UNIVERSITY OF CAPE COAST

EFFECTS OF *FALCIPARUM* MALARIA ON LEVELS OF SELECTED BIOMARKERS OF CARDIOVASCULAR DISEASE RISK IN TYPE 2 DIABETICS AND NON-DIABETIC CONTROLS

BY

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Thesis submitted to the Department of Biomedical and Forensic Sciences of the School of Biological Sciences, University of Cape Coast in partial fulfilment of the requirements for award of Doctor of Philosophy degree in Zoology (Parasitology Option).

NOVEMBER 2013

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere.

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SUPERVISORS' DECLARATION

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ABSTRACT

Falciparum malaria and Type 2 diabetes (T2D) affect millions of people globally but the effect of the dual conditions on the cardiovascular health of the affected has not been investigated. To better understand this, a study was designed to evaluate the effect of *Plasmodium falciparum* malaria on levels of selected biomarkers of cardiovascular disease (CVD) risk in diabetics compared with non-diabetic controls by measuring anthropometric indices, blood pressure (Bp), fasting levels of glucose, glycosylated haemoglobin, lipid profile, insulin, leptin, adiponectin, C-reactive protein, total antioxidants power (TAP) and lipid peroxides of 200 diabetics and non-diabetic controls before and during *P. falciparum* malaria. Insulin resistance and β-cell secretory function were assessed. Associations among the various parameters were investigated. At baseline, the two groups differed significantly ($P < 0.05$) in some of the anthropometric indices and levels of most biomarkers except insulin and TAP. Some of the parameters associated linearly in the two study groups with gender-specific variations. During malaria, levels of anthropometric indices and Bp did not change in the defined study groups. In diabetics, apart from TAP that decreased significantly ($P < 0.05$), the levels of all the other biomarkers increased. Non-diabetic controls had decreased ($P < 0.05$) TAP and leptin levels but elevated levels in the others. Anthropometric indices did not associate ($P > 0.05$) with the levels of measured biomarkers in malaria. *Falciparum* malaria increased the CVD risk of respondents in the two study groups. However, the two groups differed in their response to malaria.

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DEDICATION

To my family, teachers and in memory of my late father and grandfathers

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LIST OF ACRONYMS

ADA: American Diabetes Association

AGES: Advanced Glycation End-Products

ANOVA: Analysis of Variance

ATP: Adenosine Triphosphate

BAT: Brown Adipose Tissue

BMI: Body Mass Index

CEPC: Circulating Endothelial Progenitor Cells

CHD: Coronary Heart Disease

CHOL: Total Cholesterol

CNS: Central Nervous System

CRH: Central Regional Hospital

CRP: C-reactive Protein

CVD: Cardiovascular Disease

DAG: Diacylglycerol

ELISA: Enzyme-linked Immunosorbent Assay

FBF: Forearm Blood Flow

FBG: Fasting Blood Glucose

FFA: Free Fatty Acid

GHS: Ghana Health Service

GLUT4: Glucose Transporter-4

GSS: Ghana Statistical Service

$HbA1_c$: Glycosylated Haemoglobin

HOMAB: Homeostatic Model Assessment of Beta Cell Secretion

HOMAIR: Homeostatic Model Assessment of Insulin Resistance

HSD: Honest Significant Difference

IDF: International Diabetes Federation

IL: Interleukin

IMT: Intima-Media Thickness

iNOS: Inducible Nitric Oxide Synthase

LDL: Low-density Lipoprotein Cholesterol

LMW/HMW: Low/High Molecular Weight

LPS: Lipopolysaccharide

MLGRD: Ministry of Local Government and Rural Development

NADPH: Reduced Nicotinamide Adenine Dinucleotide Phosphate

$NADP^+$: Oxidised Nicotinamide Adenine Dinucleotide Phosphate

NF-$_k$B: Nuclear Factor Kappa B

NO: Nitric Oxide

OPD: Out-patient Department

PDX-1: Pancreatic-duodenum Homeobox-1

PKC: Protein Kinase C

RBP4: Retinol-binding Protein-4

RDT: Rapid Diagnostic Test

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

SAPK2/p38: Stress-activated Protein Kinase-2/p38

TAC: Total Antioxidant Capacity

TAP: Total Antioxidant Power

T2D: Type 2 diabetes

TNF: Tumor Necrosis Factor

TRG: Triglycerides

UNICEF: United Nations Children's Fund,

WAT: White Adipose Tissue

WC: Waist Circumference

WHO: World Health Organization

WHR: Waist-to-Hip Ratio

CHAPTER ONE: INTRODUCTION

Dysregulation of insulin has been implicated in the pathogenesis of type 2 diabetes (T2D). Insulin resistance and beta-cell dysfunction remain the major determinants in the pathogenesis of diabetes (Badman & Flier, 2007). Various factors have been identified to be responsible for the development of insulin resistance-induced diabetes mellitus. These factors include abdominal obesity, dyslipidaemia and hyperglycaemia (Escobedo *et al*., 2009) which are components of the metabolic syndrome (Cornier *et al*., 2008). Recently, energy supplus concept of insulin resistance has been proposed (Ye, 2013).

Various studies have concluded that adipose tissue plays a central role in the metabolic syndrome theory possibly due to its adipocytokine-secretory capability (Escobedo *et al*., 2009; Smith & Haslam, 2007; Koerner, Kratzsch & Kiess, 2005).

Some of the adipocytokines secreted by the adipose tissue include leptin, adiponectin, resistin, tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) (Koerner *et al*., 2005; Vendrell *et al*., 2004). Although the majority of these biomarkers are involved in energy homeostasis (Cinti *et al*., 2005), possibly through modulation of insulin action (Considine *et al*., 2008), most have also been implicated in inflammation-induced pathogenesis of diabetes (Kozłowska *et al*., 2010; Kusminski, McTernan & Kumar, 2005). Changes in the levels of these biomarkers have been observed in diabetic patients but whether the pattern of these changes will be affected in diabetic patients in the presence of malaria remains to be determined. The major adipocytokines,

leptin and adiponectin, play versatile roles and their circulating levels are expected to change in response to *Plasmodium falciparum* (*P. falciparum*) infection although no such change was observed in a small sample study involving non-diabetic *P. falciparum*-infected patients compared with uninfected subjects (Blümer *et al*., 2005). Due to their abundance and pleiotropic roles, leptin and adiponectin levels are likely to vary in diabetics before and during *P. falciparum* infection.

Oxidative stress and inflammation have been implicated in the pathogenesis of diabetes mellitus, insulin resistance and associated cardiovascular diseases (De Rooij *et al*., 2009). Several studies have associated high levels of inflammatory markers such as IL-6 and C-reactive proteins (CRP), with diabetes (De Rooij *et al*., 2009; Considine *et al*., 2008; Kusminski *et al*., 2005; Vendrell *et al*., 2004).

Inflammation has been strongly linked to atherosclerosis (Rader & Daugherty, 2008). Inflammatory markers mediate their action through release of oxidizing molecules and/or direct oxidation of cellular components, giving rise to increased oxidant but decreased antioxidant status (Stanek *et al*., 2010). Thus, inflammation increases the oxidative stress status of an organism. Low grade chronic inflammation has been associated with insulin resistance and other cardiometabolic risk factors (De Rooij *et al*., 2009). This observation indicates that irrespective of the extent of inflammation, the potential for detrimental effects on the organism is high. In the present study, oxidative stress is distinguished from inflammation. Oxidative stress was evaluated by monitoring changes in levels of lipid peroxides and total antioxidants before

and during *P. falciparum* infection in diabetics compared with non-diabetic controls.

Generally, infection has been observed to be accompanied by inflammation and thus increased oxidative stress. For instance, infection with the *Plasmodium* parasite is associated with increased oxidative stress through increased reactive oxygen species levels as part of the host's defense mechanism (Postma, Mommers, Eling & Zuidema, 1996). Interestingly, increased level of reactive oxygen species can be pathogenic depending on the site of action (Postma *et al.*, 1996). Several inflammatory markers such as CRP, IL-6 and TNFα have been identified (Stanek *et al.*, 2010; De Rooij *et al.*, 2009; Rader & Daugherty, 2008; Kusminski *et al.*, 2005) but CRP has been the most extensively studied and found to be the most reliable marker for assessing cardiovascular risk (Blake & Ridkler, 2003; Liuzzo & Rizzello, 2001). In addition, the level of CRP is regulated by other inflammatory markers like IL-6 and TNFα (Blake & Ridkler, 2003). In view of the above reasons, CRP has been used in this study as the main inflammatory marker for the assessment of cardiovascular risk posed by malaria in diabetics compared with non-diabetic controls.

Infection-induced inflammation is accompanied by altered lipid metabolism (Khovidhunkit *et al.*, 2004). The observed pattern of lipid changes is both qualitative and quantitative and similar to that observed in T2D (Khovidhunkit *et al.*, 2004). Such changes promote the accumulation of proatherogenic lipids with accelerated degradation of lipids with antiatherogenic properties (Annema *et al.*, 2010). These infection-induced lipid

alterations may pose additional challenge to the management of T2D in malaria endemic settings.

Scientific information on altered lipid metabolism due to malaria is scanty. The few studies conducted on the subject have revealed contradictory results. Some studies reported increased levels of cholesterol (Das, Thurnham and Das, 1996) with others observing reduced cholesterol levels (Al-Omar, Eligail, Al-Ashban & Shah, 2010; Simpson *et al*., 2010). None of these studies was conducted in diabetics. With increased incidence of T2D in a malaria-endemic country like Ghana (Danquah *et al*., 2010), investigations into changes in lipid levels in response to malaria may help to deepen our understanding of malaria-induced lipid changes and its consequence to the host organism. This will allow for the rational design of therapeutic strategies in the management of the two conditions. As a result, the present study compared the lipid profiles of diabetics and non-diabetic controls before and during *P. falciparum* infection.

A common feature of diabetes is the relatively high blood glucose levels observed. Blood glucose serves as the main energy source for *P. falciparum* parasite (Slavic, Krishna, Derbyshire & Staines, 2011). Indeed, in some instances, hypoglycaemia has been observed in severe malaria cases (Maitland & Marsh, 2004). This raises the question as to whether hypoglycaemia observed in severe *P. falciparum* infection in non-diabetics can be seen in uncontrolled diabetes. Although the hypoglycaemia is unexpected, one would expect a decline in blood glucose levels in uncontrolled diabetics who may get severe malaria. This line of thinking appears to be challenged by an observation that *P. falciparum* infection rather increased gluconeogenesis

(Blümer *et al.*, 2005) in non-diabetic respondents. This finding is yet to be confirmed in diabetics. As such, the levels of glucose and glycosylated haemoglobin as well as that of insulin were ascertained before and during *P. falciparum* infection in the current study.

Analysing the changes in the above biomarkers could deepen our understanding of the role that *P. falciparum* infection may play in the overall cardiovascular disease (CVD) risk of diabetics and non-diabetic controls.

Objective of the Study

The overall objective of the study was to evaluate the effect of *P. falciparum* infection on selected markers of lipid metabolism, inflammation and oxidative stress in diabetics and non-diabetic controls in relation to CVD risk. The specific objectives were to:

1. Evaluate the relationship among measures of anthropometry, blood pressure and selected markers of CVD risk before and during *P. falciparum* infection in diabetics and non-diabetic controls;

2. Investigate the effect of *P. falciparum* infection on levels of blood glucose, glycosylated haemoglobin and lipid profile of diabetics and non-diabetic controls;

3. Evaluate the impact of *P. falciparum* infection on the levels of adiponectin, insulin and leptin in diabetics and non-diabetic controls;

4. Investigate the effect of *P. falciparum* infection on levels of lipid peroxides, C-reactive protein and total antioxidants in diabetics compared with non-diabetic controls.

Hypothesis

Malaria dysregulates glycaemic and lipemic control and alters the pathogenesis of diabetes through increased oxidative stress and inflammation with resultant increased risk of cardiovascular disease (Simpson *et al*., 2010; Khovidhunkit *et al*., 2004).

Statement of the Problem

Many developing countries, including Ghana, face a double burden of both infectious and chronic diseases (Unwin *et al*., 2001). However, scientific information on the effect each has on the other is limited. A better understanding of the interaction between such infectious and chronic diseases may contribute to better management of the dual conditions or either condition in the presence of the other.

Malaria has been and continues to be with us in spite of efforts at controlling its spread and reducing treatment costs. The negative impact of malaria on the economy of Ghana and the income of poor household has been recognized (Akazili, 2002; Asante & Asenso-Okyere, 2003). Apart from the malaria burden, Ghana faces increased incidence of diabetes possibly due to increased prevalence of obesity in recent times as a result of various genetic and environmental factors (Danquah *et al*., 2010). This trend increases the potential for the coexistence of diabetes mellitus and malaria in the same individual. Either diabetes or malaria influences lipid metabolism in a manner that may increase the risk of the affected individual to cardiovascular-related illness. However, whether the coexistence of the two diseases in the same individual will exert synergistic or antagonistic effect on lipid metabolism

remains to be determined. Also, the overall impact of the two conditions on CVD risk of the affected is unknown.

Therefore, the current work sought to provide the urgently needed scientific information on the above conditions by monitoring changes in levels of selected biomarkers of CVD risk involved in energy homeostasis, inflammation and oxidative stress before and during malaria in diabetics compared with non-diabetic controls.

Justification of the Study

Several reports have provided evidence of existence of both infectious and chronic diseases in Ghana (GHS, 2011; Danquah *et al.*, 2010). The possibility that an individual may experience a co-morbidity of a given infectious and a chronic disease is high. The risk factors for mono-morbidity may differ from those of co-morbidities. Thus, the co-morbidity of *P. falciparum* infection and T2D may present additional cardiovascular-related risks not found in either disease condition alone.

In the light of predicted increased incidence of diabetes in sub-Saharan Africa (Danquah *et al.*, 2010) and for that matter, malaria-endemic countries like Ghana, investigations into co-morbidities of *P. falciparum* infection and diabetes mellitus may contribute to improved management of diabetes in the presence of *P. falciparum* infection. Also, findings from the current work could serve as a guide for any future studies that may be conducted on the impact of interaction of malaria with diabetes and its consequence to the overall health of the affected.

Ultimately, it is hoped that this work would identify novel biomarkers that can serve as therapeutic targets and allow for rational treatment regimen to be designed and adopted for the management of diabetes in malaria-endemic regions.

CHAPTER TWO: REVIEW OF RELATED LITERATURE

The review briefly covers glucose as well as lipid metabolism and how their dysregulation can result in diabetes. The various mechanisms employed by glucose and lipid to induce diabetes have been discussed. As much as possible, effort has been made to predict how the possible interaction among malaria, glucose and lipid metabolism may impact on the overall health of the affected. Since the study is in humans, more emphasis has been placed on studies involving human subjects although relevant studies in animals have not been neglected. The current study involves malaria and T2D, hence, a brief review of each of the conditions has been covered although T2D is a bit more emphasised. Throughout the review, emphasis has been placed on studies involving selected biomarkers such as insulin, leptin, adiponectin, CRP, lipid peroxides and antioxidants and how malaria-induced alteration of their levels could alter the current approach in managing T2D in malaria-endemic regions. Finally, the basic principles underlying the analytical techniques used in assessing the various biomarkers in the current study are discussed.

Glucose Homeostasis

Glucose, after absorption is disposed by different mechanisms that depend on either insulin or not, based on the tissue of destination (Cherrington, 1999; Defronzo, 1997). Postabsorptive glucose utilisation occurs at three main sites; the brain, the splanchnic (liver and gastrointestinal tissues) and the peripheral (muscle and adipose) tissue (Mandarino, Bonadonna, McGuinness

& Wasserman, 2001). The brain utilises about 50% of all glucose in the body with the liver and gastrointestinal tissues making use of approximately 25% of the glucose (Cherrington, 1999). The remaining 25% of body glucose is used by the muscle and adipose tissues (Mandarino *et al*., 2001). Apart from the muscle and adipose tissue which utilise glucose by insulin-dependent mechanisms, glucose entry into brain and splanchnic tissue is independent of insulin (Defronzo, 2004). The major glucose consumer, the brain, gets saturated at plasma glucose concentration of about 2.22 mmol/L (Defronzo, 2004).

After a glucose-rich meal, plasma glucose level rises, stimulating the release of insulin. The joint effects of high levels of blood glucose and insulin drive the uptake of glucose by splanchnic and peripheral tissues with inhibition of gluconeogenesis (Mari, Wahren, DeFronzo & Ferrannini, 1994). As shown in figure1 below, glucose production and uptake in postabsoprtive state are fairly matched in a normal human body (Defronzo, 1997).

About 80-85% of glucose use by peripheral tissues takes place in the muscle with a minute proportion (4-5%) being used by adipocytes (Bays, Mandarino & DeFronzo, 2004). Adipocytes are crucial for the sustenance of total body glucose homeostasis because they secrete adipocytokines which influence insulin sensitivity of muscle and liver tissues (Koerner *et al*., 2005; Bays *et al*., 2004).

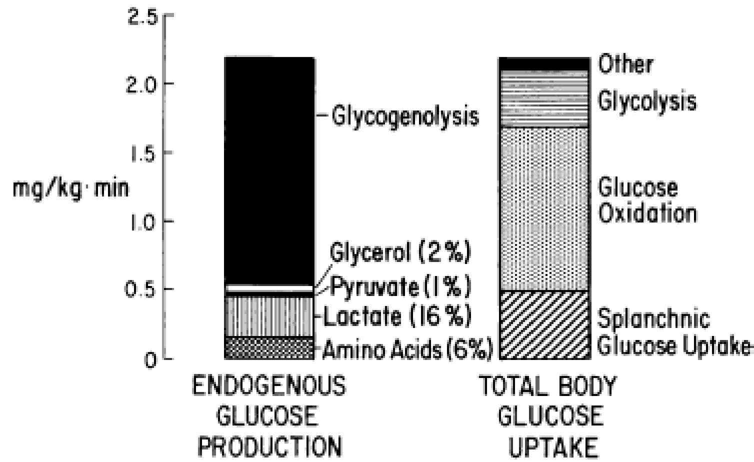

Figure 1: Glucose production and glucose use in the normal human in the postabsorptive state. (Reproduced from DeFronzo, 1997).

Studies have shown that glucose homeostasis in type 2 diabetics is disrupted through several mechanisms. These include abnormalities in glucose utilisation by the liver and gastrointestinal tissues, impaired insulin secretion and reduced sensitivity of peripheral tissues to insulin action (Lin & Sun, 2010). The possible interaction between a pair or among all the three mechanisms to result in full blown diabetes is well documented (Bjornholm & Zierath, 2005). In normal non-diabetic glucose tolerant population, fasting plasma insulin concentration rises gradually to a peak before declining to adopt a so-called horseshoe or inverted U-shape (Defronzo, 1997). Due to the resemblance of this shape to Starling's curve of the heart, it has been called Starling's curve of the pancreas (Defronzo, 2004). In type 2 diabetics, fasting plasma insulin concentrations also adopt the Starling's curve of the pancreas except that the peak value is 2-2.5-fold greater than in normal controls (Defronzo, 2004). This rise in plasma insulin level in diabetics has been explained as an adaptive strategy by the pancreas to compensate for the gradual

decline in glucose homeostasis (Defronzo, 2004). Diabetics have an already established imbalance between glucose production and utilisation. As such, the beta cells of the pancreas overwork to secrete more insulin so as to facilitate glucose use to restore the expected balance between rate of production and utilisation.

Indeed, it has been realized that this strain on the beta cells is unsustainable when fasting plasma glucose exceeds 7.8 mmol/L (Gastaldelli, Ferrannini, Miyazaki, Matsuda & DeFronzo, 2004). Thus, with increased fasting plasma glucose level, insulin secretion increases due to the initial compensatory action of beta cells. With time, the beta cells are unable to secrete adequate insulin to match the persistent hyperglycaemia and the fasting insulin levels decline sharply (Defronzo, 2004). This pattern of insulin secretion, found in various population studies has been associated with obesity (Alkhouri *et al.*, 2010; Bergman, Finegood & Kahn, 2002; Kahn, 2001). Various causes, including the number of beta cells, have been adduced to this pattern of insulin secretion.

The number of beta cells of the pancreas is maintained through a dynamic balance between islet neogenesis and apoptosis (Bonner-Weir, 2001). This delicate balance has been found to be disturbed by various abnormalities. The abnormalities occur as a result of various genetic (Gautier *et al.*, 2001; Vauhkonen *et al.*, 1997) and environmental (Shimabukuro, Zhou, Levi & Unger, 1998) factors that result in beta-cell dysfunction and a corresponding reduction in insulin secretion (Watanabe *et al.*, 1999). Two main mechanisms have been identified as the means by which environmental factors impair insulin secretion. These mechanisms involve poisoning of beta cells as a result

of increased levels of glucose and lipids in the blood, giving rise to the so-called glucotoxicity and lipotoxicity states (Poitout & Robertson, 2002).

Glucotoxicity

A number of *in vitro* and *in vivo* studies have established that persistent hyperglycaemia blights glucose-induced insulin gene expression and insulin secretion (Donath, Gross, Cerasi & Kaiser, 1999; Lu, Seufert & Habener, 1997; Poitout, Olson & Robertson, 1996; Olson *et a*l., 1995; Sharma, Olson, Robertson & Stein, 1995). Kilpatrick & Robertson (1998) categorized the undesirable effects of persistent hyperglycaemia on β-cells into three stages; glucose desensitization, cell exhaustion and glucotoxicity. Glucose desensitization refers to the quick and reversible loss of sensitivity of the exocytotic machinery of β-cells upon short stimulation by elevated glucose (Kilpatrick & Robertson, 1998). This phenomenon is considered as an adaptive strategy even upon inhibition of insulin secretion (Kilpatrick & Robertson, 1998). In the case of cell exhaustion, there is a reduction of the amount of intracellular insulin that can be readily released upon protracted exposure to a secretagogue. Glucotoxicity signifies the gradual, systematic and permanent effects of chronic high blood glucose levels on pancreatic β-cell function (Kilpatrick & Robertson, 1998). Thus, glucotoxicity results from uncontrolled cell exhaustion and glucose desensitization.

Mechanisms underlying the glucotoxicity effect have come from reduced activity of the pancreatic-duodenum homeobox-1 (PDX-1) (Olson *et al.*, 1995) and activator of insulin promoter elements 3b1 (AIPE3b1) (Poitout *et al.*, 1996; Sharma *et al.*, 1995). PDX-1, a homeodomain protein found in β-

cells and D-cells of islet of Langerhans is also present in neuroendocrine cells of the gut (Elrick & Docherty, 2001). PDX-1 has been found to be indispensable in pancreatic development (Offield *et al*., 1996; Jonsson, Carlsson, Edlund & Edlund, 1994) with various mutations of PDX-1 being associated with type 2 diabetes (Macfarlane *et al*., 2000a; Hani *et al*., 1999; Stoffers, Zinkin, Stanojevic, Clarke & Habener, 1997). Apart from the above roles, PDX-1 regulates expression of several islet genes such as those of insulin (Ohlsson, Karlsson & Edlund, 1993), somatostatin (Leonard *et al*., 1993), GLUT 2 (Waeber, Thompson, Nicod & Bonny, 1996), glucokinase (Watada *et al*., 1996; Sharma *et al*., 1995) and islet amyloid polypeptide (Carty, Lillquist, Peshavaria, Stein & Soeller, 1997; Bretherton-Watt, Gore & Boam, 1996; Serup *et al*., 1996) through binding to sequences in the promoter regions.

However, the exact role of PDX-1 in insulin gene transcription is unknown (Elrick & Docherty, 2001) considering the observation that the levels of insulin mRNA remains high when PDX-1 activity is either low (Kajimoto *et al*., 1997) or almost absent (Ahlgren, Jonsson, Jonsson, Simu & Edlund, 1998).

Interestingly, PDX-1 has been found to be involved in the activation of the insulin promoter (Macfarlane *et al*., 1994; Petersen, Serup, Leonard, Michelsen & Madsen, 1994; Melloul, Neriah & Cerasi, 1993) and elevation of mRNA levels of insulin (Macfarlane *et al*., 2000b) in response to glucose. Glucose mediates its action on PDX-1 through various cell signaling pathways that include phosphatidylinositol 3-kinase, stress-activated protein kinase-2/p38 (SAPK2/p38) (Macfarlane *et al*., 1997) and protein kinase C-ζ (Furukawa *et al*., 1999). The protein kinase-C (PKC) signaling pathway, activated by hyperglycaemia (Geraldes *et al*., 2009), has been linked to a

number of abnormal cellular functions such as endothelial dysfunction (Serghides *et al.*, 2011) and increased vascular contractility (Marso, 2002).

Biochemically, glucotoxicity has been linked to oxidative stress. In an *in vitro* study using the insulin-secreting cell, HIT-T15, Matsuoka *et al.* (1997) observed production of reactive oxygen species in the presence of reducing sugar. In a similar study, reduced transcription of the insulin gene upon chronic exposure to elevated glucose was reported but the effect was blocked in the presence of antioxidants such as aminoguanidine and *N*-acetyl-cysteine (Tanaka, Gleason, Tran, Harmon & Robertson, 1999). In other studies, persistent exposure to high glucose levels resulted in increased level of advanced glycation end-products (AGES), impaired β-cell function, and apoptosis and these observations were not seen in the presence of aminoguanidine and *N*-acetyl-cysteine (Jonas *et al.*, 1999; Tajiri, Moller & Grill, 1997). These observations suggest that the detrimental effect of hyperglycaemia on β-cell function is mediated at least in part through oxidative stress. Indeed, AGES have been implicated in various diabetes-related vascular complications (Yamagishi, 2011; Takeuchi & Yamagishi, 2009).

What will be much more intriguing is whether this phenomenon of glucose-induced toxicity observed in mammalian cells can be operational in the presence of disease-causing parasites such as the *P. falciparum*.

In a recent review, it was observed that blood still remains a reliable reservoir of glucose for the intra-erythrocytic stage of the malaria parasite (Slavic *et al.*, 2011). As a result, the parasite relies on blood glucose as its core energy source. Based on this, it has even been suggested that depriving the

malaria parasite of this major energy source could be exploited for therapeutic gains (Staines *et al*., 2010).

Indeed, impaired glucose supply has been found to induce drastic reduction in intracellular adenosine triphosphate (ATP), cytoplasmic pH (Saliba & Kirk, 1999) and depolarization of plasma membrane of *P. falciparum* (Allen & Kirk, 2004). These observations point to the reliance of the malaria parasite on glycolysis for survival and multiplication (Slavic *et al*., 2011). Glucose is supplied to the parasite by transporters on the host and parasite plasma membrane (Slavic *et al*., 2011). These transporters which ferry glucose along a concentration gradient are saturable. It has been established that the parasitophorous vacuole membrane of malaria parasite is highly permeable, allowing free entry of glucose and other solutes with maximum molecular weights of 1.4 kDa (Slavic *et al*., 2011).

However, one is not sure about the extent to which the malaria parasite can accommodate the hyperglycaemia presented in uncontrolled diabetics. On the other hand, it may be possible that the hyperglycaemic condition is so favourable to the parasite to an extent that it aids its rapid growth and multiplication to virulent levels. In this case, the malaria parasite must have a mechanism by which it averts the detrimental effects of chronic hyperglycaemia as envisaged for uncontrolled diabetes.

It has long been known that severe malaria can result in hypoglycaemia (Maitland & Marsh, 2004). In this regard, it may be questioned whether severe malaria accompanied by hypoglycaemia can be observed in uncontrolled diabetes as the hyperglycaemia may offset the potential high parasitaemia-induced hypoglycaemia observed in severe forms of malaria. Thus, in such

individuals, malaria could be a healthy development as it may ameliorate the potential detrimental effects of hyperglycaemia. Notwithstanding this, available evidence is suggestive of increased susceptibility of diabetics to malaria (Danquah *et al*., 2010). This susceptibility could result from impaired immunity to enhanced parasite multiplication in diabetics as opposed to non-diabetic controls.

Mechanisms of glucose-induced cellular toxicity

Elevated glucose levels can cause oxidative stress through several mechanisms. Glucose reacts with proteins through enzymatic and non-enzymatic means. Non-enzymatic glycation of proteins can result in products that increase the oxidant status of the organism (Baynes & Thorpe, 1999). In addition, unbound glucose activates aldose reductase activity and the polyol pathway which decrease NADPH/$NADP^+$ ratios. Also, excess glucose may activate protein kinase C (PKC) via a number of mechanisms, such as *de novo* synthesis of diacylglycerol (DAG), activation of phospholipase C and inhibition of DAG kinase (Evans, Goldfine, Maddux & Grodsky, 2002) as shown in figure 2.

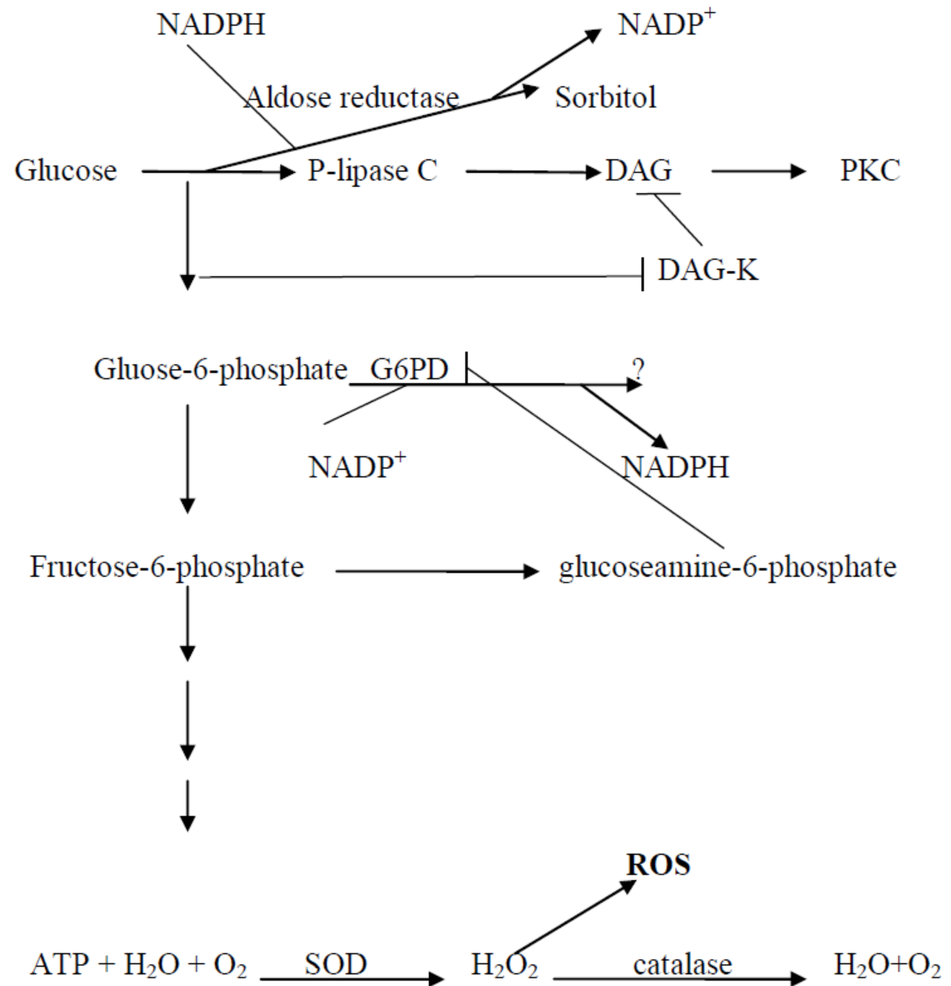

Figure 2: Schematic presentation of some of the pathways that contribute to oxidative stress in response to hyperglycaemia. ***NADPH/NADP$^+$ (reduced/oxidised nicotinamide adenine dinucleotide phosphate), P-lipase C (phospholipase C), DAG (diacylglycerol), PKC (protein kinase C), G6PD (glucose-6-phosphate dehydrogenase, ROS (reactive oxygen species), H_2O_2 (hydrogen peroxide), SOD (superoxide dismutase), inhibition (—|).***

Thus, apart from the direct effect of elevated glucose in apoptosis, glucose exerts some of its detrimental impacts by destabilizing the redox balance of an organism.

PKC activates mitochondrial NADPH oxidase (Morgan *et al*., 2007), which converts NADPH to $NADP^+$ and thus, increased oxidant status of the cell. Increased oxidative degradation of glucose gives rise to enhanced mitochondrial production of superoxide anion (Thallas-Bonke *et al*., 2008). The superoxide anion is then converted to hydroxyl radical and hydrogen peroxide by the enzyme, superoxide dismutase. Both hydroxyl radical and hydrogen peroxide are potent oxidants and can aggravate the oxidative stress of the cell.

Chemically, radicals are reactive species with unpaired electrons. As such, these radicals attain stability by abstracting electrons from other molecules and make such molecules less stable. This enhances the oxidative status of the cell, posing a huge challenge in terms of redox balance.

In *P. falciparum* infection, oxidative stress is increased particularly as a defense mechanism by the host to get rid of the parasite. The extent of oxidative stress in such conditions will be dependent on the level of infecting parasites and this poses additional challenge to the host's redox homeostasis. In diabetes, there is chronic inflammation accompanied by oxidative stress. Therefore, diabetics with plasmodium infection may be expected to exhibit elevated levels of inflammatory and oxidative stress markers. However, scientific studies in humans to test this hypothesis are limited possibly because the possible role of *P. falciparum* infection in modifying the CVD risks of diabetics in malaria endemic areas has been overlooked. With the current increasing diabesity trend coupled with the continued threat posed by *P. falciparum* infections in Africa, it may be important to evaluate the possible contributions of *P. falciparum* infections to the CVD risks of diabetics.

Adipose Tissue Metabolism

The adipose tissue, originally thought as a static storage compartment for triglycerides (Scherer, 2006), is now viewed as a functionally dynamic organ involved in various disease conditions (Ouchi, Parker, Lugus & Walsh, 2011; Halberg, Wernstedt-Asterholm & Scherer, 2008; Desruisseaux, Nagajyothi, Trujillo, Tanowitz & Scherer, 2007). As a storage organ, the adipose tissue is central to whole-body energy homeostasis (Rosen & Spiegelman, 2006).

In mammals, adipose tissues are found in different sites throughout the body particularly in areas of loose connective tissue, although adipose-specific depots can be found in various organs (Casteilla, Penicaud, Cousin & Calise. 2001). Apart from the location, adipose tissue differs in size and colour making it a heterogeneous organ (Sethi & Vidal-Puig, 2007). Based on the colour, it is classified into white adipose tissue (WAT) and brown adipose tissue (BAT) with associated functional variations (Sethi & Vidal-Puig, 2007). In humans, BAT is ultimately replaced with WAT from childhood to adulthood. Considering the principal role of WAT in storage of triglycerides and subsequent release as FFA in times of need (Sethi & Vidal-Puig, 2007), a dysfunctional WAT may have serious repercussions for human health and wellbeing.

Adipose tissue is a complex tissue consisting of preadipocytes, adipocytes, and stromal vascular cells (Lara-Castroa, Fua, Chunga & Garve, 2007). Adipocytes secrete molecules that influence their own differentiation. Differentiated adipocytes communicate with other cells of the adipose tissue

and distant organs such as the brain, skeletal muscle and liver through the secretion of various bioactive molecules like leptin, adiponectin, visfatin, fatty acids, resistin and several cytokines (Lara-Castroa *et al.*, 2007; Berg & Scherer, 2005). The dynamic roles played by the adipose tissue in various conditions may be attributed to the levels of myriad bioactive molecules secreted by adipocytes. The levels of these adipocyte-secreted bioactive molecules may vary with a given disease condition. Of the numerous adipocyte-secreted bioactive molecules involved in energy homeostasis, leptin and adiponectin will be considered in this study.

Lipotoxicity

Lipotoxicity refers to the malfunction or death of non-adipose tissues, when such tissues accumulate lipids beyond their storage capacity (Garbarino & Sturley, 2009). Normally, fatty acid homeostasis is achieved by a balance between biosynthetic and degradative processes. In mammalian cells, such as humans, free fatty acids (FFAs) are produced by the *de-novo* biosynthetic pathway or released by the action of lipases that hydrolyze triglycerides and phospholipids. In addition, FFAs can be transported into cells through various mechanisms in response to increased cellular demands or extracellular levels of FFAs (Schaffer, 2002). FFAs obtained from the above processes may be degraded for energy generation or utilized in various anabolic pathways including membrane biosynthesis, synthesis of lipid signaling molecules, post-translational modification of proteins and transcriptional regulation (Schaffer, 2002). When cells obtain more FFAs than required, the excess is esterified to triglyceride for storage in lipid droplets. The mammalian adipocytes, unlike

other cell types, are endowed with an extraordinary capacity to store large quantities of excess FFAs in cytosolic lipid droplets. Other cell-types lack this exceptional lipid-storage ability. When the cellular lipid-storage capacity of non-adipose tissue is overwhelmed, cellular dysfunction or death associated with various pathologies ensues and this condition is termed lipotoxicity.

Accumulated FFAs in blood has been linked to several conditions such as obesity, type 1 and type 2 diabetes mellitus. In such conditions, built-up FFAs and triglycerides can be taken up by the liver, cardiac and muscle tissues (Abel, Litwin & Sweeney, 2008). Excessive lipid accumulation in these tissues has been found to result in cell death and associated dysfunction in the affected organs. It may be important to note that lipid overload in various tissues can be caused by varied genetic and environmental factors. The exact effect depends on the type of cell affected.

In humans, excess lipid in cardiomyocyte has been linked to heart failure and sudden death (Osanai & Okumura, 2007). Lipid overload in the pancreas and skeletal muscle is associated with impaired insulin secretion (Clayton *et al*., 2001; Yoshikawa *et al*., 2001) and sensitivity (Petersen *et al*., 2012; Badin *et al*., 2011; Itani, Ruderman, Schmieder & Boden, 2002) respectively. In the case of liver and kidney, non-alcoholic steatohepatitis (Garg & Misra, 2002) and renal failure (Thomas, Harris, Walls, Furness & Brunskill, 2002) have been observed. Lipid overload exerts its detrimental effects by interfering with various signal transduction pathways involved in oxidative stress (Piro *et al*., 2002; Listenberger, Ory & Schaffer, 2001), apoptosis and mitochondrial membrane transport (Garbarino & Sturley, 2009; Maedler *et al*., 2001).

The extent of lipid overload in the body can be evaluated by estimating the level of plasma or serum triglycerides and FFA. Interestingly, it appears that the negative impact of lipid overload is not executed directly by triglyceride or FFA but by their metabolites which are more toxic (Unger, 2005). Ceramide, a condensation product of palmitoyl-CoA and serine under the influence of serine palmitoyl transferase, is a major culprit. In a rat model, increased ceramide formation was associated with apoptosis and increased expression of inducible nitric oxide synthase (iNOS) in heart (Zhou *et al.*, 2000) and pancreatic islet (Shimabukuro, Higa *et al.*, 1998) cells. Agents such as aminoguanidine and nicotinamide that impaired ceramide formation or iNOS expression blocked the lipoapoptosis in pancreatic islet of the rat (Shimabukura, Ohneda, Lee & Unger, 1997). These observations suggest that diabetics with uncontrolled glycaemia and lipidaemia could be faced with accelerated oxidative-induced metabolic trauma, which may result in enhanced cardiovascular risks.

Changes in lipid levels in response to *Plasmodium* infection have been observed in various studies involving non-diabetic human subjects (Al-Omar *et al.*, 2010; Simpson *et al.*, 2010; Das *et al.*, 1996). These findings point to a potential dynamic role of adipose tissue in infection. As such, it may be interesting to study the changes in diabetics compared with non-diabetics so as to better appreciate the contribution of malaria to the overall adipose tissue metabolism in general and lipid levels alterations in particular, in the two groups of subjects. The expected alterations in lipid levels may be mediated through relevant adipocytokines such as leptin and adiponectin secreted by the adipose tissue.

Leptin

Leptin has been studied extensively in various conditions since its discovery as a satiety signal in 1994 through positional cloning (Zhang *et al*, 1994). It is a 167-amino acid sequence protein with structural similarities with the cytokine family (Koerner *et al.*, 2005). It is mainly produced by the adipocytes although other tissues like the liver, placenta, ovaries, skeletal muscle, pituitary and stomach have been found to express it (Muoio & Lynis, 2002). Leptin suppresses food intake and stimulates energy expenditure by interacting with its receptors in the hypothalamic region of the brain (Koerner *et al.*, 2005).

Leptin receptors exist in varied isoforms which differ with species (Koerner *et al.*, 2005; Fruhbeck, 2001). In addition, it interacts with other hormones like insulin and has been found to be important in the regulation of immune cells, blood cells, pancreatic beta cells, muscle and adipocytes (Bjorbaek & Kahn, 2004). These features of leptin make it a versatile molecule in the context of human development and diseases. Indeed, leptin has been found to contribute to the regulation of puberty and reproduction, maternal, placental and fetal function, alteration of insulin sensitivity in muscle or liver, averts ectopic deposition of lipid and links the immune and endocrine systems (Koerner *et al.*, 2005).

With respect to human diseases, leptin has been linked to diabetes mellitus (Munzberg & Myers, 2005; Banks, 2004), atherosclerosis (DeLany, 2008), cancer (Rose, Komninou & Stephenson, 2004) and bone mass (Takeda, 2005; Wosje, Binkley, Kalkwarf & Specker, 2004). Thus, the role of leptin

appears to have been mostly studied in chronic diseases with little or no information on infectious diseases.

In Ghana and other parts of sub-Saharan Africa where infectious disease burden continues to be a major health challenge, interaction of infectious with chronic diseases may be inevitable. This is particularly so when most of these chronic diseases appear to attenuate the body's defense system. In such situations, individuals with such chronic diseases may be more susceptible to the existing infectious diseases and the interaction of the two may present a novel challenge that requires some investigations. For instance, leptin expression is known to be influenced by acute inflammation (Fantuzzi, 2005).

Inflammatory conditions can be induced by both chronic and infectious agents. As a result, alteration of the degree of inflammation due to the coexistence of chronic and infectious disease conditions such as malaria and diabetes mellitus may reflect in the level of leptin in circulation. However, the pattern of the alteration is unknown and this information may be crucial for an effective management of diabetes mellitus in the presence of malaria in the clinical setting.

In studies using rodents, the levels of leptin gene transcription and leptin protein in adipose tissue increased after 5-8 hours in response to intra-peritoneal challenge with tumor necrosis factor-α (TNFα), interleukin-1 (IL-1) or lipopolysaccharide (LPS) (Grunfeld *et al.*, 1996). Similar findings were observed in a similar study using mice (Sarraf *et al.*, 1997).

Using abdominal subcutaneous adipose tissue from healthy normal to overweight females co-incubated with TNFα, IL-6 and interleukin-1β (IL-1β), Bruun, Pedersen, Kristensen & Richelsen, (2002) found that only TNFα could exert significant inhibitory effect on both leptin production and leptin gene expression with the other cytokines exhibiting no detectable effect. As such, the authors postulated that the long-term decline in leptin secretion and transcription induced by TNFα could influence circulating leptin levels and adipose tissue to brain signaling, a situation that would be important to obesity-related diseases.

Indeed, TNFα has long been associated with obesity, a known risk factor for cardiovascular disease. Thus, with chronic inhibitory effect of TNFα on leptin levels, leptin-signaling may be attenuated leading to further weight gain due to underestimation of body fat stores. Such a condition can have negative impact on glucose homeostasis and insulin action.

Interestingly, infection generally promotes enhanced production of pro-inflammatory cytokines such as TNFα and IL-6 (Khovidhunkit *et al.*, 2004). What is not certain is whether *P. falciparum* infection *in vivo* will give rise to similar observations made *in vitro* with TNFα challenge in human adipose tissue.

Adiponectin

Adiponectin was discovered by four different groups in the mid 90s (Hu, Liang & Spiegelman, 1996; Maeda *et al.*, 1996; Nakano, Tobe, Choi-Miura, Mazda & Tomita, 1996; Scherer, Williams, Fogliano, Baldini & Lodish, 1995) resulting in different initial nomenclature (Koerner *et al.*, 2005). It is a

30-kDa monomeric polypeptide of 247 amino acid residues. The amino acid sequence is made up of a signal sequence, a hypervariable region, a collagen-like domain and a C-terminal globular domain. Whereas the globular domain enables the protein to exert its effects, the cystein-rich collagenous domain is important in the formation of polymeric complexes of 180-kDa hexamer, low molecular weight (LMW) and 400-600 kDa multimer, high molecular weight (HMW) isoforms (Koerner *et al.*, 2005). The globular domains of three monomers which associate into 75-90 kDa trimers have also been detected (Iwaki *et al.*, 2003). The various isoforms differ in biological activity, with the HMW being thought to be more active in human serum than total adiponectin (Fisher *et al.*, 2005).

Adiponectin, a major secretory product of adipocytes, exhibits autocrine/paracrine effects in the adipose tissue but endocrine functions on distal tissues (Lara-Castroa *et al.*, 2007). Autocrine functions of adiponectin include enhancement of adipocyte differentiation, insulin sensitivity (Fu, Luo, Klein & Garvey, 2005) and regulation of adiponectin metabolism (Bauche *et al.*, 2006). Other autocrine functions such as the inhibition of adipocytes-dependent secretion of interleukin-6, interleukin-8, monocyte chemotactic protein-1 and macrophage inflammatory protein-1α/β have been observed (Sell, Dietze-Schroeder, Eckardt & Eckel, 2006).

Adiponectin is synthesized by white adipose tissue. Biological activity of adiponectin is mediated through interaction with its receptors, AdipoRI and AdipoR2 (Yamauchi *et al.*, 2003). Adiponectin exhibits antiatherogenic, anti-inflammatory and antidiabetic properties (Nakatani, Hirose, Yamamoto, Saito

& Itoh, 2008). Several studies have reported inverse correlation between serum levels of adiponectin and variables of adipocity and insulin resistance (Yamamoto, Hirose, Saito, Nishikai & Saruta, 2004; Tschritter *et al*., 2003; Yamamoto *et al*., 2002; Weyer *et al*., 2001). Others have even found that hypoadiponectinaemia could predict onset of type 2 diabetes and cardiovascular diseases (Nakashima *et al*., 2006; Pischon *et al*., 2004; Spranger *et al*., 2003).

Inflammation precedes atherosclerosis, a complex process mediated by inflammatory markers such as TNFα (Hansson, Robertson & Soderberg-Naucler, 2006). The atherosclerotic process commences when the endothelium is injured by inflammatory insult in response to appropriate stimuli. Subsequently, leukocytes get attached to the endothelium and move into the arterial wall to transform macrophages. The transformed macrophages together with migrated smooth muscle cells interact with modified low-density lipoprotein cholesterol (LDL) to form lipid-laden foam cells which hardens into atherosclerotic plaque to block blood vessels with its associated negative effects. Adiponectin protects against the development of cardiovascular disease (Berg & Scherer, 2005) by suppressing the secretion of TNF-α (Fantuzzi, 2005), migration of smooth muscle cells (Goldstein & Scalia, 2004) and foam cell formation (Goldstein & Scalia, 2004) in the atherogenic process. Indeed, Yamauchi *et al*. (2003) established that adiponectin could protect ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. Various studies in humans have demonstrated the protective role of adiponectin against cardiovascular disease (Jansson *et al*., 2003; Shimabukuro *et al*., 2003).

Efficient and effective vascular endothelial function is critical to the normal function of the cardiovascular system. As a result, various indices such as forearm blood flow (FBF) and intima-media thickness (IMT) have been developed to assess the functional status of the vascular endothelial system. Determination of the FBF during reactive hyperemia is a reliable technique applied in the assessment of the functional status of the endothelium. Peak FBF was demonstrated to correlate with indices of adiposity such as body mass index (BMI) and waist circumference (Shimabukuro *et al.*, 2003).

In a prospective study involving 281 respondents with coronary artery disease, Heitzer, Schlinzig, Krohn, Meinertz & Münzel (2001) demonstrated that maximal FBF could predict the risk of cardiovascular event. Indeed, respondents who experienced cardiovascular events exhibited reduced vasodilator responses to acetylcholine and sodium nitroprusside but benefited more immensely from vitamin C infusion (Heitzer *et al.*, 2001). IMT of the carotid artery has been shown to associate with prevalence of cardiovascular disease (Mannami, Konishi, Baba, Nishi & Terao, 1997) and enhanced risk of cardiovascular incident (O'Leary *et al.*, 1999).

Adiponectin correlates negatively with IMT (O'Leary *et al.*, 1999) but positively with FBF (Jansson *et al.*, 2003). These findings indicate that, adiponectin protects against cardiovascular disease by interfering with the initiation, activation, migration and foam cell formation in the atherogenic process.

In a randomized controlled trial involving 820 chronic kidney disease respondents who were followed for a 10-year period, increased mortality was associated with high baseline adiponectin level (Menon *et al.*, 2006).

Similar observations have been made in other large sample studies involving adults with or without cardiovascular disease (Wannamethee, Whincup, Lennon & Sattar, 2007; Laughlin, Barrett-Connor, May & Langenberg, 2006) and type 2 diabetics above 70 years (Singer *et al.*, 2012).

Interestingly, in the cardiovascular health study, a large sample study involving respondents aged 65 years and above, Kizer *et al.* (2012) reported a U-shaped association between adiponectin level and mortaltity, notably in the apparently normal cohort. In addition, the optimal level of adiponectin observed in their study group was 12.4 mg/L (Kizer *et al.* (2012). In breast cancer patients, however, low adiponectin was associated with increased mortality (Duggan *et al.*, 2010). These findings appear to suggest that the prognostic value of adiponectin level depends on the underlying health status of an individual. Considering the involvement of adiponectin in inflammation, diabetes and cardiovascular diseases, it may be worthwhile to study the changes that may occur in response to malaria in both diabetics and normal controls. Although lower levels of adiponectin has been observed in diabetics (Lindsay *et al.*, 2002; Weyer *et al.*, 2001), the changes in levels of adiponectin in response to malaria is yet to be studied extensively. The present study will contribute to this vital information needed to fully appreciate the relevance of malaria to cardiovascular disease risk of respondents.

Inflammation in Cellular Homeostasis

Inflammation remains the body's major defense mechanism to injury or infection in order to re-establish cellular homeostasis and function. However, the process can be detrimental to cellular health and homeostasis depending on the extent, duration and site of the inflammatory action. Low-grade chronic inflammation has been associated with obesity-related health problems in both animal and human studies (Holland *et al*., 2011; De Rooij *et al*., 2009; Hotamisligil, Shargill & Spiegelman, 1993) with even a causal role being suggested (Holland *et al*., 2011; Uysal, Wiesbrock, Marino & Hotamisligil, 1997). Obesity-related increased inflammatory status is thought to emanate from immune cells in adipose tissue (Weisberg *et al*., 2003; Xu *et al*., 2003).Thus, the connection between immunity and adipose tissue metabolism is crucial in the pathogenesis of obesity-related disease conditions such as diabetes mellitus (Genco, Grossi, Ho, Nishimura & Murayama, 2005).

Immune responses are grouped into innate and adaptive responses (Janeway & Medzhitov, 2002). The innate immunity, also called natural immunity, deals with the immediate non-specific response of the immune system to infectious agents. This response is possible due to pathogen recognition receptors (PRRs) on the surface of existing immune cells which get activated by the presence of pathogens. The PRRs exist in different forms or types (Mills, 2011; Janeway & Medzhitov, 2002) possibly for recognition of different kinds of infectious agents. Likewise, cells that constitute the innate immunity are of different kinds such as eosinophils, macrophages, dendritic cells, mast cells and natural killer cells (Sun, Ji, Kersten & Qi, 2012). Innate immunity modulates the adaptive immune response of an organism to a

particular pathogen because the specificity and intensity of the adaptive response to the given pathogen is to some extent determined by the innate immune response to that pathogen. Thus, the adaptive or acquired immunity of an organism can be viewed as a superior form of its innate immunity.

Acquired or adaptive immunity involves highly complex defense machinery executed by different but highly specific cell types that require a relatively longer time for development, stimulation and differentiation against a specific foreign antigen. Unlike the innate immunity, adaptive immune cells keep memory of pathogens encountered so that the re-encounter of the same pathogen incites a faster and stronger reaction (Sun *et al*., 2012).

A distinctive feature of inflammation is the permeation of immune cells into target cell or tissue. The type of immune cells that infiltrate the target tissue depends on the type of inflammation. Generally, inflammation can be categorized into two; acute and chronic inflammation. In acute inflammation, the type of immune cells that infiltrate the target tissue is of the innate origin. Thus, acute inflammation triggers a swift but non-specific innate immune response to re-establish cellular homeostasis and function.

When acute inflammatory response is unsuccessful in re-establishing the needed homeostatic condition, due to the continued presence of the insult, then chronic inflammation ensues. In chronic inflammation, immune cells from both the innate and adaptive origins permeate the target tissue (Wang, Gao, Zakhari & Nagy, 2012) to orchestrate the removal of the inflammatory inducer in order to restore the required homeostatic condition.

Interestingly, the inflammatory process is highly dynamic in that the cell types involved differs with the trigger of inflammation. For instance,

immune cells that may be involved in inflammation caused by bacteria may differ from those that will deal with inflammation caused by virus or any other injury. Also, alcohol-induced inflammation (Wang *et al.*, 2012) differs from obesity-induced inflammation (Sun *et al.*, 2012) although both conditions may result in fatty liver. As a result, it becomes pertinent to isolate the specific inducer of the inflammatory process in a given tissue so as to better appreciate its overall impact on cellular function and homeostasis.

A number of inflammatory markers including CRP, IL-6 and TNFα have been identified (Stanek *et al.*, 2010; De Rooij *et al.*, 2009; Rader & Daugherty, 2008; Kusminski *et al.*, 2005). However, CRP remains the most reliable and widely studied marker for assessing cardiovascular disease risk (Blake & Ridkler, 2003; Liuzzo & Rizzello, 2001). Also, the level of CRP is regulated by IL-6 and TNFα (Blake & Ridkler, 2003). As a result, CRP is employed in the current study as the main inflammatory marker for the assessment of cardiovascular disease risk posed by malaria in diabetics compared with non-diabetic controls. Since the the current study relates to obesity-associated conditions like type 2 diabetes, more emphasis will be placed on data related to obesity-associated inflammation.

Inflammation in Diabetes Mellitus

Diabetes mellitus is a metabolic disease affecting millions of people worldwide. Although inflammation in diabetes will most likely be chronic than the acute type due to the chronic nature of the diabetic condition, evidence from both animal and human studies suggest the existence of the dual types of inflammation. Indeed, there cannot be a chronic condition in isolation without

it initiating as an acute condition. In other words, chronicity in any given condition is established solely by the persistence of its acute format. To this end, the inflammation observed in diabetes mellitus commences initially as the acute format before progressing gradually to the chronic stage as a result of failure of the cellular mechanism to resolve the acute process.

Although the exact process of initiation of inflammation in obesity, a major risk factor for T2D, is yet to be elucidated (Sun *et al.*, 2012), results from several animal studies have implicated lipids in the inflammatory condition (Lee *et al.*, 2011; Kleemann *et al.*, 2010; Radonjic *et al.*, 2009). A number of studies in rats have reported increased adiposity, body weight (Kleemann *et al.*, 2010; Li, Yu, Pan & Unger, 2002;) and stimulated insulin resistance (Samuel *et al.*, 2004; Kraegen *et al.*, 1991) in response to short-term high fat diet, a diet that consists of 60% calories from fat. Using gene microarray analyses, Radonjic *et al.* (2009) and Kleemann *et al.* (2010) observed altered expressions of genes linked to inflammation. Lately, Lee *et al.* (2011) have established conditions necessary for development of insulin resistance in rat following three days of high fat diet by ruling out at least any potential role for macrophages. The above findings provide ample evidence for the link among obesity, inflammation, insulin resistance and potential onset of diabetes mellitus at molecular and cellular levels. Also, these observations reiterate the central role of dietary composition, a modifiable risk factor, in the pathogenesis of obesity, insulin resistance and overt diabetes in at least, animal models.

In spite of the fact that human obesity is not developed in three days but over several months and years, the above findings are still relevant in

understanding the development of human obesity and related disease conditions. Development of obesity in humans can be likened to continued high fat diet feeding in animals (Sun *et al.*, 2012) as the condition results in increased adipose tissue mass, size and subsequent progression to hyperlipidaemia, insulin resistance, glucose intolerance and diabetes (Sun *et al.*, 2012). Thus, obesity results from positive energy balance.

Inflammation in diabetes may originate from adipose tissue and non-adipose tissue apoptosis as a result of lipotoxicity. Elevated adiposity has been linked to renal failure (Thomas *et al.*, 2002), cell death (Alkhouri *et al*, 2010; Murano *et al.*, 2008; Cinti *et al.*, 2005), increased endoplasmic reticulum stress (Sha, He, Yang & Qi, 2011; Hotamisligil, 2010; Ozcan *et al.*, 2004) and hypoadiponectinaemia (Sun *et al.*, 2012). It is believed that the negative effects of increased adipocity are mediated through inflammatory signaling (Shi *et al.*, 2006).

Indeed, macrophages, which are important innate immune cells that function to remove foreign antigens, dead or damaged cells and also act as antigen-presenting cells have been found to permeate or undergo expansion in adipose tissue in obesity (Weisberg *et al.*, 2003; Xu *et al.*, 2003). Since macrophages play a critical role in acute inflammation, their proliferation in obese adipose tissue is thought to signify inflammation in such tissues although a universal consensus on the exact relevance of macrophage-mediated inflammation continues to be elusive (Sun *et al.*, 2012).

Interestingly, other immune cells such as mast cells (Liu *et al.*, 2009), lymphoid cells (Winer *et al.*, 2009) and myeloid-derived suppressor cells (Xia *et al.*, 2011; Gabrilovich & Nagaraj, 2009; Ostrand-Rosenberg & Sinha, 2009)

have been found in increased abundance in obese adipose tissue with varied effects on insulin sensitivity (Sun *et al.*, 2012). All these reports point to a role for inflammation in obesity-induced health conditions. In addition, the involvement of these different immune cells, some of which function to suppress the actions of other immune cells, appear to indicate that inflammation, even in chronic state, is fairly controlled.

Thus, it is the balance between the number of pro- and anti-inflammatory immune cells at any given point in time in a given cell or tissue that matters. Hence, in inflammation-induced disease conditions, the number and strength of pro-inflammatory immune cells must outweigh their anti-inflammatory counterparts to perpetuate the disease state.

Indeed, the recognition of inflammation, assessed by elevated serum levels of pro-inflammatory markers such as CRP, IL-6 and TNFα in human diseases like diabetes is well documented (De Rooij *et al.*, 2009; Considine *et al.*, 2008; Genco *et al.*, 2005; Kusminski *et al.*, 2005; Vendrell *et al.*, 2004). What still remains a challenge is the extent and impact of the expected inflammation in such conditions in the presence of malaria, a scenario that appears to be peculiar to diabesity-engulfed malaria endemic regions of the globe. This is crucial for cogent development of any intervention strategy aimed at managing this expected novel kind of inflammation. Also, it may be interesting to investigate the specific immune cells that may participate in these conditions and their respective roles in tilting the balance in favour of inflammation particularly at the molecular level, a scope not covered by the current work. In this research, inflammation will be assessed by monitoring changes in serum levels of CRP before and during malaria attack.

Inflammation in malaria

Malaria, caused by *P. falciparum,* is an acute systemic disease thought to be the most dangerous form of human malaria (Postma *et al*., 1996) and responsible for the greatest human suffering possibly by any single pathogen in recent times (Clark, Budd, Alleva & Cowden, 2006). The mechanism by which the disease exerts its detrimental impact is complex and has been debated for years based on two main concepts: the hypoxia-driven and the cytokine-driven hypotheses (Clark *et al*., 2006).

Recently, a third model, the tissue factor model, which seeks to explain human malaria in terms of parasite sequestration associated with expression of tissue-factor in the endothelium and related coagulation abnormalities has been proposed (Francischetti, Seydel & Monteiro, 2008). Whereas the hypoxia-driven concept argues that the disease burden and potential death results from inadequate supply of oxygen to vital organs, the cytokine-inclined hypothesis is of the view that, the signs, symptoms and probable deadly outcome of *falciparum* malaria is due to the disproportionate production and release of inflammatory cytokines (Clark *et al*., 2006).

Although the hypoxia-driven hypothesis is touted to be unique to *falciparum* malaria (Clark *et al*., 2006) and more acceptable to malariologists (Planche, Dzeing, Ngou-Milama, Kombila & Stacpoole, 2005), the concept is still dependent on the negative effects of excessive release of inflammatory cytokines. Thus, the difficulty in accepting the cytokine-driven hypothesis by the malaria research community is due to the fact that excessive inflammatory cytokine release is common to other systemic infections such as sepsis (Cain, Meldrum, Harken & Mcintyre, 1998) and influenza (Beigel *et al*., 2005).

Notwithstanding the seeming controversy surrounding the peculiarity of malaria-induced inflammation, pathogenesis of the disease is thought to be mediated at least in part through the action of released inflammatory cytokines (Iwalewa, McGaw, Naidoo & Eloff, 2007; Clark *et al*., 2006; Odeh, 2001). Thus, irrespective of the model proposed to be responsible for malaria pathogenesis, the central role of inflammatory cytokines cannot be underestimated.

Indeed, various inflammatory mediators like CRP (Yapi *et al*., 2010) and TNFα (Lyke *et al*., 2004) have been associated with *P. falciparum* infection in humans with their relative levels rising with increased parasitaemia (Thevenon *et al*., 2010; Yapi *et al*., 2010). The enhanced levels could probably be due to increased expression levels as demonstrated by various molecular studies (Oakley, Gerald, McCutchan, Aravind & Kumar, 2011; Parroche *et al*., 2007; Coban *et al*., 2005; Krishnegowda *et al*., 2005; Pichyangkul *et al*., 2004).

What is not clear is the extent to which the inflammatory mediators will be released in the presence of both malaria and diabetes, a challenge that the current work seeks to address. In addition, the long and short term effects of the inflammation induced by the dual conditions of malaria and diabetes may be worthy of investigation.

Inflammation in diabetics with malaria

The pattern and extent of inflammation in the dual conditions of diabetes and malaria in the same individual may differ from that observed in either condition alone. This is based on the view that diabetes is a chronic condition compared with malaria which is considered as an acute infectious

condition although the possibility of chronicity cannot be ruled out. Indeed, cytokines, which are soluble factors that mediate inflammation, are many and can be grouped into acute and chronic forms (Feghali & Wright, 1997). As a result, one would expect that acute conditions like malaria would induce production and release of acute inflammatory cytokines for the chronic conditions to also be associated with the chronic inflammatory cytokines.

However, the pleiotropic nature of these soluble molecules in relation to their functions makes it uneasy to have such a clear-cut distinction for acute and chronic inflammatory cytokines. Nonetheless, their primary function and the duration required for their release in response to a given stimulus makes it feasible for some level of distinction to be made between acute and chronic forms of inflammatory cytokines. It must be noted that cytokines are not always pro-inflammatory but can be anti-inflammatory as well (Feghali & Wright, 1997).

Thus, the balance between pro- and anti-inflammatory roles played by the cytokines should be of interest. Interestingly, their anti-inflammatory roles are beneficial to the health and wellbeing of the organism (Kurtzhals *et al.*, 1998) but their pro-inflammatory roles are detrimental. Unfortunately, most scientific literature has discussed cytokines in a negative perspective in relation to disease pathogenesis probably due to their association with disease progression (Yapi *et al.*, 2010; Thevenon *et al.*, 2010). In this work, the pro-inflammatory roles of cytokines will be the focus unless otherwise indicated.

In relation to infectious diseases, increased levels of acute inflammatory cytokines like IL-1, IL-6 and TNFα have been associated with malaria (Maitland & Marsh, 2004; Day *et al.*, 1999). These cytokines have several

features in common in that their genes are mapped to different chromosomes of the human genome, induce fever through increased synthesis of prostaglandins and trigger acute phase reactant protein production (Feghali & Wright, 1997).

Fever is a common symptom in most infectious conditions and can be beneficial or detrimental depending on the duration and nature of the triggering agent (Angulo & Fresno, 2002). Fever is one of the important symptoms of malaria in clinical setting. Thus, malaria, being an acute condition may be associated more with increased but decreased levels of pro- and anti-inflammatory cytokines respectively.

As expected, low plasma levels of interleukin-10 (IL-10), an anti-inflammatory cytokine, was found in different forms of malaria with the degree of reduction of the anti-inflammatory cytokine correlating with disease severity (Kurtzhals *et al.*, 1998). Indeed, the role of IL-10 could be more prominent in chronic conditions where its anti-inflammatory features will be required to regulate the perpetual negative effects of pro-inflammatory reactions (Feghali & Wright, 1997). It is therefore not surprising that IL-10 is considered as a cytokine associated with chronic conditions (Feghali & Wright, 1997).

In the case of T2D, IL-6 and TNFα have been implicated in its pathogenesis (Liu *et al.*, 2009; Meigs, Hu, Rifai & Manson, 2004; Pradhan, Manson, Rifai, Buring & Ridker, 2001). The involvement of IL-6 and TNFα in T2D does not make T2D an acute condition; rather, it exemplifies the nature of the inflammation involved possibly in the initial uncontrolled stages of the disease.

Indeed, every chronic condition starts as an acute type with chronicity being perpetuated only by the persistent presence of the triggering agent. As a

result, persistent elevation of blood glucose as observed in uncontrolled diabetes may elicit acute phase response which involves acute inflammatory cytokines.

Thus, with time, proper management of the condition should lead to a switch from acute to possibly the chronic inflammatory type of cytokines. Indeed, low grade chronic inflammation has been linked to insulin resistance (De Rooij *et al.*, 2009), which is a characteristic feature of T2D. The intensity of the observed inflammation could be considered to be of low grade probably due to attenuation of the inflammation by anti-inflammatory effects of the cytokines associated with chronic conditions (Sun *et al.*, 2012; Wu *et al.*, 2011; Feghali & Wright, 1997). In this regard, the inflammation observed in T2D reflects a balance between cytokines involved in acute and chronic conditions and this depends on the duration and management of the T2D condition.

In the presence of malaria, this balance may be tilted in favour of the acute pro-inflammatory cytokines since the malaria parasite elicits that kind of immune response (Maitland & Marsh, 2004; Day *et al.*, 1999). What is not clear is the extent of inflammation that will be induced by the parasite in diabetics compared with non-diabetic controls.

Although, it may be logical to expect a much more heightened inflammation in the phase of elevated blood glucose and malaria parasite as a result of synergistic effect of the dual conditions, the possibility of an antagonistic outcome and thus attenuated inflammatory response cannot be ruled out. This puzzle, which needs to be unravelled, is the essence of the current work.

Oxidative Stress in Diabetes and Malaria

Life is perpetuated by carefully regulated biochemical processes that take place in living entities. When the regulatory machinery of these biochemical processes is compromised, over time, a disease condition ensues. Oxidative stress is one of such phenomena that results due to a disparity between reductive and oxidative processes that take place in a given cell or organism. The day to day activities of the human system is controlled by a careful balance between synthetic and degradative processes.

Biochemically, either of these processes can generate intermediates that can be reductive or oxidative. As such, oxidative stress reflects a situation where a relatively higher concentration of oxidised than reduced molecules exist in the cell. The presence of such oxidised molecules is not always deleterious to the cell, rather, the level and the location of such oxidised entities is the issue (McEligot, Yang & Meyskens, 2005).

Oxidative stress has been studied extensively in relation to several diseases including malaria, cancer and diabetes (De Rooij *et al.*, 2009; Considine *et al.*, 2008; Naidu, Suryakar, Swami, Katkam & Kumbar, 2007; Kusminski *et al.*, 2005; Vendrell *et al.*, 2004; Briganti & Picardo, 2003; Postma *et al.*, 1996). The disease results from oxidative damage caused to cellular molecules such as proteins, lipids, carbohydrates and nucleic acids (Ogino & Wang, 2007; Guha, Kumar, Choubey, Maity & Bandyopadhyay, 2006). The damage could be due to chemical modifications of such molecules through oxidation, making them unable to perform their respective functions effectively to ensure normal cellular homeostasis. Compounds known, collectively, as reactive oxygen species (ROS) and reactive nitrogen species

(RNS) have been implicated in oxidative damage of cellular molecules and organelles.

ROS/RNS are generated in the human body by various metabolic pathways and are involved in various physiological processes such as aging, immune function, cell growth and other signal transduction pathways (Naidu *et al*., 2007; McEligot *et al*., 2005). ROS/RNS can also be generated by inter action with external insults like ultraviolet and other ionizing radiations. Chemically, ROS/RNS are heterogeneous group of compounds consisting of hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxide (OH^-), hydroxyl radical ($OH^\bullet$), oxides of nitrogen (NO_x) and peroxynitrite ($ONOO^-$) (Ogino & Wang, 2007). Reaction of ROS/RNS with other biomolecules results in chemical modification of affected molecules that can be monitored through detection of such products in biological specimen.

Due to the heterogeneous nature of ROS/RNS, their reaction with the various macromolecules gives rise to a variety of products depending on the type of macromolecule involved resulting in varied detection methods (Cao & Prior, 1998). For instance, reaction of ROS/RNS with carbohydrates gives rise to products that differ from those produced by reaction of ROS/RNS with other macromolecules such as proteins and nucleic acids.

In spite of the heterogeneous nature of ROS/RNS, they appear to exhibit a common reaction pattern to some extent, which involves the so-called free radical mechanism (Ogino & Wang, 2007; McEligot *et al*., 2005). In this way, their reaction with macromolecules creates instability in the affected molecules. For instance, a reaction of a given macromolecule with the $OH^\bullet$ results in the transfer of the radical status to the macromolecule which then

continues to react with nearby molecules in a chain manner till the radical is quenched by an appropriate antioxidant to terminate the chain reaction. Thus, the product of a reaction between ROS/RNS and a given macromolecule appears more oxidative and the accumulation increases the oxidative status of the cell.

In the physiological state, the oxidative status of a cell is carefully regulated by appropriate level and activity of antioxidants. As a result, the level of oxidised products in the cell is controlled within a narrow range. This implies that a persistent increase in the levels of such oxidised products due to enhanced activity of ROS/RNS connotes dysregulation of the expected redox balance in the cell, a situation that is associated with various pathological states (De Rooij *et al.*, 2009; Considine *et al*., 2008; Naidu *et al*., 2007; Kusminski *et al*., 2005; Vendrell *et al*., 2004; Briganti & Picardo, 2003). Such a situation normally coexists with reduced levels and activities of antioxidants.

Cells are endowed with spectacular intrinsic reductant or antioxidant systems that antagonize the negative effects of ROS/RNS and keep the cell in a fairly reduced state although the reducing ability can also be acquired extrinsically (McEligot *et al*., 2005) through the diet.

Based on the mechanism of action, Plavec *et al*. (2008) classified the reductant system into three main groups: preventive, scavenging and enzyme antioxidants. Whereas the preventive antioxidants such as ferritin, transferrin, myoglobin, caeruloplasmin, metallothionine and albumin avert the creation of new ROS/RNS, the scavenging antioxidants including reduced glutathione, ascorbic acid, α-tocopherol, bilirubin, uric acid and β-carotene terminate the radical chain reaction by eliminating the ROS/RNS when formed. Enzyme

antioxidants like catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase catalyse reactions that detoxify harmful ROS/RNS generated in metabolic processes.

In spite of the seeming dominance of proteins in the composition of the intrinsic antioxidant system of the cell, some researchers have suggested that proteins may even be more susceptible to oxidative damage than the other macromolecules (Gieseg, Duggan & Gebicki, 2000). This observation exemplifies the heterogeneous and complex nature of chemical entities that constitute proteins and the inherent relatively lower antioxidant capacity of some of the chemical constituents of proteins compared with other macromolecules.

In relation to human disease, oxidative stress has been found to play a key role in both infectious and chronic diseases. A role for oxidative stress has been proposed for the pathogenesis of malaria (Guha *et al*., 2006; Postma *et al*., 1996), a disease that induce multiple organ dysfunction (Clark *et al*., 2006; Clark, Alleva, Mills & Cowden, 2004; Maitland & Marsh, 2004).

In terms of diabetes, the role of oxidative stress is well documented (Lapolla *et al*., 2007) as the hyperglycaemia; a distinctive feature of the disease is a known cause of enhanced oxidation (Lapolla *et al*., 2007; Tajiri *et al*., 1997).

Various studies (Lapolla *et al*., 2007; Guha *et al*., 2006) on oxidative stress have considered the phenomenon in terms of either malaria or diabetes alone. Scientific literature on oxidative stress in relation to both diabetes and malaria in the same individual appears lacking. With the current trend of increased diabesity in malaria-endemic regions like Ghana (Danquah *et al*.,

2010), the consequence of comorbidity of both malaria and diabetes in relation to oxidative stress should be of serious concern to the scientific community, a feat being attempted by the current study.

It is important to acknowledge that no single study may be exhaustive in assessing all the various antioxidants and pro-oxidants systems in the human body in relation to any given disease and the present study is no exception. As a result of this, the current study monitors the changes in serum total antioxidants capacity and lipid peroxide levels of participants in response to *P. falciparum* infection. No attempt is made at measuring the individual antioxidant levels or all the various biomarkers of oxidation of macromolecules in human sera.

Despite the seeming universal acceptance of a role for oxidative stress in disease pathogenesis, no such consensus appears feasible on the exact biomarker(s) representative of the total antioxidant status of a biological sample. The antioxidant status modulates the overall redox balance of the cell.

Whereas some researchers argue for the measurement of individual antioxidants levels in blood (Sies, 2007; Huang, Ou & Prior, 2005), a task that appears daunting for any single research work, due probably to the chemical heterogeneity and high number of antioxidants molecules in blood, others are in favour of estimation of a single parameter called the total antioxidant capacity (TAC), deemed to be a more representative marker of true redox status of biological entities (Suresh, Annam, Pratibha & Prasad, 2009; Nagy *et al.*, 2006; Besler & Comoglu, 2003; Cao & Prior, 1998).

In addition, no single constituent of blood antioxidant system may completely reveal its protective efficiency against oxidative damage due

possibly to interactions that take place among the various antioxidant compounds *in vivo* (Suresh *et al*., 2009). Thus, TAC is believed to exhibit the overall impact of all the antioxidants present in blood and other body fluids (Suresh *et al*., 2009; Nagy *et al*., 2006; Besler & Comoglu, 2003).

However, opponents of TAC measurement contend that the heterogeneous nature of the various chemical groups that constitute antioxidants systems of blood and other body fluids makes it impossible for any single, reliable and truly representative parameter to be measured as an indicator of cellular oxidative status, making the use of the words total and capacity inappropriate (Sies, 2007; Huang *et al*., 2005).

Indeed, it is argued that all the available protocols that determine TAC levels of blood fail to fully account for the enzyme antioxidant systems and rather measure antioxidant levels of other biomolecules with minor contribution to the blood antioxidant systems (Sies, 2007; Huang *et al*., 2005) . Such workers call for a new choice of word to replace TAC since TAC in their view, is misleading.

As the expected new term is yet to be made available to the scientific community, coupled with the admission by the opponents of TAC, that the various methods measure at least some antioxidant levels of blood, TAC then becomes an indicator of antioxidant status in the current study. It must be noted that, TAC may also be referred to as total antipoxidant power (TAP) in this thesis.

Relationship between Oxidative Stress and Inflammation

Inflammation and oxidative stress must be interrelated as inflammatory cytokines appear to exhibit their detrimental actions through the induction and production of reactive oxygen species. For instance, enhanced production of hydroxyl radical, caused by malaria is postulated to be responsible for mitochondrial pathway-dependent apoptosis and hepatic dysfunction in Swiss albino mice (Guha *et al*., 2006).

In human studies, malaria has been found to elicit acute inflammatory response (Maitland & Marsh, 2004; Day *et al*., 1999) with the level of inflammatory markers increasing with increased parasitaemia (Thevenon *et al*., 2010; Yapi *et al*., 2010). Thus, the presence of the *Plasmodium* parasite of malaria causes the release of inflammatory cytokines as well as oxidative stress markers. Similarly, the role of inflammation and oxidative stress in the pathogenesis of type 2 diabetes is well acknowledged (De Rooij *et al.*, 2009; Considine *et al*., 2008).

Evidence of a link between oxidative stress and inflammation has been provided at the molecular and cellular levels where the transcription and subsequent translation of the anti-oxidant enzyme, catalase, was found to be suppressed by TNFα, a known pro-inflammatory cytokine (Beier, Volkl & Fahimi, 1997). This observation implies that inflammatory cytokines reduce the antioxidant capacity of cells by repressing production and activity of anti-oxidant enzymes that detoxify the poisonous effects of pro-oxidant markers and thus exacerbate the negative impact of ROS.

As a result, in chronic conditions like diabetes mellitus where the release of inflammatory cytokines appears perpetual, oxidative burst and

reduced antioxidant capacity should be more pronounced. Indeed, the coexistence of reduced antioxidant but increased levels of ROS in plasma and sera of diabetic patients compared with controls has been well documented (Vairamon, Babu & Viswanathan, 2009; Besler & Comoglu, 2003; Rahbani-Nobar *et al.*, 1999).

However, information on the extent of inflammation and oxidative stress in the presence of malaria and diabetes in the same individual appears scanty in current scientific literature. Although one may expect further increased levels of both oxidative stress and inflammatory markers in diabetics with malaria compared with those without malaria or apparently healthy controls, the complexities of both processes make it difficult to predict with certainty. This uncertainty may be resolved by monitoring changes in the levels of biomarkers of oxidative stress, antioxidant status and inflammation in diabetic and non-diabetic controls before and during malaria. This task is a major objective of the current study.

Malaria in Human

Of the more than 100 species of the malaria parasite, *Plasmodium,* discovered so far, only five have been found to infect human beings (White, 2008; Weatherall *et al.*, 2002). These human host parasites of malaria include the *P. falciparum, P. malariae, P. vivax, P. ovale and P. knowlesi.* Malaria is a complex illness considered to be the most harmful and widespread parasitic infection globally (Haldar, Murphy, Milner & Taylor, 2007). It affects over 500 million people with associated 1-2 million deaths annually (Milner, Montgomerry, Seydel & Rogerson, 2009; Haldar *et al*, 2007). About 90% of

these infections and mortalities occur in sub-Saharan Africa (Asante & Asenso-Okyere, 2003) particularly in children under five years of age (Snow, Guerra, Noor, Myint & Hay, 2005).

The enormous malarial cases result in over 800 million days of illness per annum (Breman, Alilio & Mills, 2004) and therefore not surprising that the disease is almost synonymous with poverty in that the most economically poor regions of the globe continue to suffer the greatest burden of malaria (Mocumbi, 2004).

Indeed, malaria causes poverty and perpetuates the vicious cycle of poverty (Mocumbi, 2004; Sachs & Malaney, 2002; Gallup & Sachs, 2001), making it difficult for malaria-endemic countries to escape from the poverty net. For instance, in the case of Africa, malaria deprives it of an estimated U.S. $12 billion per annum in lost Gross Domestic Product (GDP) (Mocumbi, 2004) and consumes 40% of public health expenditure (Asante & Asenso-Okyere, 2003).

With respect to Ghana, the disease is known to be responsible for more than 40% of out-patient visits in public health facilities (Asante & Asenso-Okyere, 2003) with substantial negative impact on the per capita income of poor households (Akazili, 2002), a situation that aggravates the already precarious income gap between the rich and the poor. Therefore, any improvement in efforts at controlling malaria can have a serious impact on the economic wellbeing of poor households. The various control measures notably, the pharmacological methods, have often targeted some of the stages in the life cycle of the parasite.

Life cycle of *Plasmodium* parasite

The life cycle of the malaria parasite is complex with the various species of the *Plasmodium* parasite that infect humans exhibiting a similar life cycle. The parasites live partly in humans and in female *Anopheles* mosquitoes (Haldar *et al*, 2007).

In the mosquito vector, the parasite goes through a developmental phase called sporogony during which it multiplies mainly asexually to produce sporozoites, which migrate to the salivary gland of the mosquito vector. When the infected mosquito induces an infective bite on a human host, the sporozoites get injected into the subcutaneous tissue before entering the bloodstream where they circulate for few minutes prior to invasion of hepatocytes.

As demonstrated over a decade ago, the sporozoites migrate through a number of hepatocytes before further development ensues (Mota *et al*., 2001). In the hepatocytes, the sporozoites multiply asexually and develop into thousands of merozoites capable of infecting red blood cells (RBC) upon release into the bloodstream from the liver. After successful invasion of RBC by the released merozoites, further asexual multiplications continue inside the erythrocyte.

Clinical manifestation of the disease is thought to be due to the intra-erythrocytic multiplication of the malaria parasite (Haldar *et al*, 2007; Weatherall *et al*., 2002). Some of the merozoites divide into schizonts with others differentiating into male and female gametocytes. When a female anophelex mosquito bites an infected host, these gametocytes are taken up by the mosquito. In the mosquito midgut, the male gametocyte goes through

nuclear division to give rise to flagellated microgametes for fertilization of the female macrogamete to produce ookinete. The ookinete moves through the mosquito gut wall to the exterior to form the oocyst. It is the oocyst which ruptures to release the sporozoites into the mosquito to complete the sporogenic phase of the parasite's life cylce.

The duration of the intra-erythrocytic cycle is 48-72 hours depending on the species of the parasite (Weatherall *et al*., 2002). Apart from this, the various species differ in invasion requirements, average parasite density, incubation period and other infection characteristics. The phase of the growth cycle of the *Plasmodium* parasite that occurs in human host is called the schizogony. Thus, the schizogenic growth of the parasite involves intra- and exo-erythrocytic components.

Interestingly, the major malaria parasite species responsible for about 95% of all malaria-associated morbidities and mortalities globally and Ghana in particular is the *P. falciparum* (Kreuels *et al*., 2010; Guinovart, Navia, Tanner & Alonso, 2006; Greenwood, Bojang, Whitty & Targett, 2005; Hoffman, Subramanian, Collins & Venter, 2002). As described elsewhere, various molecular studies have identified several genes or gene products and other factors that interact to ensure survival of the parasite in its human and mosquito hosts and eventual induction of the malarial disease in humans (Aly, Vaughan & Kappe, 2009; Haldar *et al*, 2007; Weatherall *et al*., 2002). The present work focuses on the effect of malaria in relation to cardiovascular disease risk of infected persons, particularly if such persons are diabetic.

Due to the dominance of *P. falciparum* in malaria-related issues in Ghana (Danquah *et al*., 2010; Kreuels *et al*., 2010), complications associated

with malaria in relation to the overall cardiovascular disease risk could be attributed mainly to *P. falciparum* infectoins. To this end, the current study concentrates on the malaria caused by *P. falciparum* infection.

Malaria and Cardiovascular Disease

Although a direct link of malaria to cardiovascular disease is yet to be established, a careful look at the pathogenesis of the disease reveals that the biological mechanism adopted by the malaria parasite in unleashing its negative impact on susceptible hosts appears to involve some molecules known to mediate cardiovascular dysfunction (DeMast *et al*., 2007; Mohanty *et al*., 1997).

For instance, a characteristic feature of *P. falciparum* pathogenesis is the ability of the parasite to alter the surface properties of infected RBCs such that the infected RBCs become more rigid and adhesive. As a result, the RBCs infected with late stages of the parasite get stuck to capillary and postcapillary venules of the endothelium in the deep microvasculature, through a process called cytoadherence.

In addition, such infected RBCs can adhere to uninfected RBCs, to give rise to red cell rosettes. Cytoadherence leads to parasite sequestration in various organs, allowing it to escape detection in blood film examination, clearance by the spleen and the host's immune system. As such, the parasite continues to grow and multiply to extremely high levels, obstructing microcirculation, energy production and stimulating cytokine release and all these contribute to the multiple organ dysfunction normally observed in severe malaria (Clark *et al*., 2006; Miller, Baruch, Marsh & Doumbo, 2002).

Indeed, it is widely acknowledged that the neurological damage, coma and fatal outcome normally associated with cerebral malaria is a consequence of hypoxia caused by impaired microcirculation as a result of cytoadherence and sequestration (DeMast *et al*., 2007; Taylor *et al*., 2004; Pongponratn *et al*., 2003; Newton, Taylor & Whitten, 1998; Mohanty *et al*., 1997; Turner, 1997) of parasitized RBCs.

This sequestration-dependent hypoxia-driven cardiovascular effect of malaria was challenged almost a decade ago when the pathogenesis of malaria was compared with other conditions with similar clinical presentations (Clark *et al*., 2004). The point of controversy hinged on the inability of the sequestration-driven hypothesis to explain the lack of residual neurological defect observed in respondents who recovered from coma and other neurological symptoms due to cerebral malaria (Clark *et al*., 2004; Warrell *et al*., 1982).

This view stems from a comparison of the purported sequestration-dependent hypoxic condition induced by malaria with that observed in stroke patients where a reduction of just about 15-20 mm Hg in perfusion pressure to any part of the brain for 20-30 minute resulted in irreversible neurological damage (Adams, 1989).

Thus, whereas stroke from hypoxia leaves a residual neurological damage after recovery, no such finding has as yet been made for cerebral malaria patients who recover from hypoxia-induced coma believed to have been caused by sequestered parasitized RBCs. As such, Clark *et al.* (2004) argue that the site of parasitized RBC sequestration must vary between malaria-susceptible patients and healthy malaria-tolerant individuals. This view

appears to be ironically supported by some of the pioneering proponents of sequestration-driven hypoxia, Olweny *et al.* (1986), who reported comparable parasite load between cerebral malaria and severe non-cerebral malaria patients.

Thus, since parasite load does not appear to vary between cerebral malaria and severe non-cerebral malaria patients, then, a plausible explanation to their varied clinical presentations could be ascribed to a possible difference in location of sequestered parasitized RBCs in the vascular tree (Clark *et al.*, 2004). Indeed, the phenomenon of sequestration in malaria pathogenesis is not and has never been in doubt (Gyan *et al*., 2009; Clark *et al*., 2004; Olweny *et al*., 1986).

However, the assertion that sequestration in a specific site of the brain is a prerequisite for the neurological signs and symptoms associated with cerebral malaria is the issue of controversy since over 30% of 32 children diagnosed of cerebral malaria showed extremely minimal sequestration in the brain (Clark *et al*., 2003). The seeming controversy can be resolved if other factors apart from sequestration are considered in the malaria pathogenesis.

Interestingly, the involvement of inflammatory cytokines in the pathogenesis of cerebral malaria cannot be underestimated (Clark *et al*., 2004). Induction of inflammatory cytokine production and its association with endothelial dysfunction notably in cerebral malaria is well documented (Conroy *et al*., 2010; Yapi *et al*., 2010; Angulo & Fresno, 2002; Lucas *et al*., 1997; Medana, Hunt & Chaudhri, 1997).

Endothelial dysfunction, considered as the impaired ability of blood vessels to dilate in response to a given pharmacological agent or shear stress is

associated with reduced NO production or bioavailability (Deanfield, Halcox & Rabelink, 2007). In addition, endothelial dysfunction is linked to increased endothelial activation, intercellular adhesion molecule-1 (ICAM–1), and lactate but decreased L-argenine concentrations in blood (Deanfield *et al*., 2007; Yeo *et al*., 2007; Lopansri *et al*., 2003; Anstey *et al*., 1996).

Evidence of extensive endothelial activation and a role for ICAM-1 in fatal malaria which was provided about two decades ago (Turner *et al*., 1994) has been confirmed by a number of studies in recent times (Kima, Higginsa, Lilesa & Kaina, 2011; Conroy *et al*., 2009; Lovegrove *et al*., 2009; Yeo *et al*., 2008). Other studies have provided data to suggest that malaria exerts its negative effect on endothelial function through altered vascular reactivity (Nwokochal, Ajayi & Ebeigbe, 2011) to impaired mobilization of circulating endothelial progenitor cells (CEPC) (Gyan *et al*., 2009), especially in cerebral malaria patients. As a results, patients with reduced CEPC levels experienced increased malaria severity compared with those with relatively high CEPC levels (Gyan *et al*, 2009).

Low CEPC levels have been found to be associated with increased CVD risk (Schmidt-Lucke *et al*., 2005; Hill *et al*., 2003). Thus, cerebral malaria patients have increased risk of cardiovascular event due to reduced synthesis of new endothelial cells to possibly replace worn-out ones.

Impaired bioavailability of NO may be due to factors such as impaired synthesis, transport and activity. As such, conditions associated with impaired bioavailability of NO could also be linked to reduced synthesis, transport or activity.

With NO bioavailability being a prerequisite for normal endothelial function (Gramaglia *et al*., 2006; Tousoulis *et al*., 2006; Voetsch, Jin & Loscalzo, 2004), it may be thought that increased levels should be beneficial to cardiovascular function. Interestingly, expression of inducible nitric oxide synthase, one of the enzymes involved in the synthesis of NO was observed to increase in response to malaria (Wheeler *et al*., 1997; Anstey *et al*., 1996). This observation probably exemplifies the functional versatility of the molecule (Annan *et al*., 2000) since it has been found to possess both apoptotic and anti-apoptotic properties as well as various immunomodulatory properties (Korhonen, Lahti, Kankaanranta & Moilanen, 2005). On the other hand, these seeming contradictory reports in respect of NO function may reflect the compartmentalized nature of the various isoforms of the enzyme responsible for production of the molecule. As such, NO from inducible nitric oxide synthase may exhibit a physiological role that may be at variance with that produced by the other isoforms in a given condition.

The current study does not focus on NO or any of the above biomarkers of endothelial dysfunction as a measure of cardiovascular risk. Rather, changes in CRP, lipid profile and antioxidants levels are being monitored before and during *P. falciparum* infection. Indeed, changes in levels of these biomarkers in response to malaria (Al-Omar *et al*., 2010; Simpson *et al*., 2010; Das *et al*., 1996), notably, in diabetics may contribute immensely to the current understanding of malaria in the context of the increasing trend of diabesity (Danquah *et al*., 2010). This is very important because malaria and diabetes possess inflammatory cytokine induction capabilities and a transient

inflammation has the potential to induce intense reduction in endothelial function (Hingorani *et al.*, 2000), and thus, increased cardiovascular risk.

Malaria Situation in Ghana

Various reports from the Ghana Health Service (GHS) suggest that malaria remains the number one disease affecting the Ghanaian populace (GHS 2010; GHS 2011). According to the GHS, the total number of reported malaria cases at the out-patients departments (OPD) of the various public health facilities in the country increased from 3,694,671 in 2009 to 3,740,055 in 2010, representing 32.5% and 34% of total OPD cases in the respective years with observed regional disparities (GHS, 2010). Although the contribution of malaria to total OPD cases appears to have declined from the over 40% in 2003 (Asante & Asansu-Okyere, 2003) to the current levels, the reported 34% is far above expectation, particularly, with the advent of improved malaria diagnostics (GHS, 2010). This seemingly high number of cases could be due to the fact that most of the reported malaria cases treated at the various health facilities in Ghana lack parasitological confirmation from the appropriate laboratories. Lack of well equipped laboratories and properly trained laboratory personnel in most health facilities may explain our continued over-reliance on subjective clinical diagnosis of malaria (GHS, 2010).

Interestingly, statistics from the National Malaria Control Programme suggests that only about 10% of presumptive malaria cases diagnosed at the various hospitals and clinics are really malaria. The nationwide proportion of laboratory confirmed malaria cases at the various health facilities has improved to 18.9% according to the 2011 annual report of the GHS (GHS, 2011). These

observations seem to point to a possible misdiagnosis and overtreatment of malaria in the various public health facilties in the country.

However, this information ought to be handled with caution due possibly in part to the endemic status of Ghana as far as the malaria condition is concerned, coupled with the inherent challenges associated with the available laboratory-based malaria diagnostic tools such as microscopy and rapid diagnostic test (RDT) kits.

For instance, the sensitivity and specificity of microscopy have been reported to be operator-dependent and the currently available RDT kits have optimal sensivity of just about 90% at minimum parasite levels of 100 parasites per microlitre blood (Praveen *et al.*, 2008; Ivo, Inoni, Meza, John & Blaise, 2007). As expected, sensitivity of RDT declines sharply at parasite densities below this threshold, making its use possibly in non-severe malaria cases observed normally in adult population a bit difficult. In view of the above shortcomings, it may be more prudent to combine both clinical and laboratory diagnostic techniques, in making sampling decisions, an approach adopted by the current study.

Interestingly, sample size determination for a study such as the current one becomes very crucial in the face of dire financial constraints. Again, such estimates ought to be based on empirical data such as those provided by laboratory-confirmed malaria cases rather than the presumptive treatment-based figures reported by the various health facilities in the country. In this way, the available limited recources can be prudently utilized whilst avoiding

unnecessary oversamplying and its associated costs. These considerations informed the sample size estimation for the current work.

Malaria situation in the Central Region

As expected, malaria remains the most commonly reported disease condition at the various health facilities in the region. Information from the Central Regional Directorate of the Ghana Health Service indicate that, of the 523,075 reported malaria cases in the region in 2009, only 102, 215 (19.45%) were confirmed by laboratory tests at the various clinics and hospitals in the region. It is worthy of note, however, that the proportion of confirmed malaria cases in the region is higher than the reported national figure. Of the total malaria cases, about 27% (138,598 cases) occurred in children under five years.

Interestingly, the current work targets a segment of the adult population from 30 years and above for whom malaria prevalence data is unavailable. To this end, available data in respect of the entire adult population above 18 years were employed in prevalence estimation and subsequent sample size calculation for the current study.

According to the United Nations Children's Fund, (UNICEF), the segment of the Ghanaian population under 18 years constitutes about 45% of the estimated 23,837,000 in 2009 (UNICEF, 2010). This proportion is not substantially different from the regional figure of 46% obtained from the 2010 Population and Housing Census report of the Ghana Statistical Service (GSS) (GSS, 2012). In view of this, the national proportion of the adult population as reported by UNICEF was used in determining malaria prevalence and

subsequent sample size estimation for the current study since the GSS report was unavailable at commencement of the study. Using the above information, the laboratory-confirmed malaria prevalence in 2009, for the adult population in the Central Region was estimated to be 7%. This figure was applied in estimating the sample size using a simple formula that requires prevalence, significance and confidence levels (Naing, Winn & Rusli, 2006).

Indeed, except in pregnancy, the adult population is not considered a vulnerable group with respect to malaria although the entire Ghanaian populace is at risk of the disease. Unlike diabetes mellitus with clearly defined diagnostic criteria and specific signs and symptoms, malaria presents common signs and symptoms with a lot of other communicable diseases (Clark *et al.*, 2004). This challenge makes the use of laboratory-confirmed prevalence values in determining sample size for the current study more prudent.

Diabetes Mellitus

Diabetes mellitus is a chronic heterogeneous metabolic disorder caused by various genetic and environmental factors that culminate in defective insulin action, secretion or both with a characteristic increase in blood glucose levels (IDF, 2012; Bastaki, 2005; Florez, Hirschhorn & Altshuler, 2003). The disease has been with mankind since ancient times and continues to affect millions of people worldwide since no cure has yet been found. The widely varied causes of the disease may have made it probably more difficult to find a cure, hence, the increased emphasis on management in the current medical literature. The condition has varied prevalence across various countries. The reported national

prevalence ranges from 1.5% in Mali in the African region to as high as 24.92% in Kiribati in the Western Pacific region (IDF, 2011).

According to the International Diabetes Federation (IDF), the condition affects about 371 million adults worldwide, giving a global prevalence of 8.3% (IDF, 2012) with differences in prevalence, morbidity and mortality across various continents. About 50% of these cases are yet to be diagnosed. In Africa, the condition affects over 15 million people and this is projected to double by 2030. The proportion of individuals with undiagnosed diabetes in Africa stands at 81% of already diagnosed cases, representing the highest in the world (IDF, 2012) as depicted in figure 3 below.

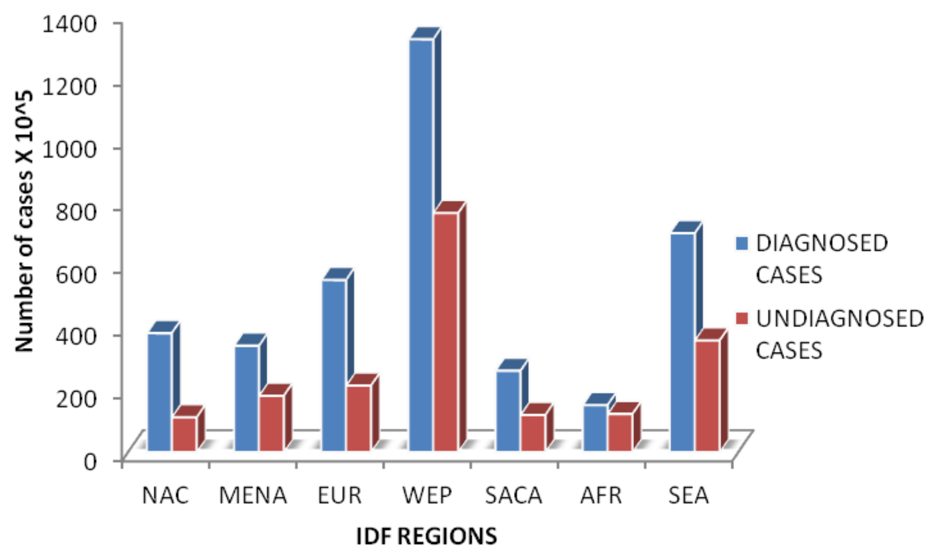

Figure 3: Global estimates of diagnosed and undiagnosed cases of diabetes in various IDF regions. ***Data obtained from IDF, 2012. NAC (North America and Caribbean), MENA (Middle East and North Africa), EUR (Europe), WEP(Western Pacific), SACA (South and Central America), AFR (Africa), SEA (South-East Asia), IDF (International Diabetes Federation).***

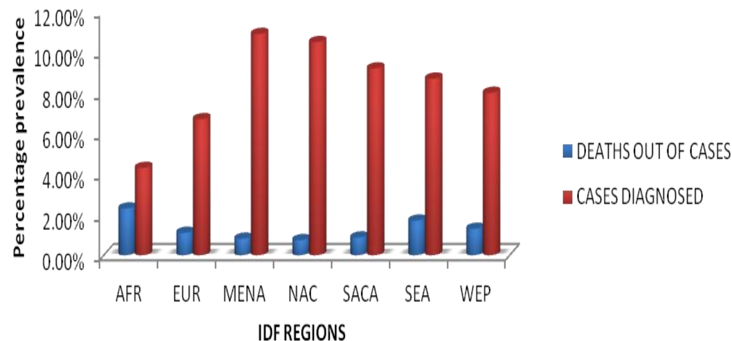

Figure 4: Comparison of prevalence of diabetes with diabetes-related mortality across various IDF regions. ***Data obtained from IDF, 2012. NAC (North America and Caribbean), MENA (Middle East and North Africa), EUR (Europe), WEP(Western Pacific), SACA (South and Central America), AFR (Africa), SEA (South-East Asia), IDF (International Diabetes Federation). Percentage mortality was estimated from the proportion of diagnosed cases.***

In 2012, diabetes caused more than 4.8 million deaths and 471 billion US dollars in treatment cost worldwide but 400,000 deaths and 2.5 billion US dollars in treatment expenditure in South, East and West Africa (IDF, 2012). As shown in figures 4 and 5, Africa recorded the highest mortality due to diabetes but the lowest prevalence and mean diabetes-related expenditure compared with other regions of the globe (IDF, 2012).

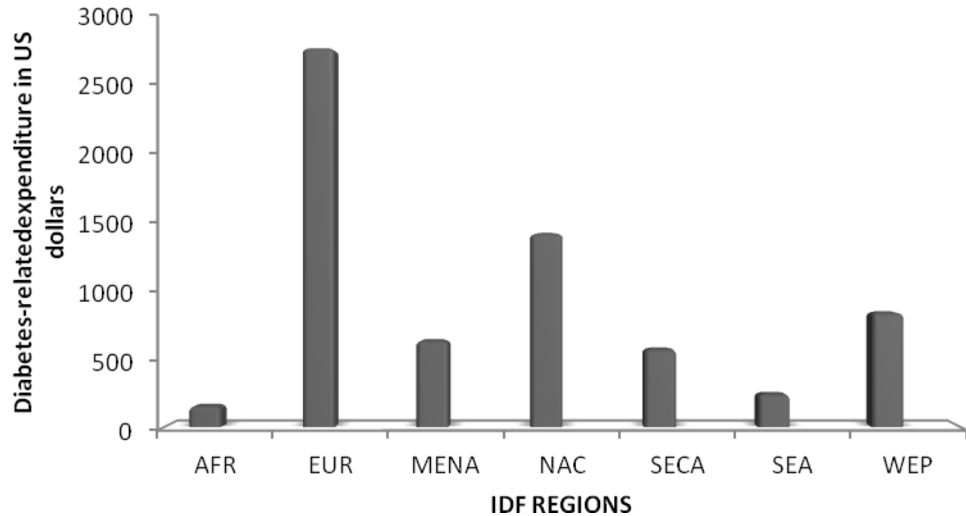

Figure 5: Comparison of mean diabetes-related expenditure across the various IDF regions. ***Data obtained from IDF, 2012. NAC (North America and Caribbean), MENA (Middle East and North Africa), EUR (Europe), WEP(Western Pacific), SACA (South and Central America), AFR (Africa), SEA (South-East Asia), IDF (International Diabetes Federation).***

In Ghana, the estimates follow the global trend with a relatively higher proportion of men being affected than women within the age group 20-79 years (IDF, 2012). According to the IDF, there were 517,430 diabetes cases in 2011, giving a national prevalence of 4.09% in the adult population with as much as 78% of the cases being undiagnosed (IDF, 2012). The mean diabetes-related expenditure for the period was estimated conservatively as 113 USD per case, giving a total expenditure of about 58.5 million US dollars. Although, the expenditure per case in Ghana is relatively lower than the mean expenditure per case for the African continent, the total financial burden is huge particularly when as much as 78% of cases remain undiagnosed. This implies that Ghana may incur additional millions of US dollars in diabetes-related cost.

These expenditures are significant and could exert huge economic strain on the limited finances of Ghana in terms of its health-related cost. All this statistics indicates that diabetes as a health condition is no respecter of person, country or continent.

Unfortunately, low-income countries such as those in the sub-Saharan Africa are projected to experience the highest incidence of the condition and this may further strain the health budget, hinder their developmental drive and widen the developmental gap between them and their developed counterparts. This is because Africa is still grappling with the infectious disease burden and the advent of chronic disease outburst as predicted for diabetes, may aggravate the already bad economic situation. Thus, developing low-income or low-middle-income countries such as Ghana have a double challenge of both chronic and infectious disease burden, dual challenges which could greatly hinder economic growth and development.

Types of Diabetes

Diabetes mellitus can be classified into three main categories: type 1, type 2 and gestational diabetes.

Type 1 diabetes

This form of diabetes constitutes 5-10% of all diabetes cases globally with its incidence predicted to increase in all regions of the globe (Lin & Sun, 2010; Gillespie, 2006). This prediction indicates an expected increased environmental contribution to the evolution of the disease (Gillespie, 2006). It is relatively rare in parts of the African continent except North Africa. Available data indicate that the incidence of this form of diabetes is highest in

Europe followed by Middle-East and North Africa, North America and Caribbean, Western Pacific, South and Central America, South-East Asia and Africa in that order as shown in figure 6 (IDF, 2011).

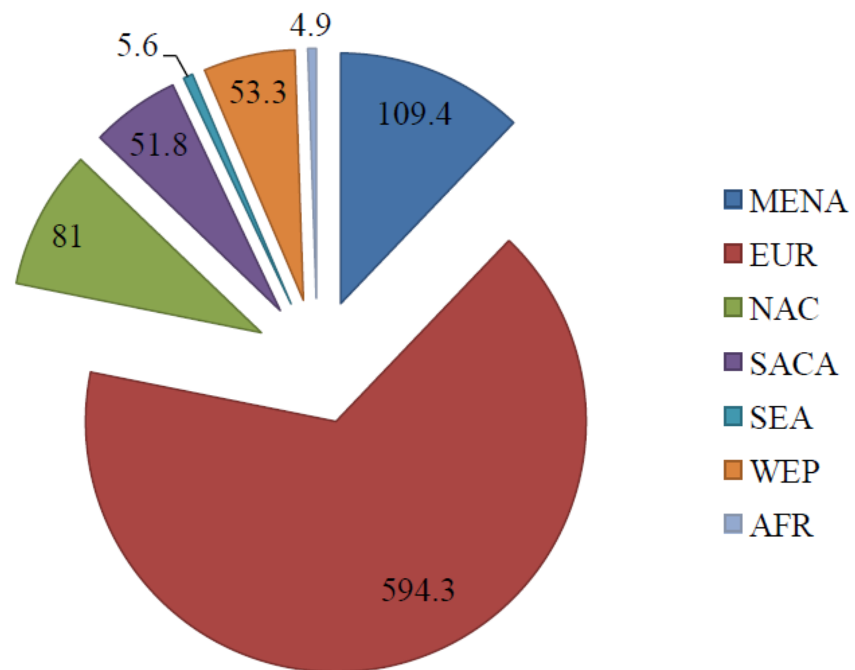

Figure 6: Incidence type 1 diabetes (0-14 years) per 100,000 population across various IDF regions. ***Data obtained from IDF, 2012. NAC (North America and Caribbean), MENA (Middle East and North Africa), EUR (Europe), WEP (Western Pacific), SACA (South and Central America), AFR (Africa), SEA (South-East Asia), IDF (International Diabetes Federation).***

About 90% of this form of diabetes results from destruction of pancreatic ß-cells due to errors in immunologic communication and recognition (Bastaki, 2005) but the cause of the ß-cell destruction in the remaining 10% is still unknown. As a result, the pancreatic ß-cells of such individuals get recognized as foreign unwanted antigen and get destroyed by self antibodies, to result in the observed insulin deficiency. The

autoimmunologic basis of type 1 diabetes has been evidenced by the detection of certain antibodies such as anti-glutamic acid decarboxylate (anti-GAD), insulin antibodies and islet cell antibody (ICA) that are believed to be responsible for the ß-cell destruction (Bastaki, 2005). These observations have been buttressed by molecular evidence through identification of a number of genes found to be associated with the condition (Brorsson *et al.*, 2009; Howson *et al.*, 2009; Gillespie, 2006).

Individuals with type 1 diabetes tend to be lean, diagnosed at a much younger age and require insulin therapy for survival (Florez *et al.*, 2003). Recent evidence, however, indicates that type 1 diabetes can also occur in older people (Largay, 2012) with type 2 forms being diagnosed in children (DeFerranti & Osganian, 2007).

In Ghana, just like most African countries, statitistics in relation to this form of diabetes appears unavailable. Since the current study is limited to type 2 diabetes mellitus, the unavailability of statistical information on type 1 diabetes may have little or no effect on the study. It is worthy of note, however, that the lack of statistics on a given condition in a particular locality does not mean the non-existence of the disease in the locality. Rather, it is a reflection of the lack of interest in the condition probably due to the apparently rare nature relative to other disease conditions. This posture, particularly for Ghana, is quite worrying as it may create the enabling environment for the upsurge of type 1 diabetes in the country. Also, it could make it difficult for proper identification of type 1 diabetics for appropriate intervention. In view of the above, some research needs to be done to ascertain at least, the prevalence of this form of diabetes in the country.

Type 2 diabetes

This form of diabetes which is the most predominant constitutes about 90-95% of all diabetes cases worldwide (Lin & Sun, 2010; Gillespie, 2006). Unlike type 1 diabetes in which significant contribution of genetics has long been documented (Pociot & McDermott, 2002), in the case of T2D, environmental influence is considered to be more important. This view appears to be buttressed by the current observation of increased incidence of the condition across various regions of the globe although genetic contribution to its evolution has been acknowledged (Lin & Sun, 2010). Population growth, aging, urbanization, sedentary lifestyle, obesity and adoption of westernized diet have been liable for the observed increased number of cases (Lin & Sun, 2010; Wild, Roglic, Green, Sicree & King, 2004; Zimmet, Alberti & Shaw, 2001; Bloomgarden, 1996). In view of this, individuals diagnosed with the condition are normally 40 years or older, overweight or obese and do not require insulin necessarily for survival (Lin & Sun, 2010).

In maturity onset diabetes of the young (MODY), a subtype of T2D that occurs at a much younger age without obesity (Fajans, Bell & Polonsky, 2001), a strong familial inheritance pattern has been documented. MODY does not require insulin for prevention of ketosis seen in typical type 1 patients and usually presents clinically as asymptomatic hyperglycaemia in young adults less than 25 years (Florez *et al*., 2003).

Several genes have been discovered to be associated with MODY (Florez *et al*., 2003) as well as typical T2D (Florez *et al*., 2003; Elbein, Hoffman, Teng, Leppert & Hasstedt, 1999). Despite the discovery of several diabetogenic genes associated with T2D (Florez *et al*., 2003), sedentary

lifestyle and weight gain probably remain the greatest environmental risks to the development of the condition. As a result, the risk of development of T2D can be lowered by regular exercise, appropriate diet and effective weight loss (Lin & Sun, 2010).

Gestational diabetes

This form of diabetes is associated with pregnancy. It is normally detected in the second or third trimester in about 4% of all pregnancies (IDF, 2012; Bastaki, 2005; ADA, 2001). Individuals with this form of diabetes carry 30-50% increased risk of developing T2D (Bastaki, 2005).

Pathogenesis of Type 2 Diabetes Mellitus

Progression to overt hyperglycaemia seen in T2D is preceded by deficient insulin secretion, defective control of hepatic glucose production and impaired insulin sensitivity (Stumvoll, Goldstein & van Haeften, 2005). With time, the function of β-cells decline in a gradual fashion till eventual failure ensues. Hence, onset of T2D is an indication of dysregulated multiorgan homeostasis. The affected organs include the skeletal muscle, pancreas, adipose tissue, liver and the central nervous system (CNS) (Lin & Sun, 2010).

According to Bjornholm & Zierath (2005), the skeletal muscle is responsible for 75% of insulin-mediated total body glucose transport. Effective insulin-mediated glucose uptake into cells depends on efficient insulin signal transduction system. Initiation of insulin signaling requires binding of insulin to its receptors and subsequent phosphorylation of various substrates including insulin receptor substrates (IRS1-4) (Lin & Sun, 2010).

The binding of phopshorylated IRS to phosphatidylinositol 3-kinase,

subsequent recruitment and phosphorylation of appropriate enzyme molecules involved in the complex insulin signaling pathway, results in eventual transfer of glucose across the plasma membrane (Lin & Sun, 2010). Molecular evidence from transgenic mice points to IRS-1 or IRS-2 as the main receptor substrate for insulin-mediated activities (White, 2003). Indeed, IRS-2 knockout mice develop T2D coupled with skeletal muscle insulin resistance (Lin *et al.*, 2004) but these abnormalities were not observed in IRS-1 knockout counterpart (Sun *et al.*, 1995; Araki *et al.*, 1994).

These findings in mice studies indicate the absolute relevance of effective insulin signaling in glucose homeostasis but relative importance of the various IRS in the development of insulin resistance and subsequent presentation of T2D. Interestingly, impaired insulin-mediated glucose transport in skeletal muscle has long been found in skeletal muscle of human T2D subjects (Zierath, Krook & Wallberg-Henriksson, 2000), an observation that implicates abnormal insulin signaling in the pathogenesis of human T2D.

Development of T2D is not limited to just abnormal insulin sensitivity as described in skeletal muscle but involves insulin synthesis and release. Physiologically, insulin synthesis is the primary function of the pancreatic β-cells with the α-cells responsible for glucagon. The action of insulin and glucagon are antagonistic to each other. Primarily, insulin remains the major hormone for clearance of glucose from blood into cells and subsequent alteration to glycogen for storage. As a result, diabetes can be viewed as a hormonal imbalance between insulin and glucagon activities because reduced insulin levels trigger concomitant increased levels of glucagon and consequent

hyperglycaemia. It is therefore not surprising that factors that impair β-cell function are associated with T2D (Hui & Perfetti, 2002).

Indeed, mechanisms that induce apoptosis of β-cells, decrease β-cell mass or impair capacity of β-cells to assuage insulin resistance also contribute to evolution of T2D (Rhodes, 2005). The various mechanisms identified so far include lipotoxocity (Poitout & Robertson, 2002), oxidative stress (Ogino & Wang, 2007; Kaneto *et al*., 2006), glucotoxicity (Donath & Halban, 2004), inflammation (De Rooij *et al.*, 2009; Donath, Storling, Maedler & Mandrup-Poulsen, 2003), stress of endoplasmic reticulum (Harding & Ron, 2002) and defects in mitochondrial activity (Lowell & Shulman, 2005). The exact impact of these outlined mechanisms appears to be organ-specific.

In the adipose tissue of T2D patients, reduced expression levels of glucose transporter-4 (GLUT4), has long been observed (Shepherd & Kahn 1999). Findings from later studies in adipocyte selective *GLUT4* knockout mice seems to suggest that GLUT4 could probably be required to suppress or prevent generation of other circulating factors such as retinol-binding protein-4 (RBP4) (Yang *et al*., 2005; Abel *et al*., 2001), for inter-organ cross-talk (Lin & Sun, 2010). This view seems to be supported by raised levels of RBP4 found in sera of obese diabetic humans as well as insulin-resistant mice (Yang *et al*., 2005). Again, the finding seems to support a long held view of requirement of glucose for effective lipid oxidation and explains the observed metabolic inflexibility reported by Kelley and Mandarino over a decade ago in T2D patients with respect to their reduced ability to switch from fatty acid degradation to glucose in reaction to insulin trigger (Kelley & Mandarino, 2000).

In fact, Minokoshi, Kahn & Kahn (2003) reported insulin resistance, glucose intolerance and overt T2D with a severely declined whole body glucose transport to a disproportionate level in response to white adipose tissue–specific *GLUT4* inactivation. As pointed out in a recent review by Lin & Sun (2010), this finding exemplifies a vital and an extremely under-recognized contribution of adipocyte to the pathogenesis of T2D.

A dysregulated link between glucose and lipid metabolism could result from dysregulated hepatic insulin signaling considered to be an important machinery in the development of T2D (Fritsche, Weigert, Haring & Lehmann, 2008). IRS-1 and IRS-2 are corresponding vital participants in hepatic insulin signaling. Dysfunctional IRS proteins have been found to give rise to abnormal glucose and lipid homeostasis seen in T2D (Dong *et al*., 2006).

Recent observations from animal research, have demonstrated that overeating and obesity diminish the capacity of the CNS to detect and integrate information from nutrient, neural and hormonal signals in relation to glucose homeostasis in hepatocyte and periphery tissue (Sandoval, Obici and Seeley, 2009). Indeed, the link between CNS and glucose and lipid homeostasis in relation to T2D has long been established (Ahima, 2006; Koerner *et al*., 2005; Munzberg & Myers, 2005; Banks, 2004). This link, probably, is established through leptin, an adipocyte that represses food intake but stimulates energy expenditure via interaction with the CNS (Koerner *et al*., 2005). It is therefore not surprising that, leptin resistance which signifies failure of the CNS to detect and respond appropriately to satiety signals is associated with obesity-related health conditions like T2D (Brennan & Mantzoros, 2006). Putting together, T2D as a condition signifies underlying

multi-organ dysfunction. It may, however, be interesting to investigate how infectious diseases such as malaria may modulate the levels and possibly function of adipokines such as leptin and adiponectin in relation to diabetes pathogenesis.

Risk Factors and Complications of Diabetes

A risk factor in an individual for a given disease refers to any condition that increases the prossibility of development of the disease in that individual with the factor compared to those without the factor. Normally, risk factors precede the actual onset of the disease condition. Interestingly, the available literature on modifiable diabetes-associated risk factors appears to be skewed more towards T2D as opposed to the type 1 form of the disease. Probably, the relative early and spontaneous onset of type 1 diabetes compared with T2D makes it a bit more challenging for such risk factors associated with the former to be identified. Also, it may be plausible that the huge emphasis on genetic contribution to its development, notably, a number of genes within the major histocompatibility complex (MHC) (Erlich *et al*., 2008), might have thwarted research efforts at identifying appropriate modifiable risk factors. In spite of the above probable challenges, any condition other than genetic factors, that has the potency to induce a strong and lasting immune response, particularly, in early stages of development such as childhood viral infection, could increase the chance of developing type 1 diabetes if improper immune recognition occurs.

On the other hand, T2D lends itself readily to modifiable risk factor identification studies considering the strong environmental contriubution to its

development (Lin & Sun, 2010). Various genetic and environmental factors have been associated with the disease. Whereas the genetic factors may not be amenable to modification, the environmental factors can be modified to attenuate the onset of the disease. Indeed, a successful phenotypic expression of the so called diabetogenic genes will obviously require favourable environmental conditions. As such, one can argue that there is no such thing as purely genetic. Rather, the appropriate interaction of both genetic and enviromenatl factors give rise to a given disease condition or attenuate its onset.

Indeed, the effects of the various genes that have been identified to increase susceptibility to T2D through candidate gene linkage (Ali *et al*., 2013; Tabor, Risch & Myers, 2002), genome-wide linkage (Rotimi *et al*., 2006), genome-wide association scan (Zeggini *et al*., 2008; Scott *et al*., 2007) and candidate-wide association scan (Gaulton *et al*., 2008) approaches in different populations, can only be felt in favourable environmental conditions. Inspite of the numerous T2D susceptibiltiy genes identified so far, a recent review by Vimaleswaran & Loos (2010), observed that, their total contribution to the pathogenesis of the disease is small. This observation does not only inspire continued search for additional susceptibility genes, it also acknowledges the major environmental contribution to the development of the condition.

Obesity has been identified as the major environmental risk factor in relation to the evolution of T2D (Escobedo *et al*., 2009). It is a component of the metabolic syndrome, assemblage of factors that include hyperlipidaemia, hypertension, blighted glucose control and insulin resistance which herald the onset of explicit diabetes or cardiovascular disease (Cornier *et al*., 2008). A

recent meta-analysis of 92 published genome-wide reports on obesity, cardiovascular disease and T2D corroborated earlier findings that the three conditions share a common genetic environment (Wu *et al.*, 2012). It is therefore not surpring that, all the components of metabolic syndrome are known risk factors for T2D. In fact, T2D is a risk factor for cardiovascular disease (Lin & Sun, 2010; Escobedo *et al.*, 2009; Brennan & Mantzoros, 2006; Kengne, Amoah & Mbanya, 2005). As a result, T2D is sometimes considered a component of the so-called metabolic syndrome depending on the reference disease condition (Cornier *et al.*, 2008).

Obesity defined in terms of body mass index (BMI), the ratio of weight in kilogram to the square of the height in metre squared, does not distinguish central fat deposition from other forms. The failure of BMI to account for body fat distribution can lead to exaggerated risk estimation for the physically active and highly muscular individuals such as sportsmen and women. Indeed, this limitation has long been observed in various clinical studies that reported stronger association of central obesity with diabetes compared with general obesity as determined by BMI (Jensen, 2006; DeFronzo, 2004; Molarius & Seidell, 1998).

To this end, waist-to-hip ratio (WHR) and waist circumference (WC) have become the tools of choice for determination of central obesity (Janssen, Katzmarzyk & Ross, 2004; Molarius & Seidell, 1998), especially, when WC seems to better account for obesity-associated health risk than BMI (Janssen *et al.*, 2004).

Conversely, WC is unable to differentiate total abdominal fat from total body fat and abdominal subcutaneous fat (Vazquez, Duval, Jacobs &

Silventoinen, 2007; Ford, Mokdad & Giles, 2003). Interestingly, the predictive value of all the three measures of obesity is strongly influenced by age, racial, gender and ethnic factors (Chiu, Austin, Manuel, Shah & Tu, 2011; Stommel & Schoenborn, 2010; Abate & Chandalia, 2003; Ford *et al*., 2003; Nakagami *et al*., 2003). In additoin, various epidemiological studies (Vazquez *et al*., 2007; Hartemink, Boshuizen, Nagelkerke, Jacobs & van Houwelingen, 2006; Janssen, Heymsfield, Allison, Kotler & Ross, 2002) have failed to conclusively corroborate the statistically significant differential prognostic value reported by some clinical studies (Katzmarzyk, Heymsfield & Bouchard, 2013 ; Janssen *et al*., 2004; Molarius & Seidell, 1998) for the three measures of adiposity in relation to diabetes. Rather, a strong correlation has been observed among the measures of obesity with a correlation coefficient of 0.8 reported for BMI and WC (Janssen *et al*., 2004).

In view of the above findings, all the three measures of obesity have been employed in the current study with the view to test for the reported correlation among them and to ascertain the possible existence of any variation in their prognostic values in the Ghanaian context in relation to other populations.

Other reported risk factors of T2D such as sedentary lifestyle, diet, insulin resistance, leptin resistance, hypoadiponectinaemia, gestational diabetes and impaired glucose tolerance or impaired fasting glucose tolerance have all been linked to obesity in one way or the other (IDF, 2011; Vazquez *et al*., 2007; Jensen, 2006; Bastaki, 2005; Janssen *et al*., 2004). As such, a detailed review of the above individual factors could result in undue repetition of information.

Once overt diabetes develops and remains uncontrolled or poorly controlled for a relatively long period of time, several complications can develop. Some of the complications include myocardial infarction, stroke, vascular, renal and kidney diseases (Giacco & Brownlee, 2010; Kengne *et al.*, 2005), with varying reported country-specific prevalences.

Interestingly, the various diabetes-associated complications have been observed to be perpetuated by oxidative stress through enhanced production of superoxide in the mitochnodria of affected cells and tissues (Giacco & Brownlee, 2010; Brownlee, 2005). Uncontrolled mitochondrial superoxide release exerts its damaging effects via at least one of five main biochemical pathways. The implicated pathways include glyceraldehyde-3-phosphate-mediated activation of the polyol pathway, increased production of intracellular AGES, abnormal expression levels of receptors for AGES, enhanced activation of PKC and the hexoseamine pathway (Giacco & Brownlee, 2010).

The detailed mechanisms by which enhanced activation of each of the above pathways contributes to the pathogenesis of diabetic complications have been comprehensively reviewed (Giacco & Brownlee, 2010; Brownlee, 2005; Brownlee, 2001).

AGES exert their pathogenic effects through modification of target proteins. This modification changes the function and interaction of the affected proteins with other matrix components. Eventually, the modified proteins get attached to AGE receptors on surface of selected vascular cells such as endothelial and smooth muscle cells to provoke further production of ROS which stimulates nuclear factor kappa B ($NF\text{-}_{k}B$) to induce myriad pathological alterations in transcription (Goldin, Beckman, Schmidt & Creager, 2006).

Whereas hyperglycaemia-induced overactivation of the polyol pathway depletes reduced glutathione stores to amplify vulnerability of target cells to oxidative damage, abnormal stimulation of PKC pathway gives rise to anomalous signaling with consequent cellular apoptosis and related myopathies (Geraldes *et al*., 2009).

What may be more intriguing for future investigations is the response of the above pathways to malaria and its overall impact on diabetic complication, particularly, when oxidative stress is reported to be the foundation for activation of the implicated pathways (Giacco & Brownlee, 2010).

Although, the current study does not specifically address the above challenge, it attempts to gain insight into the potential impact of malaria on oxidative stress by monitoring the levels of lipid peroxidation with and without malaria in diabetics compared with non-diabetic controls. Access to such information may deepen our understanding of the possible role of malaria in oxidative stress-induced pathogenesis of diabetic complication to enable development of rational therapeutic strategy for its management.

Management of Diabetes and Related Complications

T2D and associated complications are managed through several strategies that can be grouped into pharmacological and non-pharmacological approaches. Whereas pharmacological strategies employ drugs as the therapeutic agent, the non- pharmacological methods rely on diet, physical activity and weight loss to achieve the desired objective of improved quality of life for diabetics. Non-pharmacological strategies encourage appropriate

physical activity, weight loss and increased intake of complex carbohydrate, polyunsaturated fat, omega-3-containing fish, fruits and vegetables (Ajala, English & Pinkney, 2013; Campbell, Dickinson, Critchley, Ford & Bradburn, 2012; Teixeira-Lemos, Nunes, Teixeira & Reis, 2011). Although this option is normally a first-line treatment for newly diagnosed T2D patients with relatively low risk of complications, recent evidence suggest that the effectiveness of a number of antidiabetic drugs is enhanced when combined with appropriate dietary approaches (Cornier *et al.*, 2008).

Based on mechanism of action, the currently available pharmacologic antidiabetic agents can be grouped into three; sensitizers of insulin, inhibitors of insulin secretion and inducers of insulin secretion (Bösenberg & van Zyl, 2008).

Whereas the insulin sensitizers attenuate insulin resistance by stimulating sensitivity of cells to insulin action through modification of insulin signaling in hepatic and adipose tissue for enhanced clearance of glucose from the bloodstream, the inhibitors of insulin secretion also called glucosidase inhibitors, work to prevent further degradation of disaccharides and oligosaccharides to glucose. Since insulin secretion appears to be induced by glucose in a dose-dependent manner (Buchwald, 2011; Matsui-Hirai *et al.*, 2011), reduced glucose concentration results in reduced concentration of released insulin.

Phamarcologic agents that induce insulin secretion are artificially designed enzyme-resistant molecules that mimic the natural hormones released to enhance insulin secretion due to food intake (Bösenberg & van Zyl, 2008).

As such, induction of insulin secretion is sustained to ensure effective clearance of glucose from the bloodstream.

As expected, each class of antidiabetic phamarcologic agent has one form of side effect or the other that makes it unsuitable for certain categories of diabetics; an observation that is not associated with non-phamarcological approaches (Ajala *et al*., 2013; Teixeira-Lemos *et al*., 2011; Bösenberg & van Zyl, 2008). Effective management of the condition can therefore achieved by a careful blend of appropriate pharmacological and non-pharmacological strategies.

Basic Principles of Analytical Techniques used in this Study

Spectrophotometry and enzyme-linked immunosorbent assay (ELISA) are the main analytical tools employed in assessing the various biomarkers in blood samples of respondents in the current study. These techniques were chosen because they are versatile, relatively simple, amenable to automation, less expensive and suitable for the specific biomarkers being assessed in the current study. Above all, the techniques are readily available for routine analytical work in our laboratories although tedious sample preparation procedures may be required. The versatility of spectrophotometric techniques stems from the spectra of wavelengths with which biomolecules can interact with electromagnetic spectrum. The electromagnetic spectrum is an array of wavelengths spanning the different characteristics exhibited by light as it interacts with matter. The interaction of light with a given biomolecule brings about peculiar changes in absorption features that may identify the specific biomolecule or determine its quantity (Owen-Reece, Smith, Elwell &

Goldstone, 1999). This is because each biomolecule is unique in terms of structure and interaction with surrounding matter. Spectroscopic techniques exploit this uniqueness for identification and quantitation of biomolecules. This means that in principle, the level of any biomolecule can be determined in a suitable medium by appropriate spectroscopic technique.

With respect to the current study, the type of spectroscopic technique employed is the spectrophotometry. This technique is based on the ability of biomolecules in liquid medium to give a characteristic absorption within the visible range of the electromagnetic spectrum. The amount of light absorbed by a given biomolecule in a given medium is directly proportional to the number of absorbing molecules. Based on this principle, various instruments have been developed to measure the levels of various biomolecules in body fluid. To minimize errors that arise from sample preparations, various automated analyzers have been developed to suitably and reliably measure levels of biomolecules of medical relevance. One of such machines is the BT 3000 autoanalyzer, manufactured by JAS Diagnostics, Inc., USA, which is designed to quantify a number of biomolecules of medical importance in clinical settings. With the appropriate reagents, the autoanalyzer can reliably quantify different biomolecules based on different reactions within a very short time, making it suitable for routine clinical practice. For instance, levels of blood glucose and lipids are determined by enzyme-catalysed reactions (Sblendorio & Palmieri, 2008) whereas glycosylated haemoglobin level is measured by antigen-antibody precipitation method with the same analyzer.

With respect to glucose, the automated assay relies on hexokinase-catalysed phosphorylation of glucose to glucose-6-phosphate (Tonyushkina & Nichols, 2009). Subsequently, glucose-6-phosphate is oxidised under the influence of glucose-6-phoshate dehydrogenase enzyme that uses oxidised nicotinamide adenine dinucleotide (NAD^+) as the cofactor to give rise to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). The resulting NADH induces a hyperchromic shift at 340 nm and the increase in absorbance is proportional to the concentration of glucose in sample.

Meanwhile, glycosylated haemoglobin assay relies on interaction between latex particles and mouse antihuman HbA1c monoclonal antibody (Javier Gella, Serraa & Generx, 1991) to form latex-HbA1c-mouse antihuman HbA1c antibody complex. The addition of goat anti-mouse immunoglobulin G (IgG) polyclonal antibody gives rise to a precipitate with characteristic absorption at 660 nm. The amount of precipitate is proportional to the amount of HbA1c absorbed onto the surface of the latex particles.

Interestingly, total cholesterol assay utilises cholesterol esterase-catalysed degradation of cholesterol esters to free cholesterol and fatty acids (Sblendorio & Palmieri, 2008). The released cholesterol is then oxidised by a second enzyme, cholesterol oxidase that removes hydrogen groups to produce cholest-4-en-3-one and hydrogen peroxide. The released hydrogen peroxide reacts with hydroxybenzioc acid (HBA) and 4-aminoantipyrine (4-AAP) to produce water and red coloured quinoneimine with maximum absorbance at 540 nm. The intensity of the colour developed expressed as absorbance is directly proportional to the concentration of total cholesterol in sample.

In terms of serum triglycerides, the BT 3000 autoanalyzer utilises lipase-dependent hydrolysis of triglycerides into glycerol and fatty acids (Sblendorio & Palmieri, 2008). Glycerol is then phosphorylated to glycerol-3-phosphate in ATP-dependent reaction catalysed by glycerol kinase. The glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DHAP) by glycerophosphate oxidase in the presence of molecular oxygen. Finally, the released hydrogen peroxide is reacted with 4-AAP and 3,5-dichloro-2-hydroxybenzene under the influence of peroxidase enzyme to yield a coloured quinoneimine. The intensity of the developed colour which is proportional to the concentration of triglyceride in sample is measured at 510 nm.

The assay principle for determination of high-density lipoprotein cholesterol (HDL) employs a modified polyvinyl sulfonic acid (PVSA) and polyethylene-glycolmethyl ether (PEGME) coupled precipitation method patented by Xiao (2002). By this principle, low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL) and chylomicron (CM) participate in a reaction with PEGME and PVSA to obstruct access to LDL, VLDL and CM but not HDL by cholesterol esterase (CEST) and cholesterol oxidase (COX) enzymes. As a result, HDL is selectively degraded to fatty acids and hydrogen peroxides in a series of reactions catalysed by both COX and CEST enzymes. The hydrogen peroxide released is then reacted with 4-AAP and N,N-Bis(4-sulfobutyl)-3-methylaniline under the influence of the peroxidase enzyme to give a coloured quinone product with characteristic

absorption at 600 nm. The intensity of the coloured quinone expressed as absorbance is directly proportional to the concentration of HDL in the sample.

However, it must be noted that no single analyzer is designed to measure every biomolecule of interest. To this end, other techniques such as ELISA have been developed for qualitative and quantitative determination of some biomolecules (Premjeet *et al*., 2011; Lequin, 2005). ELISA is based on the coupling of antigen-antibody reaction to appropriate enzymatic reactions that give rise to coloured products with characteristic absorbance at specific wavelengths. Thus, the ELISA technique also relies on light-absorption properties of biomolecules. In the current work, quantitative sandwich ELISA technique was used. The assay relies on microplate pre-coated with antibody specific for the biomarker of interest. The specific biomarker in the sample sandwiches between the immobilized antibody and a second antibody. Upon addition of a conjugated enzyme which recognizes and binds the second antibody, a chromogen substrate is added after a series of washing steps. The intensity of the colour developed which is proportional to the concentration of the biomarker of interest in sample is then measured at a specific wavelength.

Considering the fact that the current study aims at investigating effects of *P. falciparum* malaria on levels of certain biomolecules, there is the need to briefly discuss the diagnostic principle of malaria in Ghanaian health facilities.

Malaria parasite detection in blood film by microscopy has been accepted as the gold standard (WHO, 2010) as against other diagnostic techniques such the polymerase chain reaction (PCR), rapid diagnostic test (RDT) (Hopkins *et al*., 2007) and imaging techniques (Sio *et al*., 2007).

In Ghanaian health facilities, microscopy and RDT are the routine malaria diagnostic methods. Indeed, microscopy is time-consuming and operator-dependent making the use of relatively simpler and faster RDT attractive. Microscopic determination of malaria parasite relies on the direct detection of the parasite in Giemsa-stained thin and thick films on microscopic slide.

However, RDTs rely on the release of histidine-rich protein 2 (HRP2), lactate dehydrogenase (pLDH) or aldolase by the malaria parasite into the blood stream of affected individuals (Heutmekers *et al.*, 2012). Therefore, appropriate monoclonal antibodies against these parasite antigens allow for diagnosis of malaria via detection of the *Plasmodium*-specific antigens in blood. Currently available RDTs kits have nitrocellulose membrane pre-coated with a monoclonal antibody against a specific parasite antigen in a cassette format. Upon the addition of an appropriate buffer, infected red blood cells are lysed to release the HRP2, pLDH or aldolase antigen, to bind to mouse antibody attached to colloidal gold to form a complex. This antigen-antibody complex then travels the length of the nitrocellulose strip so that another motif of the antigen can attach to a capture antibody specifically positioned as a transverse line along the strip to form a visible purple-red line. The unbound conjugated antibodies continue to travel along the strip until they bind to goat anti-mouse antibodies to form the control line (Heutmekers *et al.*, 2012).

Whereas the HRP2 is specific to *P. falciparum*, pLDH and aldolase antigens are released by other species like *P. malariae, P. vivax* and *P. ovale* (Heutmekers *et al.*, 2012). More so, HRP2 has higher sensitivity although

persistent presence after treatment makes it unsuitable for monitoring treatment failures. With more than 95% of malaria cases in Ghana attributable to *P. falciparum* (GHS, 2010) coupled with its high sensitivity in diagnosing active infection, the HRP2 RDT specific to *P. falciparum* was employed in the current study.

CHAPTER THREE: METHODOLOGY

Study Site

The research was conducted at the Diabetic Clinic of the Central Regional Hospital (CRH) in the Cape Coast Metropolis. CRH serves as the referral hospital for the various health posts, centers and hospitals in the region. In addition, it has a well organized Diabetic Clinic with patients from various parts of the region. The above reasons influenced the choice of this study site.

The Central Region is bordered by the Ashanti and Eastern regions to the north, Western region to the west, Greater Accra region to the east, and to the south by the Atlantic Ocean. It covers an estimated area of 9,826 km^2 with Cape Coast as its capital (MLGRD, 2006).

According to the 2000 Population and Housing Census, the population of the Central Region stood at 1,593,823 with annual growth rate of 2.1% (MLGRD, 2006). This gives an estimated population of 1,921,639 for the region in 2009. With respect to the Cape Coast Metropolis, the population was 82,291 according to the 2000 Population and Housing Census (MLGRD, 2006). Applying the regional population annual growth rate of 2.1% would translate into an estimated population of 99, 217 in 2009 for the Cape Coast metropolis. The recently released report of the 2010 Population and Housing Census conducted by the Ghana Statistical Service (GSS) estimated the

regional population as 2,201,863 with that of the Cape Coast metropolis being 169,894 (GSS, 2012).

Central Region has two ecological zones: the forest and the coastal zones. Climatically, the weather is typically tropical and is influenced by South West Monsoon winds (March-October) and North East Trade winds (November-February). The temperatures average between 21-32 °C throughout the year with an annual rainfall value of between 750 and 1000 mm (MLGRD, 2006). The people are mainly farmers and fishermen in the informal sector of the economy with a relatively small proportion of the working population in the formal sector (MLGRD, 2006).

Study Design

The study was a prospective cohort study that compared diabetics with or without malaria with their non-diabetic counterparts. As shown in figure 7 below, there were two main groups of respondents who were followed for two years for *P. falciparum* infection.

After the follow-up period, each group was divided into two to give a total of four groups. Group 1 was made up of normal non-diabetics who did not get malaria after the follow-up; Group 2 consisted of normal non-diabetics who had malaria; Group 3 was made up of diabetics who had malaria with Group 4 being made up of diabetics who did not get malaria at the end of follow-up. A total sample size of 200 was computed from the laboratory-confirmed regional malaria prevalence for adult population based on a formula reported by Naing *et al*. (2006).

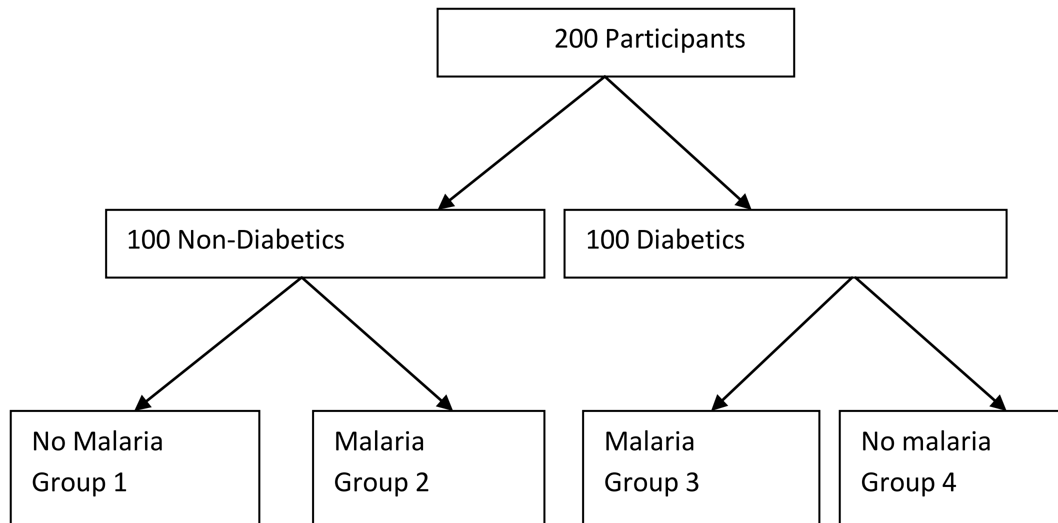

Figure 7: Flow chart of the study design

Ethical Approval

Ethical approval for the study was granted by the Committee on Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences and Komfo Anokye Teaching Hospital, Kumasi. All protocols followed were in line with the ethical requirements of the Ghana Health Service, CRH and the World Medical Association declaration of Helsinki (WHO, 2001). In addition, written informed consent was obtained from all participants.

Selection of Participants

Study participants were recruited based on the following inclusion criteria:

- willingness to participate, at least 30 years, non-alcoholic, non-smoker, not suffering from any cardiovascular disease, not suffering from hepatitis B, C, HIV or any known viral or bacterial disease, not pregnant, not nursing a baby under 2 years, no evidence of hepatic

dysfunction, absence of severe arthritis, lupus, inflammatory bowel disease, or any medical condition that can significantly influence the levels of any of the biomarkers measured.

All potential participants were thoroughly screened through a comprehensive medical examination by a competent team of health personnel. Prospective participants who met the inclusion criteria, after a thorough medical examination which included physical examination and questionnaire-based interviews and were willing to participate were enrolled for the commencement of the study in 2011.

For the diabetic group, participants were selected randomly using the database of registered diabetics at the Central Regional Hospital (CRH). Recruitment of all participants in the diabetic arm of the study was done at CRH. With respect to the controls, they were age matched with the diabetic group. Controls were recruited from the general inhabitants' of the Cape Coast metropolis. Enrolled respondents had 10 ml of venous blood taken using the venipuncture technique before and during malaria after an overnight fast. All blood samples were taken by experienced licensed technicians at the CRH. Blood sample was seperated into plasma and serum. Serum samples were aliquoted and stored at -80 ^{o}C until analysis.

Confidentiality was strictly adhered to in accordance with rules and regulations governing health-related research at CRH and the Ghana Health Service. Enrolled participants had their anthropometric indices, blood pressure, and other biochemical indices measured. Also, semi-structured questionnaires were employed to collect relevant additional health-related information.

Anthropometric and Blood Pressure Measurements

Weight was measured to the nearest 0.1 kg with height to the nearest 0.1 cm. Body mass index was computed as the ratio of weight in kilogramme to the square of the height in metre (kg/m^2). Weight and height were measured in light clothing without footwear.

Waist circumference was measured in centimetres with an inflexible tape measure at the midpoint between the lower margin of the last rib and the top of the iliac crest (WHO, 2008). In terms of hip circumference, it was measured around the widest portion of the buttocks. Waist-to-hip ratio was then computed by dividing waist circumference by the hip circumference.

Blood pressure was measured by a standard mercury sphygmomanometer on the right arm of respondents in sitting position after resting for at least five minutes.

Determination of Fasting Blood Glucose

Fasting blood glucose level was measured by the hexokinase method using the BT 3000 autoanalyzer, with reagents from JAS Diagnostics, Inc., USA.

Determination of Glycosylated Haemoglobin

Glycosylated haemoglobin (HbA1c) level was determined at 660 nm by the BT3000 autoanalyzer with reagents manufactured by JAS Diagnostics, Inc., USA based on the latex-HbA1c-mouse antihuman HbA1c antibody agglutination method.

Determination of Lipid Profile

Lipid profile of respondents was measured same day by the BT 3000 autoanalyzer. Lipid profile included total cholesterol, triglycerides, high-density lipoprotein cholesterol (LDL) and low-density lipoprotein cholesterol (HDL).

Total cholesterol and triglycerides were determined by the cholesterol-oxidase and glycerol-kinase methods respectively.

HDL was determined by a modified polyvinyl sulfonic acid (PVSA) and polyethylene-glycolmethyl ether (PEGME) coupled precipitation method patented by Xiao (2002).

LDL was calculated by the Friedewald formula as TC - (measured HDL-C + TG/2.2) in mol/L (Friedewald, Levy & Fredrickson, 1972).

Determination of Insulin

Serum insulin was determined by a commercially available direct sandwich enzyme-linked immunosorbent asssay (ELISA) kit procured from Calbiotech (Calbiotech Inc., USA).

Specifically, serum samples and insulin standards together with all other reagents were brought to room temperature. Exactly 25 µl each of already prepared insulin standards supplied in separate vials in concentrations (µIU/ml) of 6.25, 12.5, 25, 50 and 100 was placed in appropriate wells pre-coated with anti-insulin antibody. The same volume of serum sample was also placed in wells, followed by the addition of 100 µl of enzyme conjugate. The enzyme conjugate was prepared by thoroughly mixing 500 µl of 20X enzyme conjugate

concentrate with 9500 µl of assay diluent. After 10 seconds of thorough mixing, the setup was incubated for another 60 minutes. Each of the wells was then washed 6 times each with 300 µl of wash buffer by the use of a plate washer (Thermo electron co-operation, Finland). The wash buffer was obtained by diluting the supplied 20X concentrate with 475 ml of distilled water. Exactly 100 µl of TMB substrate was then added to each of the wells followed by incubation at room temperature for 15 minutes. Exactly 50 µl of the supplied stop solution was then added to each of the wells followed by immediate reading of absorbance at 450 nm. Appropriate standard curve was prepared from the corresponding absorbance and concentration values obtained from the wells containing standard insulin. The concentration of insulin in sample was subsequently estimated from a standard curve.

Estimation of Insulin Resistance

Level of insulin resistance in respondents before and during malaria was determined by the homeostatic model assessment of insulin resistance (HOMAIR) using a formula developed by Matthews and colleagues (Matthews *et al*., 1985). The formula requires fasting glucose and insulin levels as shown below:

HOMAIR = fasting insulin (µU) x fasting glucose (mmol/L)/ 22.5

Estimation of Beta Cell Function

The homeostatic model assessment of beta cell secretion (HOMAB) formula developed by Matthews and colleagues (Matthews *et al*., 1985) was used in assessing the extent of insulin secretion by the beta cells of respondent.

This formula also relies on levels of fasting glucose and insulin of respondents. The specific formula used is indicated below:

HOMAB = 20 x fasting insulin (μU/mL)/ (fasting glucose - 3.5)

Determination of Leptin

Leptin was determined by commercially available sandwich ELISA kits procured from Assaypro, USA (Assaypro Inc., USA).

All reagents and samples were brought to room temperature. Lyophilized 64 ng leptin standard was reconstituted with 2 ml of mix diluent to give a stock concentration of 32 ng/ml. Subsequent dilutions were made by mixing 100 μl of preceding leptin standard with 300 μl mix diluent to generate the required standard concentrations of 8, 2, 0.5 and 0.125 ng/ml. The various standard concentrations were prepared in duplicate. The blank had only the mix diluents with 0 ng/ml leptin. Exactly 50 μl of the various standard concentrations was placed in each microplate well pre-coated with murine antibody specific to human leptin, in duplicates. The frozen serum samples were thawed to room temperature and 20 μl of each of the samples was diluted with 140 μl mix diluent. Exactly 50 μl each of standards and samples were added to each of the wells of the microplate, sealed with the sealing tape and incubated for 2 hours. The plates were then washed six times with the plate washer and 50 μl of biotinylated leptin antibody was added to each of the wells, sealed and incubated for another 2 hours. The microplate was washed six times after the second incubation period before addition of 50 μl SP-conjugate to each well, sealed and incubated again for 30 minutes. The plate was then washed as earlier described and 50 μl of the chromogen substrate was added to

each well and sealed for a final incubation for 30 minutes. Finally, 50 µl of 0.5 N HCl stop solution was added to each well for the colour to change from blue to yellow. The intensity of the developed colour was measured as absorbance at 450 nm using the multiscan microplate reader (Thermo Scientific, Finland). Concentration of leptin in sample was determined from a standard curve.

Determination of Adiponectin

Serum adiponectin was determined by commercially available sandwich ELISA kits purchased from Assaypro, USA (Assaypro Inc., USA).

Adiponectin standard was prepared by diluting 800 ng lyophilized adiponectin standard with 4 ml of mix diluent to give a concentration of 200 ng/ml. The stock was further diluted by adding 4 ml mix diluent to obtain a concentration of 50 ng/ml. Serial dilutions were then made by mixing 100 µl of preceding standard adiponectin solution with 100 µl mix diluent to obtain consecutive concentrations of 25, 12.5, 6.25, 3.125, 1.562 and 0.781 ng/ml. The blank contained only the mix diluent. After the serum samples were thawed, 2 µl of each sample was diluted with 998 µl mix diluents to obtain the required sample dilution of 1:500 in accordance with the manufacturer's instructions. After thorough mixing, 50 µl of each of the diluted serum sample was then pipetted into each of the microplate wells pre-coated with murine antibody against human adiponectin. Also, 50 µl of the different concentrations of standard adiponectin solution were pipetted into the wells in duplicate. The wells were sealed with the transparent sealing tape and incubated for 1 hour, followed by thorough washing of 6 times with the prepared washing buffer using the wellwash 4 mk2 (Thermo electron co-operation, Finland). Exactly 50

µl of the biotinylated adiponectin-specific polyclonal antibody was added to each of the wells, sealed and incubated again for another 1 hour. The microplate was then washed as indicated above before adding 50 µl of Sp-conjugate per well. The set up was then incubated for 30 minutes before washing as described. Colour development was then initiated by the addition of 50 µl chromogen substrate to each well and incubated for 10 minutes. Finally, 50 µl of 0.5 N HCl stop solution was added to each well for the colour to change from blue to yellow. The absorbance of adiponectin in standard and samples were measured by the multiscan microplate reader (Thermo Scientific, Finland) at 450 nm. Concentration of adiponectin in sample was then estimated from a standard curve.

Determination of C-reactive Protein (CRP)

Serum CRP was determined by commercially available sandwich ELISA kits procured from Assaypro, USA (Assaypro Inc., USA).

Specifically, 20 ml of 10-fold concentrated buffer (EIA diluent concentrate) was diluted with 180 ml distilled water and used to prepare the standard, antibody, enzyme and sample according to manufacturer's instructions. CRP standard was prepared by adding 1 ml EIA diluent to 16 ng standard CRP. Serial dilutions were prepared in a 1:2 proportion of EIA diluents (100µl) and the preceding CRP standard (100 µl) to obtain concentrations of 8, 4, 2, 1, 0.5 and 0.25 ng/ml. The blank had 100µl EIA diluent only. Biotinylated CRP antibody was spun down briefly and 120 µl of the antibody was diluted with 5880 µl of EIA diluent. Exactly 60 µl of a 100-fold Streptavidin-peroxidase conjugate (Sp-conjugate) was diluted with 5940

µl EIA diluent. All preparations were carried out at room temperature. Frozen fasting serum samples were thawed and brought to room temperature. Ten microlitres (10µl) of serum was diluted with 990 µl of EIA diluent to obtain a 1:100 dilution. A further dilution was prepared by mixing 10 µl of the diluted sera with 90 µl EIA diluent to obtain the required 1:1000 dilution suggested by the manufacturer's protocol. Exactly 50 µl of 1:1000 diluted serum samples was placed in each well pre-coated with murine antibody against human CRP. The same volume of various concentrations of CRP standard was also placed in each well in duplicate. The wells were covered with a sealing tape that accompanied the kits and incubated for 2 hours. The plate was washed six times with the wellwash 4 mk2 (Thermo electron co-operation, Finland) using 300 µl wash buffer per wash. The buffer was prepared by diluting 50 ml of 20-fold wash buffer concentrate with 950 ml distilled water. After washing, 50 µl of biotinylated CRP antibody was added to each well and incubated for 30 minutes. The microplates were washed again as described and 50 µl of the Sp-conjugate was added to each well and incubated for another 30 minutes. The microplates were washed again six times with the plate washer. Exactly 50 µl of the chromogen substrate was then added to each well and incubated for another 10 minutes. When the blue colour developed, the reaction was stopped by the addition of 50 µl 0.5 N HCl solution to each well. The absorbance was then read by the multiscan microplate reader (Thermo Scientific, Finland) at 450 nm and the concentration of CRP in each sample determined from a standard curve.

Determination of Total Antioxidant Power/Capacity

Total antioxidant power also called total antioxidant capacity was determined in serum sample by a commercially available assay kit procured from Oxford Biomedical (Oxford Biomedical Research, USA) following manufacturer's instructions. The kit employs microplate-based colorimetric technique based on the reduction ability of copper (II) ion to copper (I) ion.

Specifically, supplied trolox standard was prepared by adding 2 ml 200 proof ethanol directly to the powdered trolox in a vial using needle and syringe. The vial was then vortexed for 1 minute for complete dissolution of the powdered substance. Appropriate serial dilutions were prepared in a 1:1 ratio with deionised water to obtain trolox concentrations (mM) of 2, 1, 0.5, 0.25, 0.125 and 0 for preparation of standard curve. Exactly 15 µl of properly thawed serum sample was diluted with 585 µl of supplied dilution buffer to obtain the required 1:40 dilution. After thorough mixing, 200 µl of diluted sample or standard where appropriate, was placed in each well of the microplate. Reagent blanks which contained only the dilution buffer were prepared according to manufacturer's instructions. The plate was then read at 450 nm. Exactly 50 µl copper solution was added to each well and incubated for 3 minutes. After incubation, 50 µl of the supplied stop solution was added to each of the wells and absorbance was read again immediately. The difference in absorbance between the second and the first readings was computed to obtain the net absorbance for samples, standards, and blanks. The concentration of total antioxidants in sample expressed as trolox equivalent in µM was then estimated from a standard curve.

Determination of Lipid Peroxides

Lipid peroxidation products in serum samples were measured spectrophotometrically as 2-thiobarbituric acid reactive substances (TBARS) reported generally in malondialdehyde (MDA) equivalent. The assay kit was purchased from Oxford Biomedical Research (Oxford Biomedical Research Inc., USA).

Specifically, reagents and samples were thawed to room temperature in accordance with manufacturer's instructions. Indicator solution was prepared by adding 20 ml of supplied acid reagent to 2 g of powdered 2-thiobarbituric acid and shook for a while for complete dissolution. A 20 µM MDA Standard Stock solution was prepared by diluting 80 µl of 10 mM Malonaldehyde Tetrabutylammonium Salt with 39920 µl of deionized water (dH_2O) to obtain the required 1:500 ratio per the manufacturer's instructions. Various concentrations of the stock MDA solution was prepared by diluting 0 µl, 20 µl, 40 µl, 100 µl, 400 µl and 600 µl of stock MDA solution with 800 µl, 780 µl, 760 µl, 700 µl, 600 µl, 400 µl, 200 µl and 0 µl of dH_2O respectively, to obtain corresponding MDA concentrations of 0 µM, 0.5 µM, 1 µM, 2.5 µM, 5 µM, 10 µM, 15 µM and 20 µM for the prepration of MDA standards. Exactly 200 µl of each thawed serum sample and MDA standards was mixed with 600 µl of indicator solution in appropriate separate test tubes and allowed to react for 20 minutes. The blanks were prepared by mixing 200 µl of the various serum samples with 600 µl acid reagent and allowed to also react for 20 minutes as above. At the end of the reaction, the contents of the test tubes were transferred into appropriate spectrophotometric cuvettes for the reading of absorbance at

532 nm. A net absorbance for samples and standards was obtained by subtracting the absorbance of the blank from its respective sample and duplicated standards. A standard curve was then generated by plotting the mean absorbance of the MDA standards against their respective concentrations. The free MDA concentration in the various samples was then determined from the standard curve.

Diagnosing Malaria in Study Participants

Malaria was assessed clinically and parasitologically. Thus, respondents who reported of malaria were assessed by the medical team at the Diabetes Clinic of the CRH for the requisite clinical signs and symptoms through standard medical procedure.

Parasitological confirmation of malaria parasite in blood was then obtained mainly by the use of rapid diagnostic test, CareStart™ Malaria HRP2Pf test kit (Access Bio Inc., USA), with microscopy being employed in less than 10% of the cases. In the current work, a two-band RDT specific to the HRP2 antigen of *P. falciparum* was employed. Apart from the test cassette, the package also contained alcohol pad, lancet, plastic pipette and a plastic buffer vial.

Specifically, respondents who were clinically diagnosed of malaria had the tip of the 4th finger pricked carefully with the lancet after it had been cleaned with the alcohol swab. The finger tip was squeezed gently for oozing of blood. The blood was sucked to the 5 µl mark of the plastic pipette in accordance with manufacturer’s instructions. The sucked blood was then released into the sample hole marked ‘S’and two drops of the buffer was put

into the assay hole marked 'A'. The set-up was left to react for 20 minutes before reading for interpretation. The presence of *P. falciparum* in blood was confirmed when two red-purple lines were observed on the membrane at or near the labels 'C' and 'T' on the plastic cassette. This signified a positive result. A negative result was therefore indicated by a single red-purple line at the 'C' label only. In case the line appeared only in the 'T' segment, the result was described as invalid and the test repeated. Respondents that gave positive RDT results had venous blood samples taken for analysis of additional biomarkers before being treated appropriately.

In few instances that microscopy was used both thick and thin films which are routine procedures at CRH were employed. The preparation and staining procedure used in this study has been described in detailed elsewhere (WHO, 2010).

Data Analysis

All data were analysed by using the SPSS software version 16. Apart from age, all other measured parameters were log-transformed due to skewed distribution of original values. Results are presented as mean ± standard deviation (SD). Baseline mean values between study groups (diabetics and controls) or sexes were compared with 2-tailed independent sample t-test. The mean level of education in study groups and sexes were compared across categories of formal education with one-way analysis of variance (ANOVA). Bivariate Pearson correlation was then used to assess linear relationships between any pair of the measured parameters. A simple linear regression analysis was used to determine the extent of linear association obtained from

the correlational tests. Stepwise linear regression test was used to identify independent predictors of baseline leptin.

Because only 50% of the total respondents had malaria after follow-up, mean levels of the various biomarkers of respondents before and during malaria were treated as independent samples and compared by one-way ANOVA with Tukey's post hoc Honest Significant Difference (HSD) test. Subsequent correlation and regression analyses were carried out just as was done for the baseline data. In all analyses, a p-value of <0.05 was considered statistically significant.

CHAPTER FOUR: RESULTS

General characteristics of study participants

The study involved 100 diabetics and 100 non-diabetic controls aged 32-80 years who were followed over a two-year period for *P. falciparum* infection. In terms of gender, males constituted 29% with females accounting for the remaining 71% of total respondents.

In spite of the disproportionate gender distribution of respondents, mean levels of formal education did not differ significantly ($P > 0.05$) between study groups and between genders (table 1 and 2).

Table 1: Level of formal education among respondents

Educational level	Diabetic	Non-diabetic	P-value
None	8	9	0.289
Basic	58	44	
Secondary	6	7	
Tertiary	28	40	

Figures represent percentage of individuals in the two study groups and by gender. The p-value represents difference in mean levels of formal education between respondents in the study groups and genders.

Table 2: Level of formal education of respondents by gender

Educational level	Male	Female	P-value
None	3.5	8.6	0.787
Basic	49.1	52.9	
Secondary	10.5	5.0	
Tertiary	36.8	33.6	

Figures represent percentage of individuals by gender. The p-value represents difference in mean levels of formal education between respondents.

Age, glycaemic indicators, HOMAIR, HOMAB and blood pressure

Table 3 indicates the age, glycaemic indicators, homeostatic model assessment of insulin resistance (HOMAIR), homeostatic model assessment of beta cell secretion of insulin (HOMAB) and blood pressure of respondents before malaria according to study group. Although respondents in the two study groups did not differ ($P > 0.05$) in mean age and blood pressure, the diabetics recorded significantly ($P < 0.001$) higher levels of FBG, $HbA1_C$, and HOMAIR but lower level of HOMAB than their non-diabetic counterpart. As expected, the mean FBG level for the diabetic group was slightly higher than the upper reference level in spite of a normal $HbA1_C$ level.

Stratification of data into gender between study groups revealed that diabetic males and females had significantly ($P < 0.05$) higher levels of FBG, $HbA1_C$ and HOMAIR but lower level of HOMAB than their non-diabetic counterpart (table 4). Also, diabetic males but not females were significantly ($P < 0.001$) older than their non-diabetic controls (table 4).

Table 3: Age, glycaemic indicators, HOMAIR, HOMAB and blood pressure of respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
Age (years)	53.43± 6.57	52.88 ± 10.29	0.778
FBG (mmol/L)	6.68 ± 1.46	4.54 ± 1.17	<0.001*
$HbA1_C$ (%)	5.12 ± 1.03	4.07 ± 1.02	<0.001*
HOMAIR	1.59 ± 0.19	1.08 ± 0.18	<0.001*
HOMAB	43.90 ± 25.31	116.90 ± 21.72	<0.001*
Diastolic (mmHg)	81.10 ± 11.50	82.76 ± 11.50	0.146
Systolic (mmHg)	137 ± 11.71	133.08 ± 11.21	0.34

Figures represent mean ± standard deviation; FBG = Fasting Blood Glucose; $HbA1_c$ = Glycosylated haemoglobin, HOMAIR = homeostatic model assessment of insulin resistance, HOMAB = homeostatic model assessment of beta cell secretion, *= significant p-value.

Further stratification of data in study groups into their respective male and female genders showed a significantly (P = 0.023) higher level of FBG only in non-diabetic male compared with their female counterpart (table 5). Levels of all the remaining parameters were comparable between the two sexes in each study group (table 5).

Table 4: Inter-group gender comparison of age, glycaemic indicators, HOMAIR, HOMAB and blood pressure of respondents

Parameter	Diabetic	Non-diabetic	p-value
	MALE		
Age (years)	57.92 ± 11.50	48.79 ± 12.10	<0.001*
FBG (mmol/L)	7.08 ± 0.90	4.83 ± 0.70	<0.001*
$HbA1_C$ (%)	5.02 ± 1.57	3.93 ± 1.12	<0.001*
HOMAIR	1.43 ± 0.19	1.02 ± 0.15	0.021*
HOMAB	38.33 ± 21.60	91.41 ± 22.10	<0.001*
Diastolic (mmHg)	80.93 ± 11.50	83.89 ± 11.40	0.359
Systolic (mmHg)	136.96 ± 11.50	130.20 ± 11.40	0.189
	FEMALE		
Age (years)	55.37 ± 12	53.09 ± 12	0.182
FBG (mmol/L)	6.50 ± 0.80	4.45 ± 0.70	<0.001*
$HbA1_C$ (%)	5.17 ± 1.36	4.12 ± 1.22	<0.001*
HOMAIR	1.66 ± 0.20	1.12 ± 0.19	0.003*
HOMAB	48.17 ± 27.80	130.44 ± 21.10	<0.001*
Diastolic (mmHg)	81.17 ± 11.50	82.40 ± 11.50	0.558
Systolic (mmHg)	137.15 ± 11.80	133.97 ± 11.20	0.338

FBG = Fasting Blood Glucose; $HbA1_c$ = Glycosylated haemoglobin, HOMAIR = homeostatic model assessment of insulin resistance, HOMAB = homeostatic model assessment of beta cell secretion, *= significant p-value.

Table 5: Intra-group comparison of age, glycaemic indicators, HOMAIR, HOMAB and blood pressure of respondents

Parameter	Male	Female	P-value
	DIABETIC		
Age (years)	54.68 ± 17	52.85 ± 17	0.615
FBG (mmol/L)	7.08 ± 1.50	6.50 ± 1.44	0.316
$HbA1_C$ (%)	5.02 ± 1.23	5.16 ± 1.36	0.659
HOMAIR	1.43 ± 0.17	1.66 ± 0.20	0.424
HOMAB	38.33 ± 21.60	48.17 ± 27.80	0.302
Diastolic (mmHg)	80.93 ± 11.50	81.17 ± 11.50	0.928
Systolic (mmHg)	136.96 ±11.50	137.15 ± 11.80	0.967
	NON-DIABETIC		
Age (years)	49.65 ± 10.30	54.01 ± 13	0.063
FBG (mmol/L)	4.83 ± 1.20	4.45 ± 1.20	0.023*
$HbA1_C$ (%)	3.93 ± 1.20	4.12 ± 1.20	0.279
HOMAIR	1.01 ± 0.15	1.12 ± 0.19	0.381
HOMAB	91.41 ± 22.10	130.44 ± 21.10	0.056
Diastolic (mmHg)	83.89 ± 11.40	82.40 ± 11.50	0.592
Systolic (mmHg)	130.20 ± 11.40	133.97 ± 11.20	0.311

Figures represent mean ± standard deviation; FBG = Fasting Blood Glucose; $HbA1_c$ = Glycosylated haemoglobin; HOMAIR = homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

Anthropometric indices

BMI, WC and WHR were used as measures of anthropometry to ascertain the relationship among them with respect to the current study. Interestingly, WC, weight and BMI were significantly ($P < 0.05$) higher in the diabetic than the non-diabetic group but WHR was lower in diabetics than control (table 6). The mean BMI levels of both groups fell within the overweight category.

Table 6: Anthropometric indices of respondents by study group

Index	Diabetic	Non-diabetic	P-value
WC (cm)	99.06 ± 11.40	92.81 ± 11.30	0.002*
Hip (cm)	159.24 ± 11.30	103.11 ± 11.10	0.343
WHR	0.90 ± 0.07	0.95 ± 0.07	<0.001*
Weight (kg)	76.45 ± 12.20	68.34 ± 12.50	0.001*
Height (m)	1.62 ± 0.11	1.63 ± 0.11	0.272
BMI (kg/m^2)	28.50 ± 8.65	26.09 ± 5.34	0.025*

Figures represent mean ± standard deviation; BMI = body mass index; WHR = waist-to-hip ratio; WC = waist circumference; *= significant p-value.

Comparison of male and female genders in the diabetic group demonstrated that apart from the weight, all the other anthropometric measures were significantly ($P < 0.05$) higher in females than in males (table 7).

Table 7: Intra-group gender comparison of anthropometric indices of respondents

Index	Male	Female	P-value
	DIABETIC		
WC (cm)	91.69 ± 11.20	102.85 ± 11.30	<0.001*
Hip (cm)	97.61 ± 11	108.59 ± 11.3	<0.001*
WHR	0.86 ± 0.08	0.92 ± 0.07	0.002*
Weight (kg)	73.54 ± 12	78.02 ± 12.3	0.216
Height (m)	1.59 ± 0.10	1.69 ± 0.10	<0.001*
BMI (kg/m^2)	25.50 ± 9.2	30.93 ± 12.2	<0.001*
	NON-DIABETIC		
WC (cm)	89.33 ± 10.90	93.86 ± 11.40	0.111
Hip (cm)	96.41 ± 10.90	105.17 ± 11	<0.001*
WHR	0.99 ± 0.06	0.93 ± 0.06	0.004*
Weight (kg)	73.23 ± 12.30	66.77± 12.50	0.073
Height (m)	1.72 ± 0.10	1.6 ± 0.10	<0.001*
BMI (kg/m^2)	24.64 ± 11.90	25.94 ± 12.20	0.252

Figures represent mean ± standard deviation; BMI = body mass index; WHR = waist-to-hip ratio; WC = waist circumference; *= significant p-value.

A similar comparison within the non-diabetic group rather revealed the male gender recording significantly ($P < 0.05$) higher values for height and WHR but lower ($P < 0.001$) value for hip than their female counterpart (table7).

Further comparison of data showed that whereas male diabetics had significantly ($P < 0.001$) lower mean WHR than their non-diabetic counterpart, female diabetics recorded significantly ($P < 0.001$) higher mean WC, weight and BMI than female controls (table 8).

Lipid profile and atherogenic indices of respondents without malaria

Table 9 depicts the lipid profile and atherogenic indices of respondents in diabetic and non-diabetic control groups. Apart from the mean LDL cholesterol level that was significantly ($P = 0.029$) lower in diabetic than their non-diabetic counterpart; mean levels of all the other components of lipid profile were comparable ($P > 0.05$) between the two study groups.

With respect to the atherogenic indices, mean CHOL/LDL and TRG/HDL ratios were significantly ($P < 0.05$) higher for diabetics in comparison with their non-diabetic colleagues (table 9).

Comparison of males with females in the diabetic study group showed significant ($P = 0.026$) difference between the sexes in respect of LDL to HDL ratio only (table 10).

Table 8: Inter-group gender comparison of anthropometric indices of respondents

Parameter	Diabetic	Non-diabetic	P-value
	MALE		
WC (cm)	91.69 ± 11.20	89.33 ± 10.90	0.409
Hip (cm)	97.61 ± 11	96.41 ± 10.90	0.666
WHR	0.86 ± 0.08	0.99 ± 0.09	<0.001*
Weight (kg)	73.54 ± 12	73.23 ± 12.30	0.941
Height (m)	1.69 ± 0.10	1.72 ± 0.10	0.063
BMI (kg/m^2)	25.50 ± 9.20	24.64 ±11.90	0.488
	FEMALE		
WC (cm)	102.85 ± 11.30	93.86 ± 11.40	<0.001*
Hip (cm)	108.59 ± 11	105.17 ± 11.30	0.113
WHR	0.92 ± 0.07	0.93 ± 0.06	0.316
Weight (kg)	78.02 ± 12.30	66.77 ± 12.50	<0.001*
Height (m)	1.59 ± 0.10	1.60 ± 0.10	0.15
BMI (kg/m^2)	30.93 ± 12.20	25.94 ± 12.20	<0.001*

Figures represent mean ± standard deviation; BMI = body mass index; WHR = waist-to-hip ratio; WC = Waist Circumference; *= significant p-value.

Table 9: Lipid profile and atherogenic indices of respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
CHOL	5.30 ± 1.30	5.21 ± 1.20	0.595
LDL	3.06 ± 1.01	3.36 ± 1.03	0.029*
HDL	1.26 ± 0.11	1.26 ± 0.13	0.822
TRG	1.12 ± 0.23	1.02 ± 0.20	0.141
CHOL/LDL	1.78 ± 0.60	1.57 ± 0.30	0.002*
CHOL/HDL	4.07 ± 1.10	4.22 ± 1.01	0.33
TRG/HDL	2.14 ± 1.20	2.00 ± 0.90	0.39
TRG/LDL	0.99 ± 0.60	0.81 ± 0.50	0.033*
LDL/HDL	2.65 ± 0.90	2.83 ± 0.90	0.20

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; TRG=triglyceride; CHOL=total cholesterol; *= significant p-value.

Table 10: Lipid profile and atherogenic indices by gender of diabetic respondents

Parameter	Male	Female	P-value
CHOL	5.24 ± 1.30	5.32 ± 1.30	0.756
LDL	3.19 ± 1.40	3.00 ± 1.30	0.341
HDL	1.23 ± 0.10	1.28 ± 0.11	0.141
TRG	1.14 ± 0.20	1.12 ± 0.20	0.878
CHOL/LDL	1.68 ± 0.30	1.83 ± 0.70	0.269
CHOL/HDL	3.83 ± 0.80	4.24 ± 1.20	0.099
TRG/HDL	2.34 ± 1.30	2.00 ± 1.20	0.236
TRG/LDL	1.09 ± 0.8	0.94 ± 0.6	0.259
LDL/HDL	2.37 ± 0.73	2.84 ± 1.00	0.026*

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG = triglycerides; CHOL = total cholesterol; *= significant p-value.

Within the non-diabetic group, mean LDL cholesterol, and CHO/HDL levels were significantly ($P < 0.05$) lower in males than females. However, levels of mean triglyceride and CHOL/LDL were significantly ($P <0.05$) higher in the males than females (table 11).

Table 11: Lipid profile and atherogenic indices by gender of non-diabetic respondents

Parameter	Male	Female	P-value
CHOL	4.93 ± 1.20	5.32 ± 1.20	0.081
LDL	2.96 ± 0.13	3.50 ± 0.13	0.012*
HDL	1.27 ± 0.10	1.26 ± 0.10	0.791
TRG	1.18 ± 0.20	0.97 ± 0.20	0.036*
CHOL/LDL	1.66 ± 0.20	1.54 ± 0.30	0.04*
CHOL/HDL	3.7 ± 0.60	4.43 ± 1.01	<0.001*
TRG/HDL	1.93 ± 1.00	2.03 ± 0.8	0.632
TRG/LDL	0.95 ± 0.70	0.76 ± 0.40	0.096
LDL/HDL	2.26 ± 0.60	3.04 ± 1.01	<0.001*

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG = triglycerides, CHOL = total cholesterol; *= significant p-value.

Interestingly, within the male gender, no statistically significant (P > 0.05) difference was observed between diabetic and their non-diabetic counterparts (table 12) with respect to the various lipid parameters.

Table 12: Lipid profile and atherogenic indices of male respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
CHOL	5.24 ± 1.30	4.93 ± 1.20	0.254
LDL	3.19 ± 1.40	2.96 ± 0.13	0.345
HDL	1.23 ± 0.10	1.27 ± 0.10	0.228
TRG	1.14 ± 0.20	1.18 ± 0.20	0.759
CHOL/LDL	1.68 ± 0.30	1.66 ± 0.20	0.782
CHOL/HDL	3.83 ± 0.80	3.7 ± 0.60	0.335
TRG/HDL	2.34 ± 1.30	1.93 ± 1.00	0.197
TRG/LDL	1.09 ± 0.8	0.95 ± 0.70	0.491
LDL/HDL	2.37 ± 0.73	2.26 ± 0.60	0.559

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; TRG=triglycerides, CHOL=total cholesterol.

In the female gender, mixed statistically significant ($P < 0.05$) differences were observed between diabetics and their non-diabetic counterparts with respect to the various lipid parameters (table 13). Non-diabetics had significantly ($P < 0.05$) higher mean level of LDL but lower ($P < 0.05$) levels of CHOL/LDL and TRG/HDL than the diabetic group (table 13).

Table 13: Lipid profile and atherogenic indices of female respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
CHOL	5.32 ± 1.30	5.32 ± 1.20	0.979
LDL	3.00 ± 1.30	3.50 ± 0.13	0.002*
HDL	1.28 ± 0.11	1.26 ± 0.10	0.522
TRG	1.12 ± 0.20	0.97 ± 0.20	0.056
CHOL/LDL	1.83 ± 0.70	1.54 ± 0.30	0.001*
CHOL/HDL	4.24 ± 1.20	4.43 ± 1.01	0.348
TRG/HDL	2.00 ± 1.20	2.03 ± 0.8	0.89
TRG/LDL	0.94 ± 0.6	0.76 ± 0.40	0.034*
LDL/HDL	2.84 ± 1.00	3.04 ± 1.01	0.285

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG = triglycerides; CHOL = total cholesterol; * = significant p-value.

Insulin, leptin, adiponectin, CRP, antioxidants and peroxide levels of respondents without malaria

As indicated in table 14, levels of insulin and total antioxidants power did not differ significantly ($P > 0.05$) between the study groups. However, the diabetic group exhibited significantly ($P < 0.001$) higher levels of mean CRP

and peroxides but lower ($P < 0.001$) levels of leptin and adiponectin compared with their non-diabetic controls (table 14).

Table 14: Baseline insulin, leptin, adiponectin, CRP, antioxidants and peroxide levels of respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
Insulin (µIU/ml)	5.70 ± 1.69	5.33 ± 1.72	0.425
Leptin (ng/ml)	21.24 ± 4.07	191.51 ± 24	<0.001*
Adiponectin (ng/ml)	2444.56 ± 49	5089.79 ± 16	<0.001*
C-reactive protein (ng/ml)	3746.28 ±33.10	1536.39 ± 47.40	<0.001*
Antioxidant power (µM)	226.36 ± 26.40	251.71±18.30	0.416
Peroxide (µM)	16.82 ± 2.01	9.91 ± 1.70	0.01*

= significant p-value.

In terms of gender, females recorded significantly ($P < 0.001$) higher level of leptin than their male counterpart in both diabetic and non-diabetic controls (table 15). The mean levels of all the remaining biomarkers were comparable ($P > 0.05$) between the sexes in the two study groups (table 15).

A further comparison of diabetic males with their non-diabetic counterparts revealed that the latter had significantly ($P < 0.05$) higher levels of leptin and adiponectin but lower level of CRP and peroxides than the former (table 16).

Table 15: Intra-group gender comparison of baseline insulin, leptin, adiponectin, CRP, antioxidant and peroxide levels of respondents

Parameter	Male	Female	P-value
	DIABETIC		
Insulin (µIU/ml)	5.79 ±1.78	5.65 ± 1.69	0.849
Leptin (ng/ml)	7.57 ± 0.50	38.28 ± 2.33	<0.001*
Adiponectin (ng/ml)	2361.57 ± 31.80	2493.45 ± 21.20	0.804
C-reactive protein (ng/ml)	2767.58 ± 37.40	4453.49 ± 29.90	0.093
Antioxidant power (µM)	206.68 ± 32.60	238.45 ± 23.01	0.537
Peroxide (µM)	16.46 ± 1.80	16.36 ± 2.10	0.89
	NON-DIABETIC		
Insulin (µIU/ml)	5.62 ± 1.54	5.22 ± 1.75	0.561
Leptin (ng/ml)	116.52 ± 22.90	241.05 ± 22.40	<0.001*
Adiponectin (ng/ml)	4962.49 ± 10.80	5148.73 ± 12.01	0.339
C-reactive protein (ng/ml)	1065.61 ± 48.20	1826 ± 46.30	0.155
Antioxidant power (µM)	233.13 ± 20.10	260.08 ± 17.50	0.47
Peroxide (µM)	9.46 ± 0.80	9.45 ± 0.2	0.93

= significant p-value.

The trend observed in the male respondents was similar for the females in the two study groups (table 17). Diabetic females showed significantly ($P < 0.001$) lower levels of leptin and adiponectin but higher level of CRP and peroxides compared with their non-diabetic colleagues.

Table 16: Baseline insulin, leptin, adiponectin, CRP, antioxidants and peroxide levels in male respondents by study group

Parameter	Diabetic	Non-diabetic	P-value
Insulin (µIU/ml)	5.79 ±1.78	5.62 ± 1.54	0.838
Leptin (ng/ml)	7.57 ± 0.50	116.52 ± 22.90	<0.001*
Adiponectin (ng/ml)	2361.57 ± 31.80	4962.49 ± 10.80	0.003*
C-reactive protein (ng/ml)	2767.58 ± 37.40	1065.61 ± 48.20	0.02*
Antioxidant power (µM)	206.68 ± 32.60	233.13 ± 20.10	0.669
Peroxide (µM)	16.46 ± 1.80	9.46 ± 0.80	0.007*

= significant p-value.

Table 17: Baseline insulin, leptin, adiponectin, CRP, antioxidant and peroxide levels in females by study group

Parameter	Diabetic	Non-diabetic	P-value
Insulin (µIU/ml)	5.79 ±1.78	5.22 ± 1.75	0.451
Leptin (ng/ml)	7.57 ± 0.50	241.05 ± 22.40	<0.001*
Adiponectin (ng/ml)	2361.57 ± 31.80	5148.73 ± 12.01	<0.001*
C-reactive protein (ng/ml)	2767.58 ± 37.40	1826 ± 46.30	0.001*
Antioxidant power (µM)	206.68 ± 32.60	260.08 ± 17.50	0.533
Peroxide (µM)	16.46 ± 1.80	9.45 ± 0.2	0.009*

= significant p-value.

Baseline associations

Bivariate Pearson correlation was used to assess possible linear relationship between any pair of studied parameters. As expected, a number of pairs of parameters exhibited some form of linear relationship with varying strengths of the observed associations (tables 18-23).

For instance, BMI correlated positively and relatively more strongly with leptin ($r = 0.633$ versus $r = 0.562$, $P < 0.001$) and WC ($r = 0.864$ versus $r = 0.722$, $P < 0.001$) in diabetic (figure 8-9, table 18) than their non-diabetic counterpart (figure 10-11, table 19). In a stepwise linear regression analysis, BMI and WC were independent predictors of baseline serum leptin levels, explaining 50.5 % and 31% of the observed respective variations for diabetic and control respondents. Also, the two study groups differed in the pairs of parameters that exhibited the observed correlations. In the diabetic group, $HbA1_C$ exhibited some form of correlation with FBG, LDL, CHOL and TRG (table 18) which were not observed in the non-diabetic group (table 19). Instead, FBG correlated ($P < 0.05$) with insulin, CRP and antioxidants in the non-diabetic group (table 19), an observation that was conspicuously absent in the diabetic group (table 18).

Interestingly, further categorization of data in each study group by gender (table 20-23) appears to show a similar trend in terms of strengths of correlations and specific pairs of parameters that exhibited the correlations in male and female respondents.

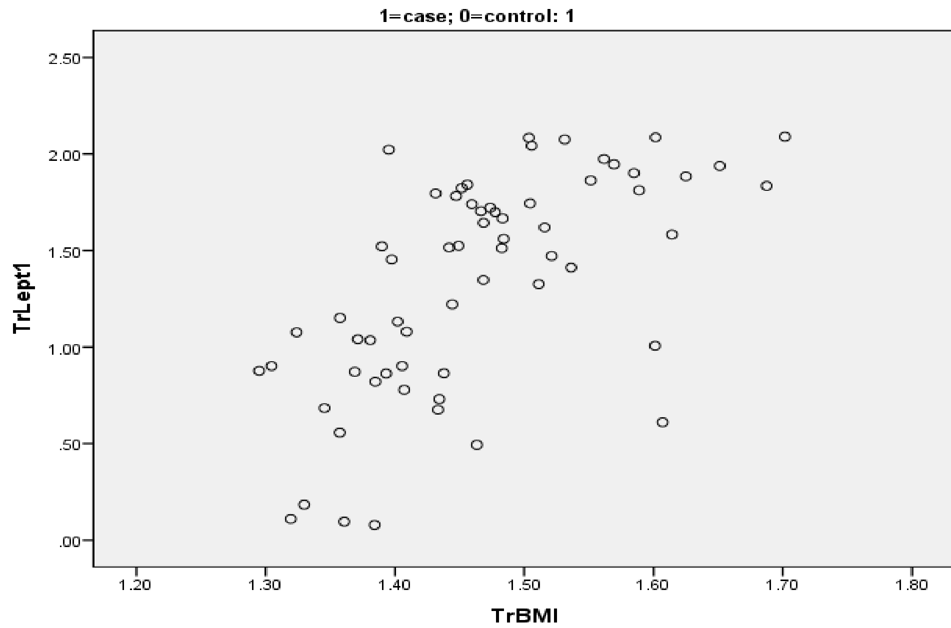

Figure 8: A simple scatter plot of the correlation between baseline serum leptin and BMI of diabetic respondents (R = 0.63, P < 0.001). ***BMI = body mass index*.**

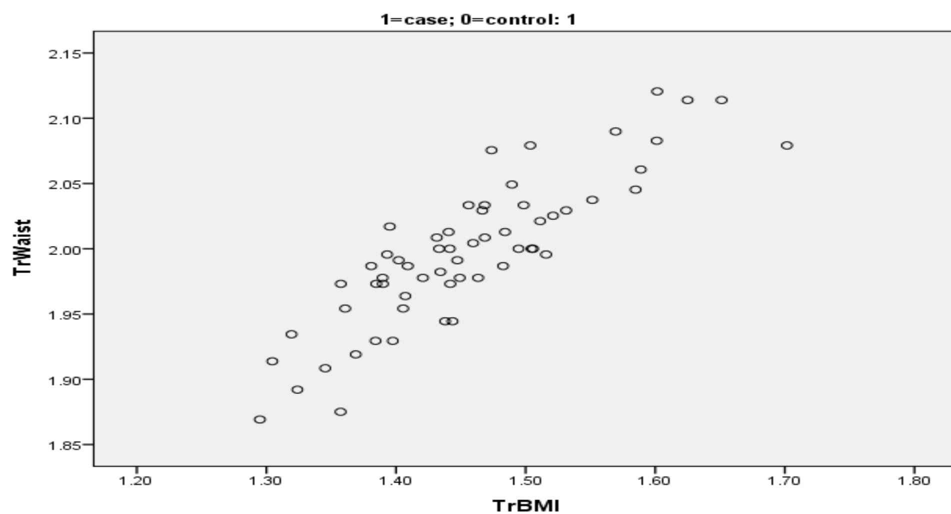

Figure 9: A simple scatter plot of the correlation between WC and BMI of diabetic respondents (R = 0.633, P < 0.001). ***WC = waist circumference, BMI = body mass index*.**

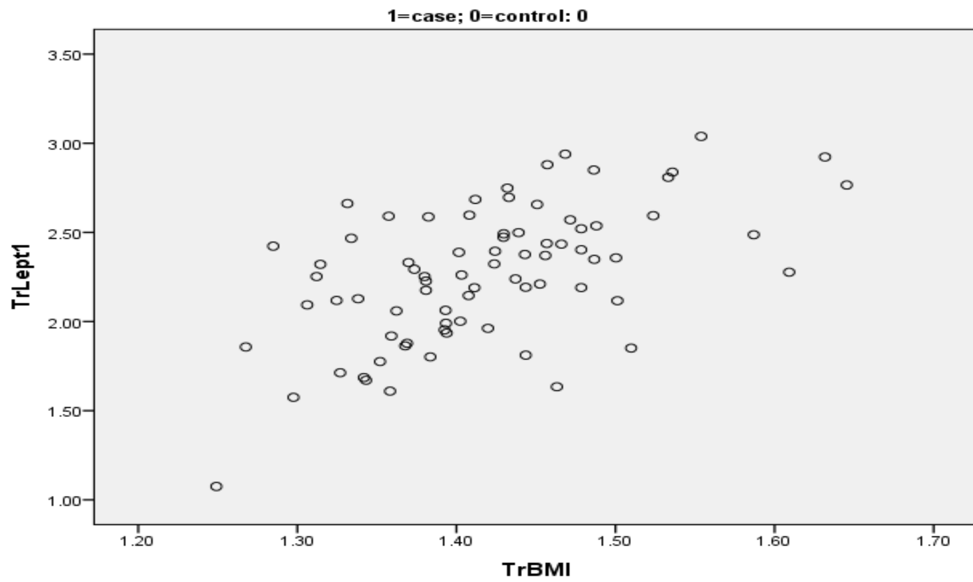

Figure 10: A simple scatter plot of the correlation between leptin and BMI of control respondents (R = 0.562, P < 0.001). ***BMI = body mass index*.**

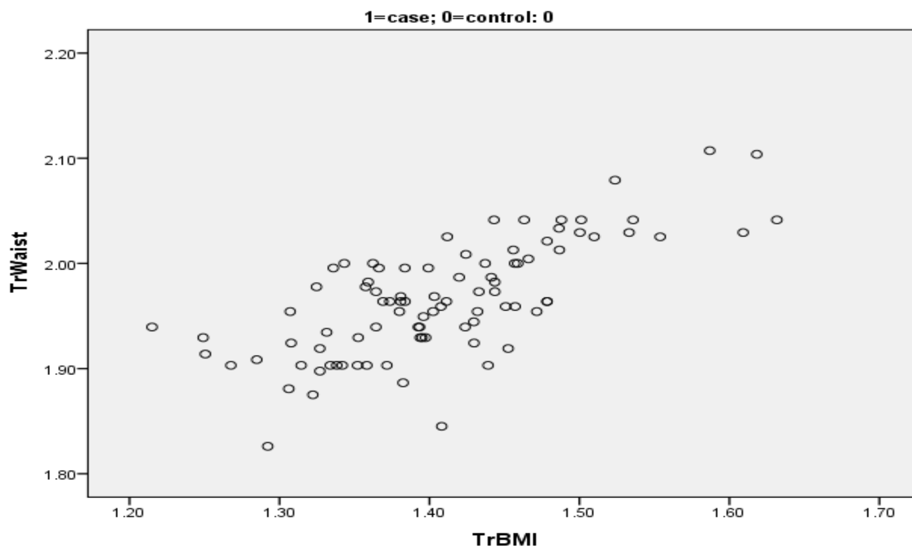

Figure 11: A simple scatter plot of the correlation between WC and BMI of control respondents (R = 0.722, P < 0.001). ***WC = waist circumference, BMI = body mass index*.**

Table 18: Observed correlations among measured parameters in diabetic group

Parameter	Correlation coefficient (r)	P-value
Duration and C-reactive protein	-0.252	0.028*
FBG and $HbA1_C$	0.346	0.001*
$HbA1_C$ and LDL	-0.219	0.035*
$HbA1_C$ and CHOL	0.263	0.011*
LDL and Systol	0.257	0.024*
$HbA1_C$ and TRG	0.236	0.023*
HOMAIR and Diastol	-0.248	0.033*
HOMAB and Insulin	0.262	0.039*
Leptin and Insulin	0.331	0.003*
Insulin and WC	0.299	0.023*
CHOL and Antioxidant power	-0.267	0.02*
Leptin and Antioxidant power	0.237	0.038*
Leptin and BMI	0.63	<0.001*
Leptin and WC	0.633	<0.001*
BMI and WC	0.864	<0.001*
Antioxidant power and BMI	0.304	0.016*

BMI = body mass index; WC = waist circumference; $HbA1_C$ = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; FBG = fasting blood glucose; systol = systolic blood pressure; HOMAIR = homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

Table 19: Observed correlations among measured parameters in control

Parameter	Correlation coefficient (r)	P-value
FBG and Insulin	0.296	0.006*
FBG and C-reactive protein	-0.287	0.011*
FBG and Antioxidant power	-0.269	0.018*
Leptin and Insulin	0.28	0.013*
Leptin and Adiponectin	0.249	0.029*
Leptin and BMI	0.562	<0.001*
C-reactive protein and BMI	0.269	0.019*
Age and BMI	-0.214	0.034*
WC and BMI	0.722	<0.001*
C-reactive protein and WC	0.252	0.034*
WHR and TRG	0.243	0.043*
LDL and BMI	-0.24	0.017*
LDL and Systol	0.222	0.029*
HDL and C-reactive protein	-0.329	0.003*
HDL and WC	-0.215	0.042*
CHOL and BMI	-0.239	0.018*

BMI = body mass index; WC = waist circumference; HbA1$_C$ = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; FBG = fasting blood glucose; systol = systolic blood pressure; WHR = waist-to-hip ratio; *= significant p-value.

Table 20: Observed correlations among measured parameters in diabetic male

Parameter	Correlation coefficient (r)	P-value
FBG and WC	-0.461	0.031*
$HbA1_C$ and LDL	-0.374	0.046*
HDL and WC	0.445	0.043*
LDL and Systol	0.402	0.042*
HOMAB and HDL	-0.421	0.032*
LDL and Antioxidant power	-0.406	0.036*
CHOL and Antioxidant power	-0.532	0.004*
TRG and Antioxidant power	-0.493	0.009*
Leptin and Insulin	0.577	0.001*
Leptin and BMI	0.449	0.028*
Adiponectin and WC	-0.58	0.006*
WC and BMI	0.858	<0.001*
Systol and WHR	-0.54	0.014*

BMI = body mass index; WC = waist circumference; HbA1c = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; FBG = fasting blood glucose; systol = systolic blood pressure: WHR = waist-to-hip ratio; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

Table 21: Observed correlations among measured parameters in diabetic female

Parameter	Correlation coefficient (r)	P-value
FBG and $HbA1_C$	0.391	0.001*
$HbA1_C$ and CHOL	0.248	0.048*
HDL and WHR	0.326	0.024*
Leptin and BMI	0.539	<0.001*
Leptin and WC	0.655	<0.001*
BMI and WC	0.823	<0.001*
Antioxidant power and WC	0.351	0.033*
HOMAB and insulin	0.417	0.013*

BMI = body mass index; WC = waist circumference; $HbA1_C$ = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; FBG = fasting blood glucose; WHR = waist-to-hip ratio; HOMAB = homeo-static model assessment of beta cell secretion; *= significant p-value.

Table 22: Observed correlations among measured parameters in non-diabetic male

Parameter	Correlation coefficient (r)	P-value
FBG and C-reactive protein	-0.446	0.025*
HDL and Diastol	-0.541	0.009*
HDL and Systol	-0.548	0.008*
Antioxidant power and Insulin	0.526	0.01*
Leptin and BMI	0.658	<0.001*
Leptin and WC	0.489	0.029*
WC and BMI	0.67	0.001*
Diastol and BMI	-0.455	0.029*
C-reactive protein and WC	0.547	0.013*

BMI = body mass index; WC = waist circumference; HbA1c = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; FBG = fasting blood glucose; systole = systolic blood pressure; diastol = diastolic blood pressure; *= significant p-value.

Table 23: Observed correlations among measured parameters in non-diabetic female

Parameter	Correlation coefficient (r)	P-value
FBG and Leptin	0.407	0.002*
FBG and Insulin	0.324	0.011*
HDL and WC	-0.306	0.01*
LDL and Systol	0.252	0.03*
LDL and BMI	-0.25	0.033*
HDL and C-reactive protein	-0.382	0.005*
CHOL and BMI	-0.276	0.018*
CHOL and Systol	0.304	0.008*
Adiponectin and CHOL/LDL	-0.358	0.008*
Leptin and BMI	0.466	<0.001*
Leptin and Diastol	0.28	0.016*
Leptin and Insulin	0.341	0.012*
Leptin and Antioxidant power	0.28	0.016*
WC and BMI	0.719	<0.001*
TRG and Age	0.26	0.025*

BMI = body mass index; WC = waist circumference; HbA1c = glycosylated haemoglobin; CHOL = total cholesterol; TRG = triglycerides; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; FBG = fasting blood glucose; systol = systolic blood pressure; *= significant p-value.

FBG, HbA1c, HOMAIR and HOMAB levels of respondents with malaria

After two years of follow–up for malaria, 50% (100 respondents) of the total enrolled respondents had malaria. These malaria-infected respondents were made up of 70 diabetics and 30 non-diabetic controls. Interestingly, comparison of anthropometric indices and blood pressure measurements at baseline and during malaria did not change significantly (P > 0.05) within and between study groups (data not shown). However, diabetics with malaria exhibited significantly (P <0.001) higher levels of FBG and HbA1c but lower (P <0.001) level of HOMAB than their non-diabetic counterparts infected with malaria (table 24). HOMAIR level was comparable (P >0.05) between the study groups.

Table 24: FBG, HbA1c, HOMAIR and HOMAB levels of respondents by study group during malaria

Parameter	Diabetic	Non-diabetic	P-value
FBG (mmol/L)	7.12 ± 0.06	5.27 ± 0.06	<0.001*
$HbA1_C$ (%)	5.67±1.03	4.63 ± 1.07	<0.001*
HOMAIR	3.58 ± 0.34	3.32 ± 0.55	0.563
HOMAB	69.74 ± 6.09	189.15 ± 21.40	<0.001*

Figures represent mean ± standard deviation; FBG = fasting blood glucose; $HbA1_c$ = glycosylated haemoglobin; HOMAIR = homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

In table 25, these parameters are compared with their baseline levels by analysis of variance test. As shown, the mean levels of all the measured parameters differed significantly ($P < 0.001$) among the four groups.

Table 25: Analysis of variance comparison of FBG, HbA1c, HOMAIR and HOMAB levels of respondents before and during malaria

	DIABETIC		**NON-DIABETIC**			
Parameter	**DM (N=70)**	**BM (N=100)**	**DM (N=30)**	**BM (N=100)**	**F_{value}**	**P-value**
FBG	7.12 ± 0.06	6.68 ± 1.46	5.27 ± 0.06	4.54 ± 1.17	35.56	<0.001*
HbA1c	5.67 ± 1.03	5.12 ± 1.03	4.63 ± 1.07	4.07 ± 1.02	16.98	<0.001*
Insulin	10.57 ± 5.27	5.79 ± 1.78	15.45 ± 8.60	5.33 ± 1.72	11.62	<0.001*
HOMAIR	3.58 ± 0.34	1.59 ± 0.19	3.32 ± 0.55	1.08 ± 0.18	8.47	<0.001*
HOMAB	69.74 ± 6.09	43.90 ± 25.30	189.15 ± 21.40	116.90 ± 21.70	6.68	<0.001*

Figures represent mean ± standard deviation; FBG = fasting blood glucose **(mmol/L)***; $HbA1_c$ = glycosylated hemoglobin* **(%)***; HOMAIR = homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; N = number of participants; DM = during malaria; BM = before malaria; * = significant p-value.*

Using Tukey's post hoc honest significant difference (HSD) test, it was observed that in the diabetic group, apart from the HOMAIR level that increased significantly ($P = 0.009$; table 26) during malaria, the levels of all the other parameters were comparable ($P > 0.05$) with their baseline levels. Indeed, the HOMAIR level in diabetics increased by over 120% during malaria compared with baseline value.

In the non-diabetic group, Tukey's post hoc HSD test revealed that the mean levels of all the measured parameters increased significantly ($P < 0.05$; table 26) during malaria compared with baseline levels with the exception of HOMAB that was comparable ($P = 0.169$; table 26) before and during malaria. The mean HOMAIR levels in non-diabetics who had malaria increased by more than 200% compared to their baseline level.

Table 26: Tukey's post hoc HSD pair wise comparison of mean FBG, HbA1c, HOMAIR and HOMAB levels of respondents with and without malaria

Parameter	Malaria	No malaria	P-value
	DIABETIC		
FBG (mmol/L)	7.12 ± 0.06	6.68 ± 1.46	0.377
HbA1c (%)	5.67 ± 1.03	5.12 ± 1.03	0.139
HOMAIR	3.58 ± 0.34	1.59 ± 0.19	0.009*
HOMAB	69.74 ± 6.09	43.90 ± 2.53	0.364
	NON-DIABETIC		
FBG (mmol/L)	5.27 ± 0.06	4.54 ± 1.17	0.02*
HbA1c (%)	4.63 ± 1.07	4.07 ± 1.02	0.032*
HOMAIR	3.32 ± 0.55	1.08 ± 0.18	0.009*
HOMAB	189.15 ± 21.40	116.90 ± 21.70	0.169

Figures represent mean ± standard deviation; FBG = fasting blood glucose; $HbA1_c$ = glycosylated haemoglobin, HOMAIR = homeostatic model assessment of insulin resistance, HOMAB = homeostatic model assessment of beta cell secretion; * = significant p-value.

Lipid profile and atherogenic indices of respondents with malaria

Apart from the CHOL/LDL and CHOL/HDL values that were significantly ($P < 0.05$) higher in diabetics than non-diabetic controls, all the other measured indices remained comparable ($P > 0.05$) between the two study groups (table 27).

ANOVA comparison of the lipid profile of respondents before and during malaria revealed that with the exception of mean LDL/HDL level that did not show significant ($P = 0.127$; table 28) difference across groups, all the other indices exhibited significant ($P < 0.05$; table 28) differences across the four groups of respondents.

Table 27: Lipid profile and atherogenic indices by study group of respondents with malaria

Lipid Index	Diabetic	Non-diabetic	P-value
CHOL	5.77 ± 0.30	5.36 ± 0.10	0.595
LDL	3.05 ± 0.31	3.26 ± 1.05	0.399
HDL	1.21 ± 0.30	1.24 ± 0.31	0.24
TRG	1.45 ± 0.11	1.22 ± 0.10	0.184
CHOL/LDL	1.96 ± 0.70	1.7 ± 0.70	0.009*
CHOL/HDL	4.9 ± 1.50	4.36 ± 1.40	0.007*
TRG/HDL	2.86 ± 0.13	2.51 ± 0.16	0.14
TRG/LDL	1.15 ± 0.60	1.02 ± 0.70	0.254
LDL/HDL	2.65 ± 1.01	2.69 ± 1.10	0.838

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL=low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG=triglycerides; CHOL=total cholesterol; * = significant p-value.

Table 28: Analysis of variance comparison of lipid profile and atherogenic indices of respondents before and during malaria

	DIABETIC		NON-DIABETIC			
Lipid Index	**DM (N=70)**	**BM (N=100)**	**DM (N=30)**	**BM (N=100)**	**F_{value}**	**P-value**
CHOL	5.77± 0.30	5.30± 1.30	5.36± 0.10	5.21± 1.20	5.21	0.002*
LDL	3.05± 0.31	3.06± 1.01	3.26± 1.05	3.36± 1.03	3.66	0.013*
HDL	1.21± 0.30	1.26± 0.11	1.24± 0.31	1.26± 0.13	3.21	0.023*
TRG	1.45± 0.11	1.12± 0.23	1.22± 0.10	1.02± 0.20	9.40	<0.001*
CHOL/LDL	1.96± 0.70	1.78± 0.61	1.70± 0.70	1.57± 0.30	6.00	0.001*
CHOL/HDL	4.9± 1.50	4.07± 1.10	4.36± 1.40	4.22± 1.01	260	<0.001*
TRG/HDL	2.86± 1.30	2.14± 1.20	2.51± 0.16	2.00± 0.90	9.57	<0.001*
TRG/LDL	1.15± 0.60	0.99± 0.60	1.02± 0.70	0.81± 0.50	5.42	0.001*
LDL/HDL	2.65± 1.01	2.65± 0.90	2.69± 1.10	2.83± 0.90	1.92	0.127

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL=low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG=triglycerides; CHOL=total cholesterol; * = significant p-value; DM = during malaria; BM = before malaria; N = number of respondents.

A Tukey's post hoc pair wise comparison of groups showed significantly ($P < 0.05$; table 29) increased mean levels of CHOL, TRG, CHOL/HDL and TRG/HDL but decreased level of HDL due to malaria in the diabetic group. The other remaining indices were comparable ($P > 0.05$; table 29) before and during malaria.

With respect to the non-diabetic group, Tukey's post hoc test revealed that malaria induced a significant ($P = 0.041$; table 30) increase only in the level of TRG with the levels of all the other indices remaining unchanged significantly ($P > 0.05$; table 30) compared with baseline levels.

Table 29: Tukey's post hoc HSD pair wise comparison of mean lipid profile and atherogenic indices of diabetic respondents with and without malaria

Lipid Index	Malaria	No Malaria	P-value
CHOL	5.77 ± 0.30	5.30 ± 1.30	0.004*
LDL	3.05 ± 0.31	3.06 ± 1.01	0.952
HDL	1.21 ± 0.30	1.26 ± 0.11	0.029*
TRG	1.45 ± 0.11	1.12 ± 0.23	0.005*
CHOL/LDL	1.96 ± 0.70	1.78 ± 0.61	0.094
CHOL/HDL	4.9 ± 1.50	4.07 ± 1.10	0.002*
TRG/HDL	2.86 ± 1.30	2.14 ± 1.20	<0.001*
TRG/LDL	1.15 ± 0.60	0.99 ± 0.60	0.525
LDL/HDL	2.65 ± 1.01	2.65 ± 0.90	0.514

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; TRG=triglycerides; CHOL=total cholesterol; * = significant p-value.

Table 30: Tukey's post hoc HSD pair wise comparison of mean lipid profile and atherogenic indices of non-diabetic respondents with and without malaria

Lipid index	Malaria	No malaria	P-value
CHOL	5.36 ± 0.10	5.21 ± 1.20	0.78
LDL	3.26 ± 1.05	3.36 ± 1.03	0.452
HDL	1.24 ± 0.31	1.26 ± 0.13	0.483
TRG	1.22 ± 0.10	1.02 ± 0.20	0.041*
CHOL/LDL	1.7 ± 0.70	1.57 ± 0.30	0.19
CHOL/HDL	4.36 ± 1.40	4.22 ± 1.01	0.621
TRG/HDL	2.51 ± 0.16	2.00 ± 0.90	0.136
TRG/LDL	1.02 ± 0.70	0.81 ± 0.50	0.34
LDL/HDL	2.69 ± 1.10	2.83 ± 0.90	0.85

Figures represent mean ± standard deviation in mmol/L except the ratios; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TRG = triglycerides; CHOL = total cholesterol, * = significant p-value.

Insulin, leptin, adiponectin, CRP, total antioxidants power and peroxide levels of respondents with malaria

Diabetics exhibited significantly ($P < 0.05$) higher levels of CRP and peroxides but lower ($P = 0.02$) level of insulin than their non-diabetic colleagues (table 31). The levels of the remaining parameters were comparable ($P > 0.05$) between the two study groups.

Table 31: Insulin, leptin, adiponectin, C-reactive protein, TAP and peroxide levels of respondents with malaria by study group

Parameter	Diabetic	Non-diabetic	P-value
Insulin (µIU/ml)	10.57 ± 0.73	15.45 ± 2.04	0.02*
Leptin (ng/ml)	71.46 ± 7.08	64.45 ± 16.17	0.2
Adiponectin (ng/ml)	10695.19 ± 522.02	10113.10 ± 582.57	0.34
C-reactive protein (ng/ml)	5945.35 ± 399.06	4684.97 ± 354.63	0.035*
TAP (µM)	46.29 ± 13.02	49.76 ± 9.67	0.869
Peroxide (µM)	24.01 ± 1.01	19.53 ± 1.96	0.032*

= significant p-value; TAP = Total Antioxidants Power

In table 32, ANOVA comparisons of mean levels of these parameters, before and during malaria showed significant ($P < 0.001$) difference across the four groups of respondents.

Table 32: Analysis of variance comparison of mean insulin, leptin, adiponectin, C-reactive protein, TAP and peroxides levels of respondents before and during malaria

	DIABETIC		**NON-DIABETIC**			
Parameter	**DM (N = 70)**	**BM (N = 100)**	**DM (N = 30)**	**BM (N = 100)**	**F_{value}**	**P-value**
Insulin (μIU/ml)	10.57 ±0.73	5.79 ±1.78	15.45 ±2.04	5.33 ±1.72	11.62	<0.001*
Leptin (ng/ml)	71.46 ±7.08	7.57 ±0.50	64.45 ±16.17	191.51 ±24.14	38.94	<0.001*
Adiponectin (ng/ml)	10695.19 ± 522.02	2444.56 ±309.03	10113.10 ± 582.57	5089.79 ± 144. 5	494.41	<0.001*
C-reactive protein (ng/ml)	5945.35 ±399.06	3746.28 ±331.47	4684.97 ± 354.63	1536.39 ± 47.40	6.08	0.001*
TAP (μM)	46.29 ±13.02	226.36 ± 26.4	49.76 ±9.67	251.71 ±67.61	47.16	<0.001*
Peroxide	24.01 ±1.01	16.82 ±1.91	19.53 ±1.96	9.91 ±1.56	14.61	<0.001*

Figures represent mean ± standard deviation; * = significant p-value; DM = During Malaria; BM = Before Malaria; TAP = Total Antioxidants Power; N = number of respondents.

Table 33: Tukey's post hoc HSD pair wise comparison of mean levels of insulin, leptin, adiponectin, CRP, TAP and peroxides of respondents with and without malaria

Parameter	Malaria	No Malaria	P-value
	DIABETIC		
Insulin (µIU/ml)	10.57 ± 0.73	5.79 ± 1.78	0.011*
Leptin (ng/ml)	71.46 ± 7.08	7.57 ± 0.5	0.002*
Adiponectin (ng/ml)	10695.19 ± 522.02	2444.56 ± 309.03	<0.001*
CRP (ng/ml)	5945.35± 399.06	3746.28 ± 331.47	0.038*
TAP (µM)	46.29 ± 13.02	226.36 ± 26.4	<0.001*
Peroxide	24.01 ± 1.01	16.82 ± 1.91	0.014*
	NON-DIABETIC		
Insulin (µIU/ml)	15.45 ± 2.04	5.33 ± 1.72	0.006*
Leptin (ng/ml)	64.45 ± 16.17	191.51 ± 24.14	<0.001*
Adiponectin (ng/ml)	10113.10 ± 582.57	5089.79 ± 144. 5	<0.001*
CRP (ng/ml)	4684.97 ± 354.63	1536.39 ± 47.40	0.032*
TAP (µM)	49.76 ± 9.67	251.71 ± 67.61	<0.001*
Peroxide (µM)	19.53 ± 1.96	9.91 ± 1.56	0.021*

Figures represent mean ± standard deviation; * = significant p-value; TAP = Total Antioxidants Power; CRP = C-reactive protein.

As expected, results of Tukey's post hoc HSD test indicated that apart from the TAP levels that decreased significantly ($P < 0.001$; table 33), the levels of all the remaining biomarkers increased significantly ($P < 0.05$) in diabetics with malaria compared with their baseline levels (table 33).

In the non-diabetic group, Tukey's HSD test detected significant ($P < 0.05$) reductions in leptin and TAP levels (table 33) with the levels of the remaining biomarkers increasing significantly ($P < 0.05$) due to malaria.

Malaria-induced associations among measured parameters

As expected, the two study groups (tables 34 and 35) differed in the pairs of parameters that exhibited linear associations due to malaria with the exception of the correlation between insulin and HOMAB or HOMAIR that was apparent in both study groups. For instance, HDL correlated negatively ($r = -0.433$; $P = 0.019$) with insulin in diabetics (table 34) compared with controls (table 35) that exhibited rather positive correlation ($P < 0.05$) between HDL and HbA1c or CHOL. As can be seen in tables 34 and 35, other varied correlations were found between various pairs of measured parameters in the two study groups during malaria.

Table 34: Observed correlations among measured parameters in diabetic respondents with malaria

Parameter	Correlation coefficient (r)	P-value
FBG and HbA1c	0.438	<0.001*
HbA1c and CHOL	0.342	0.004*
HDL and Insulin	-0.433	0.019*
CHOL/HDL and Adiponectin	0.319	0.045*
Leptin and HOMAIR	0.302	0.041*
Insulin and HOMAB	0.435	0.001*
Insulin and HOMAIR	0.901	<0.001*

HDL=high-density lipoprotein cholesterol; CHOL=total cholesterol; HbA1c = glycosylated haemoglobin; HOMAIR= homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

Table 35: Observed correlations among measured parameters in non-diabetic respondents with malaria

Parameter	Correlation coefficient (r)	P-value
FBG and Insulin	0.554	0.049*
HbA1c and HDL	0.474	0.009*
HDL and CHOL	0.435	0.018*
TRG and Leptin	0.863	0.003*
TRG and C-reactive protein	0.605	0.029*
Leptin and Adiponectin	-0.694	<0.001*
Leptin and CHOL	0.672	0.047*
Leptin and C-reactive protein	-0.53	0.004*
Insulin and HOMAB	0.683	<0.001*
Insulin and HOMAIR	0.978	<0.001*
HOMAIR and HOMAB	0.497	0.013*

HDL=high-density lipoprotein cholesterol; TRG=triglycerides, CHOL=total cholesterol; HbA1c = glycosylated haemoglobin; HOMAIR= homeostatic model assessment of insulin resistance; HOMAB = homeostatic model assessment of beta cell secretion; *= significant p-value.

CHAPTER FIVE: DISCUSSION

General characteristics of respondents

The study was a prospective cohort type involving 100 type 2 diabetics and 100 non-diabetic controls who were followed for malaria. Respondents were disproportionately distributed between the sexes with more than twice as many females as males in each study group contrary to the reported IDF-estimated T2D prevalence for Ghana, that suggest that a relatively higher proportion of males than females have the disease (IDF, 2011). This disproportionate gender distribution in the current study which has been observed in previous ones (Markanday, Brennan, Gould & Pasco, 2013; Acquah *et al*., 2011; GHS, 2011; Danquah *et al*., 2010) could be attributed to the general reluctance of males to avail themselves for medical research in general and most importantly, studies that require invasive procedures for blood sampling such as the current one. In spite of the uneven gender distribution, respondents did not differ in levels of formal education. This finding attests to the homogeneity of respondents in the current study in relation to level of formal education contrary to the disparity in levels of formal education between genders reported in the 2010 population and housing census (GSS, 2012). The seeming varied results could be attributed to differences in sample size and age of respondents in the current study compared to the census. The current study was limited to adults aged 30 years and above but census-defined adult population starts from age 18 years.

Age, Glycaemic Indicators, HOMAIR, HOMAB and Blood Pressure

Although mean age of respondents in the two study groups were comparable, stratification of data by gender revealed that diabetic males but not females were older than their non-diabetic counterpart. This male-specific variation in age, which was seen inspite of similar proportion of males in the study groups, corroborates earlier report in Kumasi (Danquah *et al.*, 2010). Age at diagnosis used as a proxy for age of onset of the diabetic condition has been inconclusively found to differ between the sexes. Whereas some workers reported lower age of onset of the condition for men (Kolb *et al.*, 2008), others made divergent observations (Hegele *et al.*, 2000; Harris *et al.*, 1997).

Interestingly, a number of studies (Nakhjavani *et al.*, 2012; Zhao *et al.*, 2012) have also found comparable age of onset of disease between the sexes just like the current study though the reported age in the current study reflects the actual age of respondents but not age of onset of the condition. These inconclusive findings can be ascribed to differences in genetic, racial, ethnic and environmental factors associated with the pathogenesis of T2D in different populations (Ali *et al.*, 2013; Lin & Sun, 2010; De Rooij *et al.*, 2009; Florez *et al.*, 2003).

Although higher levels of baseline FBG and HbA_{1c} found in diabetics compared with controls were not surprising, the observed mean levels for diabetic respondents in the current study were lower than those reported by other researchers in Nigeria (Ikekpeazu, Neboh, Maduka, Nwagbara & Nwobodo, 2010), China (Zhao *et al.*, 2012), India (Nakhjavani *et al.*, 2012) and United States of America (Bays, Goldberg, Truitt & Jones, 2008; Fonseca, Rosenstock, Wang, Truitt & Jones, 2008; Goldberg, Fonseca, Truitt & Jones,

2008). The relatively low levels of the measured baseline glycaemic indicators in the current study compared with others could be the result of differences in characteristics of study participants in terms of duration of disease, adherence to prescribed treatment regimen, analytical method employed in measuring the glycaemic parameters and other indicators. It could also be a reflection of the actual positive responsiveness of the diabetic respondents in the current study to the treatment regimen.

As expected, FBG and $HbA1_C$ levels increased in both study groups during malaria although the increase was only significant in the non-diabetic group. This observation which agrees with that of Blümer *et al.* (2005) has been ascribed to increased gluconeogenesis due to *P. falciparum* infection in non-diabetic adults. The comparable levels of FBG and $HbA1_C$ of diabetics before and during malaria may signify minimal gluconeogenesis probably due to metformin-induced inhibition of hepatic gluconeogensis acknowledged in recent times (Foretz *et al.*, 2010; Kim *et al.*, 2008) considering that metformin remains the preferred antidiabetic drug in diabetic respondents of the current sudy. However, the slight increase in FBG and $HbA1_C$ of diabetics in response to *P. falciparum* infection could point to a possible dwindling effect of metformin, suggesting probably that severe malaria could induce an appreciable increase in gluconeonesis and blood glucose level. In this regard, further studies would be needed to ascertain the role of malaria parasite load in diabetic gluconeogenesis and its overall impact on the wellbeing of the affected.

In a recent longitudinal study in adults followed for 10-14 years that aimed at assessing the association between $HbA1_C$ level and risk of CHD, Pai *et al*. (2013) reported an average risk ratio 1.67 for every 1% rise in $HbA1_C$ level in apparently healthy adults. In an earlier study, $HbA1_C$ was found to associate positively with IMT, a surrogate marker for CVD risk, in non-diabetic adults (Bobbert, Mai, Fischer-Rosinsky, Pfeiffer & Spranger, 2010). These findings suggest that the observed raised levels of FBG and $HbA1_C$ due to *P. falciparum* infection in the current study could reflect increased CVD risk in respondents. Moreover, Pearson correlational test revealed a positive association between $HbA1_C$ and total cholesterol in diabetic but HDL in non-diabetic respondents in the presence of malaria. This observation may indicate a possible variation in the risk posed by malaria in the two groups of respondents. Whereas increased risk could be indicated for the diabetic group that of the non-diabetic group would require further studies to ascertain the clinical relevance and possible threshold below which the association between HDL and HbA1c would be beneficial.

Respondents in the current study had relatively higher systolic and diastolic levels of blood pressure compared with other studies (Nakhjavani *et al*., 2012; Zhao *et al*., 2012). Interestingly, levels of blood pressure found between diabetic and control respondents were similar. Most of the diabetic respondents were documented hypertensives on appropriately prescribed antihypertensive drugs but this revelation was not apparent in the control group although the mean blood pressure values of the two study groups were at borderline. This observation suggests that the blood pressure values of the non-

diabetic controls are approaching clinically hypertensive levels, a situation that requires urgent attention if it must be avoided.

Indeed, the 2011 annual report of the Ghana Health Service acknowledged hypertension as a risk factor for non-communicable diseases in Ghana (GHS, 2011), with incidence increasing by 10-fold over a 20-year period from 1990. This increased trend in both rural and urban settings has been acknowledged in recent reviews on Ghana and other African countries (Addo *et al.*, 2012; Bosu, 2010; BeLue *et al.*, 2009). As a result, the observed near-hypertensive values, notably, for the controls in the current study may be a true reflection of awaiting future cases of hypertension. Indeed, this assertion seems to be buttressed by the observed positive correlation between systolic blood pressure and baseline LDL levels in this study group, a finding that corroborates earlier report (Oda & Kawai, 2009). This is important because both elevated LDL level and high blood pressure are established risk factors for cardiovascular mortality (King, Mainous, Matheson & Everett, 2012; Hansen *et al.*, 2011).

Insulin resistance, considered the preeminent risk factor for development of T2D (Petersen *et al*, 2012) has been linked strongly to a number of cardiovascular and metabolic aberrations (Muniyappa, Lee, Chen & Quon, 2008). It can be assessed by various methods including HOMAIR (Muniyappa *et al.*, 2008). Increased HOMAIR as a reliable predictor of cardiovascular morbidity and mortality is amply documented in diverse cross-sectional and longitudinal studies (Ausk, Boyko & Ioannou, 2010; Rutter *et al.*, 2008; Bonora *et al.*, 2007; Song *et al.*, 2007; Shinohara *et al.*, 2002). The

utility of HOMAIR in predicting morbidities in different populations varies by cut-off values (Esteghamati *et al.*, 2012; Qu, Li, Rentfro, Fisher-Hoch & McCormick, 2011; Esteghamati *et al.*, 2009; Lee *et al.*, 2006) in spite of a seemingly and reluctantly adopted value of 2.6 (Ascaso *et al.*, 2003). As anticipated, mean baseline level of HOMAIR was higher in the diabetic than the control group, in accordance with previous studies (Esteghamati *et al.*, 2012; Qu *et al.*, 2011; Esteghamati *et al.*, 2009; Lee *et al.*, 2006). However, the mean value of 1.59 recorded for the diabetic group in the current study fell below the disputed 2.6 threshold (Ascaso *et al.*, 2003) and alternative values predicted by various reports (Esteghamati *et al.*, 2012; Qu *et al.*, 2011; Esteghamati *et al.*, 2009; Buccini & Wolfthal, 2008) for different populations. Since no Ghanaian-specific threshold values are currently available, the current findings should be interpreted with caution. A cursory view of the baseline HOMAIR data may suggest a probable absence of insulin resistance in both diabetic and control groups but the significant difference in HOMAIR values between the two study groups warrant further research to ascertain the clinical relevance.

Interestingly, HOMAIR values increased considerably in *P. falciparum* infection, to levels above the disputed threshold of 2.6 (Ascaso *et al.*, 2003) in both study groups but lower than a value of 3.8 reported by Qu *et al.* (2011) for adult Hispanic population in the United States of America. Since the malaria-induced mean HOMAIR value is greater than the reluctantly but largely accepted threshold, it can be argued that conventionally, malaria caused insulin resistance in respondents of the current study. With insulin resistance being a

known risk factor for CVD, the increased HOMAIR levels could signify increased risk of respondents to CVD. Although malaria is considered as an acute infectious disease which can be treated effectively with appropriate phmarcological agent to assuage the CVD risk, the possibility of chronic infection or multiple episodes in a given individual cannot be ruled out due to the endemic nature of the infection in Ghana. With insulin resistance established as a major mechanism for development of T2D, multiple episodes of malaria attack may predispose the controls to T2D. Apart from the overt insulin resistance, persistent malaria-induced hyperglycaemia can result in beta cell apoptosis as a result of glucotoxicity (Kilpatrick & Robertson, 1998), a situation with obvious T2D as a consequence. Therefore, further studies would be needed to exemplify this possibility and identify the possible CVD risk that may be posed by asymptomatic infections as well as the parasitaemia threshold in diabetics needed to induce significant increase in FBG. This would further deepen our understanding of malaria-diabetes interaction and contribute to improved management of diabetes in malaria-endemic areas of the globe.

Indeed, beta cell secretory function assessed by HOMAB was severely impaired in diabetics compared to controls at baseline and follow-up, an observation that appears to implicate beta cell dysfunction in diabetes pathogenesis in the current study. This coexistence of beta cell dysfunction and insulin resistance was amply demonstrated in a Ghanaian populace in Accra over a decade ago (Amoah, Schuster, Gaillard & Osei, 2002). In non-diabetics with malaria, HOMAIR positively correlated with HOMAB, a finding that suggests a probable increased beta cell secretion as a compensatory measure

for the apparent reduction in insulin action on affected cells and tissues and thus increased CVD risk. The compensatory reaction of beta cells to *P. falciparum* infection could be harmful as it can increase the risk of non-diabetic respondents in the current study to T2D. Thus, with multiple episodes of *falciparum* malaria, there could be multiple compensatory insulin secretion by beta cells to a point that may overwhelm the secretory capacity of the beta cell (Donath & Halban, 2004), with a consequent development of T2D (Rhodes, 2005).

Anthropometric Indices

Three measures of anthropometry were employed in this study to ascertain the relation among them and other measured parameters. Although respondents in both the diabetic and control study groups were generally overweight or obese, the degree of general fat deposition as determined by BMI was greater for diabetics than their non-diabetic controls. Assessment of central fat desposition revealed higher but lower values for diabetics and controls in terms of WC and WHR respectively. The observed trend for BMI and WC appear to corroborate earlier findings that strongly linked higher values of these parameters of adiposity to T2D (Escobedo *et al.*, 2009; Agyemang *et al.*, 2008; Cornier *et al.*, 2008; Vazquez *et al.*, 2007; Jensen, 2006) although the specific threshold differed with population (Chiu *et al.*, 2011; Stommel & Schoenborn, 2010). In a large multiracial study to define BMI thresholds above which T2D could be diagnosed for different racial groups in Canada, Chiu *et al.* (2011) reported a value of 26 kg/m^2 for respondents of the black race. Despite the acknowledged probable differences

in characteristics between respondents in the current study and those of Chiu *et al.* (2011), the observed mean BMI for respondents in the current study appears to support the findings of the Canadian study (Chiu *et al.*, 2011). Indeed, mean BMI for the diabetic group was greater than the reported threshold with controls being just at borderline. Further stratification of data into gender rather showed that the mean BMI of the control females was closer to the threshold than their male counterpart although the difference between them was not significant. With the mean BMI of diabetic males approaching the 26 kg/m^2 threshold, that of their female counterpart fell within the obesity category (Agyemang *et al.*, 2008) as reported in a previous study (Acquah *et al.*, 2011). This female-related obesity could be ascribed to the positive perception of elderly females to weight gain in the Ghanaian society (Benkeser, Biritwum & Hill, 2012).

However, Frank *et al.* (2013) showed that measures of central obesity rather than general obesity were strongly linked to T2D in the Ghanaian population although the specific index differed with gender. They observed the strongest association between diabetes and WHR with cutoff values of 0.88 and 0.90 reported for females and males respectively (Frank *et al.*, 2013). Interestingly, the observed mean WHR in the present study seems to be at variance with the gender-specific thresholds reported by Frank *et al.* (2013). Diabetic males had lower mean WHR but the females exhibited higher mean WHR than the proposed gender-specific thresholds (Frank *et al.*, 2013). The difference between the current study and that of Frank *et al.* (2013) could be due to variations in sample size, age strata and lifestyle of respondents (Sert, Morgul & Tetiker, 2010). For instance, male control respondents had higher

WHR than their diabetic counterpart probably because the diabetics engaged more in physical activity and adhered strictly to the appropriate dietary advice in an effort to forestall aggravation of their hyperglycaemic condition. This view seems to be buttressed by the comparable WHR value recorded for female respondents in the two study groups. Interestingly, BMI rather correlated strongly and positively with WC in the current study in support of earlier reports (Katzmarzyk *et al*., 2013; Jensen, 2006; Janssen *et al*., 2004). These measures of adiposity also correlated positively with leptin, an important adipocytokine known to play a central role in adipose tissue metabolism (Lara-Castroa *et al*., 2007; Koerner *et al*., 2005). This positive association of baseline leptin level with the measures of adiposity reinforces the adepogenic role of leptin. Overall, the specific relationship between BMI and other measures of central obesity appears to be influenced by several factors which differ with different populations in varying environmental conditions. In the current study, leptin seems to drive fat deposition.

Lipid Profile and Atherogenic Indices

The higher mean baseline serum LDL cholesterol level observed for non-diabetic respondents compared with their diabetic counterpart is at variance with a number of previously published reports (Samatha, Venkateswarlu & Prabodh, 2012; Adinortey *et al*., 2011; Smith & Lall, 2008). The difference between the findings of the current study and previous reports could be attributed to differences in sample size, diabetic duration, and treatment effect. Indeed, meformin, a widely used antidiabetic drug, is known to improve lipid profile as well (Rojas & Gomes, 2013; Eleftheriadou,

Grigoropoulou, Katsilambros & Tentolouris, 2008). To this end, the analogous and normal levels of the other components of lipid profile and their ratios between the two study-groups can be attributed to treatment effect in respect of the diabetics since majority of them were on metformin.

This seeming treatment effect was maintained in malaria with respect to LDL level as the two study groups had comparable LDL levels. Also, within each study group, LDL level at baseline did not depart significantly from the level observed in *P. falciparum* infection. This observation appears contrary to previous studies in Nigeria (Nnodim & Emejulu, 2012) and India (Krishna, Chandrika, Suchetha, Manasa & Shrikant, 2009), that observed significantly increased LDL and a Korean study (Sin *et al*., 2001) that found decreased LDL level in malaria-infected patients compared with non-infected controls. Unlike the current study that involves diabetics and non-diabetics infected with *P. falciparum* malaria, previous studies either excluded diabetics (Krishna *et al*., 2009) or included malaria caused by other species of *Plasmodium* (Nnodim & Emejulu, 2012; Sin *et al*., 2001). Apart from parasite species, the differences in charateristics of respondents in the current study and previous ones could explain at least partly, the seemingly varied observations.

Triglyceride level increased appreciably in *P. falciparum* infection compared with baseline level in both study groups but malaria-induced increased CHOL and decreased HDL levels were confined to only the diabetic group. These revelations, which generally point to increased risk of CVD due to malaria in the current study, also exemplify the varied mechanisms that drive CVD risk in the two study groups. Thus, malaria appears to increase CVD risk via increased triglyceride and CHOL but decreased HDL levels in

diabetics. With respect to the non-diabetics, increased CVD risk due to malaria appears to be driven mainly by raised triglyceride level. This observation also calls for different approaches to manage the malaria-induced lipid abnormalities in the two study groups.

The comparable baseline lipid profile and lipid ratios between study groups and between genders in the present study corroborates previous reports (Acquah *et al.*, 2011; Eghan & Acheampong, 2003), except that the current mean levels are lower. This observation points to a relatively reduced baseline lipid-profile-dependent CVD risk of respondents in the present study compared with previous reports (Acquah *et al.*, 2011; Bello-Sani, Bakari & Anumah, 2007; Eghan & Acheampong, 2003). On the other hand, these findings could reflect a gradual elevation of lipid levels in sera of non-diabetic respondents due probably, to increased sedentary lifestyle (Young, 2010).

As expected, a number of lipid ratios increased in diabetics infected with *P. falciparum* compared with their non-diabetic counterpart. A similar trend was found between baseline and malaria-induced lipid ratios obtained for the diabetic but not the control group. Of the different lipid ratios, CHOL-HDL ratio, found to be the best predictor of cardiovascular event (Sert *et al.*, 2010) increased in diabetics due to *P. falciparum* infection. This finding further portrays a much increased CVD risk in diabetics due to malaria than their non-diabetic controls.

The positive correlation found between HDL and $HbA1_c$ in only malaria-infected non-diabetics requires further investigations to determine its clinical relevance. Thus, taking it on the face value, it could be argued that malaria may rather reduce the CVD risk of non-diabetics through the elevation

of HDL, probably driven by $HbA1_c$. However, one is not sure about the functional integrity of this malaria-induced HDL. Further studies will therefore be required to ascertain the functional integrity of the HDL induced by *P. falciparum* infection.

Insulin Level

Insulin is an important hormone with numerous functions in a variety of cells. To this end normal insulin level and function are necessary for homeostasis at the molecular, cellular and organismal levels. Abnormal insulin level and function are the bedrocks of a number of medical conditions (Rojas & Gomes, 2013; Young, 2010; Giovannucci, 2005; Bonora *et al*., 1998; Bonora *et al*., 1997) particularly, diabetes mellitus (Rojas & Gomes, 2013; Badman & Flier, 2007). Baseline serum insulin levels were comparable between study groups and sexes in the current study. This finding appears contrary to a number of earlier studies that reported higher levels of insulin in diabetics than their non-diabetic counterparts (Festa, Williams, Hanley & Haffner, 2008; Amoah *et al*., 2002). In fact, the mean baseline insulin levels in the present study were lower than that reported in an earlier study in Ghana (Amoah, *et al*., 2002). The difference in trend and levels of insulin could be due to discrepancies in stage of the disease of diabetics in the current work compared with previous ones (Festa *et al*., 2008; Amoah *et al*., 2002).

In T2D individuals, increased insulin level is initially observed followed by gradual decline due probably to beta cell dysfunction (Lin, Liou, Hsiao & Hwu, 2011; Badman & Flier, 2007). Hence, it is possible that diabetics in the current study had transitioned from the hyperinsulinemic stage

to the hypoinsulinemic or normoinsulinemic stage as opposed to those of Amoah *et al*. (2002) and Festa *et al* (2008) who were newly diagnosed diabetics. To this end, treatment effect could be responsible for the comparable insulin levels observed between the two study groups. This seems to be supported by the substantial decline in the secretory capacity of beta cells (HOMAB) in diabetics compared with controls in the current work although the degree of impairment differed with previous studies (Festa *et al*., 2008; Amoah *et al*., 2002). Since insulin level is dependent on the functional integrity of pancreatic beta cells, the observed severely blighted secretory function of beta cells in the diabetics should have elicited a sharp disparity in their insulin levels compared to their non-diabetic group.

Indeed, serum insulin level modestly correlated positively with beta cell secretory function only in the diabetic group, but not in the control, confirming the proposition that effect of treatment may have resulted in the analogous levels of serum insulin observed between the diabetics and their control counterpart. Thus, the moderate positive correlation points to a possible involvement of factors other than the secretory function of the beta cells as the likely culprit for the observed insulin levels.

Intriguingly, baseline insulin correlated positively with leptin in both study groups with varied strengths. This positive association with leptin, corroborates earlier report (DeCourten *et al*., 1997) suggesting that the two hormones possibly exert positive regulatory effect on each other. However, Hennige *et al*. (2006) observed a phosphorylation-dependent hampering of insulin action by leptin. Although the observed inhibition related to insulin

signaling, a similar effect could operate at the molecular level to elicit reduced insulin synthesis and release (Koh, Park & Quon, 2008). This phenomenon could explain the generally low baseline insulin levels observed in the current study. Thus, a negative feedback mechanism in which insulin possibly facilitated synthesis and release of leptin to a level that rather hampered the corresponding synthesis and release of insulin could be operational in the current study.

With *P. falciparum* infection, insulin levels rose appreciably compared with baseline levels in both study groups. The increase was much higher in non-diabetics as opposed to their diabetic counterpart. This finding, which corroborates earliear ones in Sudanese children and pregnant women infected with malaria (Elbadawi *et al.*, 2011; Eltahir *et al.*, 2010) may point to increased CVD risk in respondents. This view appears to be buttressed by the exceptionally high positive correlation found between levels of serum insulin and HOMAIR in malaria-infected respondents. With insulin resistance demonstrated to be central to development of CVD (Rask-Madsen & Kahn, 2012), the positive association between malaria-induced increased insulin level and HOMAIR could be a worry to cardiovascular health of respondents. In addition, it suggests that recurrent malaria attack could increase a person's chance of getting T2D through increased HOMAIR.

In diabetics with malaria, insulin correlated negatively with HDL, posing additional CVD risk to diabetic respondents. Thus, increased insulin secretion due to malaria possibly impaired the synthesis and release of HDL in diabetics.

Leptin Level

Another important biomolecule found to be related to the pathogenesis of T2D is leptin. Leptin, an anti-obesity molecule, is known to circulate at very low levels in lipodystrophies (Garg, 2011) but high levels in obese and T2D individuals suspected of being resistant to the molecule (Knight, Hannan, Greenberg & Friedman, 2010). Its potential as anti-obesity and anti-diabetic agent is being exploited (Coppari & Bjørbæk, 2012; Mittendorfer *et al., 2011*) for therapeutic gains. The ideal leptin level in humans differs with study, age, gender and race (Coppari & Bjørbæk, 2012; Ruhl *et al.*, 2004; Nicklas, Toth, Goldberg & Poehlman, 1997) with no consensus on possible reference levels reached so far in the scientific community.

In the present study, baseline leptin level for the control was 9-fold higher than the diabetic group and 2-5-fold higher in female than the male. Male non-diabetics recorded about 15-fold higher levels of leptin than their diabetic counterpart. Although the gender-specific variation in leptin level has been reported (Ruhl *et al.*, 2004) and ascribed to gender variation in body composition, the magnitude of the disparity in the current study suggests possible additional factors such as treatment effect. Indeed, the popular anti-diabetic drug, metformin, has long been found to reduce circulating leptin levels (Glueck *et al.*, 2001) although the extent of reduction is yet to be evaluated in the Ghanaian context and in terms of gender. To this end, the huge disparities in baseline leptin levels between diabetics and controls and between genders in the current study could be due to possible differences in gender-specific response to metformin. This view seems more plausible in view of the

comparable magnitude of some of the measures of adiposity such as WC and BMI observed between diabetic and non-diabetic males. Thus, differences in location of fat deposition alone could not be responsible for the huge disparity in levels of leptin observed.

Interestingly, other regulatory roles of leptin in terms of fat deposition and interaction with other hormones was still evident in the defined study groups as it correlated positively with BMI and insulin. This observation suggests that in spite of the reported derangement of metabolism (Lin & Sun, 2010; Escobedo *et al*., 2009; Takeuchi & Yamagishi, 2009; Badman & Flier, 2007) of several molecules in T2D, interaction between leptin and insulin remained intact in diabetics of the current study. However, the relatively high levels of baseline leptin obtained, especially, in the control group could be an indication of a possible leptin resistance although further studies would be required to fully clarify this observation.

Considering the traditional role of leptin as a satiety signal in opposing obesity development, the observed high baseline serum leptin levels in the current study, signifying possible resistance, could explain at least in part the current spate of obesity (Agyemang *et al*., 2008) and associated hypertension (Bosu, 2010) reported in the Ghanaian society. This development is quite worrying because, leptin resistance and hypertension are linked to cardiovascular morbidity and mortality (Koh *et al*., 2008; Beltowski, 2006b; Seufert, 2004).

In a number of large sample longitudinal studies (Söderberg *et al*., 2007; Wannamethee, Lowe *et al*., 2007; McNeely *et al*., 1999), high leptin

levels persistently predicted increased risk of T2D development in males but not females, a finding that appears to suggest that males in the control group of the current study could be at increased risk of developing T2D compared to their female counterpart. This relative protection of females from the negative effect of relative hyperleptinaemia needs further investigation to acertain its clinical relevance.

On the other hand, the relatively low baseline leptin level in diabetics of the current study could indicate a level below the threshold required to induce the needed antiobesity effect. This could explain the higher BMI values observed for diabetics than controls of the present study.

With *P. falciparum* malaria, leptin level in diabetics surged almost 10 times the baseline value with that of the controls declining to about 66% of baseline level. These varied responses of the two study groups with respect to leptin levels due to *falciparum* malaria may imply varied consequences for the two groups. In a number of studies in mice (Robert *et al*., 2008; Ariyasinghe *et al*., 2006), outcome of malaria was influenced greatly by nutritional factors, probably mediated by leptin. Indeed, in an experimental study in mice, malaria resulted in a 5-fold increase in serum leptin level (Pulido-Mendez, Santis & Rodriguez-Acosta, 2002).

In humans, leptin is postulated to mediate unfavourable birth weight associated with placental malaria (Walther *et al*., 2010; Kabyemela, Fried, Kurtis, Mutabingwa & Duffy, 2008), and several cardiovascular-related out-comes (Lieb *et al*., 2009; Koh *et al.,* 2008; Beltowski, 2006a). Therefore, the malaria-induced increased leptin level in diabetics of the current study may

signify increased risk of CVD but the reduced leptin level in the non-diabetics requires further studies to establish its relevance to the overall health of the affected. Nonetheless, the reduced leptin level in non-diabetic respondents could be a reflection of the negative effect of adiponectin and CRP on leptin as the two adipokines exhibited a strong negative correlation with leptin. Also, the negative association between leptin and CRP may appear to suggest that leptin exerts anti-inflammatory effect during *P. falciparum* infection, an observation that requires further studies at the molecular level for confirmation and identification of possible clinical relevance.

Adiponectin Level

A number of studies have reported association between high and low levels of adiponectin with diabetes and cardiovascular disease (Kizer *et al.*, 2012; Nakashima *et al.*, 2006) although the specific thresholds differed with study. In spite of ethnic and racial variations of adiponectin levels, blacks generally appear to have low levels of adiponectin relative to others irrespective of age (Gardener *et al.*, 2013; Degawa-Yamauchi *et al.*, 2003). In comparison with published reports (Gardener *et al.*, 2013; Nakashima *et al.*, 2006), baseline adiponectin levels were generally lower in the current study. It has long been reported that adiponectin expression levels correlate strongly and positively with circulating levels of adiponectin but negatively with TNF-α mRNA levels (Kern, Di Gregorio, Lu, Rassouli and Ranganathan, 2003), a finding that suggests an antagonistic relation between adiponectin and inflammation. As a result, the relatively low level of adiponectin in

respondents of the present study could signify generally low adiponectin mRNA levels, probably, due to the effect of inflammation.

As expected, diabetics recorded lower values than their non-diabetic counterparts with no gender-specific variation in the study groups. Although the observed trend of baseline adiponectin levels in the two study groups corroborates those of previous studies (Gardener *et al.*, 2013; Nakashima *et al.*, 2006), the lack of gender-specific variation in the current study appears to deviate from others that reported higher levels for females (Eglit, Lember, Ringmets, Rajasalu, 2013; Isobe *et al.*, 2005; Kern *et al*, 2003). This seeming deviation could be due to the relatively small number of respondents in the current study compared with previous ones.

Nonetheless, adiponectin correlated negatively with WC and CHOL/LDL ratio in male diabetics and female non-diabetic controls respect-tively. This observation exemplifies the reported anti-obesity characterisistic of adiponectin (Ong *et al.*, 2011; Kern *et al*, 2003). It also appears to show that gender variation in fat distribution greatly influenced the observed relationship between adiponectin and other measured parameters in the current study.

Interestingly, malaria caused elevation of serum adiponectin levels in both study groups. Earlier report of elevated adiponectin level in vascular wall as a response to vascular injury positions adiponectin as a probable marker for already advanced artherosclerosis (Teoh, Strauss, Szmitko & Verma, 2006). Therefore, the elevated adiponectin level in malaria-infected respondents in the current study could signify enhanced risk to CVD, especially, with a recent finding that adiponectin level associated with all cause mortality (Kizer *et al.*,

2012) in the adult. Thus, the increased serum level of adiponectin due to malaria in the current study could indicate a possible response to vascular injury caused by the *P. falciparum* parasite. In this case, the elevated serum adiponectin level could indicate increased CVD risk.

Interestingly, the negative correlation between adiponectin and leptin in non-diabetics infected with malaria shows that even in the presence of the malaria parasite, the traditional role of adiponectin as a suppressor of leptin synthesis was maintained. To this end, the elevated levels of adiponectin may have varied consequences for the two study groups. Thus, whereas elevated CVD risk can probably be predicted for the diabetic respondents, that of the control respondents would need additional studies to ascertain its significance to cardiovascular health.

C-reactive protein Level

To ascertain the extent of inflammation and its relationship with other measured parameters in respondents of the current study, CRP was measured as a marker of inflammation. As expected, the extent of baseline inflammation as determined by CRP level observed in diabetics was about twice higher than their control counterpart without any intra-group gender variation. This trend agrees with earlier reports (Stanek *et al.*, 2010; De Rooij *et al.*, 2009; Rader & Daugherty, 2008; Kusminski *et al.*, 2005). Indeed, inflammation is considered as the basis for various medical conditions (Hansson *et al.*, 2006*;* Iwalewa *et al.*, 2007).

In a series of meta-analytical reports by the Emerging Risk Factor Collaboration (ERFC) research group aimed at elucidating the role of circulat-

ing CRP level in CVD risk, the group observed that the association of CRP with cardiovascular disease may be provoked mainly by systemic inflammation (ERFC, 2010; 2012), induced probably by traditional risk factors. In one of such studies by the C-Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) group, that analysed human genetic data by the mendalian randomisation technique, no causal association was observed between circulating CRP levels and coronary heart disease (CCGC, 2011). These meta-analytical findings appear contrary to earlier prospective studies that provided clinical evidence of beneficial effect of statin, an anti-lipidaemic agent, in individuals with raised CRP levels (Ridker *et al.*, 2008; Ridker *et al.*, 2009).

To resolve this raging controversy, Boekholdt & Kastelein (2010), observed that conclusions of the meta-analytical studies failed to take into consideration the varied isoforms of biologically active CRP which could exhibit innovative thrombotic and proinflammatory features. Indeed, the currently measured circulating pentameric isoform of CRP (pCRP), can be converted to its monomeric isoform (mCRP) postulated to possess novel inflammatory properties (Ridker *et al.*, 2009). Nonetheless, the varied associations observed in the current study between CRP and FBG, components of lipid profile and WC, notably, in the control group seem to support the findings of the prospective studies (Ridker *et al.*, 2008; Ridker *et al.*, 2009) contrary to the meta-analytical studies (ERFC, 2010; CCGC, 2011; ERFC, 2012). Baseline CRP correlated positively with WC but negatively with HDL in the control group only with no such associations observed for the diabetic

group. Since WC is known to be a better predictor of health risk (Janssen *et al*., 2004) coupled with the reported protective features of HDL against cardiovascular disease (Skretteberg *et al*., 2012), their association with CRP in the control group points to a possible role of CRP in cardiovascular health. In diabetics, HDL does not exhibit anti-inflammatory properties or reverse transport cholesterol (Kastelein *et al*., 2007). These impaired HDL functions which could not even be remedied by pharmacological intervention (DeGoma, deGoma & Rader, 2008), may explain the observed high CRP level and lack of association between CRP and HDL in the diabetic arm of the current study.

As expected, CRP level in malaria increased in both study groups, in line with a number of previous reports (Agrawal, Jain & Biswas, 2013; Nahrevanian, Gholizadeh, Farahmand & Assmar, 2008). The involvement of CRP, an established marker of CVD-related inflammation, in malaria pathogenesis could explain the varied presentations of malaria in the various forms of CVD in a number of case report studies (Sunil, Diwan, Mahajan, Shilpa & Chetan, 2011; Nieman *et al*., 2009). To this end, the raised CRP level in the current study due to *falciparum* malaria may indicate increased risk to CVD. This view appears to be further supported by the strongly positive correlation between CRP and TRG in the current study. Thus, malaria appears to mediate its CVD risk increase through mechanisms that involve increased TRG and CRP, possibly corroborating earlier findings (Ridker *et al*., 2008; Ridker *et al*., 2009) that individuals with raised CRP levels benefitted from antilipidaemic therapy since the involvement of hyperlipidaemia in CVD is well documented (Kannel & Vasan, 2009).

Total Antioxidant Capacity

Various pathological conditions have been linked to oxidative stress (Stanek *et al.*, 2010; De Rooij *et al.*, 2009). Oxidative stress may be induced by several factors but its induction via inflammation as the basis of most chronic diseases in humans is well documented (Martinon, 2010; Thomson, Puntmann & Kaski, 2007). The phenomenon coexists with reduced antioxidant capacity since its impact is diminished by the action of antioxidants (Plavec *et al.*, 2008). However, the heterogeneous nature of antioxidant molecules makes it extremely difficult to measure them individually as proposed by others (Sies, 2007; Huang *et al.*, 2005). To this end, total antioxidant power also called total antioxidant capacity (TAC) believed to represent the overall antioxidant status (Suresh *et al.*, 2009) at any given moment was employed. In the current study, baseline TAC levels were comparable between diabetics and controls with no gender-specifc variation. This observation supports other published report in Iran (Taheri, *et al.*, 2012) but deviates from that of Savu *et al.* (2012) and Gowri, Biju & Saurabh (2013), who found rather higher and lower TAC levels in diabetics than controls respectively. These varied findings could reflect differences in characteristics of study participants and the heterogeneous and versatile nature of TAC. Indeed, TAC is known to be influenced by several environmental factors such as food, medication and physical activity (Rojas and Gomes, 2013; Hannley, 2012; Ristow *et al.*, 2009).

In a large sample prospective study, individuals fed on diets with relatively high TAC levels showed reduced risk of cardiovascular disease (Rautiainen *et al.*, 2012), whilst exercise was rather observed to ameliorate the

health-promoting effects of antioxidants through increased production of ROS (Ristow *et al*., 2009).

The relevance of lipid in oxidative stress reported in previous studies (Piro *et al*., 2002; Listenberger *et al*., 2001) was revealed in the current study by the correlations observed between baseline TAC levels and leptin and most of the components of lipid profile. This pattern was, however, apparent in the diabetic group only, signifying a role for oxidative stress in the evolution of T2D. Total antioxidant levels correlated negatively with triglycerides, total and LDL cholesterols in diabetic males but positively with WC in females, revealing a possible gender-specific variation in effect of baseline antioxidants in the current study. Thus, it appears that baseline total antioxidant levels counter-regulated circulating levels of triglycerides, total and LDL cholesterols in males whilst promoting central fat deposition in females. In the controls, antioxidant levels correlated negatively with FBG, corroborating the reported role of glucose (Geraldes *et al*., 2009) in oxidative stress.

Baseline total antioxidant levels seemed much more important for level of insulin observed in control males only as the two correlated strongly and positively with each other in the present study. This male-specific positive association of baseline total antioxidants with insulin level only in the control group requires further studies to postulate its clinical relevance, particularly, when hypersinsulinaemia is linked to CVD risk (Rojas & Gomes, 2013; Badman & Flier, 2007).

In the face of malaria, antioxidant power plummetted in both study groups by about 80% of their baseline figures, supporting the expectation that

malaria pathogenesis is driven at least in part by oxidative stress (Postma *et al.*, 1996). With the established protective role of antioxidants against CVD in various studies (Costa, Carvalho, Duarte, Bastos & Remião, 2013; Wang, Chun & Song, 2013), the drastic decline in total antioxidant levels during malaria in the current study may be a sign of increased susceptibility to CVD via increased oxidative stress since total antioxidant capacity/power is a reflection of the balance between total oxidation and reduction reactions.

Lipid Peroxides Level

The relevance of lipid peroxidation in the pathogenesis of several human diseases including CVD has never been doubted as exemplified by various studies (Giacco & Brownlee, 2010; Dalle-Donne, Rossi, Colombo, Giustarini & Milzani, 2006; Rumley, Woodward, Rumley, Rumley & Lowe, 2004; Walter *et al.*, 2004). The level of baseline lipid peroxides normally measured as thiobarbituric acid reactive substances (TBARS) and expressed as malonaldehyde (MDA) equivalent was higher in diabetics than non-diabetic controls in the current study. This observation which is in support of a number of previously published works (Madhikarmi, Murthy, Rajagopal & Singh, 2013; Stranges *et al.*, 2008; Turk *et al.*, 2002) reinforces the central role of oxidative stress in the overall health of an organism. In a three-year follow-up study of 634 respondents, Walter *et al.* (2004), found that serum lipid peroxides could predict cardiovascular event in patients with stable CHD independent of other risk factors. In another study involving 1,709 respondents, elevated lipid peroxide level associated with diabetes but not CHD (Stranges *et al.*, 2008). These reports, together with the

current finding, confirm the pivotal role of lipid peroxidation in T2D pathogenesis and further suggest that the consequence of serum lipid peroxide levels depends on the underlying health status of the individual.

As expected, *P. falciparum* infection resulted in a further increase in levels of lipid peroxides in both study groups, in line with the findings of Prasannachandra & D'Souza (2006). In mice studies, Henriques & Domínguez (2012) and De-Oliveira *et al*. (2010) reported elevated levels of lipid peroxides in erythrocyte membrane and liver respectively, confirming the multi-organ target approach adopted by the malaria parasite in exerting its detrimental effects on host organism.With increased lipid peroxidation, the cardiovascular health promotion role of antioxidants wanes (Costa *et al*., 2013; Wang *et al*., 2013), increasing susceptibility to CVD.Therefore, the elevated level of lipid peroxides may be an indication of increased risk to CVD due to *P. falciparum* malaria in respondents of the current study.

CHAPTER SIX: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

T2DM and malaria which affect millions of people globally have been widely acknowledged to effect their pathogenic actions through several mechanisms including inflammation, oxidative stress, reduced antioxidant activities and altered metabolism of lipid and carbohydrates. These varied mechanisms have yielded several biomarkers that have been employed in studying the pathogenic strategies adopted by either malaria or T2DM in inducing its negative impact. Interestingly, the incidence of T2DM which used to be more prevalent in developed economies is currently predicted to increase in less developed economies. Most importantly, majority of the less developed economies expected to experience the greatest burden of the imminent increased incidence of T2DM, are located in malaria-endemic regions of the globe. This makes the coexistence of malaria and T2DM in the same individual inevitable and this interaction poses new health challenges that ought to be investigated to ensure improved management of T2DM in malaria-endemic regions. The current work therefore sought to examine the CVD risk posed by *falciparum* malaria in adults with type 2 diabetes compared with their non-diabetic controls using a prospective cohort study design. Approrpiate statistical analyses of the data obtained from the various biomarkers that were measured have yielded appropriate conclusions and recommendations that may contribute to improved understanding of the interaction between *falciparum*

malaria and T2DM and hopefully lead to improved management of the dual conditions in affected individuals.

Conclusions

Diabetics had higher levels of baseline glycaemic indicators and markers of insulin resistance coupled with reduced beta cell function compared with non-diabetic controls. Diabetics had higher BMI and WC but lower WHR than controls. These differences in anthropometric measures did not influence the levels of insulin, total antioxidants and most of the components of lipid profile of respondents in the current study. A strong positive correlation was obtained among the various measures of anthropometry, notably, BMI and WC.

At baseline, a higher degree of inflammation coupled with lower levels of leptin and adiponectin were observed in diabetics than controls. The observed interrelationships among the different measured parameters exhibited gender-specific variations. The levels of the various biomolecules measured and their associations observed in the current study differed from those reported in literature. Generally, high but low respective baseline levels of leptin and adiponectin were observed in the current study.

The current study has demonstrated extensively that although *falciparum* malaria elevated the overall CVD risk of diabetics and non-diabetic controls, the mechanisms differed between the two study groups.

In diabetics, *P. falciparum* infection increased CVD risk through elevation of CHOL, TRG, HOMAIR, insulin, leptin, adiponectin, CRP and peroxide levels but decreased levels of HDL and total antioxidant power.

With respect to the non-diabetic group, elevated CVD risk was exerted by malaria via increased levels of FBG, HbA1c, TRG, HOMAIR, insulin, adiponectin, CRP and peroxide levels but decreased levels of leptin and total antioxidant power. The potential CVD risk posed by HbA1c in this study group may be attenuated by its positive association with HDL.

Malaria-induced levels of the measured biomarkers did not associate with anthropometric indices.

The specific biomarkers and degree of change in levels at baseline and during *P. falciparum* infection differed between the two study groups. This suggests differences in response and requirements for possible varied managerial approaches in dealing with the malaria-induced CVD risk in the two study groups.

Recommedations

Based on the above findings, the following recommendations are made:

1. A further study involving diabetics and controls from different regions be conducted to determine the extent to which the current findings can be replicated nationwide to obtain more evidence which could be applied to formulate appropriate policy on the management and prevention of CVD risk posed by *falciparum* malaria. In this recommended study, the health benefits of the association between HbA1c and HDL as well as that between CRP and leptin ought to be assessed in non-diabetic respondents. The possible effect of recurrent episodes of symptomatic and asymptomatic malaria on the overall

cardiovascular health of Ghanaians should also be considered in the proposed larger study. Above all, molecular evidence of the relatively low adiponectin but high leptin levels can be sought for in the proposed study.

2. Meanwhile, diabetics who get infected with malaria ought to have their insulin, HDL, triglycerides and total cholesterol levels monitored even as the malaria is treated. Antioxidant supplements, if possible, could also be incorporated in the treatment regimen. For non-diabetics, triglyceride levels need to be monitored, if the person is already on lipid-lowering drug. Also, antioxidants supplements could be incorporated in the treatment regimen.

REFERENCES

Abate, N. & Chandalia, M. (2003). The impact of ethnicity on type 2 diabetes. *Journal of Diabetes and its Complications, 17*, 39–58.

Abel, E. D., Litwin, S. E. & Sweeney G. (2008). Cardiac remodeling in obesity. *Physiological Reviews, 88*, 389–419.

Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B., Boss, O., Hadro, E., …, Kahn, B. B. (2001). Adipose -selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature, 409*, 729–733.

Acquah, S., Boampong, J. N., Adusu, J., Achampong, E. K., Setorglo, J. & Obiri-Yeboah, D. (2011). Lipid and lipoprotein levels in type 2 diabetes patients attending the Central Regional Hospital in the Cape Coast Metropolis of Ghana. *International Journal of Health Research, 4*(2), 75-82.

Adams, J. H. (1989). Cerebral infarction - its pathogenesis and interpretation. *Journal of Pathology, 157*, 281-282.

Addo, J., Agyemang, C., Smeeth, L., de-Graft, A. A., Edusei, A. K. & Ogedegbe, O. (2012). A review of population-based studies on hypertension in Ghana. *Ghana Medical Journal, 46*(2), S4-S11.

Adinortey, M. B., Gyan, B. E., Adjimani, J., Nyarko, P., Sarpong, C., Tsikata, F. Y. *et al.* (2011). Dyslipidaemia associated with type 2 diabetics with micro and macrovascular complications among Ghanaians. *Indian Journal of Clinical Biochemistry, 26*(3), 261–268.

Agrawal, V., Jain, V. & Biswas, S. (2013). Evaluation of C-reactive protein as a biochemical marker for assessing disease severity in malaria. *IOSR Journal of Dental and Medical Sciences*, *8*(2), 23-26.

Agyemang, C., Owusu-Dabo, E., de Jonge, A., Martins, D., Ogedegbe, G. & Stronks, K. (2008). Overweight and obesity among Ghanaian residents in The Netherlands, how do they weigh against their urban and rural counterparts in Ghana? *Public Health Nutrition, 12*(7), 909–916.

Ahima, R. S. (2006). Adipose tissue as an endocrine organ. *Obesity* (*Silver Spring*) *14*(Suppl 5), 242S–249S.

Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K. & Edlund, H. (1998). Beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes and Development, 12*, 1763–1768.

Ajala, O., English, P. & Pinkney, J. (2013). Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. *American Journal of Clinical Nutrition, 97,* 505–516.

Akazili, J. (2002). Costs to households of seeking malaria care in the Kassena-Nankana District of Northern Ghana. In 3rd MIM Pan-African

Conference on Malaria, Arusha, Tanzania, 17-22, November 2002. Bethesda, MD, Multilateral Initiative on Malaria.

Ali, S., Chopra, R., Manvati, S., Singh, Y. P., Kaul, N., Behura, A., *et al*. (2013). Replication of type 2 diabetes candidate genes variations in three geographically unrelated Indian population groups. *PLoS One*. 8 (3), e58881. doi:10.1371/journal.pone.0058881.

Alkhouri, N., Gornicka, A., Berk, M. P., Thapaliya, S., Dixon, L. J., Kashyap, S., *et al*. (2010). Adipocyte apoptosis, a link between obesity, insulin resistance and hepatic steatosis. *Journal of Biological Chemistry, 285*(5), 3428–3438.

Allen, R. J. & Kirk, K. (2004). The membrane potential of the intraerythrocytic malaria parasite *Plasmodium falciparum*. *Journal of Biological Chemistry, 279*, 11264-11272.

Al-Omar, I.A., Eligail, A.M., Al-Ashban, R.M. & Shah, A.H. (2010). Effect of falciparum malaria infection on blood cholesterol and platelets. *Journal of Saudi Chemical Society, 14*, 83–89.

Aly, A. S. I., Vaughan, A. M., & Kappe, S. H. I. (2009). Malaria parasite development in the mosquito and infection of the mammalian host. *Annual Review of Microbiology, 63*, 195–221.

American Diabetes Association (ADA) (2001). Gestational diabetes mellitus. *Diabetes Care, 24* (suppl 1), S77-S79.

Amoah, A. G. B., Schuster, D. P., Gaillard, T. & Osei, K. (2002). Insulin resistance, beta cell function and cardiovascular risk factors in

Ghanaians with varying degrees of glucose tolerance. *Ethnicity and Disease, 12*(suppl3), S3-10–S3-17.

Angulo, I. & Fresno, M. (2002). Cytokines in the pathogenesis of and protect-tion against malaria. *Clinical and Diagnostic Laboratory Immunology, 9*(6), 1145-1152.

Annan, D., Sanquer, S., Sébille, V., Faye, A., Djuranovic, D., Raphaël, J. C., *et al*. (2000). Compartmentalised inducible nitric oxide synthase activity in septic shock. *Lancet, 355*, 1143–1148.

Annema, W., Nijstad, N., Tölle, M., de Boer, J. F., Buijs, R. V. C., Heeringa, P., *et al.* (2010). Myeloperoxidase and serum amyloid A contribute to impaired in vivo reverse cholesterol transport during the acute phase response but not group IIA secretory phospholipase A2. *Journal of Lipid Research, 51,* 743–754.

Anstey, N. M., Weinberg, J. B., Hassanali, M. Y., Mwaikambo, E. D., Manyenga, D., Misukonis, M. A., *et al*. (1996). Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *Journal of Experimental Medicine, 184*, 557–567.

Araki, E., Lipes, M. A., Patti, M. E., Bruning, J. C., Haag, B., Johnson, R. S. *et al*. (1994). Alternative pathway of insulin signaling in mice with targeted disruption of the IRS-1 gene. *Nature, 372*, 186–190.

Ariyasinghe, A., Morshed, S. R. M., Mannoor, M. K., Bakir, H. Y., Kawamura, H., Miyaji, *et al*. (2006). Protection against malaria due to innate

immunity enhanced by low-protein diet. *Journal of Parasitology*, 92,531-538.

Asante, A. & Asenso-Okyere, K. (2003). Economic Burden of Malaria in Ghana. A Technical Report Submitted to the World Health Organisation (WHO), African Regional Office (AFRO).

Ascaso, J. F., Pardo, S., Real, J. T., Lorente, R. I., Priego, A. & Carmena, R. (2003). Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care, 26*, 3320–3325.

Ausk, K. J., Boyko, E. J. & Ioannou, G. N. (2010). Insulin resistance predicts mortality in nondiabetic individuals in the U.S. *Diabetes Care, 33*, 1179-1185.

Badin, P-M., Louche, K., Mairal, A., Liebisch, G., Schmitz G., Rustan, A. C., *et al*. (2011). Altered skeletal muscle lipase expression and activity contribute to insulin resistance in humans. *Diabetes, 60*, 1734–1742.

Badman, M. K. & Flier, J. S. (2007). The adipocyte as an active participant in energy balance and metabolism. *Gastroenterology, 132*, 2103–2115.

Banks, W. A. (2004). The many lives of leptin. *Peptides,* 25, 331–338.

Bastaki, S. (2005). Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism, 13*, 111-134.

Bauche, I. B., Ait, E., Mkadem S., Rezsohazy R., Funahashi T., Maeda N., *et al*. (2006). Adiponectin downregulates its own production and the expression of its AdipoR2 receptor in transgenic mice. *Biochemical and Biophysical Research Communication*, *345*(4), 1414-1424.

Baynes, J. W. & Thorpe, S. R. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes, 48*, 1–9.

Bays, H. E., Goldberg, R. B., Truitt, K. E. & Jones, M. R. (2008). Colesevelam hydrochloride therapy in patients with type 2 diabetes mellitus treated with metformin: glucose and lipid effects. *Archives of Internal Medicine, 168*, 1975–1983.

Bays, H., Mandarino, L. & DeFronzo, R. A. (2004). Role of the adipocyte, free fatty acids and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *Journal of Clinical Endocrinology and Metabolism, 89*, 463–478.

Beier, K., Volkl, A. & Fahimi, H. D. (1997). TNF-alpha downregulates the peroxisome proliferator activated receptor-alpha and the mRNAs encoding peroxisomal proteins in rat liver. *FEBS Letters, 412*, 385–387

Beigel, H., Farrar, H., Han, A. M., Hayden, F. G., Hyer, R., de Jong, M. D., *et al*. (2005). Current concepts – Avian influenza A (H5N1) infection in humans. *New England Journal of Medicine, 353*, 1374-1385.

Bello-Sani, F., Bakari, A. G. & Anumah, F. E. (2007). Dyslipidaemia in persons with type 2 diabetes mellitus in Kaduna, Nigeria. *International Journal of Diabetes and Metabolism, 15*, 9-13.

Beltowski, J. (2006a). Leptin and atherosclerosis. *Atherosclerosis, 189*, 47–60.

Beltowski, J. (2006b). Role of leptin in blood pressure regulation and arterial hypertension. *Journal of Hypertension, 24*, 789–801.

BeLue, R., Okoror, T. A., Iwelunmor, J., Taylor, K. D., Degboe1, A. N., Agyemang, C., *et al*. (2009). An overview of cardiovascular risk factor burden in sub-Saharan African countries, a socio-cultural perspective. *Globalization and Health, 5*, 10. doi:10.1186/1744-8603-5- 10

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

Berg, A. H. & Scherer, P. E. (2005). Adipose tissue, inflammation and cardiovascular disease. *Circulation Research, 96*, 939–949.

Bergman, R. N., Finegood, D. T. & Kahn, S. E. (2002). The evolution of β-cell dysfunction and insulin resistance in type 2 diabetes. *European Journal of Clinical Investigation, 32*, 35–45.

Besler, H. & Comoglu, S. (2003). Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerasis. *Nutritional Neuroscience, 6*(3), 189–196.

Bjorbaek, C. & Kahn, B. B. (2004). Leptin signaling in the central nervous system and the periphery. *Recent Progress in Hormone Research*, *59*, 305-331.

Bjornholm, M. & Zierath, J. R. (2005). Insulin signal transduction in human skeletal muscle, identifying the defects in type II diabetes. *Biochemical Society Transactions, 33*, 354–357.

Blake, G. J. & Ridkler, P. M. (2003). C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *Journal of the American College Cardiology*, *41(*4 Suppl S),37S-42S.

Bloomgarden, Z. T. (1996). The etiology of type II diabetes, obesity and the treatment of type II diabetes. *Diabetes Care, 19*, 1311-1315.

Blümer, R. M.E., van Thien, H., Ruiter, A. F.C., Weverling, G. J., Thuan, D. V., Endert, E., *et al*. (2005). Adiponectin and glucose production in patients infected with *Plasmodium falciparum. Metabolism*, *54*, 60-66.

Bobbert, T., Mai, K., Fischer-Rosinsky, A., Pfeiffer, A. F.H & Spranger, J. (2010). A_{1C} is associated with intima-media thickness in individuals with normal glucose tolerance. *Diabetes Care, 33*, 203–204.

Boekholdt, S. M. & Kastelein, J. J. (2010). C-reactive protein and cardiovascular risk: more fuel to the fire. *Lancet, 375,* 95–96.

Bonora, E., Willeit, J., Kiechl, S., Oberhollenzer, F., Egger, G., Bonadonna, R., *et al*. (1997). Relationship between insulin and carotid

atherosclerosis in the general population, the Bruneck study. *Stroke, 28*, 1147-1152.

Bonora, E., Willeit, J., Kiechl S., Oberhollenzer, F., Egger, G., Bonadonna, R., *et al*. (1998). U-shaped and j-shaped relationships between serum insulin and coronary heart disease in the general population, the Bruneck study. *Diabetes Care, 21*(2), 221-230.

Bonora, E., Kiechl, S., Willeit, J., Oberhollenzer, F., Egger, G., Meigs, J. B., *et al*. (2007). Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in Caucasian subjects from the general population: the Bruneck study. *Diabetes Care, 30*, 318-324.

Bonner-Weir, S. (2001). β-cell turnover: its assessment and implications. *Diabetes, 50*, S20–S24.

Bösenberg, L. H., & van Zyl, D. G. (2008). The mechanism of action of oral antidiabetic drugs: a review of recent literature. *Journal of Endocrinology, Metabolism and Diabetes of South Africa, 13*(3), 80-87.

Bosu, W. K. (2010). Epidemic of hypertension in Ghana, a systematic review. *BMC Public Health, 10*, 418. doi:10.1186/1471-2458-10-418.

Breman, J. G., Alilio, M. S. & Mills, A. (2004). Conquering the intolerable burden of malaria: what's new, what's needed, a summary. *American Journal of Tropical Medicine and Hygiene, 71*, 1–15.

Brennan, A. M. & Mantzoros, C. S. (2006). Drug insight, the role of leptin in human physiology and pathophysiology – emerging clinical applications. *Nature Clinical Practice Endocrinology and Metabolism, 2*(6), 318–327.

Bretherton-Watt, D., Gore, N. & Boam, D. S. (1996). Insulin upstream factor 1 and a novel ubiquitous factor bind to the human islet amyloid polypeptide/amylin gene promoter. *Biochemical Journal, 313*, 495–502.

Briganti, S. & Picardo, M. (2003). Antioxidant activity, lipid peroxidation and skin diseases: what's new. *Journal of the European Academy of Dermatology and Venereology, 17*, 663–669.

Brorsson, C., Hansen, N. T., Lage, K., Bergholdt R., Brunak S., Pociot F. & Type 1 Diabetes Genetics Consortium (2009). Identification of T1D susceptibility genes within the MHC region by combining protein interaction networks and SNP genotyping data. *Diabetes, Obesity and Metabolism, 11*(Suppl 1), 60–66.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature, 414*, 813-820.

Brownlee, M. (2005). The pathobiology of diabetic complications, a unifying mechanism. *Diabetes, 54*, 1615–1625.

Bruun, J. M., Pedersen, S. B., Kristensen, K. & Richelsen, B. (2002). Effects of pro-inflammatory cytokines and chemokines on leptin production in human adipose tissue in vitro. *Molecular and Cellular Endocrinology*, *190*(1-2), 91-99.

Buccini, G. S. & Wolfthal, D. L. (2008). Valores de corte para índices de insulinorresistencia, insulinosensibilidad e insulinosecreción derivados de la fórmula HOMA y del programa HOMA2. Interpretación de los datos. *Rev Argent Endocrinology and Metabolism*, 45(1), 3-21.

Buchwald, P. (2011). A local glucose- and oxygen concentration-based insulin secretion model for pancreatic islets. *Theoretical Biology and Medical Modelling, 8*, 20. doi:10.1186/1742-4682-8-20.

Cain, B. S., Meldrum, D. R., Harken, A. H. & Mcintyre, R. C. (1998). The physiologic basis for anticytokine clinical trials in the treatment of sep-sis. *Journal of the American College of Surgeons, 186*, 337-350.

Campbell, F., Dickinson, H. O., Critchley, J. A., Ford, G. A. & Bradburn, M. (2012). A systematic review of fish-oil supplements for the prevention and treatment of hypertension. *European Journal of Preventive Cardiology, 20*(1), 107-120.

Cao, G. & Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry, 44*(6), 1309–1315.

Casteilla, L., Penicaud, L., Cousin, B. & Calise, D. (2001). Choosing an adi-pose tissue depot for sampling: factors in selection and depot specificity. *Methods in Molecular Biology, 155*, 1–19.

Carty, M. D., Lillquist, J. S., Peshavaria, M., Stein, R. & Soeller, W. C. (1997). Identification of cis- and trans-active factors regulating human islet

amyloid polypeptide gene expression in pancreatic beta-cells. *Journal of Biological Chemistry, 272*, 11986–11993.

Cherrington, A. D. (1999). Control of glucose uptake and release by the liver *in vivo*. *Diabetes, 48*, 1198–214.

Cinti, S., Mitchell, G., Barbatelli G., Murano I., Ceresi E., Faloia E., *et al*. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research,46*(11), 2347–2355.

Clark, I. A., Alleva, L. M., Mills, A. C. & Cowden, W. B. (2004). Pathogenesis of malaria and clinically similar conditions. *Clinical Microbiology Review, 17*(3), 509-539.

Clark, I. A., Awburn, M. M., Whitten, R. O., Harper, C. G., Liomba, N. G., Molyneux, M. E., *et al*. (2003). Tissue distribution of migration inhibitory factor and inducible nitric oxide synthase in falciparum malaria and sepsis in African children. *Malaria Journal, 2*, 6. doi:10.1186/1475-2875-2-6.

Clark, I. A., Budd, A. C., Alleva, L. M. & Cowden, W. B. (2006). Human malarial disease: a consequence of inflammatory cytokine release. *Malaria Journal, 5*, 85. doi:10.1186/1475-2875-5-85.

Clayton, P. T., Eaton, S., Aynsley-Green, A., Edginton, E., Hussain, K., Krywawych, S., *et al*. (2001). Hyperinsulinism in short-chain L-3-hydroxyacyl- CoA dehydrogenase deficiency reveals the importance

of bioxidation in insulin secretion. *Journal of Clinical Investigation, 108*(3), 457–465.

Chiu, M., Austin, P. C., Manuel, D. G., Shah, B. R & Tu, J. V. (2011). Deriv ing ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care, 34*, 1741–1748.

Coban, C., Ishii, K. J., Kawai, T., Hemmi, H., Sato, S., Uematsu, S., *et al.* (2005). Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *Journal of Experimental Medicine, 201*, 19–25.

Conroy, A. L, Lafferty, E. I., Lovegrove, F. E., Krudsood, S., Tangpukdee, N., Liles, W. C., *et al*. (2009). Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (noncerebral) malaria from uncomplicated malaria. *Malaria Journal, 8*, 295. doi:10.1186/1475-2875-8-295

Conroy, A. L, Phiri, H., Hawkes, M., Glover, S., Mallewa, M., Seydel, K. B., *et al*. (2010). Endothelium-based biomarkers are associated with cerebral malaria in Malawian children, a retrospective case–control study. *PLoS One 5*, e15291. doi:10.1371/journal.pone.0015291

Considine, R. V., Premkumar, A., Reynolds, J. C., Sebring, N. G., Ricks, M. & Sumner A. E. (2008). Adiponectin and leptin in African Americans. *Obesity (Silver Spring) 16*, 428–434.

Coppari, R. & Bjørbæk, C. (2012). Leptin revisited: its mechanism of action and potential for treating diabetes. *Nature Reviews Drug Discovery*, *11*, 692-708.

Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C., Steig, A. J., Stob, N. R., *et al*. (2008). The metabolic syndrome. *Endocrine Review, 29*, 777-822.

Costa, V. M., Carvalho, F. Duarte, J. A., Bastos, M. L. & Remião, F. (2013). The heart as a target for xenobiotic toxicity: the cardiac susceptibility to oxidative stress. *Chemical Research in Toxicology*, *26*(9), 1285-1311.

C-Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) (2011). Association between C reactive protein and coronary heart disease, Mendelian randomisation analysis based on individual participant data. *British Medical Journal*, *342*, d548. doi:10.1136/bmj.d548.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. & Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clinical Chemistry*, *52*(4), 601-623.

Danquah, I., Bedu-Addo, G. & Mockenhaupt, F. P. (2010). Type 2 diabetes mellitus and increased risk for malaria infection. *Emerging Infectious Diseases 16*(10):1601-1604.

Das, B. S., Thurnham, D. I. & Das, D. B. (1996). Plasma α-tocopherol, retinol and carotenoids in children with falciparum malaria. *American Journal of Clinical Nutrition,* 64, 94-100.

Day, N. P., Hien, T. T., Schollaardt, T., Loc, P. P., Chuong, L. V., Chau, T.T., *et al*. (1999). The prognostic and pathophysiologic role of pro and antiinflammatory cytokines in severe malaria. *Journal of Infectious Diseases, 180*, 1288– 1297.

Deanfield, J. E., Halcox, J. P. & Rabelink, T. J. (2007). Endothelial function and dysfunction: testing and clinical relevance. *Circulation, 115*, 1285–1295.

DeCourten, M., Zimmet, P., Hodge, A., Collins, V., Nicolson, M., Staten, M., *et al*. (1997). Hyperleptinaemia, the missing link in the metabolic syndrome? *Diabetic Medicine, 14*, 200 –208.

DeFerranti, S. D. & Osganian, S. K. (2007). Epidemiology of paediatric metabolic syndrome and type 2 diabetes mellitus. *Diabetes and Vascular Disease Research, 4*, 285-296.

DeFronzo, R. A. (2004). Pathogenesis of type 2 diabetes mellitus. *Medical Clinics of North America, 88*, 787–835.

DeFronzo, R. A. (1997). Pathogenesis of type 2 diabetes mellitus, metabolic and molecular implications for identifying diabetes genes. *Diabetes,* 5, 177–269.

DeGoma, E. M., deGoma, R. I., & Rader, D. J. (2008). Beyond high-density lipoprotein levels evaluating high-density lipoprotein function as in-fluenced by novel therapeutic approaches. *Journal of the American College of Cardiology, 51*, 2199-2211.

Degawa-Yamauchi, M., Dilts, J. R., Bovenkerk,, J. E., Saha C., Pratt, J. H. & Considine, R. V. (2003). Lower serum adiponectin levels in African-American boys. *Obesity Research, 11*(11), 1384-1390.

DeLany, J. (2008). Leptin hormone and other biochemical influences on systemic inflammation *Journal of Bodywork and Movement Therapies, 12*, 121–132.

DeMast, Q., Groot, E., Lenting, P. J., de Groot, P. G., McCall, M., Sauerwein, R. W., *et al*. (2007). Thrombocytopenia and release of activated von Willebr and Factor during early *Plasmodium falciparum* malaria. *Journal of Infectious Diseases, 196*, 622–628.

De Rooij, S. R., Nijpels, G., Nilsson, P. M., Nolan, J. J., Gabriel, R., Bobbioni-Harsch, E., *et al*. (2009). Low-grade chronic inflammation in the relationship between insulin sensitivity and cardiovascular disease (RISC) population, associations with insulin resistance and cardiometabolic risk profile. *Diabetes Care, 32*(7), 1295–1301.

De-Oliveira, A. C. A., Carvalho, R. S., Paixão, F. H. M., Tavares, H. S., Gueiros, L. S, Siqueira, C. M., *et al*. (2010). Up- and down-modulation of liver cytochrome P450 activities and associated events in two murine malaria models. *Malaria Journal*, *9*, 81. doi:10.1186/1475-2875-9-81.

Desruisseaux, M. S., Nagajyothi, F., Trujillo, M. E., Tanowitz, H. B. & Scherer, P. E. (2007). Adipocyte, adipose tissue and infectious disease. *Infection and Immunity, 75*, 1066–1078.

Donath, M. Y., Gross, D. J., Cerasi, E., Kaiser, N. (1999). Hyperglycemia-induced β-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes, 48*, 738–744.

Donath, M. Y. & Halban, P. A. (2004). Decreased beta-cell mass in diabetes, significance, mechanisms and therapeutic implications. *Diabetologia, 47*, 581–589.

Donath M. Y., Storling J., Maedler K. & Mandrup-Poulsen T. (2003). Inflammatory mediators and islet beta-cell failure, a link between type 1 and type 2 diabetes. *Journal of Molecular Medicine, 81*, 455–470.

Dong, X., Park, S., Lin, X., Copps, K., Yi, X. & White, M. F. (2006). Irs1and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. *Journal of Clinical Investigation, 116*, 101–114.

Duggan, C., Irwin, M. L., Xiao, L., Henderson, K. D., Smith, A. W., Baumgartner, R. N., *et al*. (2010). Associations of insulin resistance and adiponectinwith mortality in women with breast cancer. *Journal of Clinical Oncology, 29*, 32-39.

Eghan Jr., B. A. & Acheampong, J. W. (2003). Dyslipidemia in outpatients at General Hospital in Kumasi, Ghana, cross-sectional study. *Croatian Medical Journal, 44*(5), 576-578.

Eglit, T., Lember, M., Ringmets, I. & Rajasalu, T. (2013). Gender differences in serum high-molecular-weight adiponectin levels in metabolic syndrome. *European Journal of Endocrinology, 15168*(3), 385-391.

Elbadawi, N. E. E., Mohamed, M. I., Dawod, O. Y., Ali, K. E, Daoud, O. H., Ali, E. M., *et al*. (2011). Effect of quinine therapy on plasma glucose and plasma insulin levels in pregnant women infected with *Plasmodium falciparum* malaria in Gezira state. *Eastern Mediterranean Health Journal, 17*(9), 697-700.

Elbein, S. C., Hoffman, M. D., Teng, K., Leppert, M. F. & Hasstedt, S. J. (1999). A genomewide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes, 48*, 1175–1182.

Eleftheriadou, I., Grigoropoulou, P., Katsilambros, N. & Tentolouris, N. (2008). The effects of medications used for the management of diabetes and obesity on postprandial lipid metabolism. *Current Diabetes Reviews, 4*, 340–356.

Elrick, L. J. & Docherty, K. (2001). Phosphorylation-dependent nucleocytoplasmic shuttling of pancreatic duodenal homebox-1. *Diabetes, 50*, 2250-2252.

Eltahir, E. M., ElGhazali, G., A-Elgadir, T. M. E., A-Elbasit, I. E., Elbashir, M. I. & Giha, H. A. (2010). Raised plasma insulin level and homeostasis model assessment (HOMA) score in cerebral malaria: evidence for insulin resistance and marker of virulence. *Acta Biochemica Polonica, 57*(4), 513–520.

Erlich H., Valdes A. M., Noble J., Carlson J. A., Varney M., Concannon P., *et al*. (2008). HLA DR-DQ hap- lotypes and genotypes and type 1

diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes, 57*, 1084 –1092.

Escobedo, J., Schargrodsky, H., Champagne, B., Silva, H., Boissonnet, C. P., Vinueza, R., *et al*. (2009). Prevalence of the metabolic syndrome in Latin America and its association with sub-clinical carotid atherosclerosis, the CARMELA cross-sectional study. *Cardiovascular Diabetology, 8*(52). doi:10.1186/1475-2840-8-52.

Esteghamati, A., Ashraf, H., Esteghamati, A. R., Meysamie, A., Khalilzadeh, O., Nakhjavani, M., *et al*. (2009). Optimal threshold of homeo-stasis model assessment for insulin resistance in an Iranian population: the implication of metabolic syndrome to detect insulin resistance. *Diabetes Research and Clinical Practice, 84*, 279-287.

Esteghamati, A., Ashraf, H., Khalilzadeh, O., Zandieh, A., Nakhjavani, M., Rashidi, A., *et al.* (2012). Optimal cut-off of homeostasis model assessment of insulin resistance (HOMA-IR) for the diagnosis of metabolic syndrome, third national surveillance of risk factors of noncommunicablediseases in Iran (SuRFNCD-2007). *Nutrition and Metabolism, 7*, 26. doi:10.1186/1743-7075-7-26.

Evans, J. L., Goldfine, I. D., Maddux, B. A. & Grodsky, G. M. (2002). Oxida-tive stress and stress-activated signaling pathways, a unifying hypothe-sis of type 2 diabetes. *Endocrine Review, 23*(5), 599–622.

Fajans, S. S., Bell, G. I. & Polonsky, K. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New England Journal of Medicine, 345*, 971–980.

Fantuzzi, G. (2005). Adipose tissue, adipokines and inflammation. *Journal of Allergy and Clinical Immunology, 115*, 911–919.

Feghali, C. A. & Wright, T. M. (1997). Cytokines in acute and chronic inflammation. *Frontiers in Bioscience, 2*, d12-d26.

Festa, A., Williams, K., Hanley, A. J. G. & Haffner, S. M. (2008). β-cell dysfunction in subjects with impaired glucose tolerance and early type 2 diabetes, comparison of surrogate markers with first-phase insulin secretion from an intravenous glucose tolerance test. *Diabetes, 57*, 1638–1644.

Fisher, F. F., Trujillo, M. E., Hanif, W., Barnett, A. H., McTernan, P. G., Scherer, P. E., *et al.* (2005). Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. *Diabetologia, 48*(6), 1084-1087.

Florez, J. C., Hirschhorn, J. & Altshuler, D. (2003). The inherited basis of diabetes mellitus,implications for the genetic analysis of complex traits. *Annual Review of Genomics and Human Genetics, 4*, 257–291.

Fonseca, V. A., Rosenstock, J., Wang, A. C., Truitt, K. E. & Jones, M. R. (2008). Colesevelam HCl improves glycaemic control and reduces LDL cholesterol in patients with inadequately controlled type 2 diabetes on sulfonylurea-based therapy. *Diabetes Care*, *31*, 1479–1484.

Foretz, M., Hébrard, S., Leclerc, J., Zarrinpashneh, E., Soty, M., Mithieux, G., *et al.* (2010). Metformin inhibits hepatic gluconeogene sis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *Journal of Clinical Investigation, 120*(7), 2355–2369.

Ford, E. S., Mokdad, A. H. & Giles, W. H. (2003). Trends in waist circumference among U.S. adults. *Obesity Research, 11*, 1223–1231.

Francischetti, I. M. B., Seydel, K. B. & Monteiro, R. Q. (2008). Blood coagulation, inflammation and malaria. *Microcirculation, 15*(2), 81–107.

Frank, L. K., Heraclides, A., Danquah, I., Bedu-Addo, G., Mockenhaupt, F. P., & Schulze, M. B. (2013). Measures of general and central obesity and risk of type 2 diabetes in a Ghanaian population. *Tropical Medicine and International Health, 18*(2), 141–151.

Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*, 499–502.

Fritsche, L., Weigert, C., Haring, H. U. & Lehmann, R. (2008). How insulin receptor substrate proteins regulate the metabolic capacity of the liver – implications for health and disease. *Current Medicinal Chemistry, 15*, 1316–1329.

Fruhbeck, G. (2001). A heliocentric view of leptin. *Proceedings of the Nutrition Society, 60*, 301–318.

Fu, Y., Luo, N., Klein, R. L. & Garvey, W. T. (2005). Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation, potential role in autoregulation of adipocyte metabolism and adipose mass. *Journal of Lipid Research, 46*(7), 1369-1379.

Furukawa, N., Shirotani, T., Araki, E., Kaneko, K., Todaka, M., Matsumoto, K., *et al*. (1999). Possible involvement of atypical protein kinase C (PKC) in glucose-sensitive expression of the human insulin gene, DNA-binding activity and transcriptional activity of pancreatic and duodenal homeobox gene-1 (PDX-1) are enhanced via calphostin C-sensitive but not phorbol 12-myristate 13-acetate (PMA) and Go 6976-insensitive pathway. *Endocrine Journal,* 46, 43–58.

Gabrilovich, D. I. & Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nature Reviews Immunology, 9*, 162–174.

Gallup, J. L. & Sachs, J. D. (2001). The economic burden of malaria. *American Journal of Tropical Medicine and Hygiene, 64*, 85–96.

Garbarino, J. & Sturley, S. L. (2009). Saturated with fat, new perspectives on lipotoxicity. *Current Opinion in Clinical Nutrition and Metabolic Care, 12*, 110–116.

Gardener, H., Crisby, M., Sjoberg, C., Hudson, B., Goldberg, R., Mendez, A. J., *et al*. (2013). Serum adiponectin in relation to race eth nicity and vascular risk factors in the Northern Manhattan Study. *Metabolic Syndrome and Related Disorders, 11*(1), 46-55.

Garg, A. (2011). Lipodystrophies, genetic and acquired body fat disorders. *Journal of Clinical Endocrinology and Metabolism, 96*, 3313–3325.

Garg, A. & Misra, A. (2002). Hepatic steatosis, insulin resistance and adipose tissue disorders. *Journal of Clinical Endocrinology and Metabolism, 87*, 3019–3022.

Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M. & DeFronzo, R. A. (2004). Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia, 47*, 31–39.

Gaulton, K. J., Willer, C. J., Li, Y., Scott, L. J., Conneely, K. N., Jackson, A. U., *et al*. (2008).Comprehensive association study of type 2 diabetes and related quantitative traits with 222 candidate genes. *Diabetes, 57*(11), 3136-3144.

Gautier, J-F., Wilson, C., Weyer, C., Mott, D., Knowler, W. C., Cavaghan, M., *et al*. (2001). Low acute insulin secretory responses in adult offspring of people with early onset type 2 diabetes. *Diabetes, 50*, 1828–1833.

Geraldes, P., Hiraoka-Yamamoto, J., Matsumoto, M., Clermont, A., Leitges, M., Marette, A., *et al*. (2009). Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nature Medicine, 15*, 1298-1306.

Genco, R. J., Grossi, S. G., Ho, A., Nishimura, F., & Murayama, Y. (2005). A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *Journal of Periodontology,* 76(11), 2075-2084.

Ghana Health Service (GHS) (2011). *Ghana Health Service 2011 Annual Report*. Accra.

Ghana Health Service (GHS) (2010). *Ghana Health Service 2010 Annual Report*. Accra.

Ghana Statistical Service (GSS) (2012). *2010 population and housing census, summary results of final report*. Sakoa Press Limited, Accra.

Giacco, F. & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research, 107*, 1058-1070.

Gieseg, S., Duggan, S. & Gebicki J. M. (2000). Peroxidation of proteins before lipids in U937 cells exposed to peroxyl radicals. *Biochemical Journal, 350*, 215-218.

Gillespie, K. M. (2006). Type 1 diabetes, pathogenesis and prevention. *Canadian Medical Association Journal, 175*(2), 165-170.

Giovannucci, E. (2005). The role of insulin resistance and hyperinsulinemia in cancer causation. *Current Medicinal Chemistry - Immunology, Endocrine and Metabolic Agents, 5*, 53-60.

Glueck, C. J., Fontaine, R. N., Wang, P., Subbiah, M. T., Weber, K., Illig, E., *et al*. (2001). Metformin reduces weight, centripetal obesity, insulin, leptin and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index greater than 30. *Metabolism, 50*(7), 856–861.

Goldberg R. B., Fonseca V. A., Truitt K. E., Jones M. R. (2008). Efficacy and safety of colesevelam in patients with type 2 diabetes mellitus and in- adequate glycemic control receiving insulin-based therapy. *Archives of Internal Medicine, 168*, 1531–1540.

Goldin A., Beckman J. A., Schmidt A. M. & Creager M. A. (2006). Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation, 114*, 597– 605.

Goldstein, B. J. & Scalia, R. (2004). Adiponectin, a novel adipokine linking adipocytes and vascular function. *Journal of Clinical Endocrinology and Metabolism, 89*, 2563–2568.

Gowri, P., Biju, T. & Saurabh, R. J. (2013). Evaluation of total antioxidant capacity of saliva in type 2 diabetic patients with and without periodontal disease: a case-control study. *North American Journal of Medical Sciences, 5*(1): 51–57.

Gramaglia, I., Sobolewski, P., Meays, D., Contreras, R., Nolan, J. P., Frangos, J. A., *et al*. (2006). Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria. *Nature Medicine, 12*, 1417–1422.

Greenwood, B. M., Bojang, K., Whitty, C. J. & Targett, G. A. (2005). Malaria. *Lancet, 365*, 1487–1498.

Grunfeld, C., Zhao, C., Fuller, J., Pollack, A., Moser, A., Friedman, J. & Feingold, K. R. (1996). Endotoxin and cytokines induce expression of

leptin, the ob gene product, in hamsters. *Journal of Clinical Investigation, 97*, 2152–2157.

Guha, M., Kumar, S., Choubey, V., Maity P. & Bandyopadhyay, U. (2006). Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. *The FASEB Journal, 20*, E439–E449.

Guinovart, C., Navia, M. M., Tanner, M. & Alonso, P. L. (2006). Malaria, burden of disease. *Current Molecular Medicine, 6*, 137–140.

Gyan, B., Goka, B. Q., Adjei, G. O, Tetteh, J. K. A., Kusi, K. A., Aikins, A., *et al*. (2009). Cerebral malaria is associated with low levels of circulating endothelial progenitor cells in African children. *American Journal of Tropical Medicine and Hygiene, 80*(4), 541–546.

Halberg, N., Wernstedt-Asterholm, I. & Scherer, P. E. (2008). The adipocyte as an endocrine cell. *Endocrinology and Metabolism, 37*, 753–768.

Haldar K., Murphy S. C., Milner Jr. D. A. & Taylor T. E. (2007). Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. *Annual Review of Pathology Mechanisms of Disease, 2*, 217–249.

Hani, E. H., Stoffers, D. A., Chevre, J. C., Durand, E., Stanojevic, V., Dina, C., *et al*. (1999). Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. *Journal of Clinical Investigation, 104*, R41–R48.

Hannley, P. P. (2012). Back to the future, rethinking the way we eat. *American Journal of Medicine*, *125*(10), 947. doi: 10.1016/j.amjmed.2012.07.012.

Hansen, T. W., Li Y., Boggia, J., Thijs, L., Richart, T. & Staessen, J. A. (2011). Predictive role of the night time blood pressure. *Hypertension, 57*(1), 3–10.

Hansson, G. K., Robertson, A-K. L. & Soderberg-Naucler, C. (2006). Inflammation and atherosclerosis. *Annual Review of Pathology Mechanisms of Disease, 1*, 297–329.

Harding, H. P., & Ron, D. (2002). Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes, 51*, S455–S461.

Harris, S. B., Gittelsohn, J., Hanley, A., Barnie, A., Wolever, T. M., Gao, J., *et al*. (1997). The prevalence of NIDDM and asso- ciated risk factors in native Canadians. *Diabetes Care, 20*, 185-187.

Hartemink, N., Boshuizen, H. C., Nagelkerke, N. J., Jacobs, M. A. & van Houwelingen, H. C. (2006). Combining risk estimates from observational studies with different exposure cutpoints, a meta-analysis on body mass index and diabetes type 2. *American Journal of Epidemiology, 163*(11), 1042–1052.

Hegele, R. A., Cao, H., Harris, S. B., Zinman, B., Hanley, A. J. G. & Anderson, C. M. (2000).Gender, obesity, hepatic nuclear factor-1a G319S and the age-of-onset of type 2 diabetes in Canadian Oji-Cree. *International Journal of Obesity, 24*, 1062 – 1064.

Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T. & Münzel, T. (2001). Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation, 104*(22), 2673-2678.

Hennige, A. M., Stefan, N., Kapp, K., Lehmann, R., Weigert, C., Beck, A., *et al*. (2006). Leptin downregulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. *The FASEB Journal, 20*, 1206 –1208.

Henriques, J. R. R. & Domínguez, N. G. (2012). Modulation of the oxidative stress in malaria infection by clotrimazole. *Brazilian Journal of Pharmaceutical Sciences*, *48*(3), 520-528.

Heutmekers, M., Gillet, P., Maltha, J., Scheirlinck, A., Cnops, L., Bottieau, E., *et al*. (2012). Evaluation of the rapid diagnostic test CareStart pLDH Malaria (Pf-pLDH/pan-pLDH) for the diagnosis of malaria in a reference setting. *Malaria Journalournal,* 11:204. doi:10.1186/1475-2875-11-204.

Hill, J. M., Zalos, G., Halcox, J. P., Schenke, W. H., Waclawiw, M. A., Quyyumi, A. A., *et al*. (2003). Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *New England Journal of Medicine, 348*, 593–600.

Hingorani, A. D., Cross, J., Kharbanda, R. K., Mullen, M. J., Bhagat, K., Taylor, M., *et al*. (2000). Acute systemic inflammation

impairs endothelium-dependent dilatation in humans. *Circulation, 102*, 994–999.

Hoffman, S. L., Subramanian, G. M., Collins, F. H. & Venter, J. C. (2002). Plasmodium, human and anopheles genomics and malaria. *Nature, 415*, 702–709.

Holland, W. L., Bikman, B. T., Wang, L. P., Yuguang, G., Sargent, K. M., Bulchand, S., *et al*. (2011). Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *Journal of Clinical Investigation, 121*, 1858–1870.

Hopkins, H., Kambale, W., Kamya, M. R., Staedke, S. G., Dorsey, G. & Rosenthal, P. J. (2007). Comparison of HRP2 and pLDH-based rapid diagnostic tests for malaria with longitudinal followup in Kampala, Uganda. *American Journal of Tropical Medicine and Hygeine*, *76*(6), 1092–1097.

Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell, 140*, 900–917.

Hotamisligil, G. S., Shargill, N. S. & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha, direct role in obesity-linked insulin resistance. *Science, 259*, 87–91.

Howson, J. M. M., Walker, N. M., Clayton, D., Todd, J. A. & The Type 1 Diabetes Genetics Consortium (2009). Confirmation of HLA class II

independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A. *Diabetes, Obesity and Metabolism, 11*(Suppl 1), 31–45.

Hu, E. Liang, P. & Spiegelman, B. M. (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. *Journal of Biological Chemistry, 271*, 10697–10703.

Huang, D., Ou, B. & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841-1856.

Hui, H. & Perfetti, R. (2002). Pancreas duodenum homeobox-1 regulates pancreas development during embryogenesis and islet cell function in adulthood. *European Journal of Endocrinology, 146*, 129-141.

Ikekpeazu, E. J., Neboh, E. E., Maduka, I. C., Nwagbara, I. J. & Nwobodo, M. W. (2010). Type-2 diabetes mellitus and malaria parasitaemia: effect on liver function tests. *Asian Journal of Medical Sciences, 2*(5), 214-217.

International Diabetes Federation (IDF) (2012). *Diabetes at a glance, 2012 Africa. IDF diabetes atlas* (5th ed). Brussels, Belgium.

International Diabetes Federation (IDF) (2011). *Country estimates table 2011. IDF diabetes atlas* (5th ed). Brussels, Belgium.

Isobe, T., Saitoh, S., Takagi, S., Takeuchi, H., Chiba, Y., Katoh, N., *et al.* (2005). Influence of gender, age and renal function on plasma adiponectin level, the Tanno and Sobetsu study. *European Journal of Endocrinology, 153*, 15391–15398.

Itani, S. I., Ruderman, N. B., Schmieder, F. & Boden, G. (2002). Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C and IkB-b. *Diabetes, 51*, 2005–2011.

Ivo, M., Inoni, B., Meza, G., John, R. & Blaise, G. (2007). The sensitivity of the OptiMAL rapid diagnostic test to the presence of *Plasmodium falciparum* gametocytes compromises its ability to monitor treatment outcomes in an area of Papua New Guinea in which malaria is endemic. *Journal of Clinical Microbiology, 45*(2), 627–630.

Iwaki, M., Matsuda, M., Maeda, N., Funahashi, T., Matsuzawa, Y., Makishima, M., *et al*. (2003). Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes, 52*, 1655–1663.

Iwalewa, E. O., McGaw, L. J., Naidoo, V. & Eloff, J. N. (2007). Inflammation, the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology, 6*(25), 2868-2885.

Janeway, Jr. C. A. & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology, 20*, 197–216

Janssen, I., Heymsfield, S. B., Allison, D. B., Kotler, D. P. & Ross R. (2002). Body mass index and waist circumference independently contribute to the prediction of nonabdominal, abdominal subcutaneous, and visceral fat. *American Journal of Clinical Nutrition, 75*(4), 683–688.

Janssen, I., Katzmarzyk, P. T. & Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. *American Journal of Clinical Nutrition, 79*, 379–84.

Jansson, P. A., Pellmé, F., Hammarstedt, A., Sandqvist, M., Brekke, H., Caidahl, K., *et al.* (2003). A novel cellular marker of insulin resistance and early atherosclerosis in humans is related to impaired fat cell differentiation and low adiponectin. *The FASEB Journal, 17*, 1434–1440.

Javier Gella, F., Serraa J. & Generx J. (1991). Latex agglutination procedures in immunodiagnosis. *Pure and Applied Chemistry*, *63*(8), 1131-1134.

Jensen, M. D. (2006). Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model. *Obesity (Silver Spring)*, *14*(suppl), 20S–24S.

Jonas, J. C., Sharma, A., Hasenkamp, W., Ilkova, H., Patane, G., Laybutt R., *et al*. (1999). Chronic hyperglycemia triggers loss of pancreatic β-cell differentiation in an animal model of diabetes. *Journal of Biological Chemistry, 274*, 14112–14121.

Jonsson, J., Carlsson, L., Edlund, T. & Edlund, H. (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature, 371*, 606–609.

Kabyemela, E. R., Fried, M., Kurtis, J. D., Mutabingwa, T. K. & Duffy P. E. (2008). Fetal responses during placental malaria modify the risk of low birth weight. *Infection and Immunity*, *76*, 1527-1534.

Kahn, S. E. (2001). The importance of b-cell failure in the development and progression of type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism, 86*, 4047–4058.

Kajimoto, Y., Watada, H., Matsuoka, T., Kaneto, H., Fujitani, Y., Miyazaki, J., *et al*. (1997). Suppression of transcription factor PDX-1/IPF1/STF-1/IDX-1 causes no decrease in insulin mRNA in MIN6 cells. *Journal of Clinical Investigation, 100*, 1840–1846.

Kaneto, H., Nakatani, Y., Kawamori, D., Miyatsuka, T., Matsuoka, T. A., Matsuhisa, M., *et al.* (2006). Role of oxidative stress, endo- plasmic reticulum stress and c-Jun N-terminal kinase in pancreatic beta-cell dysfunction and insulin resistance. *International Journal of Biochemistry and Cell Biology, 38*, 782–793.

Kannel, W. B. & Vasan, R. S. (2009). Triglycerides as vascular risk factors: new epidemiologic insights. *Current Opinion in Cardiology*, *24*(4), 345-350.

Kastelein, J. J., van Leuven, S. I., Burgess, L., Evans, G. W., Kuivenhoven, J. A., Barter, P. J., *et al*. (2007). Effect of torceptrepid on carotid atherosclerosis in familial hypercholesterolemia. *New England Journal of Medicine, 356*, 1620–1630.

Katzmarzyk, P. T., Heymsfield, S. B. & Bouchard, C. (2013). Clinical utility of visceral adipose tissue for the identification of cardiometabolic risk in white and African American adults. *American Journal of Clinical Nutrition, 97*, 480–486.

Kengne, A. P., Amoah, A. G. B. & Mbanya, J-C. (2005). Cardiovascular complications of diabetes mellitus in Sub-Saharan Africa. *Circulation, 112*, 3592-3601.

Kelley, D. E. & Mandarino, L. J. (2000). Fuel selection in human skeletal muscle in insulin resistance, a reexamination. *Diabetes, 49*, 677–683.

Kern, P. A., Di Gregorio, G. B., Lu, T., Rassouli, N. & Ranganathan, G. (2003). Adiponectin expression from human adipose tissue, relation to obesity, insulin resistance, and tumor necrosis factor-α expression. *Diabetes, 52*(7), 1779-1785.

Khovidhunkit, W., Kim, M-S., Memon, R. A., Shigenaga, J. K., Moser, A. H., Feingold, K. R. & Grunfeld, C. (2004). Effects of infection and in flammation on lipid and lipoprotein metabolism, mechanisms and con sequences to the host. *Journal of Lipid Research, 45*, 1169–1196.

Kilpatrick, E. D. & Robertson, R. P. (1998). Differentiation between glucose-induced desensitization of insulin secretion and β-cell exhaustion in the HIT-T15 cell line. *Diabetes, 47*, 606–611.

Kim, Y. D., Park, K. G., Lee, Y. S., Park, Y. Y., Kim, D. K., Nedumaran, B., *et al*. (2008). Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. *Diabetes*, *57*(2), 306- 314.

Kima, H., Higginsa, S., Lilesa, W. C. & Kaina, K. C. (2011). Endothelial activation and dysregulation in malaria, a potential target for novel therapeutics *Current Opinion in Hematology, 18*, 177–185.

King, D. E., Mainous, A. G, Matheson, E. M. & Everett, C. J. (2012). Impact of healthy lifestyle on mortality in people with normal blood pressure, LDL cholesterol, and C-reactive protein. *European Journal of Preventive Cardiology, 20*(1), 73–79.

Kizer, J. R, Benkeser, D., Arnold, A. M., Mukamal, K. J., Ix, J. H., Zieman, S. J., *et al*. (2012). Associations of total and high-molecular- weight adiponectin with all-cause and cardiovascular mortality in older persons, the cardiovascular health study. *Circulation, 126*, 2951-2961.

Kleemann, R., van Erk, M., Verschuren, L., van den Hoek, A. M., Koek, M., Wielinga, P. Y., *et al*. (2010). Time-resolved and tissue-specific systems analysis of the pathogenesis of insulin resistance. *PLoS One, 5*(1), e8817.

Knight, Z. A., Hannan, K. S., Greenberg, M. L. & Friedman, J. M. (2010). Hyperleptinemia is required for the development of leptin resistance. *PLoS One 5*(6), e11376. doi:10.1371/journal.pone.0011376.

Koerner, A., Kratzsch, J. & Kiess, W. (2005). Adipocytokines, leptin—the classical, resistin—the controversical, adiponectin—the promising, and more to come. *Best Practice and Research Clinical Endocrinology and Metabolism, 19*(4), 525–546.

Koh, K. K., Park, S. M. & Quon, M. J. (2008). Leptin and cardiovascular disease: response to therapeutic interventions. *Circulation*, *117*, 3238-3249.

Kolb, H., Schneider, B., Heinemann, L., Heise, T., Lodwig, V., Tshiananga, J. K. T., *et al*. (2008). Type 2 diabetes phenotype and progresssion is significantly different if diagnosed before versus after 65 years of age. *Journal of Diabetes Science and Technology, 2*(1), 82-90.

Korhonen, R., Lahti, A., Kankaanranta, H. & Moilanen, E. (2005). Nitric oxide production and signaling in inflammation. *Current Drug Targets - In flammation and Allergy, 4*(4), 471-479.

Kozłowska, L., Rydzewski, A., Fiderkiewicz, B., Wasinska-Krawczyk, A., Grzechnik, A. & Rosołowska-Huszcz, D. (2010). Adiponectin, resistin and leptin response to dietary intervention in diabetic nephropathy. *Journal of Renal Nutrition, 20*(4), 255–262.

Kraegen, E. W., Clark, P. W., Jenkins, A. B., Daley, E. A., Chisholm, D. J. & Storlien, L. H. (1991). Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes, 40*, 1397–403.

Kreuels, B., Kreuzberg, C., Kobbe, R., Ayim-Akonor, M., Apiah-Thompson, P., Thompson B *et al*. (2010). Differing effects of HbS and HbC traits on uncomplicated falciparum malaria, anemia, and child growth. *Blood, 115*(22), 4551-4558.

Krishna, A. P., Chandrika, Suchetha K., Manasa A.& Shrikant L. P. (2009). Variation in common lipid parameters in malaria infected patients. *In dian Journal of Physiology and Pharmacology*, *53*(3), 271–274.

Krishnegowda, G., Hajjar, A. M, Zhu, J., Douglass, E. J., Uematsu, S., Akira, S., Woods, A. S. & Gowda, D. C. (2005). Induction of proinflammatory

responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum, cell signaling receptors, glycosylphosphati dylinositol (GPI) structural requirement, and regulation of GPI activity. *Journal of Biological Chemistry, 280*(9), 8606–8616.

Kurtzhals, J. A., Adabayeri, V., Goka, B. Q., Akanmori, B. D., Oliver-Commey, J. O., Nkrumah, F. K., *et al*. (1998). Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *Lancet,* 351, 1768–1772.

Kusminski, C. M., McTernan, P. G. & Kumar, S. (2005). Role of resistin in obesity, insulin resistance and type II diabetes. *Clinical Science, 109*, 243–256.

Lapolla, A., Piarulli, F., Sartore, G., Ceriello, A., Ragazzi, E., Reitano, R., *et al*. (2007). Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery dis ease. *Diabetes Care, 30*, 670–676.

Lara-Castroa, C., Fua Y., Chunga, B. H. & Garve, W. T. (2007). Adiponectin and the metabolic syndrome, mechanisms mediating risk for metabolic and cardiovascular disease. *Current Opinion in Lipidology, 18*, 263–270.

Largay, J. (2012). New-onset diabetes, how to tell the difference between type 1 and type 2 diabetes. *Clinical diabetes, 30*(1), 25-26.

Laughlin, G. A., Barrett-Connor, E., May S. & Langenberg, C. (2006). As sociation of adiponectin with coronary heart disease and mortality, the Rancho Bernardo study. *American Journal of Epidemiology, 165*, 164–174.

Lee, S., Choi, S., Kim, H. J., Chung, Y. S., Lee, K. W., Lee, H. C., *et al*. (2006). Cut off values of surrogate measures of insulin resistance for metabolic syndrome in Korean non-diabetic adults. *Journal of Korean Medical Science, 21*, 695-700.

Lee, Y. S, Li, P., Huh, J. Y., Hwang, I. J., Lu, M., Kim, J. I., *et al.* (2011). Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. *Diabetes, 60*, 2474–2483.

Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S. & Montminy, M. R. (1993). Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pan creatic islet cells. *Molecular Endocrinology, 7*, 1275–1283.

Lequin, R. M. (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clinical Chemistry, 51*(12), 2415–2418.

Li, J., Yu X., Pan, W. & Unger, R. H. (2002). Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. *American Journal of Physiology, Endocrinology and Metabolism 282*, E1334–1341.

Lieb, W., Sullivan, L. M., Harris, T. B., Roubenoff, R., Benjamin, E. J., Levy, D., *et al*. (2009). Plasma leptin levels and incidence of heart

failure, cardiovascular disease and total mortality in elderly individuals. *Diabetes Care*, *32*(4), 612–616.

Lin, K-H., Liou, T-L., Hsiao, L-C. & Hwu, C.-M. (2011). Clinical and bio chemical indicators of homeostasis model assessmentestimated insulin resistance in postmenopausal women. *Journal of the Chinese Medical Association, 74*, 442 – 447.

Lin, X., Taguchi, A., Park, S., Kushner, J. A., Li, F., Li, Y. & White, M. F. (2004). Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *Journal of Clinical Investigation*, *114*, 908–916.

Lin, Y. & Sun, Z. (2010). Current views on type 2 diabetes. *Journal of Endocrinology, 204*, 1–11.

Lindsay, R. S., Funahashi, T., Hanson, R. L., Matsuzawa, Y., Tanaka, S., Tataranni, P. A., *et al*. (2002). Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet, 360*, 57–58.

Listenberger, L. L., Ory, D. S. & Schaffer, J. E. (2001). Palmitate-induced apoptosis can occur through a ceramide-independent pathway. *Journal of Biological Chemistry, 276*, 14890–14895.

Liu, J., Divoux, A., Sun, J., Zhang, J., Clément, K., Glickman, J. N., *et al*. (2009). Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nature Medicine, 15*(8), 940–945.

Liuzzo, G. & Rizzello, V. (2001). C-reactive protein and primary prevention of ischemic heart disease. *Clinica Chimica Acta, 311*, 45-48.

Lopansri, B. K., Anstey, N. M., Weinberg, J. B., Stoddard, G. J, Hobbs, M. R., Levesque, M. C., *et al*. (2003). Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet, 361*, 676–678.

Lovegrove, F. E., Tangpukdee, N., Opoka, R. O., Lafferty, E. I., Rajwans, N., Hawkes, M., *et al*. (2009). Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLoS One, 4*(3), e4912.

Lowell, B. B. & Shulman, G. I. (2005). Mitochondrial dysfunction and type 2 diabetes. *Science, 307*, 384–387.

Lu, M., Seufert, J. & Habener, J. F. (1997). Pancreatic β-cell-specific repress sion of insulin gene transcription by CCAAT/Enhancer-binding protein. Inhibitory interactions with basic helix-loop-helix transcription factor E47. *Journal of Biological Chemistry, 272*, 28349–28359.

Lucas, R., Juillard, P., Decoster, E., Redard, M., Burger, D., Donati, Y., *et al*. (1997). Crucial role of tumor necrosis factor (TNF) receptor 2 and membrane-bound TNF in experimental cerebral malaria. *European Journal of Immunology,* 27, 1719–1725.

Lyke, K. E., Burges, R., Cissoko, Y., Sangare, L., Dao, M., Diarra, I., *et al*. (2004). Serum levels of the proinflammatory cytokines inter leukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor

alpha, and IL-12(p70) in Malian children with severe *Plasmodium falciparum* malaria and matched uncomplicated malaria or healthy controls. *Infection and Immunity, 72*, 5630–5637.

Macfarlane, W. M., Campbell, S. C., Elrick, L. J., Oates, V., Bermano, G., Lindley, *et al*. (2000a). Glucose regulates islet amyloid polypeptide gene transcription in a PDX-1 and calcium dependent manner. *Journal of Biological Chemistry, 275*, 15330–15335.

Macfarlane, W. M., Shepherd, R. M., Cosgrove, K. E., James, R. F., Dunne, M. J. & Docherty, K. (2000b). Glucose modulation of insulin mRNA levels is dependent on transcription factor PDX-1 and occurs independently of changes in intracellular Ca^{2+}. *Diabetes, 49*, 418–423.

Macfarlane, W. M., Smith, S. B., James, R. F., Clifton, A. D., Doza, Y. N., Cohen, P., *et al*. (1997). The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the tran scription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic beta-cells. *Journal of Biological Chemistry, 272*, 20936–20944.

Macfarlane, W. M., Read, M. L., Gilligan, M., Bujalska, I. & Docherty, K. (1994). Glucose modulates the binding activity of the beta-cell transcription factor IUF1 in a phosphorylation-dependent manner. *Biochemical Journal,* 303, 625–631.

Madhikarmi, N. L., Murthy K. R. S., Rajagopal, G., Singh, P. P. (2013). Lipid peroxidation and antioxidant status in patients with type 2 diabetes in relation to obesity in Pokhara – Nepal. *Journal of Diabetology*, *1*, 3

Maeda, K, Okubo, K, Shimomura, I., Funahashi, T., Matsuzawa, Y. & Matsubara, K. (1996). cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene tran script 1). *Biochemical and Biophysical Research Communication 221*(2), 286–289.

Maedler, K., Spinas, G. A., Dyntar, D., Moritz, W., Kaiser, N. & Donath, M. Y. (2001). Distinct effects of saturated and monounsaturated fatty acids on β-cell turnover and function. *Diabetes, 50*(1), 69–76.

Maitland, K. & Marsh, K. (2004). Pathophysiology of severe malaria in children. *Acta Tropica, 90*, 131–140.

Mandarino, L., Bonadonna, R., McGuinness O. & Wasserman, D. (2001). Regulation of muscle glucose uptake in vivo. In, Jefferson L. S. and Cherrington A. D. (Eds), *Handbook of physiology. The endocrine sys tem, vol. II - the endocrine pancreas and regulation of metabolism* (pp. 803–848). Oxford University Press, Oxford.

Mannami, T., Konishi, M., Baba, S., Nishi, N. & Terao, A. (1997). Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city, the Suita study. *Stroke, 28*, 518– 525.

Mari, A., Wahren, J., DeFronzo, R. A. & Ferrannini, E. (1994). Glucose absorption and production following oral glucose, comparison of compartmental and arteriovenous-difference methods. *Metabolism, 43*, 1419–1425.

Markanday, S., Brennan, S. L., Gould, H. & Pasco, J. A. (2013). Sex-differences in reasons for non-participation at recruitment, Geelong Os teoporosis Study. *BMC Research Notes, 6*, 104. doi,10.1186/1756-0500-6-104.

Marso, S. P. (2002). The pathogenesis of type 2 diabetes and cardiovascular disease. *British Journal of Diabetes and Vascular Disease, 2*, 350-356.

Martinon, F. (2010). Signaling by ROS drives inflammasome activation. *European Journal of Immunology*, *40*(3), 616–619.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Taylor, B. A., Treacher, D. F. & Turner, R. C. (1985). Homeostasis model assessment insulin re sistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia 28*, 412–419.

Matsui-Hirai, H., Hayashi, T., Yamamoto, S., Ina, K., Maeda, M., Kotani, H., *et al*. (2011). Dose-dependent modulatory effects of insulin on glucose-induced endothelial senescence *in vitro* and *in vivo*: a relation ship between telomeres and nitric oxide. *Journal of Pharmacology and Experimental Therapeutics, 337*(3), 591-599.

Matsuoka, T. A., Kajimoto, Y., Watada, H., Kaneto, H., Kishimoto, M., Umayahara, Y., *et al*. (1997). Glycation-dependent, reactive oxygen

species mediated suppression of the insulin gene promoter activity in HIT cells. *Journal of Clinical Investigation, 99*, 144–150.

McEligot, A. J., Yang, S. & Meyskens, J. F. L. (2005). Redox regulation by intrinsic species and extrinsic nutrients in normal and cancer cells. *Annual Review of Nutrition, 25*, 261–295.

McNeely, M. J., Boyko, E. J., Weigle, D. S., Shofer, J. B., Chessler, S. D., Leonnetti, D. L., *et al*. (1999). Association between base line plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care*, *22*, 65–70.

Medana, I. M., Hunt, N. H. & Chaudhri, G. (1997). Tumor necrosis factor-α expression in the brain during fatal murine cerebral malaria, evidence for production by microglia and astrocytes. *American Journal of Pathology, 150*, 1473–1486.

Meigs, J. B., Hu, F. B., Rifai, N. & Manson, J. E. (2004). Biomarkers of endo thelial dysfunction and risk of type 2 diabetes mellitus. *Journal of American Medical Association*, *291*, 1978–1986.

Melloul, D., Neriah, B. Y. & Cerasi, E. (1993). Glucose modulates the binding of an islet-specific factor to a conserved sequence within the rat I and the human insulin promoters. *Proceedings of the National Academy of Sciences, USA, 90*, 3865–3869.

Menon, V., Li, L., Wang, X., Greene, T., Balakrishnan, V., Madero, *et al*. (2006). Adiponectin and mortality in patients with chronic kidney

disease. *Journal of the American Society of Nephrology, 17*, 2599–2606.

Miller, L. H., Baruch, D. I., Marsh, K. & Doumbo, O. K. (2002). The pathogenic basis of malaria. *Nature, 415*(7), 673-679.

Mills, K. H. (2011). TLR-dependent T cell activation in autoimmunity. *Nature Reviews Immunology, 11*, 807–822.

Milner, D. A., Montgomerry, J., Seydel, K. B. & Rogerson, S. J. (2009). Severe malaria in children and pregnancy: an update and perspective. *Trends in Parasitology, 24*(12), 590-595.

Ministry of Local Government and Rural Development (MLGRD) (2006). Cape Coast Metropolitan demographic characteristics. Retrived from http://www.ghanadistricts.com/districts/?r=3&_=50&sa=2657

Minokoshi, Y., Kahn, C. R. & Kahn, B. B. (2003). Tissue-specific ablation of the GLUT4 glucose transporter or the insulin receptor challenges as sumptions about insulin action and glucose homeostasis. *Journal of Biological Chemistry, 278*, 33609–33612.

Mittendorfer, B., Horowitz, J. F., DePaoli, A. M., McCamish, M. A., Patterson, B. W. & Klein, S. (2011). Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes. *Diabetes, 60*, 1474–1477.

Mocumbi, P. (2004). Plague of my people. *Nature, 430*, 925.

Mohanty, D., Ghosh, K., Nandwani, S. K., Shetty, S., Phillips, C., Rizvi, S., *et al*. (1997). Fibrinolysis, inhibitors of blood coagulation, and monocyte derived coagulant activity in acute malaria. *American Journal of Hematology, 54*, 23–29.

Molarius, A. & Seidell, J. C. (1998). Selection of anthropometric indicators for classification of abdominal fatness—a critical review. *International Journal of Obesity and Related Metabolic Disorders, 22*, 719–27.

Morgan, D., Oliveira-Emilio, H. R., Keane, D., Hirata, A. E., Santos, da Rocha, M., *et al*. (2007). Glucose, palmitate and pro-inflammatory cytokines modulate production and activity of a phagocyte-like NADPH oxidase in rat pancreatic islets and a clonal β cell line. *Diabetologia, 50*, 359–369.

Mota, M. M., Pradel, G., Vanderberg, J. P., Hafalla, J. C., Frevert, U., Nussenzweig, R. S., *et al*. (2001). Migration of Plasmodium sporozoites through cells before infection. *Science, 291*, 141-144.

Muoio, D. M. & Lynis, D. G. (2002). Peripheral metabolic actions of leptin. *Best Practice and Research Clinical Endocrinology and Metabolism, 16*, 653–666.

Muniyappa, R., Lee, S., Chen, H. & Quon, M. J. (2008). Current approaches for assessing insulin sensitivity and resistance in vivo, advantages, limitations, and appropriate usage. *American Journal of Physiology, Endocrinology and Metabolism, 294*, E15–E26.

Munzberg, H. & Myers, M. G. Jr. (2005). Molecular and anatomical determinants of central leptin resistance. *Nature Neuroscience, 8*, 566-570.

Murano, I., Barbatelli, G., Parisani, V., Latini, C., Muzzonigro, G., Nagajyothi, F., *et al.* (2012). Response of adipose tissue to early infection with *Trypanosoma cruzi* (Brazil strain). *Journal of Infectious Diseases, 205*, 830-840.

Nagy, G., Ward, J., Mosser, D. D., Koncz, A., Gergely, P. Jr., Stancato, C., *et al*. (2006). Regulation of CD4 expression via recycling by HRES-1/RAB4 controls susceptibility to HIV Infection. *Journal of Biological Chemistry, 281*(45), 34574-34591.

Nahrevanian, H., Gholizadeh, J., Farahmand, M., Assmar, M. (2008). Patterns of co-association of C-reactive protein and nitric oxide in malaria in endemic areas of Iran. *Memórias do Instituto Oswaldo Cruz*, *103*, 39-44.

Naidu, M. S. K., Suryakar, A. N., Swami, S. C., Katkam, R. V. & Kumbar, K. M. (2007). Oxidative stress and antioxidant status in cervical cancer patients. *Indian Journal of Clinical Biochemistry, 22*(2), 140-144.

Naing, L., Winn, T. & Rusli, B.N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, *1*, 9-14.

Nakano, Y., Tobe, T., Choi-Miura, N. H. Mazda, T. & Tomita, M. (1996). Isolation and characterization of GBP28, a novel gelatin-binding

protein purified from human plasma. *Journal of Biochemistry (Tokyo), 120*(4), 803-812.

Nakagami, T., Qiao, Q., Carstensen, B., Nhr-Hansen, C., Hu G., Tuomilehto, J., *et al*. (2003). Age, body mass index and type 2 diabetes—associations modified by ethnicity. *Diabetologia, 46*(8), 1063-1070.

Nakashima, R., Kamei, N., Yamane, K., Nakanishi, S., Nakashima, A. & Kohno, N. (2006). Decreased total and high molecular weight adiponectin are independent risk factors for the development of type 2 diabetes in Japanese-Americans. *Journal of Clinical Endocrinology and Metabolism, 91*, 3873-3877.

Nakatani, H., Hirose, H., Yamamoto, Y., Saito, I. & Itoh, H. (2008). Significance of leptin and high–molecular weight adiponectin in the general population of Japanese male adolescents. *Metabolism, Clinical and Experimental, 57*, 157-162.

Nakhjavani, M., Morteza, A., Jenab, Y., Ghaneei, A., Esteghamati, A., Karimi, M., *et al*. (2012). Gender difference in albuminuria and ischemic heart disease in type 2 diabetes. *Clinical Medicine and Research, 10*(2), 51-56.

Newton, C. R., Taylor, T. E. & Whitten, R. O. (1998). Pathophysiology of fatal *falciparum* malaria in African children. *American Journal of Tropical Medicine and Hygiene, 58*, 673-683.

Nicklas, B. J., Toth, M. J., Goldberg, A. P., & Poehlman, E. T. (1997). Racial differences in plasma leptin concentrations in obese postmenopausal women. *Clinical Endocrinology and Metabolism 82*, 315–317.

Nieman A-E., de Mast, Q., Roestenberg, M., Wiersma J., Pop G., Stalenhoef A., *et al*. (2009). Cardiac complication after experimental human malaria infection: a case report. *Malaria Journal*, 8:277. doi: 10.1186/1475-2875-8-277.

Nnodim, J. & Emejulu, A. (2012). Lipid profile and lipid peroxidation status in type II diabetes with malaria in owerri Nigeria. *Novel Science International Journal of Medical Science 1*(2), 34-36.

Nwokochal, C. R., Ajayi, I. O. & Ebeigbe, A. B. (2011). Altered vascular reactivity induced by malaria parasites. *West Indian Medical Journal, 60*(1), 13-18.

Oakley, M. S., Gerald, N., McCutchan, T. F., Aravind, L. & Kumar, S. (2011). Clinical and molecular aspects of malaria fever. *Trends in Parasitology, 27*, 10, 442-449.

Oda, E. & Kawai, R. (2009). LDL cholesterol is associated with blood pressure in Japanese women. *Diabetes Care, 32*(9)*, e113.* doi: 10.2337/dc09-1025.

Odeh, M. (2001). The role of tumor necrosis factor-α in the pathogenensis of complicated *Falciparum* malaria. *Cytokine, 14*(1), 11-18.

Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., *et al*. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development, 122*, 983–995.

Ogino, K. & Wang, D. (2007). Biomarkers of oxidative/nitrosative stress: an approach to disease prevention. *Acta Med Okayama, 61*(4), 181-189.

Ohlsson, H., Karlsson, K. & Edlund, T. (1993). IPF1: a homeodomain-containing transactivator of the insulin gene. *EMBO Journal, 12*, 4251–4259.

O'Leary, D. H., Polak, J. F., Kronmal, R. A., Manolio, T. A., Burke, G. L., Wolfson, Jr. S. K. (1999). Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *New England Journal of Medicine*, *340*, 14–22.

Olson, L. K., Sharma, A., Peshavaria, M., Wright, C. V. E., Towle, H. C., Robertson, R. P., *et al*. (1995). Reduction of insulin gene transcription in HIT-T15 cells chronically exposed to a supraphysiologic glucose concentration is associated with loss of STF-1 transcription factor expression. *Proceedings of the National Academy of Sciences, USA, 92*, 9127–9131.

Olweny, C., Chauhan, S., Simooya, O., Bulsara, M., Njelsani, E. & van Thuc, H. (1986). Adult cerebral malaria in Zambia: preliminary report of clinical findings and treatment response. *Journal of Tropical Medicine and Hygiene, 89*, 123–129.

Ong, K. L., Tso, A. W. K., Xu, A., Law, L S. C., Li, M., Wat, N. M. S., *et al*. (2011). Evaluation of the combined use of adiponectin and C-reactive protein levels as biomarkers for predicting the deterioration in glycaemia after a median of 5.4 years. *Diabetologia*, *54*(10), 2552-2560.

Osanai, T. & Okumura, K. (2007).Therapeutic challenge to adiposity of the heart. *Circulation Research, 100*, 1106-1108.

Ostrand-Rosenberg, S. & Sinha, P. (2009). Myeloid-derived suppressor cells, linking inflammation and cancer. *Journal of Immunology, 182*, 4499–4506.

Ouchi, N., Parker, J. L., Lugus, J. J. & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology, 11*, 85–97.

Owen-Reece, H., Smith, M., Elwell, C. E. & Goldstone, J. C. (1999). Near infrared spectroscopy. *British Journal of Anaesthesia*, *8*(3), 418-426.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., *et al*. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science, 306*, 457–461.

Pai, J. K., Cahill, L.E., Hu, F. B., Rexrode K. M., Manson J. E., & Rimm E. B. (2013). Haemoglobin A1c is associated with increased risk of incident coronary heart disease among apparently healthy, nondiabetic men and women. *Journal of the American Heart Association, 2*, e000077 doi: 10.1161/JAHA.112.000077

Parroche, P., Lauw, F. N., Goutagny, N., Latz, E., Monks, B. G., Visintin, A., *et al*. (2007) Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *Proceedings of the National Academy of Sciences, USA, 104*(6), 1919–1924.

Petersen, K. F., Dufour, S., Morino, K., Yoo, P. S., Clinea, G. W. & Shulman, G. I. (2012). Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes. *Proceedings of the National Academy of Sciences, USA, 109*(21), 8236–8240.

Petersen, H. V., Serup, P., Leonard, J., Michelsen, B. K. & Madsen, O. D. (1994). Transcriptional regulation of the human insulin gene is dependent on the homeodomain protein STF1/IPF1 acting through the CT boxes. *Proceedings of the National Academy of Sciences, USA, 91*, 10465–10469.

Pichyangkul, S., Yongvanitchit, K., Kum-arb, U., Hemmi, H., Akira, S., Krieg, A. M., *et al*. (2004). Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a toll-like receptor 9-dependent pathway. *Journal of Immunology, 172* (8), 4926–4933.

Piro, S., Anello, M., Di Pietro, C., Lizzio, M. N., Patanè, G., Rabuazzo, A. M., *et al*. (2002). Chronic exposure to free fatty acids or high glucose

induces apoptosis in rat pancreatic islets, possible role of oxidative stress. *Metabolism, 51*(10), 1340–1347.

Pischon T., Girman C. J., Hotamisligil G. S., Rifai N., Hu F. B. & Rimm E. B. (2004). Plasma adiponectin levels and risk of myocardial infarction in men. *Journal of the American Medical Association*, *291*, 1730-7173.

Planche, T., Dzeing, A., Ngou-Milama, E., Kombila, M. & Stacpoole, P. W. (2005). Metabolic complications of severe malaria. *Current Topics in Microbiology and Immunology, 295*, 105-136.

Plavec, T., Nemec, S. A., Butinar, J., Tozon, N., Prezelj M., Kandel, B., *et al*. (2008). Antioxidant status in canine cancer patients. *Acta Veterinaria (Beograd*), 58 (2-3), 275-286.

Pociot, F. & McDermott, M. F. (2002). Genetics of type 1 diabetes mellitus. *Genes and Immunity, 3*, 235–249.

Poitout, V., Olson, L. K. & Robertson, R. P. (1996). Chronic exposure of βTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE-3b1 insulin gene transcription activator. *Journal of Clinical Investigation, 97*, 1041–1046.

Poitout, V. & Robertson, R. P. (2002). Secondary β-cell failure in type 2 diabetes—a convergence of glucotoxicity and lipotoxicity.

Pongponratn, E., Turner, G. D., Day, N. P., Phu, N. H., Simpson, J. A., Stepniewska, K., *et al*. (2003). An ultrastructural study of the

brain in fatal *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene, 69*, 345–359.

Postma, N. S., Mommers, E. C., Eling, W. M. C. & Zuidema, J. (1996). Oxidative stress in malaria, implications for prevention and therapy. *Pharmacy, World and Science, 18*(4), 121-129.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E. & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association, 286*, 327–334.

Prasannachandra, D. V. & D'Souza, B. (2006). Comparative study on lipid peroxidation and antioxidant vitamins E and C in *Falciparum* and *Vivax* malaria. *Indian Journal of Clinical Biochemistry 21*(2), 103-106.

Praveen, K. B., Nipun, S., Pushpendra, P. S., Mrigendra, P. S., Manmohan, S., Gyan, C., *et al*. (2008). The usefulness of a new rapid diagnostic test, the First Response® Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *Malaria Journal* , *7*, 126.

Premjeet, S., Deepika, G., Sudeep, B., Sonam, J., Sahil, K., Devashish, R., *et al*. (2011). Enzyme-linked immuno-sorbent assay (ELISA), basics and it's application: a comprehensive review. *Journal of Pharmacy Research, 4*(12), 4581-4583.

Pulido-Mendez, M., Santis J. D. & Rodriguez-Acosta, A. (2002). Leptin and leptin receptors during malaria infection in mice. *Folia Parasitologica*, *49*, 249-251.

Qu, H-Q., Li, Q., Rentfro, A. R., Fisher-Hoch, S. P. & McCormick, J. B. (2011). The definition of insulin resistance using HOMA-IR for Americans of Mexican descent using machine learning. *PLoS One,* 6(6), e21041. doi,10.1371/journal.pone.0021041.

Rader, D. J. & Daugherty, A. (2008). Translating molecular discoveries into new therapies for atherosclerosis. *Nature, 451*, 904 –913.

Radonjic, M., de Haan, J. R., van Erk, M. J., van Dijk, K. W., van den Berg, S. A., de Groot, P. J., *et al*. (2009). Genome- wide mRNA expression analysis of hepatic adaptation to high-fat diets reveals switch from an inflammatory to steatotic transcriptional program. *PLoS One, 4*(8), e6646. doi:10.1371/journal.pone.0006646

Rahbani-Nobar, M. E., Rahimi-Pour, A., Rahbani-Nobar, M., Adi-Beig, F., & Mirhashemi, S. M. (1999). Total antioxidant capacity, superoxdie dismutase and glutathione peroxidise in diabetic patients. *Medical Journal of the Islamic Academy of Sciences, 12*(4), 109-114.

Rask-Madsen, C. & Kahn, C. R. (2012). Tissue–specific insulin signaling, metabolic syndrome and cardiovascular disease. *Arteriosclerosis, Thrombosis and Vascular Biology, 32*, 2052-2059.

Rautiainen, S., Levitan, E. B., Orsini, N., Åkesson, A., Morgenstern, R., Mittleman, M. A. & Wolk, A. (2012). Total antioxidant capacity from diet and risk of myocardial infarction, a prospective cohort of women. *American Journal of Medicine*, *125*(10), 974. doi: 10.1016/j.amjmed.2012.03.008

Rhodes, C. J. (2005). Type 2 diabetes – a matter of beta-cell life and death? *Science, 307*, 380–384.

Ridker, P. M., Danielson, E., Fonseca, F. A., Genest, J., Gotto, A. M. Jr., Kastelein, J. J., …, JUPITER Study Group. (2008). Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *New England Journal of Medicine, 359*, 2195–2207.

Ridker, P. M., Danielson, E., Fonseca, F. A., Genest, J., Gotto, A. M. Jr., Kastelein, J. J., *et al*. (2009). Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin, a prospective study of the JUPITER trial. *Lancet, 373*, 1175–1182.

Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntop, M., *et al*. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences, USA*, *106*(21), 8665–8670.

Robert, V., Bourgouin, C., Depoix, D., Thouvenot, C., Lombard, M-N. & Grellier, P. (2008). Malaria and obesity: obese mice are resistant to cerebral malaria. *Malaria Journalournal, 7*, 81. doi: 10.1186/1475-2875-7-81.

Rojas, L. B. A. & Gomes, M. B. (2013). Metformin: an old but still the best treatment for type 2 diabetes. *Diabetology and Metabolic Syndrome, 5, 6*. doi: 10.1186/1758-5996-5-6.

Rose, D. P. Komninou, D. & Stephenson, G. D. (2004). Obesity, adipocytokines and insulin resistance in breast cancer. *Obesity Review, 5*, 153–165.

Rosen, E. D. & Spiegelman, B. M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature, 444*, 847–853.

Rotimi C. N., Chen G., Adeyemo A. A., Jones L. S., Agyenim-Boateng K., Eghan B. A. Jr., *et al.* (2006). Genomewide scan and fine mapping of quantitative trait loci for intraocular pressure on 5q and 14q in West Africans. *Investigative Ophthalmology and Visual Science, 47*(8), 3262-3267.

Ruhl C. E, Everhart J. E., Ding J., Goodpaster B. H., Kanaya A. M., Simonsick E. M., *et al.* (2004). Serum leptin concentrations and body adipose measures in older black and white adults. *American Journal of Clinical Nutrition, 80*, 576–583.

Rumley, A. G., Woodward, M., Rumley, A., Rumley, J. & Lowe, G. D. O. (2004). Plasma lipid peroxides: relationships to cardiovascular risk factors and prevalent cardiovascular disease. *QJM: An International Journal of Medicine,* 97(12), 809-816.doi: 10.1093/qjmed/hch130.

Rutter, M. K., Wilson, P. W. F., Sullivan, L. M., Fox, C. S., Sr D'Agostino, R. B. & Meigs J. B. (2008). Use of alternative thresholds: defining insulin resistance to predict type 2 diabetes mellitus and cardiovascular disease. *Circulation, 117*, 1003-1009.

Sachs, J. & Malaney, P. (2002).The economic and social burden of malaria. *Nature, 415*, 680–685.

Saliba K. J. & Kirk, K. (1999). PH regulation in the intracellular malaria parasite, *Plasmodium falciparum*. H(+) extrusion via a v-type h(+)-atpase. *Journal of Biological Chemistry, 274*, 33213-33219.

Samatha P. Venkateswarlu M. & Prabodh V. S. (2012). Lipid profile levels in type 2 diabetes mellitus from the tribal population of Adilabad in Andhra Pradesh, India. *Journal of Clinical and Diagnostic Research, 6*(4), S590-S592.

Samuel, V. T., Liu, Z-X., Qu, X., Elder, B. D., Bilz, S., Befroy, D., *et al.* (2004). Mechanism of hepatic insulin resistance in non- alcoholic fatty liver disease. *Journal of Biological Chemistry, 279*(31), 32345–32353.

Sandoval, D. A., Obici, S. & Seeley, R. J. (2009). Targeting the CNS to treat type 2 diabetes. *Nature Reviews Drug Discovery, 8*, 386–398.

Sarraf, P., Frederich, R. C., Turner, E. M., Ma, G., Jaskowiak, N. T., Rivet, D. J., *et al*. (1997). Multiple cytokines and acute inflammation raise mouse leptin levels — potential role in inflammatory anorexia. *Journal of Experimental Medicine, 185*, 171–175.

Savu, O., Ionescu-Tirgoviste, C., Atanasiu, V., Gaman, L., Papacocea, R. & Stoian, I. (2012). Increase in total antioxidant capacity of plasma de spite high levels of oxidative stress in uncomplicated type 2 diabetes mellitus. *Journal of International Medical Research, 40*(2), 709-716.

Sblendorio, V. & Palmieri, B. (2008). Accuracy of analyses for lipid profile parameters as measured with the CR3000 system. *European Review for Medical and Pharmacological Sciences*, *12*, 191-196.

Schaffer, J. E. (2002). Fatty acid transport: the roads taken. *American Journal of Physiology, Endocrinology and Metabolism, 282*, E239–E246.

Scherer, P. E. (2006). Adipose tissue: from lipid storage compartment to endocrine organ. *Diabetes, 55*, 1537–1545.

Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. & Lodish, H. F. (1995). A novel serum protein similar to C1q, produced exclusively in adipo cytes. *Journal of Biological Chemistry, 270* (45), 26746–26749.

Schmidt-Lucke, C., Rossig, L., Fichtlscherer, S., Vasa, M., Britten, M., Kamper, U., *et al*. (2005). Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: Proof of concept for the clinical importance of endogenous vascular repair. *Circulation, 111*, 2981–2987.

Scott, L. J., Mohlke, K. L., Bonnycastle, L. L., Willer, C. J., Li, Y., Duren, W. L., *et al*. (2007). A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science, 316* (5829), 1341-1345.

Sell, H., Dietze-Schroeder, D., Eckardt, K. & Eckel, J. (2006). *Biochemical and Biophysical Research Communication, 343*(3), 700-706.

Serghides, L., Kim, H., Lu, Z., Kain, D. C., Miller, C., Francis, R. C., *et al*. (2011). Inhaled nitric oxide reduces endothelial activation and parasite accumulation in the brain, and enhances survival in experi mental cerebral malaria. *PLoS One*, *6*(11), e27714.

Sert, M., Morgul, G. & Tetiker B. T. (2010). Diabetic dyslipidemia is a well-known issue, but what about lipoprotein A levels in type 2 diabetics? *International Journal of Diabetes and Metabolism, 18*, 81-87.

Serup, P., Jensen, J., Andersen, F. G., Jorgensen, M. C., Blume, N., Holst, J. J., *et al*. (1996). Induction of insulin and islet amyloid poly peptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. *Proceedings of the National Academy of Sciences, USA, 93*, 9015–9020.

Sethi, J. K. & Vidal-Puig, A. J. (2007). Adipose tissue function and plasticity orchestrate nutritional adaptation. *Journal of Lipid Research, 48*, 1253-1262.

Seufert, J. (2004). Leptin effects on pancreatic beta-cell gene expression and function. *Diabetes, 53*(suppl 1), S152–S158.

Sha, H., He, Y., Yang, L. & Qi, L. (2011). Stressed out about obesity: IRE1alpha-XBP1 in metabolic disorders. *Trends Endocrinology and Metabolism, 22*, 374–381.

Sharma, A., Olson, L. K., Robertson, R. P. & Stein, R. (1995). The reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to high glucose concentration is associated with the loss of RIPE3b1

and STF-1 transcription factor expression. *Molecular Endocrinology,* 9 (9), 1127–1134.

Shepherd, P. R. & Kahn, B. B. (1999). Glucose transporters and insulin action –implications for insulin resistance and diabetes mellitus. *New England Journal of Medicine, 341*, 248–257.

Shi, H, Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H. & Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *Journal of Clinical Investigation, 116*, 3015–3025.

Shimabukura, M., Ohneda, M., Lee, Y. & Unger, R. H. (1997). Role of nitric oxide in obesity-induced beta cell disease. *Journal of Clinical Investigation, 100*, 290–295.

Shimabukuro, M., Higa, M., Zhou, Y. T., Wang, M.Y., Newgard, C. B. & Unger, R. H. (1998). Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats: role of serine palmitoyltransferase overexpression. *Journal of Biological Chemistry, 273*, 32487–32490.

Shimabukuro, M., Higa, N., Asahi, T., Oshiro, Y., Takasu, N., Tagawa, T., *et al*. (2003). Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *Journal of Clinical Endocrinology and Metabolism, 88* (7), 3236– 3240.

Shimabukuro, M., Zhou, Y-T., Levi, M. & Unger, R. H. (1998). Fatty acid induced β-cell apoptosis: a link between obesity and diabetes. *Proceed ings of the National Academy of Sciences, USA, 95*, 2498–2502.

Shinohara, K., Shoji, T., Emoto, M., Tahara, H., Koyama, H., Ishimura, E., *et al*. (2002). Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. *Journal of the American Society Nephrology, 13*, 1894–1900.

Sies, H. (2007). Total antioxidant capacity, appraisal of a concept. *Journal of Nutrition, 137*(6), 1493-1495.

Simpson, D. C., Kabyemela, E., Muehlenbachs, A., Ogata, Y., Mutabingwa, T. K., Duffy, P. E., *et al*. (2010). Plasma levels of apoliprotein A1 in malaria-exposed primigravidae are associated with severe anemia. *PLoS One, 5*(1), e8822. doi: 10.1371/journal.pone.0008822.

Sin, C., Lim, D. J., Song, T. J., Lee, K. C., Suh, I., Yoon, S. Y., *et al*. (2001). Lipid profile changes in infection of *Plasmodium vivax*. *Korean Journal of Infectious Diseases*, *33*(1), 58-61.

Singer, J. R., Palmas, W., Teresi, J., Weinstock, R., Shea, S. & Luchsinger, J. A. (2012). Adiponectin and all-cause mortality in elderly people with type 2 diabetes. *Diabetes Care, 35*(9), 1858-1863.

Sio, S. W., Sun, W., Kumar, S., Bin, W. Z., Tan, S. S, Ong, S. H., *et al*. (2007). Malaria Count: an image analysis-based program for the accurate determination of parasitaemia. *Journal of Microbiological Methods, 68*, 11-18.

Skretteberg, P. T., Grundvold, I., Kjeldsen S. E., Erikssen, J. E., Sandvik, L., Liestøl, K., *et al*. (2012). HDL-cholesterol and prediction of

coronary heart disease, modified by physical fitness? A 28-year follow-up of apparently healthy men. *Atherosclerosis*, *220*, 250–256.

Slavic, K., Krishna, S., Derbyshire, E. T. & Staines, H. M. (2011). Plasmodial sugar transporters as anti-malarial drug targets and comparisons with other protozoa. *Malaria Journal ,* 10, 165.

Smith S. C. Jr. & Haslam D. (2007). Abdominal obesity, waist circumference and cardio-metabolic risk: awareness among primary care physicians, the general population and patients at risk—the shape of the nations survey. *Current Medical Research and Opinion, 23*, 29-47.

Smith, S. & Lall, A. M. (2008). A study on lipid profile levels of diabetics and non-diabetics among Naini Region of Allahabad, India. *Turkish Journal of Biochemistry, 33*(4), 138–141.

Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y., & Hay, S. I. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature, 434*, 214–217.

Söderberg, S., Zimmet, P., Tuomilehto, J., Chitson, P., Gareeboo, H., Alberti, K. G. & Shaw, J. E. (2007). Leptin predicts the development of diabetes in Mauritian men, but not women, a population-based study. *International Journal of Obesity (Lond)*, *31*, 1126 –1133.

Song, Y., Manson, J. E., Tinker, L., Howard, B. V., Kuller, L. H., Nathan, L., *et al*. (2007). Insulin sensitivity and insulin secretion determined by homeostasis model assessment and risk of diabetes in a multiethnic

cohort of women: the women's health initiative observational study. *Diabetes Care, 30*, 1747-1752.

Spranger, J., Kroke, A., Moehhling, M., Bergmann, M. M., Ritow, M., Boeling, H., *et al*. (2003). Adiponectin and protection against type 2 diabetes mellitus. *Lancet, 361*, 226-228.

Stanek, A., Cieślar, G., Romuk, E., Kasperczyk, S., Sieroń-Stołtny, K., Birkner, E., *et al*. (2010). Decrease in antioxidant status of plasma and erythrocytes from patients with ankylosing spondylitis. *Clinical Biochemistry, 43*, 566–570.

Staines, H. M., Derbyshire, E. T., Slavic, K., Tattersall, A., Vial, H. & Krishna, S. (2010).Exploiting the therapeutic potential of Plasmodium falciparum solute transporters. *Trends in Parasitology, 26*, 284-296.

Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L. & Habener, J. F. (1997). Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nature Genetics, 15*, 106–110.

Stommel, M. & Schoenborn, C. A. (2010). Variations in BMI and prevalence of health risks in diverse racial and ethnic populations. *Obesity, 18*(9), 1821–1826.

Stranges, S., Dorn, J. M., Donahue, R. P., Browne, R. W., Freudenheim, J. L., Hovey, K. M., *et al*. (2008). Oxidation, type 2 diabetes, and coronary heart disease: a complex interaction. Findings from a population-based study. *Diabetes Care*, *31*(9), 1864–1866.

Stumvoll, M., Goldstein, B. J., & van Haeften, T. W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. *Lancet, 365*, 1333–1346.

Sun, S., Ji Y., Kersten, S. & Qi, L. (2012). Mechanisms of inflammatory responses in obese adipose tissue. *Annual Review of Nutrition, 32*, 261-286.

Sun, X. J., Wang, L. M., Zhang, Y., Yenush, L., Myers, M. G. Jr., Glasheen, E., *et al*. (1995). Role of IRS-2 in insulin and cytokine signaling. *Nature, 377,* 173–177.

Sunil, K., Diwan, S. K., Mahajan, S. N, Shilpa, B. & Chetan, M. (2011). Case report of *Plasmodium falciparum* malaria presenting as wide complex tachycardia. *Asian Pacific Journal of Tropical Biomedicine*, S305-S306.

Suresh, D. R., Annam, V., Pratibha K. & Prasad, B. V. M. (2009). Total anti oxidant capacity – a novel early bio-chemical marker of oxidative stress in HIV infected individuals. *Journal of Biomedical Science, 16*, 61.

Tabor, H. K., Risch, N. J. & Myers, R. M. (2002). Candidate-gene approaches for studying complex genetic traits, practical considerations. *Nature Review Genetics, 3*, 391-397.

Taheri, E., Djalali, M., Saedisomeolia, A., Moghadam, A. M., Djazayeri, A. & Qorbani, M. (2012). The relationship between the activates of anti-oxidant enzymes in red blood cells and body mass index in Iranian type 2 diabetes and healthy subjects. *Journal of Diabetes and Metabolic Disorders 11*, 3. doi:10.1186/2251-6581-11-3.

Tajiri, Y., Moller, C. & Grill, V. (1997). Long term effects of aminoguanidine on insulin release and biosynthesis, evidence that the formation of advanced glycosylation end products inhibits β-cell function. *Endocri nology, 138*, 273–280.

Takeda, S. (2005). Central control of bone remodeling. *Biochemical and Bio-physical Research Communication, 328*, 697–699.

Takeuchi, M. & Yamagishi, S. (2009). Involvement of toxic AGEs (TAGE) in the pathogenesis of diabetic vascular complications and Alzheimer's disease. *Journal of Alzheimers Disease 16*, 845–858.

Tanaka, Y., Gleason, C. E., Tran, P. O. T., Harmon, J. S. & Robertson, R. P. (1999). Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proceedings of the National Academy of Sciences, USA, 96*, 10857–10862.

Taylor, T. E., Fu, W. J., Carr, R. A., Whitten, R. O., Mueller, J. S., Fosiko, N. G., *et al*. (2004). Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nature Medicine, 10*, 143–145.

Teixeira-Lemos, E., Nunes, S., Teixeira, F. & Reis, F. (2011). Regular physical exercise training assists in preventing type 2 diabetes development, focus on its antioxidant and anti-inflammatory properties. *Cardiovascular Diabetology, 10*, 12. doi:10.1186/1475-2840-10-12.

Teoh, H., Strauss, M. H., Szmitko, P. E. & Verma, S. (2006). Adiponectin and myocardial infarction: a paradox or a paradigm? *European Heart Journal*, *27*, 2266 –2268.

Thallas-Bonke, V., Thorpe, S. R., Coughlan, M. T., Fukami, K., Yap, F. Y.T, Sourris, K. C., *et al*. (2008). Inhibition of NADPH oxidase prevents advanced glycation end product–mediated damage in diabetic nephropathy through a protein kinase C-α–dependent pathway. *Diabetes, 57*, 460–469.

The Emerging Risk Factors Collaboration (2012). C-reactive protein, fibrinogen, and cardiovascular disease prediction. *New England Journal of Medicine*, *367*, 1310-1320.

The Emerging Risk Factors Collaboration (2010). C-reactive protein concetration and risk of coronary heart disease, stroke, and mortality, an individual participant meta-analysis. *Lancet, 375*, 132–140.

Thevenon, A. D., Zhou, J. A., Megnekou, R., Ako, S., Leke, R. G. F. & Taylor, D. W. (2010). Elevated levels of soluble TNF receptors 1 and 2 correlate with *plasmodium falciparum* parasitemia in pregnant women: potential markers for malaria-associated inflammation. *Journal of Immunology, 185*, 7115–7122.

Thomas, M. E., Harris, K. P. G., Walls, J., Furness, P. N. & Brunskill, N. J. (2002). Fatty acids exacerbate tubulointerstitial injury in protein-overload proteinuria. *American Journal of Physiology - Renal Physiology, 283*(4), F640–F647.

Thomson, M. J., Puntmann, V. & Kaski, J. C. (2007). Atherosclerosis and oxidant stress: the end of the road for antioxidant vitamin treatment? *Cardiovascular Drugs and Therapy*, 21(3), 195-210.

Tonyushkina, K. & Nichols, J. H. (2009). Glucose meters: a review of technical challenges to obtaining accurate results. *Journal of Diabetes Science and Technology, 3*(4), 971-980.

Tousoulis, D., Antoniades, C., Koumallos, N., Marinou, K., Stefanadi, E., Latsios, G., *et al*. (2006). Novel therapies targeting vascular endothelium. *Endothelium, 13*(6), 411–421.

Tschritter, O., Fritsche, A., Thamer, C., Haap, M., Shirkavand, F., Rahe, S., *et al*. (2003). Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes, 52*, 239-243.

Turk, H. M., Sevinc, A., Camci, C., Cigli, A., Buyukberber, S., Savli, H., *et al*. (2002). Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Acta Diabetology*, *39*(3):117-22.

Turner, G. (1997). Cerebral malaria. *Brain Pathologyogy, 7*(1), 569–582.

Turner, G. D., Morrison, H., Jones, M., Davis, T. M., Looareesuwan, S., Buley, I. D., *et al*. (1994). An immunohistochemical study of the pathology of fatal malaria: evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *American Journal of Pathology* 145 (5), 1057–1069.

Unger, R. H. (2005). Longevity, lipotoxicity and leptin: the adipocyte defense against feasting and famine. *Biochimie, 87*, 57–64.

United Nations Children's Fund (2010). At a glance: Ghana statistics. Retrieved from http://www.unicef.org/infobycountry/ghana_statistics.html.

Unwin, N., Setel, P., Rashid, S., Mugusi, F., Mbanya, J., Kitange, H., *et al*. (2001). Noncommunicable diseases in sub-Saharan Africa: where do they feature in the health research agenda? *Bulletin of the World Health Organisation*, *79*(10), 947-953.

Uysal, K. T., Wiesbrock, S. M., Marino, M. W. & Hotamisligil, G. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature, 389*, 610–614.

Vairamon, S. J., Babu, M. & Viswanathan, V. (2009). Oxidative stress markers regulating the healing of foot ulcers in patients with type 2 diabetes. *Wounds, 21*(10), 273–279.

Vauhkonen, N., Niskanen, L., Vanninen, E., Kainulainen, S., Uusitupa M. & Laakso, M. (1997). Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited: metabolic studies on offspring of diabetic probands. *Journal of Clinical Investigation, 100*, 86–96.

Vazquez, G., Duval, S., Jacobs, Jr. D. R. & Silventoinen, K. (2007). Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. *Epidemiologic Review, 29*, 115–128.

Vendrell, J., Broch, M., Vilarrasa, N., Molina, A., Gómez, J. M., Gutiérrez, C., *et al*. (2004). Resistin, adiponectin, ghrelin, leptin and proinflammatory cytokines: relationships in obesity. *Obesity Research, 12*, 962–971.

Vimaleswaran, K. S. & Loos, R. J. F. (2010). Progress in the genetics of common obesity and type 2 diabetes. *Expert Reviews in Molecular Medicine, 12*, e7. doi:10.1017/S1462399410001389.

Voetsch, B., Jin R. C. & Loscalzo, J. (2004). Nitric oxide insufficiency and atherothrombosis. *Histochemistry and Cell Biology,* 122, 353–367.

Waeber, G., Thompson, N., Nicod, P. & Bonny, C. (1996). Transcriptional activation of the *GLUT2* gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Molecular Endocrinology, 10*, 1327–1334.

Walter, M. F., Jacob, R. F., Jeffers, B., Ghadanfar, M. M., Preston, G. M., Buch J., *et al*. (2004). Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the Prevent study. *Journal of the American College of Cardiology*, *44*(10), 1996–2002.

Walther, B., Miles, D. J. C., Crozier, S., Waight, P., Palmero, M. S., Ojuola, O., *et al*. (2010). Placental Malaria is associated with reduced early life weight development of affected children independent of low birth weight. *Malaria Journal, 9*, 16. doi: 10.1186/1475-2875-9-16.

Wang, H. J., Gao, B., Zakhari, S. & Nagy, L. E. (2012). Inflammation in alcoholic liver disease. *Annual Review of Nutrition, 32*, 343-368.

Wang, Y., Chun, O. K. & Song, W. O. (2013). Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. *Nutrients*, *5*(8), 2969-3004. doi: 10.3390/nu5082969.

Wannamethee, S. G., Lowe, G. D., Rumley, A., Cherry, L., Whincup, P. H. & Sattar, N. (2007). Adipokines and risk of type 2 diabetes in older men. *Diabetes Care, 30*, 1200 –1205.

Wannamethee, S. G., Whincup, P. H., Lennon, L. & Sattar, N. (2007). Circulating adiponectin levels and mortality in elderly men with and without cardiovascular disease and heart failure. *Archives of Internal Medicine, 167*(14), 1510-1517.

Watada, H., Kajimoto, Y., Miyagawa, J., Hanafusa, T., Hamaguchi, K., Matsuoka, T., *et al*. (1996). PDX-1 induces insulin and glucokinase gene expressions in alphaTC1 clone 6 cells in the presence of betacellulin. *Diabetes, 45*, 1826–1831.

Watanabe, R. M., Valle, T., Hauser, E. R., Ghosh, S., Eriksson, J., Kohtamaki, K., *et al*. (1999). Familiality of quantitative metabolic traits in Finnish families with non-insulin-dependent diabetes mellitus: Fin land-United States Investigation of NIDDM Genetics (FUSION) Study investigators. *Human Heredity, 49*(3), 159–168.

Warrell, D. A., Looareesuwan, S., Warrell, M. J., Kasemsarn, P., Intaraprasert, R., Bunnag, D., *et al*. (1982). Dexamethasone proves deleterious in cerebral malaria, a double-blind trial in 100 comatose patients. *New England Journal of Medicine, 306*, 313–319.

Weatherall, D. J., Miller, L. H., Baruch, D. I., Marsh, K., Doumbo, O. K., Casals-Pascual, C., *et al*. (2002). Malaria and the red cell. *Hematology,* 35-57.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel R. L. & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation, 112*, 1796–1808.

Weyer, C., Funahashi, I., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. E., *et al*. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism, 86*(5), 1930-1935.

Wheeler, M. A., Smith, S. D., García-Cardeña, G., Nathan, C. F., Weiss, R. M. & Sessa, W. C. (1997). Bacterial infection induces nitric oxide synthase in human neutrophils. *Journal of Clinical Investigation, 99*, 110-116.

White, M. F. (2003). Insulin signaling in health and disease. *Science, 302*, 1710–1711.

White, N. J. (2008). *Plasmodium knowlesi*, the fifth human malaria parasite. *Clinical Infectious Diseases, 46*, 172–173.

Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes Care, 27*, 1047–1053.

Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., *et al*. (2009). Normalization of obesity-associated insulin resistance through immunotherapy. *Nature Medicine, 15*(8), 921–929.

World Health Organization (WHO) (2010). *Basic malaria microscopy: Part 1. learner's guide* (2nd ed.). Geneva, Switzerland.

World Health Organization (WHO). (2008). Waist circumference and waist-hip ratio: Report of a WHO expert consultation.

World Health Organization (WHO) (2001). World Medical Association Declaration of Helsinki. *Bulletin of the World Health Organization*, *79*(4), 373-374.

Wosje, K. S., Binkley, T. L., Kalkwarf, H. J. & Specker, B. L. (2004). Relationships between bone mass and circulating leptin concentrations in Hutterites. *Bone, 34*, 1017– 1022.

Wu, C., Gong, Y., Yuan, J., Gong, H., Zou, Y. & Ge, J. (2012). Identification of shared genetic susceptibility locus for coronary artery disease, type 2 diabetes and obesity, a meta-analysis of genome-wide studies. *Cardiovascular Diabetology, 11*, 68. doi:10.1186/1475-2840-11-68.

Wu, D., Molofsky, A. B., Liang, H. E., Ricardo-Gonzalez, R. R., Jouihan, H. A., Bando, J. K., *et al*. (2011). Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science, 332*, 243–247.

Xia, S., Sha, H., Yang, L., Ji, Y., Ostrand-Rosenberg, S. & Qi, L. (2011). Gr-1+CD11b+myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. *Journal of Biological Chemistry, 286*, 23591–2399.

Xiao, H. (2002). Method and composition for determining high-density lipo protein cholesterol. *Chinese Patent,* CN 1379235A.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., *et al*. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation, 112*(12), 1821–1830.

Yamagishi, S. (2011). Role of advanced glycation end products (AGEs) and receptor for AGEs (RAGE) in vascular damage in diabetes. *Experimental Gerontology, 46*(4), 217–224.

Yamamoto, Y., Hirose, H., Saito, I., Tomiata, M., Taniyama, M., Matsubara, K., *et al*. (2002). Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipo protein–cholesterol, independent of body mass index, in the Japanese population. *Clinical Science, 103*(2), 137-142.

Yamamoto, Y., Hirose, H., Saito, I., Nishikai, K. & Saruta, T. (2004). Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population. *Journal of Clinical Endocrinology and Metabolism, 89*, 87–90.

Yamauchi, T., Kamon, J., Waki, H., Imai, Y., Shimozawa, N., Hioki, K, *et al*. (2003). Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *Journal of Biological Chemistry, 278*(4), 2461–2468.

Yang, Q., Graham, T. E., Mody, N., Preitner, F., Peroni, O. D., Zabolotny, J. M., *et al*. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature, 436*, 356– 362.

Yapi, H. F., Ahiboh, H., Koffi, D., Yapo, A., Bla, K. B., Monnet, D. & Djaman, A. J. (2010). Assessment of inflammatory and immunity pro teins during *falciparum* malaria infection in children of Côte d'Ivoire. *American Journal of Scientific and Industrial Research, 1*(2), 233-237.

Ye, J. (2013). Mechanisms of insulin resistance in obesity. *Frontiers of Medicine*, *7*(1), 14-24.

Yeo, T. W., Lampah, D. A., Gitawati, R., Tjitra, E., Kenangalem, E., McNeil, Y. R., *et al*. (2007). Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with *falciparum* malaria. *Journal of Experimental Medicine, 204*(11), 2693–2704.

Yeo, T. W., Lampah, D. A., Gitawati, R., Tjitra, E., Kenangalem, E., Piera, K., *et al*. (2008).Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. *Proceedings of the National Academy of Sciences, USA, 105* (44), 17097–17102.

Yoshikawa, H., Tajiri, Y., Sako, Y., Hashimoto, T., Umeda, F. & Nawata, H. (2001). Effects of free fatty acids on β-cell functions: a possible involvement of peroxisome-proliferator-activated receptors α or pancreastic/duodenal homeobox. *Metabolism, 50*(5), 613–618.

Young, L. H. (2010). Diet-induced obesity obstructs insulin signaling in the heart. *American Journal of Physiology – Heart and Circulatory Physiology,* 298, H306–H307.

Zeggini, E., Scott, L. J., Saxena, R., Voight, B. F., Marchini, J. L., Hu T., *et al*. (2008). Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nature Genetics, 40*(5), 638-645.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature,* 372, 425–432.

Zhao, B., Liu, Y., Zhang, Y., Chen, Y., Yang, Z., Zhu, Y., *et al*. (2012). Gender difference in carotid intima-media thickness in type 2 diabetic patients: a 4-year follow-up study *Cardiovascular Diabetology,* 11, 51. doi:10.1186/1475-2840-11-51.

Zhou, Y-T., Grayburn, P., Karim, A., Shimabukuro, M., Higa, M., Baetens, D., *et al*. (2000). Lipotoxic heart disease in obese rats: implications for human obesity. *Proceedings of the National Academy of Sciences, USA,* 97 (4), 1784–1789.

Zierath, J. R., Krook, A. & Wallberg-Henriksson, H. (2000). Insulin action and insulin resistance in human skeletal muscle. *Diabetologia,* 43, 821–835.

Zimmet, P., Alberti, K. G. & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature,* 414, 782–787.