KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

ASSESSING RELATIONSHIP BETWEEN DIETARY PATTERN, ANTIOXIDANT MICRONUTRIENTS STATUS AND RISK OF CARDIOVASCULAR DISEASES AMONG TYPE 2 DIABETIC OUTPATIENTS AT KOMFO ANOKYE TEACHING HOSPITAL

BY

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DECLARATION

I declare that I have wholly undertaken the study reported herein under the supervision of Dr. Charles Apprey and that except portions where references have been duly cited, this dissertation is the outcome of my research.

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ABSTRACT

Cardiovascular diseases are associated with type 2 diabetes mellitus and concurrently, responsible for 68% cause of mortality among type 2 diabetics. Antioxidant micronutrients level can delay or prevent diabetic complication such as cardiovascular diseases. The study aims to assess relationship between dietary pattern, antioxidant micronutrients status and risk of cardiovascular diseases among outpatient type 2 diabetics. A cross sectional study was conducted on 152 outpatient type 2 diabetic patients. The BMI, waist circumference, fasting blood glucose and serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), very low density lipoprotein (VLDL), coronary risk, atherogenic index of plasma, and serum zinc were determined. Data was collected with questionnaires. Dietary intakes of antioxidants were assessed using 24-hour dietary recall and food frequency questionnaire. Data were analyzed using SPSS version 23. Out of the 152 study population, 37 (24.3%) were males and 115 (75.7%) were females. Generally, 74.3% of type 2 diabetes patients had high FBG and 64.6% had high HbA1c. The prevalence of single dyslipidemia (63.8%), combined dyslipidemia (15.8%) and mixed dyslipidemia (1.3%) was found among study participants. Furthermore, 35.3% of type 2 diabetic patients had high coronary risk and 5.3% had high atherosclerosis risk. Coronary risk was strongly associated with TC, ($r = 0.695$, p value < 0.000) and LDL-C, ($r = 0.783$, p value < 0.000). Moreover, atherosclerosis risk was strongly associated with TG, ($r = 0.775$, p value < 0.0001), VLDL, ($r = 0.778$, p value < 0.0001) and inversely associated with HDL-C ($r = -0.283$, $p = 0.003$). A logistic regression showed TC, TG, LDL-C had significant effects on coronary risk (TC: OR= 2.640, 95% CI= 1.879-3.708, p value < 0.0001, TG: OR= 2.549, 95% CI= 1.342-4.841, p value $= 0.004$, LDL-C: OR= 4.858, 95% CI= 2.902-8.135, p value < 0.0001 respectively). The mean intakes of zinc (5.04±2.76 mg/day), vitamin E (5.16±2.60 mg/day) and vitamin C (82.72±38.76 mg/day) were lowered among type 2 diabetic patients. Dietary vitamin E was directly associated with HbA1c, ($r = 0.220$, p value $= 0.033$), TC, ($r = 0.260$, p value $= 0.011$), LDL-C ($r = 0.267$, p value $= 0.009$) and TC/HDL-C ratio, ($r = 0.217$, p value $= 0.036$), after adjusting for age and gender. Also, when controlling for age, gender and dietary zinc; serum zinc was inversely associated with HbA1c ($r = -0.227$, p value $= 0.05$) and FBG, ($r = -0.206$, p value $= 0.033$). In conclusion, cardiovascular risk factors such as hyperglycemia, dyslipidemia, abdominal obesity, coronary risk were high among type 2 diabetic patients. Dietary intakes of antioxidant micronutrients as well as serum zinc were seen low among study participants. Poor dietary intakes of antioxidant micronutrients and reduced serum zinc, together with high cardiovascular risk factors may put outpatient type 2 diabetics at high risk of developing cardiovascular diseases.

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BMI	Body Mass Index
CVDs	Cardiovascular Diseases
CHD	Coronary Heart Disease
DM	Diabetes Mellitus
EDTA	Ethylenediamine tetraacetic acid
FBG	Fasting Blood Glucose
µg/dL	Microgram per deciliter
HbA1c	Glycated Hemoglobin
HDL-C	High Density Lipoprotein- Cholesterol
IDF	International Diabetes Federation
KATH	Komfo Anokye Teaching Hospital
LDL-C	Low Density Lipoprotein- Cholesterol
MI	Myocardial Infarction
mmol/L	Millimole per Liter
NCEP ATP	National Cholesterol Education Program Adult Treatment Panel
NHMRC	National Health and Medical Research Council
Nitro-PAPS	2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) phenol disodium salt
PAD	Peripheral Arterial Disease

RCT	Randomized Clinical-Controlled Trial
SPSS	Statistical Package for the Social Sciences
TC	Total Cholesterol
TG	Triglyceride
VLDL-C	Very Low Density Lipoprotein- Cholesterol
WC	Waist Circumference
WHO	World Health Organization

LIST OF PUBLICATIONS

Asamoah-Boakye, O., Apprey, C. and Annan, R. (2017). Effects of antioxidant micronutrients against cardiovascular disease risk in type 2 diabetes mellitus: A systematic review. *Journal of Nutritional Disorders and Therapy*, doi: 10.4172/2161-0509.1000214.

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CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus is a long-term and enervating disease that requires prolonged management and significantly predisposes to serious, chronic complications (Motala and Ramaiya, 2010). It is listed among non-communicable diseases, which cause high rate of mortality in the world, contributing to more than 60% of all deaths (Mota and Dinu, 2013). Cardiovascular diseases are complication of type 2 diabetes mellitus, and concurrently, responsible for 68% cause of mortality among type 2 diabetics (Pellegrino, 2016; Bhupathiraju and Hu, 2016).

Dietary behaviours including intakes of high saturated fat and refined carbohydrate, and lifestyles such as smoking and physical inactivity are risk factors of diabetes mellitus (Osonoi *et al*., 2016). Diabetes mellitus then can develop into these cardiovascular diseases; peripheral vascular disease, ischemia, cerebrovascular diseases and coronary artery disease (Mahan *et al*., 2012). The major contributing factors for developing heart-related diseases in individuals with diabetes include uncontrolled hyperglycemia, abdominal obesity, abnormal blood pressure (Mahan *et al*., 2012), obesity and lipid disorders (Niroumand *et al*., 2015). However, diet rich in antioxidant micronutrients helps to prevent likelihood of cardiovascular events in diabetes mellitus patients (Osonoi *et al*., 2016).

According to International Diabetes Federation (2015), 415 million persons lived with diabetes mellitus in the world as at 2015. Eighty percent of individuals having diabetes stay in countries with low socioeconomic status (IDF, 2013). Furthermore, it is estimated that in Africa, 14.2 million persons within the ages of 20-79 years have diabetes; with prevalence rate of 3.2% (Oguoma *et al*., 2017) and predicted to reach 28 million by 2030 (IDF-Africa, 2015). In sub-Saharan Africa, about 30% of patients admitted in hospital's cardiovascular disease units have diabetes mellitus, and it is responsible for mortality in two out of three diabetic patients

(Kengne *et al*., 2005). In Ghana, about 266,200 of the population in the age range 20-79 years have been diagnosed with diabetes, and the prevalence rate is about 1.9% (IDF, 2015).

The presence of insulin resistance and chronic high blood glucose can induce oxidative stress (Plourde, 2017). Also, the high level of blood glucose can non-enzymatically bind to protein to form advanced glycation end-products, which also produce reactive oxygen species, and has damaging effects on endothelial cells (Khoshju, 2017). These reactive oxygen species contribute to development of atherosclerosis by causing injury to the endothelial cells, and subsequently lead to diabetes complication such as cardiovascular diseases (Porasuphatana *et al*., 2012). Also, type 2 diabetes can result in atherogenic lipid disorders which promote atherosclerotic events, and finally lead to cardiovascular diseases (Okpa *et al*., 2015). Dietary antioxidants are emerging nutritional intervention which help in the management and prevention of comorbidities in diabetes (Zhang *et al*., 2016).

Dietary antioxidants help to prevent or delay creation of atherosclerotic plaque by interfering with oxidation of low density lipoprotein-cholesterol, and hence, protect walls of vascular tissues from oxidative damages (Zhang *et al*., 2016). Diet containing adequate antioxidant micronutrients including, vitamin E, and vitamin C provide such antiatherogenic effects in type 2 diabetics. Additionally, Zinc, a micronutrient antioxidant, helps stabilize lipids biomolecules and thus, protects against lipid peroxidation and production of free radicals, as well as help protect endothelial cells from apoptosis (Sornio *et al*., 2007). A prospective cohort research by Sornio *et al*. (2007) showed that reduced concentration of zinc level was associated with risk of developing coronary heart disease among type 2 diabetes mellitus patients. This implies that normal or high serum level of zinc can probably reduce risk of coronary heart disease in type 2 diabetics.

The nutritional management of diabetes includes intake of high fiber diets from whole grain, vegetables and fruits. These fruits and vegetables help manage blood glucose level, through

enhancing insulin action in diabetics (Li *et al*., 2014). Additionally, increase in dietary intakes of vegetables and fruits provides antioxidants micronutrients (Asif, 2014) which protect against complications in diabetes (Bajaj and Khan, 2012), through decreased oxidation of low density lipoprotein-cholesterol, and thus, improve endothelial function in diabetics (Sarmento *et al*., 2013). According to Zhang *et al*. (2016), intervention on antioxidant micronutrients has become great public health interest, regarding prevention of diabetes complications such as cardiovascular diseases. There is therefore the need to determine the antioxidant micronutrients status and risk of heart-related diseases in type 2 diabetics. Also, the outcome of this research can provide information on nutritional management strategies of heart-related disorders among type 2 diabetics in our healthcare settings.

1.1 PROBLEM STATEMENT

According to IDF (2015), diabetes mellitus has caused 4,790 deaths among adults in Ghana. At least, 6% of urban Ghanaians were diagnosed of type 2 diabetes, and was linked to age, obesity and low socioeconomic status, and frequently lead to hypertension and dyslipidemia (Danquah *et al*., 2012). According to Kengne *et al*. (2005), 5-8% type 2 diabetes individuals may have coronary heart disease, and cardiomyopathy may affect 50% of them. Also, cardiovascular disease is responsible for 68% cause of all mortality among type 2 diabetes patients (Bhupathiraju and Hu, 2016). It is obvious that diabetes is highly associated with cardiovascular disease, but the risk factors are not well-defined in sub-Saharan Africa (Kengne *et al*., 2005). The consequences of diabetes mellitus are severe complications and reduced life expectancy which can affect productivity and economic growth (Danquah *et al*., 2012). Inadequate dietary intake of antioxidant micronutrients can possibly put diabetic patients at high risk of cardiovascular diseases (Sarmento *et al*., 2013).

A study by Amissah and Amoako-Boateng (2012) showed prevalence of cardiovascular diseases among people with diabetes mellitus is 60.4% in a Teaching Hospitals in Ghana. The

increase in cardiovascular diseases in diabetic patients can be attributed to poor antioxidant micronutrients status, owing to inadequate dietary consumption of antioxidants micronutrients.

1.2 RESEARCH QUESTIONS

- Do the usual diets consumed by type 2 diabetics contain antioxidant micronutrients?
- What are the CVDs risk factors among type 2 diabetic patients?
- What are the antioxidant micronutrients status among type 2 diabetics?
- Can antioxidant micronutrients status determine risk of CVDs among type 2 diabetics?

1.3 GENERAL OBJECTIVE

- To assess relationship between dietary pattern, antioxidant micronutrients status and risk of cardiovascular diseases (CVDs) among type 2 diabetic outpatients.

1.4 SPECIFIC OBJECTIVES

- To determine CVDs-associated factors among type 2 diabetic patients.
- To assess dietary pattern of antioxidant micronutrients intake among type 2 diabetic patients.
- To determine antioxidant micronutrients status of type 2 diabetics.
- To determine relationship between lipid profile, antioxidant micronutrients status and coronary risk among type 2 diabetics.

1.5 JUSTIFICATION

An increasing evidence support dietary intakes of antioxidant micronutrients can result in decreased inflammatory markers, decreased oxidation of low density lipoprotein-cholesterol, and amelioration of endothelial function disorders among diabetes mellitus patients (Sarmento *et al*., 2013). This study would provide evidence to support claims that adequate antioxidant micronutrients status can protect type 2 diabetic patients against possible likelihood of cardiovascular events. The outcome of the research would support promotion of dietary intakes of antioxidant micronutrients in the management of diabetes mellitus.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS-DEFINITION AND CAUSES

Diabetes mellitus is defined as a metabolic disorder, characterized by high glucose in the blood due to defective insulin secretion, defective insulin action, or both. The body produces a hormone called insulin that is responsible for the metabolism of carbohydrate, protein and fat. The inability to produce insulin; with insulin insufficiency causes hyperglycemia, which lead to diabetes mellitus (Mahan *et al*., 2012). Diabetes mellitus leads to irreversible metabolic disturbances, which alter structural and functional capacity of vascular tissues, including pancreatic beta cells (Devi *et al*., 2016). Diabetes mellitus has been associated with risk factors such as physical inactivity (WHO, 2015), overweight or obesity (WHO, 2016), dyslipidemia, family history of diabetes, hypertension, and medical history of cardiovascular diseases (ADA, 2016). Diabetes mellitus is marked with common symptoms such as excessive thirst, weight loss, polyuria, and blurred vision (IDF, 2015).

2.1.1 CLASSIFICATION OF DIABETES MELLITUS

Diabetes mellitus is grouped into insulin-dependent diabetes (type 1), non-insulin dependent (type 2) diabetes, gestational diabetes (WHO, 2016) and other unclassified types of diabetes associated with defect of the pancreas by either infection, drug or chemical-induced (Mahan *et al*., 2012).

Type 1 diabetes mellitus occurs as a result of defective insulin secretion due to autoimmune damage of pancreatic beta cells (Webster-Gandy *et al*., 2006). The mechanism of autoimmune destruction is still unclear; however, it may be induced by exposure to environmental toxins or infections (Rolfes *et al*., 2009). This mostly occurs in children or adults less than 40 years (Webster-Gandy *et al*., 2006).

Type 2 diabetes mellitus is the most commonly diagnosed type and happens when there is deficient insulin secretion or tissues become resistant to insulin. Type 2 diabetes can occur unnoticed for a long period because symptoms are less marked than type 1 diabetes (IDF, 2015). The resulting hyperglycemia progresses slowly, and causes complications before a person is diagnosed of type 2 diabetes mellitus (Ahmad and Crandall, 2010). Metabolic causal determinants including lipid disorders, obesity, abdominal adiposity, abnormal blood pressure, and physical inactivity are known predisposing factors of type 2 diabetes mellitus, and cause increased likelihood of cardiovascular events (Dudek, 2014).

Gestational diabetes is physiologically progressive insensitivity to insulin during pregnancy, which leads to maternal hyperglycemia. In circumstances of uncontrolled maternal hyperglycemia, it can pose risk of adverse birth delivery such as macrosomia, resulting in cesarean section (Farrar, 2016). Unmanaged diabetes in pregnancy can increase risk of developing type 2 diabetes in women and offspring, later in life (Seller *et al*., 2016).

2.2 PREVALENCE AND BURDEN OF DIABETES MELLITUS

In 2015, about 415 million individuals had diabetes in the world, and prevalence rate was 8.8% (IDF, 2015). IDF estimated 642 million people will have diabetes across the continents by 2040.

Furthermore, it is estimated that 14.2 million people within age range 20-79 years have diabetes in Africa, with prevalence rate of 3.2% (Oguoma *et al*., 2017). In 2011, 4.5% of total population had type 2 diabetes in sub-Saharan Africa (Ganu *et al*., 2016).

In 2015, the overall population of adults (20-79 years) in Ghana was 13,880,000. In that same year, adults with diagnosed diabetes mellitus were 266,200, with prevalence rate of 1.9%.

There was also 189,900 undiagnosed people with diabetes in Ghana in 2015 (IDF, 2015).

2.3 COMPLICATION OF DIABETES MELLITUS

Diabetes mellitus is accompanied by complications which cause long-term morbidity and mortality (ADA, 2013). The persistent high blood glucose in diabetes is complicated with microvascular and macrovascular diseases (Nelms *et al.*, 2011) such as cerebrovascular accidents, coronary heart disease, cardiac autonomic neuropathy (Ferranti *et al*., 2014), myocardial infarction, congestive cardiac failure (Tracey *et al*., 2016), coronary artery diseases, peripheral artery diseases (Fowler, 2008). The microvascular diseases associated with diabetes are peripheral neuropathy, nephropathy and retinopathy (Papatheodorou *et al*., 2015). Some other less common complication that can occur are impaired wound healing, gangrene, periodontal diseases (Dudek, 2014), osteoporosis, sleep disorders and reproductive dysfunction (Ma, 2016).

Type 2 diabetes mellitus can result in macrovascular comorbidities such as hypertension and cardiovascular diseases (ADA, 2013), coronary artery diseases, peripheral artery diseases and stroke (Fowler, 2008).

2.4. 0 CARDIOVASCULAR DISEASES (CVDs)

Cardiovascular events are the main underlying factors for morbidity and deaths in developed and developing countries, accounting for 10.3% of the world's burden of diseases and 30.9 % of deaths (Francis *et al*., 2015; Yussif *et al*., 2001). Cardiovascular diseases are group of diseases that impair normal functioning of heart and blood vessels (Rolfes *et al*., 2009).

There are many different diseases which affect the blood vessels and heart but the commonly occurring which this review focuses on are coronary heart disease, stroke, peripheral arterial disease, hypertensive heart disease (WHO CVD atlas, 2016), myocardial infarction (Harris, 2013), heart failure (Kemp and Conte, 2012) and congestive heart failure (Figueora and Perters, 2006).

2.4.0.1 CORONARY HEART DISEASE

This manifests as disruption in blood supply to the coronary arteries and increases the risk of heart attack and unexpected death (Rolfes *et al*., 2009). The underlying cause is atherosclerotic plaque on arterial wall which progresses slowly and closes lumen of arteries (Webster-Gandy *et al*., 2006).

2.4.0.2 STROKE

Stroke occurs when there is an obstruction in blood flow to brain due to either blockage of arteries or bleeding of blood vessels. The obstruction is as a result of persistent atherosclerotic plaque which had formed in arterial blood vessels leading to the brain (Harris, 2013).

2.4.0.3 PERIPHERAL ARTERIAL DISEASE (PAD)

In PAD, blood supply to lower extremities of body is impeded by occlusion of peripheral atherosclerotic plague (Hernando and Conjero, 2007). The occurrence of PAD can cause hypo perfusion to tissues of lower leg, which leads to ulceration, commonly on the foot or toes (Nelms *et al*., 2010).

2.4.0.4 HYPERTENSIVE HEART DISEASE

This is abnormal blood pressure which persists with a force on the arterial walls of heart. A systolic blood pressure equal or above 120 mmHg, together with diastolic blood pressure equal or above 80 mmHg presents hypertension. Hypertension can occur asymptomatic and being unnoticed, can result in heart diseases, and peripheral vascular diseases (Mahan *et al*., 2012).

2.4.0.5 MYOCARDIAL INFARCTION (MI)

This is acute blockage of coronary artery which disrupts blood supply to heart muscles. The tissue in heart begins to die, causing loss of blood supply, due to existing atherosclerotic plaque and leads to ischemic heart disease (Harris, 2013).

2.4.0.6 HEART FAILURE

A medical condition which occurs due to impediment in adequate blood supply to heart; to maintain cardiac output that is sufficient to provide metabolic needs and venous return. The disruption of blood supply is due to heart injury from multiple causes such as diabetes, hypertension, and ischemic heart disease which cause malfunction of myocardial cells (Kemp and Conte, 2012).

2.4.0.7 CONGESTIVE HEART FAILURE

The pulmonary vascular tissues become congested and leads to decreased cardiac output. This congestion in heart impairs the function of ventricles to receive and pump blood to peripherals (Figueora and Peters, 2006).

2.4.1 DIET AND CARDIOVASCULAR DISEASES

The multifactorial risks of cardiovascular diseases are diet-related conditions such as diabetes mellitus, hypertension, abnormal blood lipids, obesity and lifestyle factors; physical inactivity and smoking (Ramirez-Prado *et al*., 2015).

Diet is a known modifiable determinant of cardiovascular diseases. It is interesting to note that dietary patterns have changed over the decades. Dietary patterns that are high in energy, low fiber, and high fat may contribute to cardiometabolic disorders; dyslipidemia, obesity, abnormal systolic blood pressure, hyperglycemia and vascular abnormalities (Appannah *et al*., 2015).

Dietary fat is an associated risk factor of developing heart-related diseases. The proportion of saturated fat and trans fatty acids in food, as well as quantity consumed can contribute to increased risk of developing heart-related diseases (Guasch-Ferre *et al*., 2015). Saturated fat is found in animal products such as cow's milk, meat, egg yolk, butter and some plants; palm kernel oil and coconut oil. Trans fat is produced from industrial hydrogenation of plant oil in

the presence of metal catalyst and high heat. Also, trans fat is made from unsaturated fat in ruminants through bacterial enzymes activities (De Souza *et al*., 2015).

However, intakes of monounsaturated and polyunsaturated sources of dietary fat is proven to decelerate the possible likelihood of cardiovascular disease (Guasch-Ferre *et al*., 2015). This is supported by meta-analysis of randomized trials by Hooper *et al*. (2015), which reported consumption of less saturated fat from 17% to 9% of total energy caused 17% decrease in risk of cardiovascular disease. Additionally, dietary patterns rich in vegetables, fruits, legumes, whole grain, nut and seeds have been shown in research to reduce the risk of cardiovascular diseases (Fleming *et al*., 2013).

2.5 DIABETES AND CARDIOVASCULAR RISK- PATHOPHYSIOLOGY

A positive correlation exists between diabetes mellitus and risk of developing atherothrombosis and cardiovascular diseases, through various mechanisms (King and Grant, 2016). The pathogenesis of diabetes and cardiovascular disease is multifactorial and complex (Dokken, 2008).

The pathogenesis of type 2 diabetes begins with insulin resistance, with subsequent high blood glucose. The pancreas compensates this defect by secreting more insulin, leading to pancreatic beta cell dysfunction, and finally insulin insufficiency (Pradeepa *et al*., 2012). Hyperglycemia prevails, and glucose cleaves without enzymes-facilitator, to protein to produce advanced glycated end products (AGES), as well as sorbitol. The accumulation of AGES and sorbitol increases oxidative stress which causes damage to endothelial cells and blood vessels (Rolfes *et al*., 2009).

Also, AGES enhance production of inflammatory markers such as cytokines, platelet-derived growth factor which up-regulate blood vessel permeability and pro-coagulant effects on the endothelium (Thomas and Foody, 2007). Moreover, insensitivity of insulin action, high blood glucose and increased oxidative stress enhance endothelial cell injury, which lead to

development of atherosclerosis. During atherosclerosis, there is raised levels of platelet activators, coagulating factors, clot formation, which can lead to development of cardiovascular diseases (King and Grant, 2016).

Meanwhile, it is well established that increased risk of cardiovascular diseases among people with diabetes is accelerated when other risk factors including abnormal blood pressure, lipid disorders, obesity and cigarette smoking are present (Martin-Timon *et al*., 2014). Atherogenic dyslipidemia, as a result of hypercholesterolemia, hypertriglyceridemia, high small dense LDL-C, low HDL-C (Taskinen and Boren, 2015) can promote occurrence of atherosclerotic events, and elevate risk of heart-related diseases (Tangvarasittichai, 2015). Atherosclerosis occurs because small dense LDL-C is pro-atherogenic and highly oxidative to cause damage to endothelial cells. Also, diabetes promotes glycation of LDL-C and HDL-C particles. Paradoxically, glycated HDL-C, which is antiatherogenic has short life time, whereas glycated LDL-C particles has long life time, to accelerate atherogenesis (Dokken, 2008).

2.5.1 DYSLIPIDEMIA

Dyslipidemia is a group of metabolic disorders classified as either one or many lipid and lipoproteins disorder: high triglyceride level, high cholesterol level, low high density lipoprotein cholesterol (HDL-C) and elevated low density lipoprotein cholesterol (LDL-C) (Kasabe *et al*., 2017). The occurrence of dyslipidemia is often accompanied by uncontrolled blood glucose, which concurrently increases risk of atherosclerosis and heart-related disease among diabetes mellitus patients (Shrestha and Khanal, 2017).

Also, the adipose tissue can be insensitive to insulin action which stimulates increased intracellular production of triglyceride with non-esterified fatty acid release (Krentz, 2003). The presence of insulin resistance increases VLDL concentration with simultaneous increase in triglyceride and decreased HDL-C levels (Nesto, 2005). The frequent dyslipidemia without

management poses greater threat for developing heart-related diseases in type 2 diabetics (Meshram *et al*., 2016).

2.5.2 ATHEROGENIC INDEX OF PLASMA (LOG TG/HDL-C) AND CORONARY RISK (TC/HDL-C RATIO)

Atherogenic Index of Plasma (AIP) is a well-established independent marker to predict cardiac occurrence of cardiovascular diseases (Sharaye, 2015). AIP is defined as a log relationship between triglyceride and high density lipoprotein cholesterol (Adu *et al*, 2015). People at risk of coronary artery disease have been shown to have elevated AIP (Rajlaxmi e*t al*., 2015). Also, a conclusion made from the study by Sushith *et al*. (2012) was that coronary artery disease was better predicted by AIP, rather than single lipid profile. This makes AIP a better substitute in assessment of atherosclerosis risk and cardiovascular diseases (Niroumand *et al*., 2015). An AIP $\leq$ 0.21 is defined as low-moderate atherosclerosis risk and AIP > 0.21 is high risk atherosclerosis, as established by Shen *et al.* (2016). However, underlying pathogenesis of log TG/HDL-C for the development of atherosclerosis is not well understood (Wen *et al*., 2015).

Moreover, the ratio of TC to HDL-C has also been established as a predictor of future cardiovascular diseases (Lafta, 2014). A study by Tohidi *et al*. (2010) has shown that the ratio of TC to HDL-C may be a better surrogate to foresee cardiovascular diseases in type 2 diabetics. The size of low density lipoprotein cholesterol is usually appraised indirectly by using TC/HDL-C. That is, TC/HDL-C determines amount of small dense LDL-C in blood. This small dense LDL-C is a good determinant of atherosclerosis and coronary diseases in type 2 diabetes mellitus (Song *et al*., 2016). TC/HDL-C ratio is used as a marker of coronary heart disease and is defined as < 3.5 as low coronary risk, whereas $\geq$ 3.5 is high coronary risk (Lima *et al*., 2011).

2.6.0 DIETARY ASSESSMENT METHODS

Dietary patterns have become significant in predicting occurrence of heart diseases (Shim *et al*., 2014). Dietary assessment methods are developed to acquire suitable data on dietary intakes, needed to promote health and also help in prognosis of cardiovascular diseases (Castell *et al*., 2015).

Dietary assessment includes food production, supply, and purchases at households and food intakes by individuals (Coulston *et al*., 2013). The 24-hour dietary recall, dietary records and food frequency questionnaire are some usual subjective tools for dietary assessment used to collect data on food intakes of individuals (Shim *et al*., 2014).

2.6.0.1 DIETARY RECORD

The individual provides written records of food and beverages, and portion sizes of each consumed food for one or more days during time of eating. The quantity of food eaten is measured with food scales or food models. Dietary record provides list on food name, cooking methods, recipes for food preparation and portion sizes. As such, dietary record provides data on food consumed during a specific period (Thompson and Subar, 2001).

2.6.0.2 FOOD FREQUENCY QUESTIONNAIRE (FFQ)

This assesses habitual food intake of selected list of foods, in terms of frequency and quantity of consumption in a specified period. The FFQ mostly focuses on dietary intake of defined nutrients which is associated with specific diseases. Also, FFQ establishes how dietary patterns relate to low intake of specified nutrients among a population (Rodrigo *et al*., 2015). Food frequency questionnaire has an advantage of providing usual dietary intake over a long period (Yanagisawa *et al*., 2016). Conversely, FFQ does not provide detailed, and accurate reflection on habitual diets of respondents, although, frequency and quantity of foods are measured (Rodrigo *et al*., 2015).

2.6.0.3 TWENTY-FOUR-HOUR DIETARY RECALL

This provides retrospective assessment of food and beverages consumed in the past day, beginning from morning to evening. The 24-hour food recall is characterized by providing information on precise description, portion sizes, food preparation methods, and where food is eaten. This dietary assessment tool provides both precise and valid data on energy and nutrients of individuals. However, it is limited by the over-reliance on memory of respondent during recalling and also has tendency to under or overestimating nutrients intake (Castell *et al*., 2015). Using Dietary Reference Intakes values, the average daily intake of zinc, vitamin E, and vitamin C were calculated for all type 2 diabetic patients (National Academy of Sciences, Food and Nutrition Board, Institute of Medicine, 2011).

Table 2.1: Range of Dietary Reference Intake of antioxidant micronutrients in type 2 diabetic patients

Dietary antioxidant micronutrients (mg/day)	**Dietary Reference Intake (DRI)**	
	Male	**Female**
Vitamin E (DRI, 2000)	15 mg	15 mg
Zinc (DRI, 2001)	11 mg	8 mg
Vitamin C (DRI, 2001)	90 mg	75 mg

(Adapted from National Academy of Sciences, Food and Nutrition Board, Institute of Medicine, 2011 in Mahan *et al*., 2012).

2.7 ANTIOXIDANT MICRONUTRIENTS AND CVD RISK IN DIABETES MELLITUS

2.7.1 VITAMIN E AND CVD RISK IN DIABETES MELLITUS

Vitamin E can occur as tocopherol and tocotrienol. The alpha tocopherol subtype of tocopherol is the most predominant in plasma of human providing health benefits (Valdes-Ramos *et al*., 2015). Vitamin E is an important antioxidant which helps protect lipid membrane against lipid peroxidation (Goud *et al*., 2016). The presence of uncontrolled hyperglycemia and lipid abnormalities predispose type 2 diabetics to stress from biomolecules oxidation through generation of free radicals, formation of advanced glycated end products, dysfunctional glutathione metabolism and oxidation of glucose (Goud *et al*., 2016).

Generation of free radicals can cause endothelial cell injuries, triggering atherosclerosis, with progressive cardiovascular diseases (Magge, 2012). Vitamin E halts propagation of chain reaction of lipid peroxyl radical formed in cells (Goldenstein *et al*., 2013). Therefore, dietary intake of vitamin E may reduce stress from oxidation and lower the likelihood of occurrence of heart-related disease in diabetes (Rafighi *et al*., 2013). Vitamin E does this by scavenging free radicals, as well as inhibiting oxidation of low-density lipoprotein cholesterol on vascular injured walls (Zhang *et al*., 2016).

2.7.2 ZINC AND CVD RISK IN DIABETES MELLITUS

Zinc is a micronutrient which influences glucose metabolism by promoting insulin sensitivity (Devi *et al*., 2016). Zinc plays such role by being involved in the synthesis, storage and secretion of insulin in pancreas (Pujar *et al*., 2014). Zinc serves as cofactor in more than 200 enzymes during metabolism of macronutrients (Olaniyan *et al*., 2012). Zinc acts as cofactor in activities of enzymatic antioxidants by removing free radicals from biomolecules, as well as stabilizing lipid membranes from lipid peroxidation (Puri *et al*., 2013).

Moreover, iron and copper are known to speed up rate of lipid peroxidation in cell membranes. Zinc strongly competes with these minerals for binding sites, and thus may prevent oxidation of lipids in diabetics (Samman and Foster, 2013). Zinc inhibits free radical formation by protecting sulfhydryl compounds of protein and enzymes from oxidative damage (Farid and Abulfaraj, 2013).

In diabetes, with uncontrolled hyperglycemia and insulin resistance, there can be altered levels of zinc which can cause tissue damage through oxidation of lipids (Puri *et al*., 2013). Plasma zinc levels were proven to decrease in type 2 diabetics in studies conducted by several investigators (Basaki *et al*., 2012; Saharia and Goswani, 2013). Moreover, decreased level of zinc is found to be associated with cardiac oxidative injury in diabetics (Song *et al*., 2005). Altered level of zinc in type 2 diabetics has been observed to be due to high zinc excretion in urine when there is persistent high blood glucose (Lee *et al*., 2016) or exogenous inadequate zinc intakes (Mahdizadeh *et al*., 2014).

2.7.3 VITAMIN C AND CVD RISK IN DIABETES MELLITUS

Vitamin C is a significant hydrophilic antioxidant present in plasma of human. It has protective role on immune function, anti-inflammatory and scavenge free radicals produced from oxidative process (Badawi *et al*., 2013). According to Song *et al*. (2009), vitamin C also donates free ion to unstable vitamin E to become stable again, and thus prevent damage from free radicals. However, vitamin C may act as a prooxidant and cause protein glycation under rare situations (Lee *et al*., 2004).

According to Valdes-Ramos *et al*. (2015), the occurrence of free radicals in biomolecules due to hyperglycemia, requires increment of vitamin C intake in type 2 diabetics. This explains why, people with diabetes mellitus normally have low level of plasma vitamin C (Lee *et al*., 2004). Studies by Carter *et al*. (2013) and Mazloom *et al*. (2011) found inverse association between fasting plasma glucose, HbA1c, and plasma vitamin C in type 2 diabetics. This makes

it important to supply vitamin C in diet so as to influence plasma concentration of total antioxidant status (Zhou *et al*., 2016).

2.8 DEFINITION OF ANTHROPOMETRIC AND BIOCHEMICAL DATA

2.8.1 BODY MASS INDEX (BMI)

Body mass index is the ratio of weight (kg) to height squared (m^2). This is usually adopted to categorize people as underweight, normal, overweight and obesity in adults. BMI values are same for both sexes (NHMRC, 2013). BMI can help predict future health outcome and functional status of individuals (Grzegorzewska *et al.*, 2016).

However, the use of BMI is limited to factors such as distribution of lean mass and body fat (Zhu *et al*., 2014), age variation, sex and ethnicity of individuals (Grzegorzewska *et al.*, 2016). Globally, being obese is a predisposing factor to increased possibility of type 2 diabetes and cardiovascular diseases (Heiss and Goldberg, 2016).

Table 2.2: The International accepted categorization of underweight, overweight and obesity in adults based on BMI

	BMI (Kg/m^2)
Categorization	**End point values**
Underweight	< 18.5
Normal range	18.5-24.9
Overweight	≥ 25.0
Pre-Obese	25.0-29.9
Obese	≥ 30.0
Obese Class I	30.0-39.9
Obese Class II	≥ 40.0

Source: Adapted from WHO 2004.

2.8.2 WAIST CIRCUMFERENCE (WC)

Waist circumference is clinically used indirectly to identify abdominal adiposity (Ayala *et al*., 2014). In overweight and obese people, it is clinically used to identify metabolic syndrome (Cetin *et al*., 2016). According to Zhu *et al*. (2014), assessing waist circumference is a powerful predictor tool to determine cardiometabolic risk factors comparable to BMI.

Abdominal obesity is also predisposing determinant for occurrence of type 2 diabetes mellitus (Saboor *et al*., 2014). Also, the presence of abdominal obesity can stimulate production of inflammatory adipokine, and thus promote risk of developing cardiovascular diseases (Heiss and Goldberg, 2016). Abdominal obesity is classified as waist circumference above 102 cm for males and more than 88 cm for females (NCEP ATP III, 2002).

2.8.3 ASSESSMENT OF BLOOD GLUCOSE

Glucose is the major end product of polysaccharide hydrolysis, found in blood circulation. The glucose is used to generate energy or transformed to glycogen for storage (Wei *et al*, 2003). However, when excess glucose is present in blood, it leads to body malfunction, causing diabetes mellitus. There is therefore the need for its monitoring in blood during clinical diagnosis (Traore *et al*, 2013). An enzymatic method is used to determine blood glucose using hexokinase, after deproteination with solution of barium hydroxide and zinc sulphate (Dohnal, 2010).

According to American Diabetes Association (2017), fasting plasma glucose can be used as diagnostic tool for prediabetes and diabetes, by showing impaired fasting glucose tolerance. Glycosylated hemoglobin is used clinically to show evidence of the concentration of blood glucose (Sherwani *et al*., 2016) over the past two to three months (Kato *et al*., 2012).

Fasting blood glucose is defined as < 126 mg/dL (< 7.0 mmol/L) as normoglycemia and ≥ 126 mg/dL as hyperglycemia (≥ 7.0 mmol/L). Moreover, HbA1c < 6.5% is defined as normoglycemia and HbA1c ≥ 6.5% is classified as hyperglycemia (ADA, 2013).

2.8.4 ASSESSMENT OF LIPID PROFILE

According to Khan *et al*. (2008), the possible likelihood of developing cardiovascular disease has been evaluated by assessing fasting blood lipids level. The presence of one or more disorders in blood lipid profile defines dyslipidemia (Samdani *et al*., 2017). The lipid disorders consist of hypercholesterolemia, hypertriglyceridemia, decreased high-density lipoprotein and elevated low-density lipoprotein. (Niroumand *et al*., 2015). Table 2.3 gives cut-off guidelines for assessing dyslipidemia.

Table 2.3: Classification of lipid profile by NCEP ATP III, (2002)

Lipid Parameter	Defining levels in mg/dL	Defining levels in mmol/L
Total Cholesterol	< 200 Normal	< 5.18 Normal
	200-239 High optimal	5.18-6.16 High optimal
	≥ 240 High	≥ 6.20 High
Triglyceride	< 150 Normal	< 1.69 Normal
	150-199 High optimal	1.69-2.24 high optimal
	200-499 Abnormal	2.25-5.64 Abnormal
	≥ 500 Very abnormal	≥ 5.65 Very abnormal
HDL-C	Men < 40 Abnormal	< 1.03 Abnormal
	≥ 40 Normal	≥ 1.03 Normal
	Women < 50 abnormal	< 1.3 Abnormal
	≥ 50 Normal	≥ 1.3 Normal
LDL-C	< 100 Normal	< 2.59 Normal
	100-129 Near normal	2.59-3.34 Near normal
	130-159 High optimal	3.37-4.12 High optimal
	160-189 Abnormal	4.14-4.89 Abnormal
	≥ 190 Very Abnormal	≥ 4.92 Very Abnormal

TC/HDL-C ratio is used as a marker of coronary heart disease and is defined as < 3.5 as low coronary risk, whereas ≥ 3.5 is high coronary risk (Lima *et al*., 2011).

Log (TG/HDL-C) was used to find for Atherogenic Index of Plasma (AIP). An AIP ≤ 0.21 is defined as low-moderate atherosclerosis risk and AIP > 0.21 is high risk atherosclerosis, as established by Shen *et al.* (2016).

2.8.4.1 TOTAL CHOLESTEROL

Cholesterol is the fat-like substance found in cell membranes. Cholesterol is carried into blood circulation by lipoproteins; HDL-C, LDL-C and VLDL. A high blood cholesterol is used as a marker for atherogenic lipoprotein; low HDL and high LDL cholesterol. These atherogenic lipoproteins are co-founding risk variables of metabolic syndrome (NCEP ATP III, 2002). Hypercholesterolemia alters permeability of arterial endothelial cells which influences influx of LDL-C particles into arterial wall. As such, high blood total cholesterol can stimulate events of atherosclerosis (Bergheanu *et al*., 2017).

2.8.4.2 TRIGLYCERIDE

In the small intestine, fats are digested to fatty acids and glycerol and later emulsified by bile to form a micelle, which is repackaged to form triglyceride. Triglycerides are picked up in the intestines and carried by chylomicron into hepatic tissue and adipose tissue (Rolfes *et al*, 2009). According to Aamir *et al*. (2015), insulin resistance is often associated with high triglyceride and this is used as marker of atherogenic dyslipidemia. Many studies have reported that occurrence of hypertriglyceridemia can predict risk of cardiovascular disease in type 2 diabetes mellitus (Khan *et al.*, 2008). It has been reported that after-meal high triglyceride can damage endothelial cells and thus put type 2 diabetics at risk of atherogenesis (Khan *et al.*, 2008).

2.8.4.3 LIPOPROTEINS CHOLESTEROL

Lipoprotein is protein transporter used to carry cholesterol in blood to various tissues. The lipoproteins assessed in serum of fasting individual is classified as low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and very low density lipoprotein (VLDL) (NCEP ATP III, 2002). VLDL and LDL-C contain apo-CIII which triggers oxidative process, leading to endothelial cells damage and atherosclerotic events (Talayero and Sacks, 2011).

Type 2 diabetes can cause increased formation of small dense LDL-C which predisposes to risk of cardiovascular diseases (Khavandi *et al*., 2017). Also, high LDL-C is a predictor of coronary heart disease in type 2 diabetes (Talayero and Sacks, 2011). However, HDL-C protects type 2 diabetics from developing atherosclerosis through its action in reverse cholesterol transport to liver for metabolism (Khan *et al.*, 2008). Pooled results of epidemiological studies have shown raised concentration of HDL-C had direct influence in reducing the risk of coronary heart disease (Hwang *et al*., 2014).

2.9.0 SYSTEMATIC REVIEW

2.9.0.1 LITERATURE SEARCH

In diabetes mellitus, there is persistent high glucose in blood and tissue insensitivity to insulin (Rafighi *et al*., 2013) which causes raised oxidative stress and subsequently, promotes development of injuries to endothelial cell (King and Grant, 2016). High glucose in blood can promote oxidative stress through formation of free radicals which result in diabetic complication (Doddigarla *et al*., 2015). This reduces their antioxidant status and it is proposed that dietary intakes of antioxidant micronutrients can play a role in lowering stress from oxidation (Tabar, 2012). Although, several reviews had been done on diabetes mellitus, there exist little data for antioxidant micronutrients relating to type 2 diabetes. According to Mahdizadeh *et al*. (2014), clinical significance of antioxidant micronutrients relating to Type 2 diabetes mellitus remains controversial which require further evaluation.

A systematic search performed from April 2012 to October, 2016 selected published studies. The search evaluated the protective role of antioxidant micronutrients (from diet and/or supplement) against CVD risk among type 2 diabetes mellitus patients. The electronic databases used in the search were Pubmed, Cochrane, PMC, google scholar and plos one. The search results identified the following articles in the respective databases: Pubmed (82), Cochrane (24), PMC (6545), google scholar (68380), and plos one (14542). Some search words phrases included antioxidant, micronutrients and risk of CVD, type 2 diabetes mellitus, dietary intakes, risk/likelihood of CVD and type 2 diabetes mellitus, serum vitamin E and type 2 diabetes mellitus, serum zinc and type 2 diabetes mellitus, serum selenium and type 2 diabetes mellitus, and serum selenium and type 2 diabetes mellitus. After thorough evaluation of search articles, most of the articles were discarded due to duplicated articles, irrelevant information, partial data, and articles that did not match inclusion criterion. After elimination of these articles, 16 articles containing full text of publications were included and evaluated. The

systematic search was to evaluate the effects/status of antioxidant micronutrients among type 2 diabetics. The exposure for the systematic review were zinc, vitamin E, and selenium, whereas main outcome was effects of antioxidant micronutrients on type 2 diabetes: reduced fasting blood glucose and HbA1c, reduced lipidemia, improved antioxidant status, reduced oxidative stress. The review included all age groups in the search strategy, although all the studies had age restriction

The search strategy and result is summarized in figure 2.1.

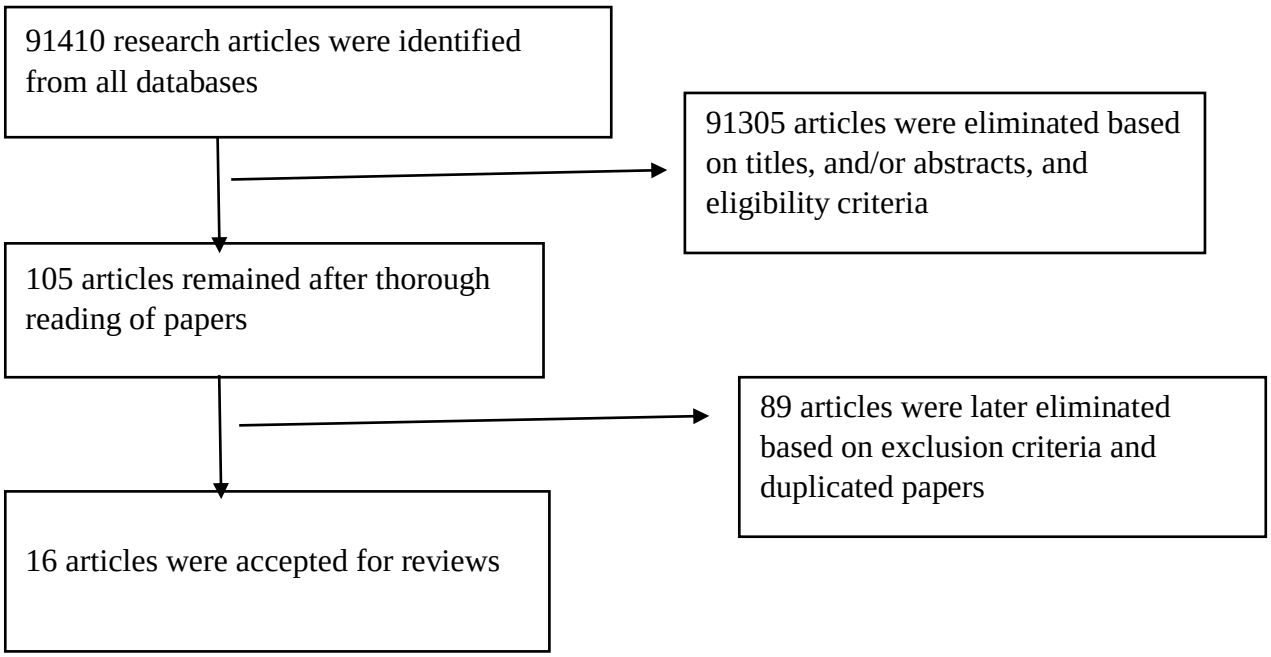

Figure 2.1: Summary of search strategy

2.9.0.2 INCLUSION CRITERIA

Study plan: cross-sectional, case-control, randomized clinical controlled trial

Study population: Human sample of all ages and gender

Outcome: Outcome measures included; status of antioxidant micronutrients among type 2 diabetics, effects of dietary supplementation of antioxidant micronutrients on type 2 diabetics.

2.9.0.3 EXCLUSION CRITERIA

Articles in press

Animal studies

Reviews

Case reports that did not meet inclusion criteria

Type 1 and gestational diabetes mellitus patients

2.9.0.4 STUDY DESIGN

Three study designs were identified in various studies. Six studies performed cross-sectional study on status of antioxidant micronutrients among type 2 diabetes mellitus patients. Another five studies used case-control to compare status of antioxidant micronutrients among people with type 2 diabetes and healthy non-diabetics. Also, five studies evaluated randomized clinical-controlled trials, where antioxidant micronutrients were incorporated in food or as supplement to type 2 diabetics and healthy non-diabetics orally, and various outcomes were measured after intervention.

2.9.0.5 STUDY POPULATION AND COUNTRY

All included articles had similar population. The subjects included were male and female type 2 diabetics and healthy controls between ages 20 and 80 years. The countries for the search were India and Iran, and other studies from Germany, Saudi Arabia, Australia, Nigeria and Nepal. This shows few studies on antioxidant micronutrients have been done in Africa in recent years.

2.9.0.6 ANTIOXIDANT MICRONUTRIENTS INCLUDED IN STUDY

The main antioxidant micronutrients considered are vitamin E, zinc and selenium. Also, other micronutrients such as copper, chromium, vitamin C, manganese, magnesium, chromium and

iron were not considered for this review but were micronutrient co-variables in the research papers used for the systematic review.

2.9.0.7 RESEARCH GAP

From the results obtained in the systematic review, it is clear ``some studies on antioxidant micronutrients remained inconclusive on association of effects of antioxidant micronutrients in reducing possible occurrence of heart-related diseases``. Therefore, further research is required to evaluate such effects of antioxidant micronutrients on type 2 diabetics. Majority of studies had no inclusion on dietary intakes of antioxidant micronutrients. Hence, there remain research gaps on relationship between dietary antioxidant intake and status among type 2 diabetics. The study is aimed at finding out if the antioxidant micronutrients intake, through the diet could ensure the build-up of sufficient antioxidants in the serum of type 2 diabetics, to protect them against cardiovascular disease risk factors. Also, there has been few studies on effects of antioxidant micronutrients in type 2 diabetes mellitus that had been done in Africa, with only two studies in Nigeria.

2.9.0.8 RESULTS ON SYSTEMATIC REVIEW

Table 2.4: Summary description of results obtained from systematic review studies

Lead Author and Country	Title	Aims	Sample size
1.Devi *et al*. (2016), India	Research on serum zinc and copper levels in type 2 diabetes mellitus	To investigate serum zinc and copper levels in type 2 diabetes mellitus patients with and without microvascular complication	120
2.Pujar *et al*. (2014), India	Correlation between serum zinc, magnesium, copper and HbA1c on type 2 diabetics at Bagalkot: A case-control study	To investigate the serum zinc, magnesium and glycated hemoglobin in patients with type 2 diabetes and healthy control	100

3.Tabar (2012), Iran	Determination of serum selenium in patients with type 2 diabetics	To investigate association between serum selenium levels in type 2 diabetics patients and healthy control treatment	80
4.Myke-Mbata *et al*. (2015), Nigeria	Variation in some trace elements in diabetes mellitus	To evaluate serum zinc, copper, chromium and magnesium as indices of chronic complication in diabetes mellitus and compare the parameters among subjects with diabetic complications.	209
5.Mahmoo-di *et al*. (2014), Iran	The effects of omega-3 plus vitamin E, zinc and vitamin C supplementation on cardiovascular risk markers in postmenopausal women with type 2 diabetes	To examine effects of omega-3 plus vitamin E, zinc and vitamin C supplementation on CVD risk markers in postmenopausal women with type 2 diabetes	69
6.Samman *et al*. (2013), Australia	Effects of zinc and alpha-linoleic acid supplementation on glycemia and lipidemia in women with type 2 diabetes: a randomized, double-blind placebo-controlled trial	To investigate effects of zinc and alpha-linoleic supplementation on markers of glycemia, and lipid levels in type 2 diabetes mellitus	48
7.Goud *et al*. (2016), India	A study on antioxidants and iron nutritional status in type 2 diabetes mellitus with and without coronary heart diseases	To investigate the antioxidant and iron nutritional status in type 2 diabetes mellitus with and without coronary heart diseases	60
8.Vafa *et al*. (2015), Iran	Effects of tocotrienol-enriched canola oil on	To investigate effects of tocotrienol on blood glucose, insulin sensitivity and	50

	glycemic control and oxidative status in patients with type 2 diabetes mellitus: A randomized double-blind placebo-controlled clinical trial	oxidative stress in type 2 diabetics	
9. Rafighi *et al*. (2013), Iran	Association of dietary vitamins C and E intake and antioxidant enzymes in type 2 diabetes mellitus patients	To investigate outcome of vitamins C and E supplementation on type 2 diabetes mellitus patients	170
10. Olaniyan *et al*. (2012), Nigeria	Serum copper and zinc levels in Nigeria type 2 diabetic patients	To determine serum levels of zinc and copper in type 2 diabetic patients and their association with age, gender, glycemic status and duration of diabetes	103
11. Lee *et al*. (2016), Germany	Effect of a fermented dietary supplement containing chromium and zinc on metabolic control in patients with type 2 diabetes: A randomized, placebo-controlled, double-blind cross-over study	To investigate the effect of a fermented dietary supplement based on fruits, vegetables, nut and fortified with chromium, zinc on metabolic control in type 2 diabetes mellitus	36
12. Farid and Abulfaraj, (2013), Saudi Arabia	Trace mineral status related to levels of glycated hemoglobin of type 2 diabetic subjects in Jeddah, Saudi Arabia.	To compare some mineral status of type 2 diabetes mellitus patients and non-diabetic healthy individuals	110
13. Udupa *et al*. (2012), India	Study of comparative effects of antioxidants on insulin sensitivity in type 2 diabetes mellitus	To examine effect of alpha lipoic acid, omega-3 fatty acid and vitamin E on insulin sensitivity, oxidative stress, lipid metabolism and	94

		glycemic control in type 2 diabetics	
14. Ramaswamy *et al*. (2016), India	Micro and macro nutrients status in type 2 diabetics evaluating the significance of cation ratios	Relationship between calcium, magnesium, zinc, chromium, and HbA1c among type 2 diabetics	73
15. Saharia and Goswani, (2013), India	To investigate association between serum zinc and HbA1c of type 2 diabetes mellitus patients in a tertiary care hospital of Assam	To investigate relationship between serum zinc and HbA1c among type 2 diabetics	100
16. Mahdizadeh *et al*. (2014), Iran	Investigation of imbalances of trace elements in individuals with type 2 diabetes	To assess serum levels of trace elements of zinc and copper, BMI, FBS and HbA1c among people with type 2 diabetes compared to healthy controls	-

Table 2.4.1: Summary of study design, main findings and knowledge gap

Study Design	Micronutrients	Outcome measures	Main findings	Knowledge gap
1.Cross-sectional	Zinc and copper	Serum zinc and copper	Serum zinc levels were lower for type 2 diabetic patients with complications than patients without complications and control group	Much research is recommended for further observation
2.Cross-sectional (case-control)	Zinc, copper and magnesium	Fasting plasma glucose, serum zinc, copper, magnesium and	There was inverse relationship between serum	Further studies are recommended to examine

		glycated hemoglobin	zinc, magnesium and HbA1c among type 2 diabetics. Study population had significantly lowered levels of zinc and magnesium compared to control	molecular mechanism of zinc, magnesium and copper in pathogenesis of diabetic complications
3.Cross-sectional	Selenium	Fasting blood glucose, HbA1c and serum selenium	There was reduction in serum selenium in type 2 diabetics which indicated a possible metabolic response to oxidative stress	There is need of daily supplementation of adequate selenium in diet of diabetics to enhance antioxidant defence system
4.Cross-sectional	Copper, zinc, magnesium and chromium	HbA1c, serum copper, zinc, magnesium and chromium	Serum magnesium and zinc were significantly lowered among type 2 diabetics, compared with healthy control, whereas copper was significantly increased among type 2 diabetics than healthy controls	Trace elements evaluation and supplementation for all ages can be helpful in management of type 2 diabetes mellitus
5.Randomized double-blind placebo controlled clinical trial	Vitamin C, vitamin E and zinc	Glycated hemoglobin, plasma insulin, plasma total cholesterol, LDL-C, apolipoproteins	LDL-Cholesterol was significantly decreased in type 2 diabetics with duration equal or less	Short duration of supplementation

		A1 and B100, HOMA-IR	than 7 years in the group receiving omega-3 fatty acid plus vitamins E and C	
6.Randomized, double-blind placebo-controlled trial (RCT)	Zinc	Food record, serum total cholesterol, triglyceride, HDL-C, random glucose, HbA1c, plasma insulin and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR)	The present RCT did not demonstrate beneficial effect of zinc, with or without alpha-linoleic acid supplementation on glycemia and lipidemia in medically-controlled type 2 diabetic patients with adequate dietary zinc and plasma zinc concentration within reference intervals	Small sample size was used. Low doses of supplementation used
7.Cross-sectional (case-control)	Vitamin E and iron	Fasting blood glucose, postprandial blood glucose, serum total cholesterol, triglyceride, HDL-C, LDL-C, VLDL, malondialdehyde, serum vitamin E, ferritin, transferrin, iron	Dyslipidemia, increased lipid peroxidation, inflammation and oxidative stress were associated with diabetics, compared to non-diabetics, and oxidative stress further exposes diabetics to cardiovascular diseases.	Small sample size was used.
8. Randomized,	Vitamin E	Fasting blood glucose, fasting	Tocotrienol can improve fasting	Suboptimal doses of

double-blind placebo-controlled trial (RCT)		insulin, total antioxidant capacity, malondialdehyde, HOMA-IR	blood glucose concentration and reduce oxidative stress parameters in type 2 diabetic patients with uncontrolled blood glucose and can be considered in combination with hypoglycemic drugs to better control type 2 diabetes. Tocotrienol had no effects on fasting insulin and HOMA-IR.	tocotrienol rich fraction was used
9.Case-control trial	Vitamins C and E	Blood pressure, HbA1c, fasting plasma glucose, plasma vitamin C, E, glutathione peroxidase and superoxide dismutase (SOD)	Three months' supplementation of vitamins C and E separately and in combination showed significant decrease in blood pressure, improved insulin action, increased level of GSH and SOD activities	Suitable diet containing vitamins C and E need to be appraised.
10.Cross-sectional	Zinc and copper	Serum zinc, copper and fasting plasma glucose	Serum zinc was significantly lowered among type 2 diabetics, compared to non-diabetics, whereas serum copper was significantly	Further studies should consider HbA1c as glycemic test.

			higher in diabetics than non-diabetics. No correlation was found between age, gender, glycemic status, and duration of diabetes with serum concentration of micro elements in individuals with type 2 diabetes	
11. Randomized, double-blind controlled trial	Zinc and chromium	Glycated hemoglobin, FBG, Fructosamine and lipid profile	The intervention study did not support evidence that a fermented plant-based dietary supplement enriched with chromium and zinc improved glucose metabolism in type 2 diabetes mellitus	No positive influence on measured outcome
12. Cross-sectional	Zinc, magnesium, chromium, copper and manganese	HbA1c, serum zinc, magnesium and chromium	Type 2 diabetes had altered metabolism of zinc, copper, chromium, magnesium and these were associated with increased glycated hemoglobin	No dietary assessment data was recorded
13. Randomized,	Vitamin E	Serum vitamin E, serum alpha-lipoic acid, FBG, HbA1c, waist	Vitamin E was significantly decreased in parameters of	No dietary intake was assessed

double-blind controlled trial		circumference and BMI	oxidative stress and insulin resistance, compared to placebo group	
14. Cross-sectional (case-control)	Zinc, magnesium, chromium and calcium	HbA1c, serum zinc, magnesium and chromium	Type 2 diabetes was associated with altered micro and macro nutrients, and serum zinc was significantly reduced in type 2 diabetic patients	No dietary assessment data
15. Cross-sectional	Zinc	HbA1c, FBG and serum zinc	There was significant reduction of serum zinc in type 2 diabetic patients, and inverse relationship between HbA1c and serum zinc	Further studies on effectiveness of intervention with zinc supplement
16. Cross-sectional (case-control)	Copper and zinc	HbA1c, serum zinc, serum copper, FBG, BMI	There was significant reduction of serum zinc in type 2 diabetic patients compared to controls	Clinical significance of trace element is still controversial

2.9.0.9 DISCUSSION ON SYSTEMATIC REVIEW

The systematic review conducted included six cross-sectional studies, five randomized double-blind controlled- clinical trials, and five case-control studies. The results obtained from six cross-sectional studies indicated that serum antioxidants micronutrients such as zinc and selenium were significantly lowered in type 2 diabetes, compared to non-diabetics. It is noted that serum zinc level had negative correlation with glycated hemoglobin levels of type 2 diabetic patients (Farid and Abulfaraj, 2013; Saharia and Goswani, 2013). This implies that an increase in zinc concentration in blood will cause decrease in glycated hemoglobin in type 2 diabetics and further contribute to reduce the possible likelihood of oxidative stress. Additionally, it was observed that type 2 diabetics with HbA1c above 8% had strong correlation with regard to altered serum zinc, compared to those with HbA1c less than 8% (Farid and Abulfaraj, 2013). However, a study by Olaniyan *et al*. (2012) had no association between serum zinc and fasting blood glucose in type 2 diabetics.

According to Farid and Abulfaraj (2013), reduced serum zinc levels in type 2 diabetics can be due to high rate of removal of zinc in urine, as a result of hyperglycemia in uncontrolled diabetes. Also, it could be that, significant reduction of antioxidant micronutrients is an indicator of metabolic response to oxidative stress occurring among type 2 diabetics (Tabar, 2012). According to Myke-Mbata *et al*. (2015), reduced concentration of antioxidant micronutrients may contribute to imbalance of antioxidant and oxidant free radicals in type 2 diabetes mellitus.

Moreover, results obtained from five case control studies showed serum zinc, vitamin E were significantly decreased in type 2 diabetics (zinc: 67.50 µg/dL, vitamin E: 0.595±0.393 mg/dL) compared to healthy groups (zinc: 89.61±61 µg/dL, vitamin E: 1.400±0.241 mg/dL) (Pujar *et al*., 2014; Goud *et al*., 2016, Udupa *et al*., 2012). Also, giving vitamins C and E supplements to type 2 diabetics for three months had significant reduction in blood glucose parameters,

compared to placebo (Rafighi *et al*., 2013). Hence, supplementation of vitamin C or vitamin E can possibly reduce insulin resistance and oxidative stress in type 2 diabetes mellitus.

The results revealed from five randomized controlled-clinical placebo trials (RCT) showed some beneficial effects when type 2 diabetic patients were provided with alpha-tocopherol and ascorbic acid. Other studies done on zinc reported zinc supplement did not improve blood glucose among type 2 diabetics. According to Vafa *et al*. (2015), 200 mg/day fortification of tocotrienol in canola oil, given to type 2 diabetic patients for eight weeks, reduced fasting blood glucose by 15.4%, compared to type 2 diabetic patients who were given pure canola oil. Similar result was observed by Udupa *et al*. (2012), who found significant improvement in blood glycemic level as well as reduced total cholesterol in type 2 diabetics, given vitamin E supplement. In addition, the findings of Vafa *et al*. (2015) showed significant improvement of total antioxidant capacity and reduced oxidative stress of type 2 diabetic patients who received tocotrienol-enriched canola oil.

In a study by Mahmoodi *et al*. (2014), it was found that type 2 diabetic patients who had the diabetes duration less than 7 years and received zinc plus vitamin C supplementation had significant decreased in HbA1c levels, compared to same type 2 diabetic group with duration more than seven years. It could be inferred from this study that oxidative stress and atherosclerosis may be long progressive complication which can weaken antioxidant defence of type 2 diabetic patients.

Notwithstanding, some RCT showed no beneficial effects on type 2 diabetic patients. A research by Samman *et al*. (2013) demonstrated no beneficial effects on glycemia and lipidemia in type 2 diabetics when zinc was supplemented for 12 weeks intervention. The same result was seen by Lee *et al*. (2016) after 15 mg supplementation of zinc.

2.9.0.10 CONCLUSION ON SYSTEMATICC REVIEW

The results support evidences that antioxidant micronutrients could significantly protect type 2 diabetics against cardiovascular events. Results analyzed in RCT studies showed supplementation of vitamin E is associated with reducing cardiovascular risks in type 2 diabetes mellitus, except that RCT studies on zinc supplementation had no significant association on CVDs risk parameters in type 2 diabetes mellitus.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.0.1 MATERIALS

Household handy measures, measuring tape, Deseco manual weighing scale, Seca 213 mobile stadiometer, EDTA tubes, fluoride tubes, eppendorf tubes, gel and clot activator vacuum tubes, fasting blood glucose reagents, total cholesterol reagent, triglyceride reagent, HDL-C reagent, lysing reagent, zinc reagent, water bath, integra 400 plus machine, eppendorf centrifuge, semi-automated spectrophotometer, well plates, multimode microplate reader,

3.1 STUDY DESIGN

A cross sectional study design was adopted.

3.2 STUDY SITE

Komfo Anokye Teaching Hospital (KATH) was the location for the study. Komfo Anokye Teaching Hospital is one of the well-equipped teaching hospitals in Ghana and the largest tertiary health institution in the middle and northern belt of Ghana. The Teaching Hospital has Diabetes Clinic which provides medical care for diabetic patients from Ashanti Region and referrals from Brong Ahafo, Northern, Upper East and Upper West Regions.

3.3 STUDY POPULATION

The study included outpatient type 2 diabetics. The prevalence rate of type 2 diabetes in urban Ghana was 6.0 percent (Danquah *et al*., 2012). Also, the diabetic clinic of Komfo Anokye Teaching Hospital recorded 9000 diabetic attendance in 2011.

3.4 ETHICAL CONSIDERATION

Approval was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST, Kumasi, (CHRPE/AP/463/16) to

undertake the study. All participants of this study signed an informed consent form, in accordance with CHRPE regulations, before conducting the study.

3.5 SAMPLE SIZE CALCULATION

The Cochrane's formula below was adopted to determine sample size for this study:

$N= Z^2 p(1-p)/d^2$

N represents sample size

Z= confidence level = 95 % (Z-score standard value = 1.96)

p = Estimated prevalence of type 2 diabetics = 6% = 0.06

d = marginal error

$N= 1.96^2 \times 0.06 (1-0.06)/0.05^2$

$N= 3.8416 \times 0.06 (0.94)/0.0025$

N= 86.7= 87. 75% × 87= 65. 65 + 87 = 152

The minimum sample size is 87, however, 75 % accretion was calculated to obtain convenient sample size of 152, to obtain a large study population.

3.6 INCLUSION CRITERIA

Outpatient type 2 diabetics who gave consent to undertake the study.

Outpatient type 2 diabetics within age ranges 35 to 80 years were included in the study.

Outpatient type 2 diabetics taking trace elements medication and vitamins supplement were included in the study.

3.6.1 EXCLUSION CRITERIA

The study excluded gestational and type 1 diabetics. Type 2 diabetics who had serious comorbidities such as HIV/AIDS and tuberculosis were also excluded.

3.7 SAMPLING PROCEDURE

A random sampling method was used to recruit study participants based on the study's inclusion criteria. Outpatient type 2 diabetics who attended the Diabetes Clinic during October and November 2016, were first educated on the purpose of the study. Afterward, participants were randomly approached and asked about their willingness to undertake the study. Out of the 250 type 2 diabetics requested to partake in the research, only 152 gave consent to participate in the study.

3.8 DATA COLLECTION

Structured questionnaires were used to gather data on patients' demographic characteristics, antioxidant micronutrients intake, physical activity, usual dietary intake, and medical history.

3.8.1 DIETARY ASSESSMENT

A food frequency questionnaire (FFQ) containing a list of 55 common foods was used to assess dietary patterns of antioxidant micronutrients intake of study participants over six months. The FFQ included five frequency categories ranging from "daily" to "never." A 24-hour duplicate recall on one weekday and a weekend was used to assess current and usual dietary intakes of study participants (This was done because majority of participants reported consuming same meals daily). Handy measures of food items were used to allow participants to quantify amount of food eaten. The weight (in grams) of foods consumed by participants were recorded using handy measures provided by University of Ghana, Food Science and Nutrition Department (2010). The composition of nutrients in foods were analyzed with Nutrient Analysis Template (Food Science and Nutrition Department, University of Ghana, 2010).

3.8.2 ANTHROPOMETRIC DATA

The weight of study participants was measured with weighing scale (Deseco manual weighing scale, India). A stadiometer (Seca 213 mobile stadiometer, Germany) was used to take height,

and BMI was calculated using weight (in kilogram) and height square (in meter). Waist circumference was taken by putting measuring tape just above the navel of study participants, and minus 0.1 cm was deducted because participants were in clothes.

3.9 BIOCHEMICAL DATA

Study participants were asked to eat their supper by 6 pm; for the blood sample to be taken the next morning. Study participants were seated 5 minutes prior to phlebotomy. Blood samples were taken from each type 2 diabetic outpatient by venipuncture after an overnight fast. The collected blood samples were put into fluoride tubes and clot activator tubes. Seven milliliters of blood samples were collected from each participant.

3.9.1 GLUCOSE TEST PRINCIPLE

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow[\text{Glucose oxidase}]{} \text{D-gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{hydroxybenzoate} + \text{4-aminoantipyrine} \xrightarrow[\text{Peroxidase}]{} \text{Quinoneimine dye} + H_2O.$$

Glucose is oxidized by glucose oxidase to produce gluconic acid and hydrogen peroxide. The hydrogen peroxide formed reacts with hydroxybenzoate and 4-aminoantipyrine in the presence of peroxidase to produce a red-violet quinoneimime dye.

3.9.1.0.1 FASTING BLOOD GLUCOSE (FBG)

Two ml of blood samples were taken using phlebotomy into fluoride tubes. The tubes were allowed to stand for about 10 minutes and centrifuged for 5 minutes in an automatic integra 400 plus centrifuging machine (Roche Diagnostics GmbH, Germany) for plasma. Ten µL plasma was put in dry test tubes and 1 mL glucose reagent was added. The test tubes were incubated for 10 minutes in water bath at $37\ ^0$C. Ten µL of deionized water was pipetted into the sterile test tube and 1 mL glucose reagent was added to make the blank. A semi-automated spectrophotometer (Biolabo Diagnostic Kenza Biochemistry Try, France) was used to read

absorbance at 510 nm, which determined concentration of blood glucose. The final colour change produced a red-violet quinoneimime dye. The same procedure was used to determine fasting blood glucose for all samples. The test principle in the glucose is summarized in the equation below.

3.9.1.1 PRINCIPLE OF GLYCOSYLATED HEMOGLOBIN MEASUREMENT

The whole blood is hemolyzed and mixed continuously for 5 minutes with weak binding cation-exchange resin. The HbA_0 adheres to resin at time of mixing. The non-glycosylated hemoglobin attaches to the resin and detach glycosylated hemoglobin in the precipitate. After mixing, a filter divides the precipitate binding the glycosylated hemoglobin from resin. The percentage of glycosylated hemoglobin is determined by measuring the ratio of absorbance of glycosylated hemoglobin fraction to total hemoglobin fraction (Travelli *et al*., 1978; Gonen and Rubenstein, 1978; Gabbay *et al*., 1977).

3.9.1.1.1 GLYCOSYLATED HEMOGLOBIN (HBA1C) TEST

Two ml of venous blood samples were taken into EDTA tubes and mixed well.

Hemolysate Preparation

A 250 µL of lysing reagent was pipetted into labelled sample tubes. Then, 50 µL of well mixed whole blood samples were put into each labelled tube and mixed well again. Labelled sample tubes were incubated for 5 minutes at room temperature to allow complete lysis of red blood cells.

Glycosylated Hemoglobin Determination

The stopper of pre-filled resin tubes was removed and 100 µL of each hemolysate tube was added. The resin separator was inserted into resin tube in a way so that the rubber sleeve of the resin separator was placed 1 cm above the surface of the resin suspension. Labelled hemolysate tubes were put on rotator and mixed continuously for 5 minutes. The labelled hemolysate tube

was removed from rotator and resin separator was pushed into labelled tubes until resin was firmly packed at the bottom and allowed for supernatant to come into separator tube. The supernatant was poured into another cuvette for absorbance to be read at 415 nm, against distilled water blank.

3.9.2 SERUM LIPID PROFILE (SERUM TOTAL CHOLESTEROL, TRIGLYCERIDE, HDL-C, LDL-C)

Three ml of blood samples were put into gel and clot activator vacuum tubes (ChannelMed, England). The tubes were allowed to stand for about 30 minutes. Centrifugation machine (Eppendorf Centrifuge 5804, Eppendorf Netheler- Hinz, Germany) at 4000 rpm for 10 minutes was used to separate the serum from sediment. The supernatant serum was subjected to HDL-cholesterol, total cholesterol and triglycerides analysis using reagents manufactured by Fortress Diagnostics Limited, United Kingdom.

3.9.2.1 SERUM HDL-C CONCENTRATION

HDL-cholesterol was determined using semi-micro assay by pipetting 200 µl of serum sample, using sterile automatic micropipette (Pipette plus, USA) into a labeled sterile test tube followed by addition of 500 µl of the HDL precipitant reagent, containing 0.55 mmol/L phosphotungstic acid and 25 mmol/L magnesium chloride (which precipitated LDL-cholesterol, to leave HDL-cholesterol as supernatant). The labeled sterile test tubes were mixed gently and incubated for 10 minutes at room temperature and then centrifuged for 10 minutes at 4000 rpm (Eppendorf Centrifuge 5804, Eppendorf Netheler- Hinz, Germany) and HDL-cholesterol content was determined using CHOD-PAP method. Hundred microliter of deionized water was pipetted into the sterile test tubes and 1 ml of HDL reagent was added to make the blank. Hundred microliter of serum HDL supernatant was pipetted into sterile test tubes and 1 ml of HDL reagent added. The test tubes contents were mixed gently and incubated for 5 minutes at 37^0C

in water bath. A semi-automated spectrophotometer (Humalyzer Junior, Human GmBH, Germany) measured concentrations of the HDL at 500 nm with a 1cm light path cuvette.

3.9.2.2 TOTAL CHOLESTEROL TEST PRINCIPLE

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty Acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHO}} \text{Cholesten-3-one + } H_2O_2$$

$$2\ H_2O_2 + \text{4-aminoantipyrine + phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + } 4\ H_2O$$

CHE = cholesterol esterase; CHO = cholesterol oxidase (Fortress Diagnostics, United Kingdom).

3.9.2.2.1 SERUM TOTAL CHOLESTEROL CONCENTRATION

Ten microliter of serum sample was pipetted using sterile automatic micropipette (Pipette plus, USA) into a labeled sterile test tube, followed by addition of 1000 µl of the cholesterol reagent containing three key enzymes; cholesterol esterase, cholesterol oxidase and peroxidase (the peroxidase reaction forms a coloured dye which has intensity directly related to concentration of cholesterol in the sample). Thousand microliter of reagent was then added to 10 µl of deionized water in a labeled test tube to form the blank. The test tube contents were then stirred gently and put in a water bath at 37^0C to incubate for 5 minutes. A semi-automated spectrophotometer (Biolabo Diagnostic Kenza Biochemistry Try, France) measured concentrations of the total cholesterol at 500 nm with a 1cm light path cuvette.

3.9.2.3 TRIGLYCERIDE TEST PRINCIPLE

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK,\ Mg^{2+}} \text{glycerol-1-phosphate} + ADP$$

$$\text{Glycerol-1-phosphate} + O_2 \xrightarrow{GPO} H_2O_2 + \text{dihydroxyacetone phosphate}$$

$$H_2O_2 + \text{p-chlorophenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

GK= glycerol kinase; GPO= glycerol phosphate oxidase (Fortress Diagnostics, United Kingdom).

3.9.2.3.1 SERUM TRIGLYCERIDE CONCENTRATION

Total triglyceride was determined by pipetting ten microliters of serum sample using sterile automatic micropipette (Pipette plus, USA) into a labeled sterile test tube followed by addition of 1000 µl of the triglyceride reagent. The test tube was then stirred gently and put in water bath at 37^0C to incubate for 5 minutes. A semi-automated spectrophotometer (Humalyzer Junior, Human GmBH, Germany) was used to determine concentration of triglyceride by reading absorbance of the coloured dye (quinoneimine dye by catalytic influence of peroxidase) at 505 nm with 1cm light path cuvette. The triglyceride reagent contains four key enzymes; lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase. The final colour produced was red-coloured quinoneimine dye. The process was repeated for all samples to determine level of HDL, TC and TG.

The concentration of LDL-cholesterol in all samples was determined with Friedewald formula; LDL-C = TC − [HDL-C+($\frac{TG}{2.2}$)] (mmol/l). Also, VLDL was determined using the formula; VLDL = ($\frac{TG}{2.2}$) (Fortress Diagnostics, United Kingdom).

3.9.3 ANTIOXIDANT MICRONUTRIENT STATUS (SERUM ZINC)

3.9.3.1 ZINC TEST PRINCIPLE

The 2-(5-Nitro-2-pyridylazo)-5-(N-Propyl-N-sulfopropylamino) phenol disodium salt (Nitro-PAPS) is the highly sensitive colorimetric reagent in the zinc reagent which is used for the direct determination of the zinc. The Nitro-PAPS reacts with zinc in alkaline medium to generate purple complexes.

3.9.3.1.2 DETERMINATION OF SERUM ZINC

Three ml of blood samples were put into gel and clot activator vacuum tubes (ChannelMed, England). The tubes were allowed to stand for about 30 minutes. Centrifugation machine (Heraeus Christ- Labofuge A, Germany) at 4000 rpm for 10 minutes was used to separate the serum from sediment. The serum was later transferred to eppendorf tubes and refrigerated at -20 ^{0}C until further analysis. Serum zinc was determined using full automated Multimode Microplate Reader (Jos-Hansen and Soehne GmbH, Germany) by pipetting 12 µL sample followed by addition of 200 µL zinc reagent 1 into well plates, and incubated at 37 ^{0}C for 5 minutes. Then, 50 µL zinc reagent 2 was added to same well plates containing sample and reagent 1 and also incubated at 37 ^{0}C for 5 minutes. A blank sample was prepared in well plate and incubated for same period of time. Five standard concentrations were prepared in serial dilution, using 0.9% sodium chloride. The standards were measured in order to plot standard curve. The reagent contains Nitro-PAPS, bicarbonate buffer pH 9.8. A fully automated Multimode Microplate Reader (Jos-Hansen and Soehne GmbH, Germany) was used to determine concentrations of serum zinc by measuring absorbance of the purple colour at 570 nm. Laboratory reference protocol for normal serum zinc used was 66-110 µg/dL (Biobase Bioindustry Company limited, China).

3.10 DATA ANALYSIS

Statistical Package for the Social Sciences (SPSS), version 23 (SPSS Inc Chicago, IL) by IBM Corporation was adopted for data analysis. Categorical variables for sociodemographic data were analysed using descriptive statistical analysis and presented as absolute and relative frequencies. Continuous variables for anthropometric and biochemical parameters were analysed to give means and standard deviations. The independent t- test was used to find if there were statistically significant difference between the mean values of the anthropometric data such as waist circumference, body mass index, and biochemical parameters such as fasting blood glucose, glycated hemoglobin, lipid profile and serum zinc among participants with and without coronary risk.

Partial correlation, controlling for age and gender was used to find association between antioxidant micronutrients intake and cardiovascular risk factors. Additionally, partial correlation, controlling for age, gender and zinc intake was used to find association between antioxidant micronutrients status and cardiovascular risk factors. Bivariate correlation was also used to find association between biochemical parameters, and coronary risk and atherogenic index of plasma. A binary logistic regression was done to calculate odds ratio in order to estimate prevalence risk of CVD risk factors and antioxidant micronutrients status. Nutrient intakes were analyzed using Nutrient Analysis Template Software. The average daily intakes of vitamin E, zinc and vitamin C were compared with Dietary Reference Intakes. All analyses were reckoned to be significant at p value ≤ 0.05.

CHAPTER FOUR

4.0 RESULTS

The study reports sociodemographic data, dietary intakes using 24-hour food recall and food frequency questionnaire, anthropometric data and biochemical data collected from 152 outpatient type 2 diabetics, who attended Komfo Anokye Teaching Hospital Diabetes clinic. The biochemical data were on fasting blood glucose, glycated hemoglobin, lipid profile, and serum zinc.

4.1 SOCIODEMOGRAPHIC CHARACTERISTICS OF THE TYPE 2 DIABETIC PATIENTS

The study involved 152 participants; 37 (24.3%) males and 115 (75.7%) females. From Table 4.1, majority of participants (65.8%) were within age range 40-59 years. Fifty-one (33.6%) of them had history of alcohol intake, and 7 (4.6%) currently consume alcohol. Ten (6.6%) had smoked before. Seventy-six (50.0%) had family history of any of the cardiovascular diseases (CVDs), including stroke, hypertensive heart disease, coronary heart disease and heart failure. Majority of study participants (126, 82.9%) had lived with diabetes for 5 years and above, while twenty-six (17.1%) participants had diabetes less than 5 years. There were 75 (49.3%) who had hypertensive heart disease, while 1 (0.7%) had both hypertensive heart disease and stroke, and 1 (0.7) also having myocardial infarction. Sociodemographic parameters were compared between type 2 diabetics with and without coronary risk. Although, there was differences in frequencies of sociodemographic parameters of type 2 diabetics with and without coronary risk, it was statistically not significant (p values > 0.05).

Table 4.1: Demographic characteristics of outpatient type 2 diabetic patients

Sociodemographic data	Total, N= 152	Without CR N=98	With CR N=54	p value
Gender				
Male	37(24.3)	25 (67.6)	12 (22.2)	0.697
Female	115(75.7)	73 (63.5)	42 (36.5)	
Age range (years)				
18-39	5(3.3)	4 (80.0)	1 (20.0)	
40-59	100(65.8)	63 (63.0)	37 (37.0)	0.717
60-80	47(30.9)	31 (66.0)	16 (34.0)	
Education level				
Primary	9(5.9)	6 (66.7)	3 (33.3)	
JHS	63(41.5)	40 (63.5)	23 (36.5)	
SHS/0 Level	36(23.7)	25 (69.4)	11 (30.6)	0.960
Tertiary	21(13.8)	13 (61.9)	8 (38.1)	
Uneducated	23(15.1)	14 (60.9)	9 (39.1)	
Past alcohol intake				
Yes	51(33.6)	34 (66.7)	17 (33.3)	
No	101(66.4)	64 (63.4)	37 (36.6)	0.723
Current alcohol intake				
Yes	7(4.6)	7 (100.0)	0 (0.00)	
No	145(95.4)	91 (62.8)	54 (37.2)	0.051
Past smoking				
Yes	10(6.6)	8 (80.0)	2 (20.0)	
No	142(93.4)	90 (63.4)	52 (36.6)	0.496
Family history of CVD(s)				
Yes	76(50.0)	44 (57.9)	32 (42.1)	
No	64(42.1)	44 (68.8)	20 (31.3)	0.149
I don't know	12(7.9)	10 (83.3)	2 (16.7)	
CVDs				
Hypertensive heart disease	75 (49.3)	46 (61.3)	29 (38.7)	
Hypertensive heart disease and stroke	1 (0.7)	1 (100.0)	0 (0.0)	0.611
Myocardial infarction	1 (0.7)	1 (100.0)	0 (0.0)	
No	75(49.3)	51 (67.1)	25 (32.9)	
Duration of DM (years)				
Less than 5	26(17.1)	15 (57.7)	11 (42.3)	
5-10	55(36.2)	38 (69.1)	17 (30.9)	0.597
11-15	35(23.0)	24 (68.6)	11 (31.4)	
More than 15	36(23.7)	21 (58.3)	15 (41.7)	

Data shown as frequency (Percentages) for categorical variables. Categorical data with 3 or more groupings were compared using chi-square and 2 groupings were compared using Fischer's exact test. DM: Diabetes Mellitus (Type 2), CVDs: Cardiovascular diseases, CR: Coronary risk (TC/HDL-C), CR <3.5 as low, CR ≥3.5 as High.

4.2 ANTHROPOMETRIC AND BIOCHEMICAL PROFILE OF TYPE 2 DIABETIC PATIENTS

Table 4.2 shows means ± standard deviation (SD) of anthropometric and biochemical parameters of outpatient type 2 diabetics with or without coronary risk.

The mean TC, TG, LDL-C, VLDL levels were significantly higher among type 2 diabetics with coronary risk (TC= 5.9±1.3 mmol/L, TG= 1.3±0.6 mmol/L, LDL-C= 3.8±0.9 mmol/L, VLDL= 0.6±0.3 mmol/L), compared to type 2 diabetics without coronary risk (TC= 4.3±1.2 mmol/L, TG= 1.0±0.4 mmol/L, LDL-C= 2.3±0.9 mmol/L, VLDL= 0.4±0.2 mmol/L) (TC: p value = 0.000, TG: p value = 0.007, LDL-C: p value = 0.000, VLDL: p value = 0.004 as shown in Table 4.2). Also, a significantly higher mean intake of zinc was seen among type 2 diabetics with coronary risk (6.2±3.1 mg/day), compared to those without coronary risk (4.9±2.4 mg/day, p value = 0.014). Although, mean intake of vitamin C was higher in type 2 diabetics without coronary risk (86.7±40.3 mg/day) compared to those with coronary risk (75.5±34.9 mg/day), it was not significant (p value = 0.075). Other parameters such as age, BMI, waist circumference, serum zinc showed no significant mean differences between type 2 diabetics with and without coronary risk.

Table 4.2: Comparison of means of anthropometric and biochemical parameters of outpatient type 2 diabetics with and without coronary risk

Parameters	Total, N=152	Without CR N= 98	With CR N= 54	p value
Age	55.5±9.2	55.7±9.3	55.1±9.0	0.692
Anthropometric data				
BMI Kg/m^2	27.7±4.6	27.6±4.7	27.9±4.5	0.715
WC (cm)	97.4±11.7	96.9±12.1	98.3±11.0	0.485
Biochemical data				
FBG mmol/L	9.4±3.5	9.3±3.6	9.6±3.4	0.550
HbA1c %, N=96	7.3±1.3	7.1±1.3 (59)	7.4±1.2 (37)	0.374
TC mmol/L	4.9±1.5	4.3±1.2	5.9±1.3	**0.000**
TG mmol/L	1.2±0.5	1.0±0.4	1.3±0.6	**0.007**
HDL-C mmol/L	1.5±0.3	1.5±0.3	1.5±0.3	0.295
LDL-C mmol/L	2.9±1.2	2.3±0.9	3.8±0.9	**0.000**
VLDL mmol/L	0.5±0.2	0.4±0.2	0.6±0.3	**0.004**
Serum zinc µg/dL	21.5±17.3	21.4±17.5	21.6±17.2	0.829
Dietary intakes (mg/day)				
Vitamin E	5.2±2.6	5.0±2.4	5.4±2.7	0.377
Zinc	5.0±2.7	4.9±2.4	6.2±3.1	**0.014**
Vitamin C	82.7±38.7	86.7±40.3	75.5±34.9	0.075

DM: Diabetes Mellitus (Type 2), BMI: Body Mass Index, WC: Waist Circumference, FBG: Fasting Blood Glucose, HbA1c: Glycated Hemoglobin TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, VLDL: Very Low Density Lipoprotein. Serum zinc: 66.0-110.0 µg/dL. *Independent t- test compared the means differences.*

Table 4.3 shows prevalence of CVDs risk factors among outpatient type 2 diabetics. One hundred and thirteen (74.3%) of type 2 diabetics were hyperglycemic. Out of 96 study subjects, 62 (64.6%) had high HbA1c. The prevalence of single, combined and mixed dyslipidemia among type 2 diabetics were 63.8%, 15.8% and 1.3% respectively. The prevalence of abdominal obesity was 66.4%, which was increased in female type 2 diabetics (79.1%),

compared to male type 2 diabetics (27.0%). Overall, type 2 diabetics with combined overweight and obesity was 110 (72.3%).

TC/HDL-C ratio which is used to assess coronary risk for CVDs showed 54 (35.5%) had high coronary risk. Also, 8 (5.3%) of the participants had high atherogenic index of plasma.

Table 4.3: Prevalence of CVDs risk factors among outpatient type 2 diabetics

CVD risk factors	Total, N	Frequency	Percentage (%)
Dyslipidemia (mmol/L)			
High TC ≥5.18	152	63	41.4
High TG ≥2.26	152	8	5.3
Low HDL-C	152	32	21.1
Women < 1.3	115	27	23.5
Men < 1.03	37	5	13.5
High LDL-C	152	23	15.1
Single dyslipidemia	152	97	63.8
Combined dyslipidemia	152	24	15.8
Mixed dyslipidemia	152	2	1.3
High TC/HDL ratio	152	54	35.5
High LogTG/HDL-C	152	8	5.3
Hyperglycemia			
FBG ≥7.0 mmol/L	152	113	74.3
HbA1c ≥ 6.5%, n=96	96	62	64.6
Abdominal obesity			
Female > 88 cm	115	91	79.1
Male > 102 cm	37	10	27.0
Total abdominal obesity	152	101	66.4
Obesity (Kg/m^2)			
Overweight	152	61	40.1
Obesity	152	49	32.2
BMI ≥ 25.0	152	110	72.3

CVDs: Cardiovascular diseases, BMI: Body Mass Index (overweight: BMI= 25.5-29.9 Kg/m^2); Obese: BMI≥ 30 Kg/m^2).

4.3 DIETARY INTAKE OF ANTIOXIDANT MICRONUTRIENTS

Table 4.4 shows average daily dietary intake of some antioxidants micronutrients among outpatient type 2 diabetics with and without coronary risk. None of the female participants met the daily dietary intake of vitamin E (0.0%), whereas only 1 (2.7%) male participant met the daily dietary intake of vitamin E (Dietary reference intake, 2000; adapted from National Academy of Sciences, 2011). Seventeen (14.8) female participants met daily intake of zinc, and 1 (2.7%) male participant met daily intake of zinc (Dietary reference intake, 2001; adapted from National Academy of Sciences, 2011).

Almost the same proportions of males and females (45.9% and 45.2% respectively) had adequate intake of vitamin C (Dietary reference intake, 2001; adapted from National Academy of Sciences, 2011). Comparison of frequencies of dietary consumption of zinc, vitamin E and vitamin C did not show any significant difference between type 2 diabetics with and without coronary risk.

Table 4.4: Dietary antioxidant micronutrient intake among outpatient type 2 diabetics with and without coronary risk

Dietary Antioxidant micronutrients intakes (mg/day)	Total, N =152	Without CR risk N= 98	With CR risk N= 54	p value
FEMALE	**115**			
Daily zinc intake				
Less than 8	98 (85.2)	65 (66.3)	33 (33.7)	0.06
Greater than or equal to 8	17 (14.8)	7 (41.2)	10 (58.8)	
Daily vitamin E intake				
Less than 15	115 (100.0)	72 (62.6)	43 (37.4)	1.000
Daily vitamin C intake				
Less than 75	63 (54.8)	36 (57.1)	27 (42.9)	0.245
Greater than or equal to 75	52 (45.2)	36 (69.2)	16 (30.8)	
MALE	**37**			
Daily zinc intake				
Less than 11	36 (97.3)	25 (69.4)	11 (30.6)	0.324
Greater than or equal to 11	1 (2.7)	0 (0.00)	1 (100.0)	
Daily vitamin E intake				
Less than 15	36 (97.3)	24 (66.7)	12 (33.3)	1.000
Greater than or equal to 15	1 (2.7)	1 (100.0)	0 (100.0)	
Daily vitamin C intake				
Less than 90	20 (54.1)	11 (55.0)	9 (45.0)	0.09
Greater than or equal to 90	17 (45.9)	14 (82.4)	3 (17.6)	

Categorical data were presented as frequency (Percentages). Categorical data were compared using Fischer's exact test. DM: Diabetes Mellitus (Type 2). Based on Dietary Reference Intake (2000 and 2001) by Food and Nutrition Board, Institute of Medicine. zinc intake (2001), and vitamin E, vitamin C (2000). p value ≤ 0.05 as significant.

4.3.1 RELATIONSHIP BETWEEN DIETARY ANTIOXIDANT MICRONUTRIENTS INTAKE AND CARDIOVASCULAR RISK FACTORS

Table 4.5 shows relationship between lipid profile, FBG, HbA1c, log TG/HDL-C and Total cholesterol/HDL-C ratio and dietary intakes of inadequate vitamin E, zinc and vitamin C when controlling for gender, age. A weak, significant positive correlation was found between, HbA1c (r = 0.220, p value = 0.033), TC (r = 0.260, p value= 0.011), LDL-C (r = 0.267, p value = 0.009), TC/HDL-C ratio (r = 0.217, p value = 0.036) and vitamin E intake, while zinc intake had weak, significant direct correlation with LDL-C (r = 0.203, p value = 0.049).

Dietary intakes of zinc and vitamin C did not show significant correlation with cardiovascular risk factors. Additionally, dietary intake of vitamin C showed very weak, not significant negative correlation with TC and HDL-C ratio and atherogenic index of plasma.

Table 4.5: Relationship between cardiovascular risk factors and dietary antioxidant micronutrients among participants using partial correlation

Biochemical parameters	Total	**Dietary antioxidant intake (mg/day)**		
		Vitamin E r (p value)	Zinc r (p value)	Vitamin C r (p value)
FBG mmol/L	152	0.159 (0.126)	0.197 (0.056)	0.027 (0.797)
HbA1c %	96	0.220 (**0.033**)	0.183 (0.077)	0.007 (0.949)
TC mmol/L	152	0.260 (**0.011**)	0.184 (0.076)	-0.008 (0.939)
TG mmol/L	152	0.073 (0.486)	0.028 (0.789)	-0.070 (0.501)
HDL-C mmol/L	152	0.158 (0.128)	0.078 (0.789)	0.075 (0.473)
LDL-C mmol/L	152	0.267 (**0.009**)	0.203 (**0.049**)	-0.016 (0.880)
TC/HDL-C ratio	152	0.217 (0.036)	0.175 (0.092)	-0.116 (0.267)
Log TG/HDL-C	152	0.077 (0.462)	-0.007 (0.949)	-0.099 (0.343)

Correlation is significant at 0.05 level (2-tailed). Controlling variables: gender and age.

4.4 RELATIONSHIP BETWEEN BIOCHEMICAL PARAMETERS AND INDEPENDENT CARDIOVASCULAR RISK FACTORS

Table 4.6 shows relationship between lipid profile, FBG, WC, HbA1c, and coronary risk and atherogenic index of plasma. A strong positive correlation was observed between total cholesterol, LDL-C, and coronary risk, which was statistically significant (TC, r = 0.695, p value = 0.000; LDL-C, r = 0.783, p value = 0.000). Also, TG and VLDL showed weak positive correlation which was significant (TG, r = 0.291, p value = 0.000; VLDL, r = 0.299, p value = 0.000).

Furthermore, triglyceride, VLDL had strong, direct relation with Log TG/HDL-C ratio, which was statistically significant (TG: r = 0.775, p value = 0.000; VLDL, r = 0.778, p value= 0.000). HDL-C had weak, inverse relationship with Log TG/HDL-C ratio (r = -0.283, p value = 0.003). Both waist circumference, FBG and HbA1c showed statistically not significant weak, positive correlation.

Table 4.6: Bivariate correlation between anthropometric and biochemical parameters, and independent CVD risk factors

Biochemical parameter	Total, N	TC / HDL-C ratio (Coronary risk)	Log TG/HDL-C (Atherogenic Index)
		r (p value)	**r (p value)**
WC (cm)	152	0.012(0.882)	0.043 (0.598)
FBG mmol/L	152	0.038 (0.641)	0.106 (0.196)
HbA1c %	96	0.130 (0.207)	0.102 (0.322)
TC mmol/L	152	0.695 (**0.000****)	-0.033(0.686)
TG mmol/L	152	0.291 (**0.000****)	0.775 **(0.000**)**
HDL-C mmol/L	152	0.144 (0.076)	-0.283 **(0.003**)**
LDL-C mmol/L	152	0.783 (**0.000****)	-0.142 (0.082)
VLDL mmol/L	152	0.299 (**0.000****)	0.778 **(0.000**)**

**Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.01 (2-tailed).*

A binary logistic regression performed showed TC, TG, LDL-C had significant effects on high coronary risk among study subjects (TC: OR= 2.6, 95% CI= 1.8-3.7, p value < 0.0001, TG: OR= 2.5, 95% CI= 1.3-4.8, p value =0.004, LDL-C: OR= 4.8, 95% CI= 2.9-8.1, p value < 0.0001 respectively). However, serum zinc, FBG and HbA1c showed no significant effects on high coronary risk in type 2 diabetics (FBG: OR= 1.0, 95% CI= 0.9-1.128, p value = 0.556, HbA1c: OR= 1.115, 95% CI= 0.840-1.588, p value = 0.376, Zinc: OR= 1.002, 95% CI= 0.935-1.128, p value = 0.576).

Table 4.7: Binary logistic regression showing relationship between biochemical parameters and high coronary risk

		High coronary risk				
			95 % CI			
Biochemical parameters	**B**	**OR**	**Lower**	**Upper**	**p value**	**Hosmer and Lemeshow test**
FBG	0.028	1.028	0.937	1.128	0.556	0.615
HbA1c	0.144	1.155	0.840	1.588	0.376	0.528
TC	0.971	2.640	1.879	3.708	**0.000**	0.132
TG	0.936	2.549	1.342	4.841	**0.004**	0.344
HDL-C	0.53	1.698	0.632	4.563	0.294	0.573
LDL-C	1.581	4.858	2.902	8.135	**0.000**	0.650
Serum zinc	0.010	1.002	0.935	1.128	0.576	0.348

Predictor variable: biochemical parameters. Dependent variable: high coronary risk ≥ 3.5. p value is significant at ≤ 0.05. OR: odd ratio

4.5 RELATIONSHIP BETWEEN SERUM ZINC AND CARDIOVASCULAR RISK FACTORS

Table 4.8 shows relationship between FBG, HbA1c, lipid profile parameters, and level of serum zinc when controlling for gender, age and dietary zinc intake. Fasting blood glucose, glycated hemoglobin had a weak, inverse correlation with serum zinc, which was significant (FBG: r = -0.206, p value = 0.05; HbA1c: r = -0.227, p value = 0.033). Lipid profile parameters had very weak, not significant negative correlation with serum zinc (p values > 0.05). Also, there was very weak, direct correlation between HDL-C and serum zinc level. This was not statistically significant (r = 0.022, p value = 0.793).

Table 4.8: Partial correlation showing relationship between serum zinc and cardiovascular risk factors among participants

CVD risk factors	Serum zinc (µg/dL) r (p value)
FBG mmol/L	-0.206 **(0.05)**
HbA1c %	-0.227 **(0.033)**
TC mmol/L	-0.051 (0.635)
TG mmol/L	-0.052 (0.633)
HDL-C mmol/L	0.022 (0.793)
LDL-C mmol/L	-0.041 (0.707)
TC/HDL-C	-0.023 (0.835)
Log TG/HDL-C	-0.037 (0.729)

Controlling variables: gender, age and dietary zinc intake. Correlation is significant at 0.05 (2-tailed).

CHAPTER FIVE

5.0 DISCUSSION

5.1 SOCIODEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

This study showed, type 2 diabetes mellitus was more prevalent among adult females (75.7%) compared to adult males (24.3%) (Table 4.1). This implies that type 2 diabetes is more prevalent among female adults than male adults in Kumasi. It could also mean that females seek health care more than males on medical condition. Similar observation was made in a study by Sarfo-Kantanka *et al*. (2017), in which type 2 diabetes was more common among females (58.9%) than males (41.1%) in Kumasi. It was also found that type 2 diabetes was most commonly associated with ages within 40-59 years. This means that type 2 diabetes occurs mostly among adult population.

Furthermore, type 2 diabetics with family history of cardiovascular diseases (CVDs, 42.1%) had higher, not significant coronary risk, compared to those without family history of CVDs (31.3%) (p value = 0.149). This implies that development of cardiovascular disease could be associated with hereditary predisposition.

5.2 PREVALENCE OF CARDIOVASCULAR DISEASE RISK FACTORS

Type 2 diabetics are susceptible to proatherogenic cardiovascular risk factors which include uncontrolled blood glucose, abdominal obesity, dyslipidemia (Mokta *et al*., 2017). From Table 4.3, 74.3% of type 2 diabetic patients had high fasting blood glucose and 64.6% had high HbA1c. High blood glucose is a risk factor for development of cardiovascular disease. This is because chronic hyperglycemia and dyslipidemia induce injury to endothelial cells, which can subsequently progress to atherosclerosis and may lead to risk of developing cardiovascular diseases (Bhutto *et al*., 2017). This means that hyperglycemia among participants can damage their endothelial cells and subsequently, predispose them to CVDs risk. The prevalence of hypercholesterolemia was 41.4%, hypertriglyceridemia, 5.3%, low HDL-C, 21.1% and high

LDL-C, 15.1%. Also, the study found the prevalence of single dyslipidemia to be 63.8%, combined dyslipidemia, 15.8% and mixed dyslipidemia, 1.3%.

In type 2 diabetes, there is alteration in lipids and lipoprotein parameters which contribute to oxidative stress and the development of atherosclerosis (Nimmanapalli *et al*., 2016). This implies that dyslipidemia among study participants make them susceptible to atherosclerosis and risk of developing cardiovascular diseases. Studies by Yadav e*t al.* (2014) and Yan *et al.* (2016) found the prevalence of single dyslipidemia were 64.1% and 67.1% respectively among type 2 diabetics. Also, Samdani *et al*. (2017) reported prevalence of combined and mixed dyslipidemia were 22.0% and 24.0% respectively. Prevalence rates of single, combined and mixed dyslipidemia of other studies are slightly higher than the finding of this study.

Furthermore, other modifiable CVDs risk factors such as abdominal obesity (66.4%), and combined overweight and obesity (72.3%) were of high prevalence among studied type 2 diabetics. Abdominal obesity and obesity are among powerful risk factors which could elevate risk of heart-related diseases in type 2 diabetes (Nimmanapalli *et al*., 2016). The findings give indication that having obesity and abdominal obesity can elevate possible occurrence of cardiovascular events in study participants.

Coronary risk and atherogenic index of plasma are used for independent assessment of cardiovascular disease risk. From Table 4.3, 35.5% of participants had high coronary risk and 5.3% had high atherosclerosis risk. This further shows that the participants are exposed to predisposing factors of heart-related diseases. Hence, type 2 diabetics showing high coronary risk and atherosclerosis risk are at increased likelihood of occurrence of atherosclerosis, leading to cardiovascular diseases.

5.3 DIETARY INTAKE OF ANTIOXIDANT MICRONUTRIENTS AMONG TYPE 2 DIABETIC PATIENTS

The dietary intakes of antioxidant micronutrients; vitamin E, zinc and vitamin C were assessed in Table 4.4. The mean dietary consumption of zinc, vitamin C and vitamin E were 5.04±2.76 mg/day, 82.7±38.7 mg/day and 5.2±2.6 mg/day respectively. A similar study by Zhou *et al*. (2016) found higher mean intakes of vitamin C (90.4±60.5 mg/day) and vitamin E (11.2±4.9 mg/day) among type 2 diabetic population, than the findings of this study. Among female type 2 diabetics, 85.2% had poor intake of zinc and none met recommended daily intakes of vitamin E, whereas 54.8% had lower intake of vitamin C. Additionally, there were poor intakes of vitamin E (97.3%), zinc (97.3%) and vitamin C (54.1%) among male type 2 diabetics.

Evidence shows that adequate intakes of antioxidant micronutrients are needed to slow or avoid occurrence of atherosclerosis and further risk of CVDs (Zhang *et al*., 2016). However, this study showed inadequate intakes of antioxidant micronutrients. Consequently, the observed poor intakes of antioxidant micronutrients such as vitamin E may possibly affect serum antioxidant micronutrients levels, and hence decrease antioxidant status among type 2 diabetic patients. For instance, inadequate zinc intake may have resulted in reduced serum zinc level among type 2 diabetics, and this could have possibly affected insulin activity and hence observed hyperglycemia in participants. Reduced antioxidant status may predispose type 2 diabetic patients to stress from oxidative free radicals and further, possible occurrence of atherosclerosis.

5.3.1 RELATIONSHIP BETWEEN ANTIOXIDANT MICRONUTRIENTS INTAKE AND CVDS RISK FACTORS

From Table 4.5, intake of vitamin E is directly associated with HbA1c, TC, LDL- and TC/HDL-C ratio. This was weak and significant (HbA1c: r = 0.220, p value = 0.033; TC: r = 0.260, p value = 0.011; LDL-C: r =0.267, p value = 0.009; TC/HDL-C: r = 0.217, p value = 0.036 respectively). This means higher intake of vitamin E may not influence elevated HbA1c, TC, LDL-C and TC/HDL-C ratio among participants. Vitamin E protects biomolecules from oxidative stress, through scavenging of free radicals and posing lower risk of diabetic complication (Rafighi *et al*., 2013). However, the study found lower intake of vitamin E. Lower dietary intake of Vitamin E could result in low serum vitamin E which could create imbalance in antioxidant status and hence reduce protective role of vitamin E against lipid peroxidation. It is also recalled that dyslipidemia and atherogenic risk are high among type 2 diabetic patients, which may increase oxidative stress. Hence, poor dietary intake of vitamin E, which can result in reduced antioxidant status may predispose them to oxidative stress and hence risk of developing atherosclerosis.

5.4 RELATIONSHIP BETWEEN LIPID PROFILE AND INDEPENDENT CVD RISK FACTORS

The study looked at which of the lipid profile parameters is related to increased coronary risk and atherogenicity, using bivariate correlation. It was found that total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) had direct, significant association on high coronary risk (TC: r = 0.695, p value = 0.000; TG: r =0.291 p value = 0.000; LDL: r = 0.783, p value = 0.000, shown in Table 4.6). This implies that an increase in TC, TG, LDL-C levels can directly influence increased in coronary risk, and hence, can contribute to increased risk of heart-related diseases in type 2 diabetics. Additionally, TG, VLDL had strong, significant direct association with atherogenic index of plasma (TG: r =

0.775, p value=0.000; VLDL: r = 0.778, p value=0.000), whereas HDL-C had weak, significant inverse association with atherogenic index of plasma (r = -0.283, p value = 0.003). This means low HDL-C levels could influence increased atherosclerosis risk in participants. Therefore, low HDL-C, high TG and high VLDL circulating in blood can induce risk of developing atherosclerosis in type 2 diabetics.

Furthermore, since correlational analysis showed association of biochemical parameters and coronary risk, then it becomes relevant to establish the cause and effects of biochemical parameters on coronary risk. The regression result in Table 4.7 showed TC, TG, LDL-C had significant effects on coronary risk for cardiovascular disease among participants. An increase in total cholesterol indicates close to 3- folds higher coronary risk for cardiovascular events in type 2 diabetics (OR= 2.6, 95% CI= 1.8-3.7, p value < 0.0001). Also, an increase in triglyceride indicates over 2- folds higher coronary risk for cardiovascular disease in type 2 diabetics (OR= 2.5, 95% CI= 1.3-4.8, p value =0.004). An increase in LDL-C shows about 5- folds higher coronary risk for cardiovascular disease in type 2 diabetics (OR= 4.8, 95% CI= 2.9-8.1, p value < 0.0001). This explains that type 2 diabetic patients are predisposed to higher coronary risk for cardiovascular disease when there are increases in above lipid parameters.

5.4.1 RELATIONSHIP BETWEEN SERUM ANTIOXIDANT MICRONUTRIENT (ZINC) AND CVDs RISK FACTORS

According to Pujar *et al*. (2014), zinc acts as cofactor for synthesis and storage of insulin in the pancreas, as well as secretion of insulin into the blood. Additionally, zinc helps insulin bind to receptors of liver cell membrane for uptake of glucose. Therefore, a deficiency in zinc could cause insulin resistance, which could lead to hyperglycemia, and subsequent oxidative damage to endothelial cells (Marreiro *et al*., 2017). Overall, mean serum zinc was reduced among type 2 diabetic patients (21.5±17.3 µg/dL). A similar study by Myke-Mbata *et al*. (2015) in Nigeria found reduced, but higher mean serum zinc level (53.41±4.35 µg/dL) among type 2 diabetics.

This could be attributed to their low dietary zinc intake which contributed to reduced serum zinc levels. Furthermore, reduced serum zinc could be attributed to insulin resistance which was evident as hyperglycemia among type 2 diabetic patients. According to Puri *et al*. (2013), high glucose in blood could lead to high excretion of zinc in urine. This might have contributed to reduced serum zinc levels, seen in type 2 diabetic patients.

Zinc in serum had inverse relationship with glycated hemoglobin and fasting blood glucose when controlling for gender, age and dietary zinc. This was weak and significant (HbA1c: r = -0.206, p value = 0.05; FBG: r = -0.227, p value = 0.033). This indicates that a decrease in serum zinc level could have been influenced by the increase in blood glucose. This could be inferred from the hyperglycemia inducing glucosuria, accompanied by loss of zinc in the urine.

CHAPTER SIX

6.0 CONCLUSION

In conclusion, risk factors of cardiovascular disease such as high coronary risk (35.5%), dyslipidemia (63.8%), hyperglycemia (74.3% FBG, 64.6% HbA1c), and abdominal obesity (66.4%) were high among study participants. Also, dietary intakes of zinc, vitamin E and vitamin C were found to be lower among type 2 diabetics, based on the recommended dietary intake. Participants had reduced level of serum antioxidant micronutrient which can predispose them to developing cardiovascular diseases. Dyslipidemia was associated with high coronary risk which increases risk of heart-related diseases among type 2 diabetics. Reduced serum level of zinc was associated with increasing blood glucose. Poor dietary and serum level of antioxidant micronutrients, together with high cardiovascular risk factors may put type 2 diabetics at increased risk of developing heart-related diseases.

LIMITATION OF STUDY

Majority of study participants could not give 3 days' recall of their dietary intakes.

Urinary analysis of zinc was not done to show evidence of increased urinary excretion of zinc.

RECOMMENDATIONS

It is recommended that further studies should involve case-control study, to determine relationship between dietary and serum antioxidant micronutrients intakes and risk of cardiovascular diseases of both healthy adults and type 2 diabetics.

Further studies should assess relationship between dietary patterns, serum vitamin E, serum vitamin C, total antioxidant capacity, and cardiac risk profile of type 2 diabetics with and without any cardiovascular diseases.

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APPENDIX

APPENDIX A

Table 4.4.1: Dietary Consumption Pattern of Antioxidant Micronutrients

Some food sources of Antioxidant micronutrients	**Dietary Consumption Pattern**				
Fruits	Daily	Weekly	Monthly	Occasionally	Never
Banana	4 (2.6)	49 (32.5)	35 (23.2)	43 (28.5)	20 (13.2)
Orange	15 (9.9)	60 (39.7)	31 (20.5)	29 (19.2)	16 (10.6)
Grapes	0 (0.0)	7 (4.6)	1 (0.7)	21 (13.9)	122 (80.8)
Mango	0 (0.0)	5 (3.3)	32 (21.2)	66 (43.7)	48 (31.8)
Pawpaw	8 (5.3)	48 (31.8)	36 (23.8)	34 (22.5)	25 (16.6)
Apple	2 (1.3)	23 (15.2)	35 (23.2)	51 (33.8)	40 (26.5)
Avocado	0 (0.0)	12 (7.9)	37 (24.5)	50 (33.1)	52 (34.4)
Vegetables					
Tomatoes	112 (74.2)	39 (25.8)	0 (0.0)	0 (0.0)	0 (0.0)
Turkey berry (abedru)	54 (35.8)	60 (39.7)	16 (10.6)	7 (4.6)	14 (9.3)
Lettuce	10 (6.6)	25 (16.6)	15 (9.9)	42 (27.8)	59 (39.1)
Kontomire	51 (33.8)	84 (55.6)	10 (6.6)	6 (4.0)	0 (0.0)
Carrot	9 (6.0)	49 (32.5)	50 (33.1)	22 (14.6)	21 (13.9)
Cabbage	6 (4.0)	42 (27.8)	48 (31.8)	35 (23.2)	20 (13.2)
Ayoyo leaves	9 (6.0)	28 (18.5)	23 (15.2	41 (27.2)	50 (33.1)
Dandelion	5 (3.3)	15 (9.9)	20 (13.2)	42 (27.8)	69 (45.7)
Some Zinc foods					
Poultry	2 (1.3)	31 (20.5)	54 (35.8)	51 (33.8)	13 (8.6)

Liver	2 (1.3)	9 (6.0)	12 (7.9)	32 (21.2)	96 (63.6)
Pork	0 (0.0)	4 (2.6)	5 (3.3)	18 (11.9)	124 (82.1)
Meat (cow/goat/bush)	3 (2.0)	31 (20.5)	21 (13.9)	60 (39.7)	36 (23.8)
Tuna fish	1 (0.7)	28 (18.5)	26 (17.2)	42 (27.8)	54 (35.8)
Shell fish (crab, oysters)	0 (0.0)	10 (6.6)	17 (11.3)	37 (24.5)	87 (57.6)
Salmon (fresh/smoked)	27 (17.9)	83 (55.0)	17 (11.3)	7 (4.6)	17 (11.3)
Some Vitamin E food sources					
Tiger nut	4 (2.6)	7 (4.6)	11 (7.3)	27 (17.9)	102 (67.5)
Plant oil (palm oil, frytoil, sunflower oil)	83 (55.0)	62 (41.1)	41 (2.6)	2 (1.3)	0 (0.0)
Margarine	1 (0.7)	1 (0.7)	3 (2.0)	15 (9.9)	131 (86.8)

1.1 QUESTIONNAIRE FOR STUDY

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

QUESTIONNAIRE ON DIETS AND CLINICAL INFORMATION FOR RISK OF CVDS AMONG TYPE 2 DIABETIC PATIENTS.

PERSONAL INFORMATION

Identification no:……. Date:……..……

Name (optional):…… Age: ……

Sex (☐ male ☐ female) Occupation :……

Level of Education? ☐ Primary ☐ JHS ☐ SHS ☐ Tertiary ☐ None

Tick in box SOCIOECONOMIC STATUS…….

- **DIET AND LIFESTYLE STATUS**

How often do you take fruits? ☐ Daily ☐ Weekly ☐Monthly ☐Occasionally ☐ Never

How many times do you eat fruit(s) per that day? ☐once ☐ 2 times ☐ 3-4 times ☐>4t ☐ None

How often do you take vegetables? ☐ Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐ Never

Do you drink alcohol currently? ☐ Yes ☐ No

Did you take alcohol in the past? ☐ Yes ☐ No

Did you smoke in the past? ☐ Yes ☐ No

How will you rate your physical activity level? ☐ Very active ☐ Active ☐ Moderately active ☐ Sedentary

What physical activity do you do? ☐ Brisk walking ☐ Stretching/strengthening exercise ☐ Jogging ☐ Other aerobics ……. ☐ None

How long do you do your exercise? ☐ < 30 mins ☐ 30 mins ☐ 1 hour ☐ >1hour ☐ None

How frequently do you exercise? ☐ Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐ None

- **CLINICAL INFORMATION**

Do you have Family history of CVDs? ☐ Yes ☐ No ☐ I don't know

Do you have any of the following CVDs complication of diabetes?

☐ Hypertension ☐ Coronary heart diseases ☐ Myocardial Infarction ☐ Stroke specify…… ☐ None

How long have you had CVDs? ☐ Less than 5years ☐ 5-10years ☐>10 years ☐ None

How do you manage your CVDs effectively? ☐ Drugs ☐ Diet ☐ Physical activity

☐ Drug and diet ☐ All 3 ☐ None ☐ Defaulted

Do you use any nutrient (vitamin or mineral) supplement? ☐ Yes ☐ No

How long have you been with diabetes? ☐ <5years ☐ 5-10years ☐ 11-15years ☐>15years

Are you on any cholesterol-lowering drugs? ☐ Yes ☐ No

Do you have any chronic liver diseases? ☐ Yes ☐ No

Have you seen dietitian before on your diet? ☐ Yes ☐ No

Do you monitor your blood glucose? ☐ Yes ☐ No

Have you been diagnosed of hypertension? ☐ Yes ☐ No

What is your current blood pressure level?.....

- **LEVEL OF AWARENESS OF CVD**

Have you had diabetes education on CVDs before? ☐ Yes ☐ No

Do you know diabetes patient is at risk of CVDs? ☐ Yes ☐ No

Do you know fruit and vegetables intakes can reduce your risk of CVD? ☐ Yes ☐ No ☐ I don't know.

Name	
Identification number & Date	
DIRECTIONS: The questionnaire is a 24-hour dietary recall to assess current dietary intake of foods**.** **Note: Two weekdays and one weekend food recall**	

weekday 1: Menu and Time	**Description of Food and beverages**	**Estimated Portion size**	**where food is eaten**
Breakfast **Time:**			
Mid-morning snack **Time:**			
Lunch **Time:**			
Mid-afternoon snack **Time**			

Supper **Time:**			
Bedtime snack **Time**			
weekday 2: Menu and Time	Description of Food and beverages	Estimated Portion size	How food is prepared/ where food is eaten
Breakfast **Time:**			
Mid-morning snack **Time:**			
Lunch **Time:**			
Mid-afternoon snack **Time**			
Supper			

Time:			
Bedtime snack **Time**			
Weekend: Menu and Time	Description of Food and beverages	Estimated Portion size	How food is prepared/ where food is eaten
Breakfast **Time:**			
Mid-morning snack **Time:**			
Lunch **Time:**			
Mid-afternoon snack **Time:**			
Supper			

Time:			
Bedtime snack **Time:**			

Name	
Identification number	
DIRECTIONS: The questionnaire is to assess the number of times of fruits and vegetables, plant oils, fish oils food you have consumed over the **past 6 months.** Where possible please provide **one answer to a question. NB: please tick in the box and provide amount you eat at a time a**	

Meal consumed	Code	Daily	Weekly (1-3 times)	Monthly	Occasionally	Never	Portion size (g)
FRUITS							
Watermelon	A1						
Banana	A2						
Citrus (Orange, tangerine,lemon)	A3						
Grape fruit	A4						
Mango	A5						
Pineapple	A6						
Pawpaw	A7						
Apple	A8						
Pear	A9						

Guava	A10						
Others	A11						
VEGETABLES							No portion
Tomatoes	B1						
Garden eggs	B2						
Kwansosaa(abedru)	B3						
Lettuce	B4						
Kontomire	B5						
Okra	B6						
Carrot	B7						
Cabbage	B8						
Other leafy dark vegetables	B9						
Ayoyo leaves	B10						
Dandelion leaves	B11						
Mushroom	B12						
STEW/ SOUP							
Tomatoes stew	C1						
Cabbage stew	C2						
Kontomire stew	C3						
Garden eggs stew	C4						
Light vegetable soup	C5						
NUTS AND OILS							
plant oils (frytol, palm oil, Corn, soy, canola, coconut, sunflower oils)	D1						
Tiger nut	D2						

Margarine							
Peanut butter							
FISH OIL							Portion
Salmon	E1						
Herrings	E2						
Anchovies	E3						
Tuna + canned oil	E4						
Shell fish (shrimps, Oysters,lobsters)	E5						
Sardine with canned oil	E6						
MEAT, POULTRY AND PRODUCTS, DIARY							
beef (cow)	F1						
Meat (goat, bush meat)	F2						
Organ meat (liver)							
Poultry (chicken, turkey)	F3						
Pork	F4						
Beans (soyabeans, dried beans, Bambara beans)	F5						
Milk, wagashi	F6						
Yoghurt /soy milk	F7						
Egg (whole)	F8						
WHOLE GRAIN CEREALS							
Wheat meal (rice)	G1						
Oat porridge with bran	G2						
Rice (brown/white)							

1.2 CONSENT FORM USED FOR THE STUDY

Participant Information Leaflet and Consent Form

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate

Title of Research:
Assessing relationship between dietary pattern, serum level of antioxidant micronutrients and risk of developing cardiovascular disease (CVD) among type 2 diabetic patients attending Komfo Anokye Teaching Hospital.

Name(s) and affiliation(s) of researcher(s):
The study is being conducted by Odeafo Asamoah-Boakye, KNUST, Kumasi and Dr. Charles Apprey of Department of Biochemistry, KNUST, Kumasi.

Background (Please explain simply and briefly what the study is about):
Diabetes mellitus is a chronic challenging disease in the healthcare system of Ghana. Uncontrolled Diabetes mellitus can lead to cardiovascular diseases which form major cause of death among people with diabetes. Dietary antioxidants such as vitamin E, zinc and selenium are growing evidences of nutritional intervention for prevention of diabetes complications. The study is to determine relationship between dietary pattern, serum level of antioxidant micronutrients and risk of developing cardiovascular disease (CVD) among type 2 diabetic patients attending Komfo Anokye Teaching Hospital.

Purpose(s) of research:
The purpose of this research is to form part of the assessment leading to the award of MPhil Human Nutrition and Dietetics.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:
The research will randomly select Type 2 diabetic patients from Diabetic Clinic at Komfo Anokye Teaching Hospital. Each participant will undergo questionnaire interview after giving consent to partake in the study. The participants will have their weight and height checked at biochemistry laboratory at Komfo Anokye Teaching Hospital using weighing scale and stadiometer respectively. Venous blood sample will be taken from each participant at biochemistry laboratory at Komfo Anokye Teaching Hospital for clinical analyses of fasting blood glucose, lipid profile, serum vitamin E, zinc and selenium. In total, the study will recruit 150 participants from Komfo Anokye Teaching Hospital.

Risk(s):
There are no serious risks if participant(s) exclude(s) or partake in the study.

Benefit(s):
Participants will receive nutritional education and counselling.
Results obtained will be used by healthcare professionals to improve on nutritional intervention strategies of diabetes which participants will benefit from.

Confidentiality:
Identification number will be used to replace participants' names. The results obtained from the study will be held private. The names of the participants will be anonymous to the public if the study is published.

Voluntariness:
Participation in this study is voluntary and participants are free to suspend their participation when they wish to. However, they would be advised to fully be involved since this is a relevant study.

Alternatives to participation:
The study will need all participants who give consent to participate.

Withdrawal from the research:
Participant (s) can withdraw out of their own will.

Consequence of Withdrawal:
There will be no consequence, except that if participant withdraws I have to recruit another participant since this is a cross sectional study.

Costs/Compensation:
For the time and inconvenience, participants will receive fruit snacks as appreciation.

Contacts:
Any concern or contributions should be addressed to Mr. Odeafo Asamoah-Boakye on 0501348592 or to Department of Biochemistry, KNUST.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 03220 63248 or 020 5453785

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'S NAME: _____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'S NAME: _____