistry analyzer and an assay kit (Creatinine slides) from VITROS Chemistry Products, USA.

The reaction scheme of the assay is as follows:

$$Creatinine + H_20 \xrightarrow{Creatinine\ amidohydrolase} Creatine$$

$$Creatine + H_20 \xrightarrow{Creatine\ amidohydrolase} Sarcosine + urea$$

$$Sarcosine + O_2 + H_20 \xrightarrow{Sarcosine\ oxidase} Glycine + formaldehyde + H_2O_2$$

$$H_2O_2 + Leuco\ dye \xrightarrow{Peroxidase} Dye + 2H_20$$

3.5.4.2 Serum direct HDL

Serum high density lipoprotein (HDL) was analysed with VITROS 350 automated biochemistry analyzer and an assay kit (dHDL slides) from VITROS Chemistry Products, USA.

3.5.4.3 Plasma glucose

Plasma glucose was analysed with VITROS 350 automated chemistry analyser and an assay kit (GLU slides) from VITROS Chemistry Products, USA.

The reaction scheme of the assay is as follows:

$$\mathrm{B-D-glucose} + O_2 + H_20 \xrightarrow{Glucose\ oxidase} D - gluconic\ acid + H_2O_2$$

$$2H_2O_2 + 4 - aminoantipyrine + 1,7 - dihydroxynaphthalene \xrightarrow{Peroxidase} Red\ dye$$

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3.5.4.4 Serum total cholesterol

Serum total cholesterol (CHOL) was analyzed with VITROS 350 automated chemistry analyzer and an assay kit (CHOL slides) from VITROS Chemistry Products, USA.

The reaction scheme of the assay is as follows:

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

$$Cholesterol\ esters + H_20 \xrightarrow{Cholesterol\ ester\ hydrolase} Cholesterol + fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} Cholest - 4 - en - 3 - one + H_20$$

$$H_2O_2 + Leuco\ dye \xrightarrow{Peroxidase} Dye + 2H_20$$

3.5.4.5 Serum triglycerides

Serum triglycerides (TRIG) was analyzed with VITROS 350 automated chemistry analyzer and an assay kit (TRIG slides) from VITROS Chemistry Products, USA.

3.5.4.6 Serum Leptin

ALPCO® ELISA kit (USA) for the quantitative determination of total leptin in human serum was used.

3.5.4.6.1 Test method

Preparations were made for the working solutions of the streptavidin-HRP conjugate by diluting 1:50 in assay buffer. Since the whole plate of 96 wells was used, 240 µL of concentrate was diluted in 12 mL of assay buffer. Calibrator, control, and serum sample were pipetted into the corresponding labeled wells; 20 µL of each. Monoclonal anti-leptin-biotin conjugate was pipetted into each well; 80 µL into each. This was incubated on a plate shaker for 1 hour at room

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temperature. The wells were washed 3 times with diluted wash buffer (300 µL/well for each wash) and the plate firmly tapped against absorbent paper to ensure that it is dry. 100 µL of diluted streptavidin-HRP conjugate was Pipetted into each well. This was incubated on a plate shaker for 30 minutes at room temperature. The wells were washed 3 times with diluted wash buffer (300 µL/well for each wash) and the plate firmly tapped against absorbent paper to ensure that it is dry. 100 µL of TMB substrate was pipetted into each well at timed intervals. This was incubated on a plate shaker for 10-15 minutes at room temperature. 50 µL of stop solution was pipetted into each well at timed intervals as for TMB timed intervals. The plate was read on a URIT 660 microwell plate reader at 450 nm within 20 minutes after the addition of the stop solution. Same procedure was followed for the controls. The controls all passed.

3.6 Ethical Approval

This study was approved by the University of Ghana's Ethical and Protocol Review Committee of the College of Health Sciences, and bears the identification number **"CHS-Et/M.3-P2.7/2017-2018"**. The risks and benefits of the study were clearly explained to the study participants, who subsequently gave their consent for inclusion in the study. Also, all the data collected from the study were handled with strict confidentiality.

3.7 Data analysis

The data were analyzed with the Statistical Package for Social Sciences (SPSS), version 24. Demographic and clinical features of the study participants were summarized with descriptive statistics. Furthermore, an independent-samples t-test was conducted to determine the significance of the differences between the CKD group and the control group with regard to clinical and biochemical parameters. The eta squared statistic was computed to determine the effect size.

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Finally, a standard multiple regression analysis was conducted to determine factors that predispose to increasing serum leptin levels in both groups. The R^2 value; adjusted R^2 value; and beta values (standardized and unstandardized) were regression coefficients that were presented. Although the R^2 value indicates the variance in the dependent variable (which in this case, is mean serum leptin levels) is explained by the model, the adjusted R^2 values (Adj. R^2) were used for making such inferences, as the sample sizes used in the regression analysis in both study groups were small. According to Tabachnick & Fidell (2007), using the R^2 values to draw inferences under such circumstances would be an overestimation of the variance explained by the model. Preliminary analyses were performed to check for violations of the assumptions of multicollinearity and singularity, normality, linearity, independence of residuals, and homoscedasticity, as well as the assumptions of sample size and outliers, as a violation of any of these assumptions affects the inferences made from the regression model (Tabachnick & Fidell, 2007). The significance of each predictor variable was assessed by determining the *p* values, and confidence intervals; *p* values that were considered significant were those that were below 0.05.

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CHAPTER FOUR

4.0 RESULTS

4.1 Demographic and clinical characteristics of the study participants

One hundred and eight (108) participants comprising – seventy-two (72) CKD subjects and thirty-six (36) controls were enrolled in this study. The demographic and clinical characteristics of these individuals are presented in Tables 1 and 2 (2a and 2b) respectively.

Table 1: Demographic features of the study participants

Demographic features	CKD group		Control group	
	Number	%	Number	%
Gender				
Male	38	52.8	19	52.8
Female	34	47.2	17	47.2
Education level				
None	1	1.4	0	0
Basic	13	18.1	2	5.6
Secondary	25	34.7	7	19.4
Tertiary	33	45.8	27	75.0

CKD: Chronic Kidney Disease, %: percentage

More than half of the participants in both groups were males. About half of the participants in the CKD group were people who have had tertiary education. An even greater number from the control group had had tertiary education. More than two-third of the participants have had some form of formal education.

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Table 2a: Clinical features (categorical) of the study participants

Clinical features	CKD group	
	Number	%
CKD stage		
Stage 3A	7	9.7
Stage 3B	21	29.2
Stage 4	30	41.7
Stage 5	14	19.4
Period with CKD		
Less than 1 year	23	31.9
1-3 years	29	40.3
More than 3 years	20	27.8
Period on dialysis		
Non-dialysis CKD	39	54.2
Less than 1 year	5	6.9
1-3 years	21	29.2
More than 3 years	7	9.7
HHD category		
LVH	20	27.8
CHF	20	27.8
IHD	32	44.4
Period with HHD		
Less than 1 year	21	29.2
1-3 years	27	37.5
Greater than 3 years-6 years	13	18.1
More than 6 years	11	15.3

CKD: Chronic Kidney Disease; HHD: Hypertensive Heart Disease; LVH: Left Ventricular Hypertrophy; CHF: Congestive Heart Failure; IHD: Ischemic Heart Disease

Majority of the CKD subjects were in stage four. Majority of them have had HHD for one to three years. Only few of the CKD subjects have been on dialysis for more than 3 years. Majority of the CKD subjects had IHD.

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Table 2b: Clinical features (continuous) of the study participants

Clinical features	Mean ± SD		p-value
	CKD group (n=72)	Control group(n=36)	
BMI	25.4 ± 2.7 Kg/m^2	22.9 ± 1.1 Kg/m^2	<0.0001*
Systolic BP	161.4 ± 19.5 mmHg	113.3 ± 4.9 mmHg	<0.0001*
Diastolic BP	99.9 ± 12.0 mmHg	75.1 ± 3.7 mmHg	<0.0001*
Fasting blood glucose	6.3 ± 2.0 mmol/l	4.7 ± 0.6 mmol/l	<0.0001*
Serum creatinine	292.8 ± 136.8 µmol/l	72.9 ± 10.4 µmol/l	<0.0001*
Total cholesterol	6.1 ± 1.4 mmol/l	4.5 ± 0.6 mmol/l	<0.0001*
Triglycerides	1.9 ± 0.7 mmol/l	1.5 ± 0.4 mmol/l	0.001*
Low-density lipoprotein cholesterol	3.9 ± 1.2 mmol/l	2.4 ± 0.5 mmol/l	<0.0001*
High-density lipoprotein cholesterol	1.3 ± 0.2 mmol/l	1.5 ± 0.2 mmol/l	0.011*
Estimated GFR	27.3 ± 12.1 ml/min/1.73m^2	117.1 ± 18.5 ml/min/1.73m^2	<0.0001*
Serum leptin	26.4 ± 14.0 ng/ml	5.7 ± 3.9 ng/ml	<0.0001*
Age	52.3 ± 13.0 years	43.8 ± 11.4years	0.031*

***significant at 0.05 level, GFR: Glomerular Filtration Rate, N: number of participants**

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4.2 Comparison of serum leptin levels between the control and study groups

Results in Table 2b indicate a significantly higher serum leptin level, creatinine, glucose, BMI among participants of the CKD group compared with those of the control group ($p < 0.0001$ (two-tailed). Estimated GFR were lower in the CKD group compared to those of the control group ($p < 0.0001$ (two-tailed). The CKD group was comparatively older than the control group ($p < 0.05$) (two-tailed).

4.3 Predictors of increasing serum leptin levels

4.3.1 Predictors of increasing serum leptin levels in the CKD group

Table 3 is a summary of the regression analysis in the CKD group. The standard multiple regression model included : being at CKD Stage 5, high-density lipoprotein cholesterol (HDL), fasting blood glucose (FBG), HHD diagnosis of more than 6 years, systolic BP, CKD diagnosis of more than 3 years, female gender, body mass index (BMI), low-density lipoprotein cholesterol (LDL), HHD diagnosis of 1-3years, congestive heart failure (CHF), IHF, dialysis treatment of 1-3 years, CKD diagnosis of 1-3 years, diastolic BP, dialysis treatment of more than 3 years, HHD diagnosis of 4-6 years, age, dialysis treatment of less than 1 year, CKD Stage 3A, and CKD Stage 3B. The model explained 79.2% of the variance in mean serum leptin levels. When the variance explained by the other variables in the model were controlled for, being at CKD stage 5 made the largest contribution (beta = 0.37, $p < 0.0001$). Significant contributions were also made by HDL (beta = 0.269, $p < 0.0001$), FBG (beta = 0.267, $p = 0.001$), HHD diagnosis of more than 6 years (beta = -0.217, $p = 0.020$), systolic BP (beta = 0.201, $p = 0.030$), female gender (beta = 0.191, $p = 0.006$), BMI (beta = 0.18, $p = 0.017$), and LDL (beta = 0.177, $p = 0.037$), meaning that they each contributed those respective proportions to the total variance in mean serum leptin levels.

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Table 3: Summary of regression analysis (CKD Group)

	Mean serum leptin levels					
Predictors	**Unstd. Beta (SE)**	**95% *CI* for Unstd. Beta**	**Std. Beta**	***p* value**	**Pr.C**	**Pr.C^2×100%**
CKD Stage 5	12.976 (2.416)	8.124 to 17.828	0.370**	< 0.0001	0.291	8.4681
HDL	19.855 (4.725)	10.365 to 29.345	0.269**	< 0.0001	0.227	5.1529
FBG	1.889 (0.512)	0.861 to 2.917	0.267**	0.001	0.200	4.00
HHD diagnosis of more than 6 years	-8.366 (3.481)	-15.359 to -1.374	-0.217*	0.020	-0.130	1.69
Systolic BP	0.144 (0.064)	0.015 to 0.272	0.201*	0.030	0.121	1.4641
Female gender	5.304 (1.842)	1.604 to 9.003	0.191*	0.006	0.156	2.4336
BMI	0.921 (0.373)	0.172 to 1.671	0.18*	0.017	0.133	1.7689
LDL	2.090 (0.973)	0.136 to 4.045	0.177*	0.037	0.116	1.3456
CKD diagnosis of more than 3 years	6.145 (3.416)	-0.716 to 13.006	0.198	0.078	0.097	0.9409

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HHD diagnosis of 1-3years	-4.287 (2.305)	-8.918 to 0.344	-0.15	0.069	-0.101	1.0201
CHF	-3.865 (2.501)	-8.889 to 1.159	-0.125	0.129	-0.084	0.7056
IHF	-3.372 (2.147)	-7.685 to 0.940	-0.121	0.123	-0.085	0.7225
Dialysis treatment of 1-3 years	2.836 (3.215)	-3.622 to 9.294	0.093	0.382	0.048	0.2304
CKD diagnosis of 1-3 years	2.221 (2.336)	-2.471 to 6.913	0.079	0.346	0.051	0.2601
Diastolic BP	-0.086 (0.099)	-0.286 to 0.114	-0.074	0.392	-0.047	0.2209
Dialysis treatment of more than 3 years	-2.621 (4.439)	-11.538 to 6.296	-0.056	0.558	-0.032	0.1024
HHD diagnosis of 4-6 years	-2.026 (2.989)	-8.029 to 3.997	-0.056	0.501	-0.037	0.1369
Age	0.35 (0.095)	-0.155 to 0.225	0.033	0.711	0.020	0.04
Dialysis treatment of less than 1 year	-1.256 (4.409)	-10.111 to 7.599	-0.023	0.777	-0.015	0.0225
CKD Stage 3A	0.577 (3.422)	-6.296 to 7.449	0.012	0.867	0.009	0.0081
CKD Stage 3B	-0.249 (2.285)	-4.839 to 4.342	-0.008	0.914	-0.006	0.0036

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N = 72; df = 71; F = 13.898; R^2 = 0.854; Adj. R^2 = 0.792; p = 0.000; **Significant at 0.01 level; *Significant at 0.05 level

4.3.2 Predictors of serum leptin levels in the control group

Table 4 is a summary of regression analysis for the control group. The standard multiple regression model included female gender, age, BMI, systolic BP, diastolic BP, FBG, TG, LDL, HDL, and eGFR. This explained 89.4% of the variance in serum leptin levels. When the variance explained by the other variables in the model were controlled for, female gender made the largest unique contribution (beta = 0.709, $p < 0.0001$). Significant contributions were also made by BMI (beta = 0.341, $p < 0.0001$), and eGFR (beta = -0.222, $p = 0.011$).

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Table 4: Summary of regression analysis (Control Group)

	Mean serum leptin levels						
Predictors	**Unstd. Beta (SE)**	**95% *CI* for Unstd. beta**	**Std. Beta**	***P* value**	**Pr.C**	**Pr.C^2**	**Pr.C^2×100%**
Female gender	5.504 (0.551)	4.369 to 6.640	0.709**	< 0.0001	0.550	0.3025	30.25
BMI	1.280 (0.282)	0.698 to 1.861	0.341**	< 0.0001	0.250	0.0625	6.25
eGFR	-0.047 (0.017)	-0.082 to -0.012	-0.222*	0.011	-0.152	0.023104	2.3104
Systolic BP	0.105 (0.057)	-0.012 to 0.223	0.131	0.076	0.102	0.010404	1.0404
Diastolic BP	0.003 (0.073)	-0.148 to 0.154	0.003	0.972	0.002	0.000004	0.0004
LDL	-1.156 (0.707)	-2.611 to 0.300	-0.131	0.114	-0.090	0.0081	0.81
TG	-0.637 (0.767)	-2.217 to 0.943	-0.071	0.414	-0.046	0.002116	0.2116
Age	0.022 (0.028)	-0.036 to 0.079	0.062	0.444	0.043	0.001849	0.1849
HDL	0.805 (1.732)	-2.763 to 4.373	0.034	0.646	0.026	0.000676	0.0676

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FBG	0.056 (0.446)	-0.862 to 0.974	0.009	0.901	0.007	0.000049	0.0049

N = 36; df = 35; F = 30.398; R^2 = 0.924; Adj. R^2 = 0.894; p = 0.000; **Significant at 0.01 level; *Significant at 0.05 level

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CHAPTER FIVE

5.0 DISCUSSION

5.1 The relationship found between CKD and leptin levels in serum

This study aimed at investigating the association between CKD and serum leptin. The study showed that the CKD group had a comparatively higher blood leptin levels than those of the control group ($p < 0.0001$). This may suggest that CKD is a significant determinant of increasing serum leptin levels. This was expected, as CKD sufferers are known to have higher levels of serum leptin, probably due to reduced clearance by the diseased kidneys (Heimbürger *et al*., 1997; Nordfors *et al*., 1998; Merabet *et al*., 1997). This, however, contradicts the work of Scholze *et al* in 2007, where worsening GFR was associated with lower serum leptin levels. The work of Scholze *et al* in 2007 was, however a 7-year longitudinal study; a long time for the body to adopt mechanisms to reduce serum leptin levels. This possible adaptive phenomenon is evidenced in the work of Wahba & Mak in 2007 where, the expression of the gene that encodes leptin is lower in the adipose tissues of CKD sufferers.

All CKD participants in this cohort were concurrently diagnosed with HHD. It has been noted that leptin may cause cardiovascular problems through an increase in cellular alkaline phosphatase activity, promoting the multiplication, calcification, and migration of vascular smooth muscles cells (Parhami *et al*., 2001). This study, however, did not measure serum levels of alkaline phosphatase. CKD is also known to be a catabolic state associated with high prevalence of inflammation, malnutrition, and atherosclerosis, involves repeated activation of acute phase proteins and cytokines, including leptin (Wright & Hutchison, 2009). Hence it could be inferred that the higher serum leptin levels observed in members of this cohort may have facilitated their HHD diagnosis.

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The significantly higher levels of leptin observed in the CKD group could have such clinical implications such as increased thrombocyte aggregations (Malyszko *et al*., 2002), increased white blood cell counts (Mabuchi *et al*., 2005), and influence eating behaviors (Aguilera *et al*., 2004). This study however did not evaluate these aforementioned factors.

5.2 Factors that predict serum leptin levels

Another objective this study sort to achieve was to identify specific factors that predispose to higher serum leptin levels in both the CKD group and the control group. Remarkably, the regression model containing the predictor variables was able to explain 79.2% of the variance in serum leptin levels among participants of the CKD group, meaning only 20.8% of the variance in serum leptin levels went unexplained by the model. Moreover, being at CKD stage 5 made the largest unique significant contribution to the model, explaining 8.47% of the observed variance, and predicting the occurrence of higher serum leptin levels. Except for the fact that more than half of the participants (54.2%) had not been put on dialysis, this observation that being at CKD stage 5 is a determinant of the occurrence of higher serum leptin levels could confer a survival advantage on this cohort of CKD sufferers, as it has been reported elsewhere that in patients undergoing haemodialysis, high levels of leptin could predict favorable outcomes (Scholze *et al*., 2007). That said, at present, it is difficult to assert whether or not the identification of CKD stage 5 as a determinant of increased serum leptin could be advantageous to this cohort, as data from which such an inference could be drawn are limited, and hence, warrants more research to fill in the gaps in knowledge.

Another key predictor of serum leptin levels that was identified, was CKD patient with HHD for more than six years, and that showed lower serum levels among this cohort of CKD patients. This is in contrast with the finding that individuals with cardiovascular conditions have significantly

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higher serum leptin levels (Reilly *et al*., 2004; Wallerstedt *et al*., 2004; Wallace *et al*., 2001), although the reverse have been reported elsewhere (Iribarren *et al*., 2007). It is noted then that the presence of HHD diagnosis for more than six years in a CKD sufferer could probably compensate for the reduced ability of the aberrant kidney to take up leptin from circulation (Heimbürger *et al*., 1997; Nordfors *et al*., 1998; Merabet *et al*., 1997). It is not clear whether this reduction in serum leptin in association with HHD diagnosis for more than six years in a CKD sufferer could predict better outcomes as stated by Scholze and colleagues (Scholze *et al*., 2007). This is because HHD in itself is not associated with favorable outcomes (Bonow & Udelson, 1992; Aronow & Kronzon, 1993; Diamond & Phillips, 2005). Moreover, hypertension has been associated with high serum leptin levels (Paolisso *et al*., 1999; Malmqvist *et al*., 2002). Consistent with that, this study found increasing serum leptin among CKD sufferers to be predicted by increasing systolic BP, but not diastolic BP.

In this study, high levels of HDL and LDL cholesterols, which are also linked with several cardiovascular events (Murray *et al*., 2003), significantly predicted increasing serum leptin levels in the CKD group, uniquely contributing 5.15% and 1.35% each to the total variance observed (table 3 above). Additionally, this study reports high fasting blood glucose, a predictor of metabolic risks (Expert Panel on the Detection, Treatment, and Evaluation of High Blood Cholesterol in Adults, 2001), as a significant determinant of higher serum leptin levels among CKD patients. Another study that reported on the relationship existing between fasting glucose and serum leptin focused on an apparently healthy population (Bedir *et al.*, 2003).

Some studies have reported a correlational association between BMI and leptin (Hirose *et al*., 1998; Díez *et al*., 2005). Others have reported BMI as a determinant of increasing serum leptin (Bedir *et al.*, 2003), together with female gender (Bedir *et al.*, 2003; Maffei *et al*., 1995; Considine

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et al., 1996). This study corroborated the fact that these two factors are predictors of higher serum leptin among both apparently healthy individuals and CKD sufferers most likely due to more adipose tissues in the obese and females and leptin is an adipose tissue derived hormone (Kiess 1998). Even though the serum leptin levels were significantly lower among the participants of the control group, the fact that female gender and BMI predicted higher levels of leptin is cause for concern, as high serum leptin levels are associated with high uric acid levels (Fruehwald-Schultes *et al*., 1999; Bedir *et al*., 2003), which is a precursor for gout and cardiovascular diseases, and is also associated with the metabolic syndrome (Ruggiero *et al*., 2006; Kim *et al*., 2009; Richette & Bardin, 2010; Roddy & Doherty, 2010). This study however did not measure uric acid levels.

The predictive ability of total cholesterol and triglycerides could not be determined as the variables violated the assumption of multicollinearity, and hence was excluded from the regression model so as not to cause an over dispersion of the results, which would have affected the integrity of the inferences drawn from this results. Hence the variables that were excluded, together with other variables that were not investigated in this study may account for the proportions that went unexplained in these models.

Furthermore, eGFR, though identified as a predictor of serum leptin (Sarnak *et al*., 2002; Okpechi *et al*., 2007), was only found to be predictive among the participants of the control group. It is suggested that if leptin levels correlate positively with eGFR, then synthetic leptin analogs could be used to rectify the low levels (FDA, 2014), but if the correlation is negative, then high flux haemodialysis is recommended to assist in clearing this high molecular weight protein (Vanholder *et al*., 2003). Neither of the two recommendations could be implemented in this cohort of CKD sufferers as the establishment of the predictive ability of eGFR on serum leptin levels was not ascertained. It is important to mention that the relationship that exist between serum leptin and

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eGFR has not been widely investigated. In an experimental mice model of early-stage type 2 diabetes, increased leptin levels were associated with higher GFR (Wei *et al.*, 2004); this contrasts what was observed in the control group of this study. Leptin has additionally been shown to be associated with focal glomerulosis and Transforming Growth factor beta (TGF-beta) secretion by glomerular endothelial cells (Wolf *et al.*, 2002). Moreover, in patients with biopsy-proven obesity-related glomerulopathy, the gene that encodes the leptin receptor were demonstrated to be overexpressed than in apparently healthy control subjects (Wu *et al.*, 2006). Besides these, most studies on eGFR have studied it in association with metabolic risk (Tomaszewski *et al*., 2007), the metabolic syndrome (Chen *et al*., 2003), resistin (Kielstein *et al*., 2003), adiponectin (Schalkwijk *et al.*, 2006), serum creatinine, serum cystatin C, and lean tissue mass (Vinge *et al.*, 1999). In light of these, it is important to reiterate that more studies be devoted to the elucidation of the relationship between GFR and serum leptin, as well as improvements in techniques for accurate GFR prediction.

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CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusion

In summary, serum leptin levels were higher among chronic kidney disease subjects with HHD. Having CKD stage 5 appeared the most significant predictor of serum leptin. In the control group, female gender was the most important predictor of blood leptin levels. Implications of raised leptin levels in CKD subjects merit further investigations.

6.2 Recommendations

From the findings made in this work, additional research should be carried out to ascertain the implications of the presence of higher serum leptin levels among individuals at CKD stage 5. Also, additional research needs to be carried out to ascertain whether concurrent lower serum levels and presence of HHD diagnosis for more than six years could predict better outcomes in CKD sufferers. Further, more studies on serum leptin in CKD sufferers, involving larger sample sizes, need to be carried out in the country to assist in improving the prognosis of CKD.

Additional research needs to be carried out on the assaying of leptin in both bound and free forms to help interpret their levels better.

6.3 Limitations

The special nature of the target study population did not permit sampling of the desired number of study participants. Moreover, due to limited resources, biochemical parameters like C-reactive protein and serum uric acid were not evaluated in relation to serum leptin, and these could possibly account for the small proportion of variance not explained by the regression model. Furthermore, eGFR in the control group may be higher than presented, as the MDRD equation has been reported to potentially under calculate GFR prediction in apparently healthy participants (Rule *et al*., 2004).

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APPENDIX I

Consent form

The prevalence rate of chronic kidney disease (CKD) in Sub-Saharan Africa is estimated to be 13.9% which is higher than the worldwide prevalence of 10%. Most CKD patients in Ghana are between the ages of 20years and 50 years, which represents an economically productive group. The classical risk factors causing increase in prevalence of CKD include diabetes, hypertension and cardiovascular diseases among others.

The high rates of cardiovascular disease (CVD) death noted among CKD patients can only be partially explained by known risk factors. Uremic factors (such as leptins) may negatively interact with biochemical functions. Leptin has been established to cause cardiovascular problems at high levels. Leptin can raise the activity of alkaline phosphatase at cellular level, and ignites the proliferation, calcification and migration of vascular smooth muscles cells. The association between leptin and CKD has to be established to aid in explaining the high prevalence of CVD among CKD patients.

This study being conducted in Ghana, will improve existing data on CKD by providing relevant information relating to leptin levels. The study will assess serum leptin levels to better demonstrate its link with CKD and CKD management. This will also help find out which factors may be linked with high levels in the Ghanaian CKD patient population. The study will also help streamline interventions to CKD patients. By this, the reduction of blood levels of leptin (through haemodiafiltration), when necessary, might give a beneficial clinical impact. There has been conflicting outcomes concerning the association between levels of leptin in blood and CKD. If leptin is found to accumulate in blood, it can be targeted in an effort to reducing the high CVD that causes mortality among CKD patients.

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This study will take a small amount of blood (5ml) from you by inserting a needle in your forearm. The risk involved in this blood collection procedure is negligible and it will cause only minimal pain and bruising. This sample will be taken just once and will be used for analyses of glucose, leptin, lipids, and creatinine levels. Some information in your folder like your medical history would also be needed for this study.

It will be appreciated if you will consent to take part in this research. Your participation in this study is not compulsory but strictly voluntary, and you can withdraw from this research at any time without any disadvantage concerning your medical care at this Hospital. All information gathered will be treated with strict confidentiality.

For further enquiries on this study, please contact;

Christian Nsormi Adekena (0202868848), Department of Chemical Pathology, Prof. Henry Asare-Anane (0246024002), Head of Chemical Pathology Department and Dr. Seth Amanquah (0244293987), Chemical Pathology Department of School of Biomedical and Allied Health Sciences, University of Ghana.

Consent

I ……

of…….give my consent for my sample to be used for the research project stated above which has been explained to me

By……

Patient's signature…….....Date……

Doctor's signature……Date……..

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APPENDIX II: Research participation information sheet

I, CHRISTIAN NSORMI ADEKENA, of the Department of Chemical Pathology, School of Biomedical and Allied Health Sciences (SBAHS) , University of Ghana wish to embark on a study entitled, *Leptin Levels in Chronic Kidney Disease Subjects with Hypertensive Heart Disease Attending Korle-Bu Teaching Hospital.*

This study is aimed at determining the association between leptin levels in blood and estimated GFR in chronic kidney disease subjects with hypertensive heart disease attending Korle-bu teaching hospital. You will be asked few routine questions about your personal details and chronic kidney disease history. The benefits of the study are to help know whether this leptin accumulates in your blood or not. This information will go a long way to help your doctors manage some of the heart diseases you have. It will be appreciated if you volunteer, though participation is entirely voluntary and strictly confidential. You have the right to decide to not partake in this work or withdraw from the study whenever you wish. Participants of the study will undergo an overnight fast after which 5 mls of blood will be withdrawn. Both procedures may involve slight discomfort. The amount of blood to be taken by phlebotomists and used for this research study will not exceed 5 mls. You can be assured of full and strictest confidentiality of your personal information. This study will adhere to all applicable protocols and will maintain quality assurance in accordance with good laboratory practice. The blood samples collected will bear an identification code to ensure anonymity, confidentiality and ease of identification. There is the possibility that you might not benefit directly from participation. However, the information obtained and conclusions drawn will be applied in the adoption of relevant health policies as well as the appropriate care and management. You will incur no costs and you will also not be paid for participating in this study. However, you will be entitled to know the outcome of the laboratory results and this will be well

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explained to you. Data will be loaded onto a lock Microsoft Excel spreadsheet. Study questionnaires will be stored in a cabinet in a locked office.

My contact numbers is 0202868848. You may call me for any further clarification. Thank you for the cooperation and anticipated compliance to the study requirements.

Signature of Participant: …… Date……..

Signature of Researcher…… Date…… 22

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Appendix III

Questionnaire for assessment of chronic kidney disease

You are likely referred here by a professional to attend to issues concerning your impaired kidney. This short questionnaire is designed to help undertake a research on leptin levels in CKD subjects.

Section I: Socio-Demographics

Age……. Sex…… weight……. Height……. Blood pressure

Occupation…….. Diagnosis…….. Other complications……. Educational level…….

Marital status: married/single/divorced

Section II: Kidney Disease

1. How long ago were you diagnosed? (Circle one) less than a year / 1 - 3 yrs / 3 - 5 yrs / 5 - 10 yrs / greater than10 yrs

2. By what means were you diagnosed? (Check those that apply)

Blood estimation of creatinine

Proteinuria

Other means: _____

3. Are you on dialysis? YES/NO

4. If yes, how long has it been since you started dialysis? Less than 6mnths / 6mnths –a year / 1 - 3 years / 3 - 5 yrs / greater than 5 years

5. Were you informed what led to the kidney disease (like. related to surgery, high blood pressure, kidney stones, severe medical illness, medication, diabetes or glomerulonephritis)?

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_____

_____

6. Check whichever applies to you:

Kidney issues during childhood?

Hospitalized for failure of kidney?

Renal failure secondary to other reasons?

Renal stones?

Infections of the bladder?

Dysuria?

Bladder surgery?

Pelvic radiation?

Chemotherapy?

History of CKD in family?

Hematuria?

Foamy urine?

Section III: Medications

1. Do you take pain medications (i.e.ibuprofen, naproxen, Motrin)? N/ Y

A. how often; if yes? Once a day / 3 times a week / once a week / once per month

2. Do you take herbal medicines? Yes / No

A. list them here if yes:

_____

_____

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Section IV: Hypertension

1. Do you have hypertension or take treatment for it? Yes / No

2. How long has it been since you were diagnosed? < 1 yr / 1 -3 yrs / 3 - 5 yrs / 5 - 10 yrs / > 10 yrs

3. Do you measure your bp in the house? Yes / No

4. How often if yes? Once a day / severally a week / weekly / monthly

5. Do you take salted food? No / once in a while / often / with every meal

6. Do you take food processed in factories? Not at all / once in a while / weekly / daily

7. How often do you exercise? Daily / 3 times a week / once per week / monthly

8. Have you ever been admitted for high bp? Yes / No

9. Have you ever got a stroke? Yes / No

10. Have you ever experienced a heart attack? Yes / No

Section V: Diabetes

1. Has diabetes or prediabetes ever been your diagnosis? Yes / No

2. Since when were you diagnosed? < 1 yr / 1 to 3 yrs / 3 to 5 yrs / 5 to 10 yrs / > 10 yrs

3. Are you on treatment for diabetes? Yes / No

-If yes, for how long? < 1 / 1 to 5yrs / 5 to 10yrs / > 10yrs

-If treatment has stopped, since when did you stop? < 1yr / 1 to 5yrs / 5 to 10yrs / > 10yrs

4. Have you ever been treated with insulin? Yes / No

- For how long did you take it; if yes? < 1yr / 1 to 5yrs / 5 to 10yrs / > 10yrs

- If treatment with insulin has stopped, since when did you stop? < 1yr / 1 to 5yrs / 5 to 10yrs / > 10yrs

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5. How controlled is your fasting blood glucose? Usually < 5.0mmol/L / 5.0-8.0 mmol/L/ 8.1-11.2mmol/L / > 11.2mmol/L / I don't know

6. Have you been told you have eye problems from diabetes? Yes / No

7. Do you take treatment for your eye problems? Yes / No

8. Are your feet numb? Yes / No

Section VI: Heart disease

1. How old were you when heart disease was first diagnosed?.....…….(please specify)

2. Were you diagnosed of heart disease before or after you've been diagnosed of chronic kidney disease? A) Before B) After

If before, has it worsened after the diagnosis of chronic kidney disease? Y/N

3. What treatments do you now have for your heart disease? A) No treatment B) aspirin C) diet D) exercise E) other (please specify)……..

Section VII: Stroke

1. Have you ever been informed by a physician you have had stroke? Y/N

If yes, how old were you when you were first informed by a physician that you had a stroke? (Please specify)……..

2. Have you had stroke after you've been diagnosed of chronic kidney disease? Y/N

3. What treatments do you have now for your stroke? A) No treatment B) aspirin C) diet D) exercise or rehabilitation E) other (please specify)……..

Section VIII: Summary

1. Please enter other relevant information here

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Information from clinical files

Hypertensive heart disease

1. A) Physician diagnosis …… [ ] B) Deviated cardiac apex on examination…… [ ]
2. A) ECG diagnosis …… [ ] B) Left ventricular hypertrophy …… [ ]
3. A) ECHO diagnosis …… [ ] C) Left ventricular hypertrophy

Chronic kidney disease

1. eGFR < 60ml/min for > 3 months …… [ ]
2. Imaging (Ultrasound scan showing small kidneys < 9cm on for right and left kidney) …. [ ]
