UNIVERSITY OF GHANA

DIET AND LEVELS OF TWO ENDOGENOUS ANTIOXIDANTS, SUPEROXIDE DISMUTASE AND CATALASE, IN THE BLOOD OF SEVENTH-DAY ADVENTIST VEGETARIANS AND NON-VEGETARIANS IN MAYERA AND DANSOMAN IN THE GREATER ACCRA REGION OF GHANA.

BY

BOADIWAA OFORI-AMANFO
(10396481)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN DIETETICS

JULY, 2015

DECLARATION

I, Boadiwaa Ofori-Amanfo, hereby declare that with the exception of references cited, which have been duly acknowledged, this dissertation was done by me under the supervision of Prof. George Asare (Maj. Rtd.) and Dr. Matilda Asante.

…………………........

OFORI-AMANFO BOADIWAA DATE…………………..

(STUDENT)

……………………….

PROF. GEORGE ASARE (Maj. Rtd.) DATE…………………….

(CO-SUPERVISOR)

……………………….

DR. MATILDA ASANTE DATE……………………

(PRINCIPAL SUPERVISOR)

ABSTRACT

Background: Vegetarianism is a dietary lifestyle that involves the restriction of diet, disallowing some or all foods of animal origin, thereby consuming mainly foods of plant origin. Vegetarian diets are composed of only plant products which include fruits and vegetables, as well as grains, legumes, nuts and seeds. The plant products they consume are rich sources of vitamins such as Vitamins A, C and E which act directly as antioxidants, as well as protein and minerals such as zinc, iron, magnesium and selenium, which function as co-factors for antioxidant enzymes, synthesized in the body. Free radicals cause cell damage and have been found to be an important contributory factor in the occurrence of diseases such as brain dysfunction, cancer, cardiovascular diseases. Little data exists on dietary composition and serum levels of endogenous antioxidants of vegetarians in Ghana.

Objectives: The aim of the study was to compare the diet and serum and plasma levels of two common intracellular antioxidants in Seventh - day Adventist vegetarians and non-vegetarians in Mayera and Dansoman, in the Greater Accra region of Ghana.

Methodology: A case – control study was carried out involving 39 vegetarians and 30 non-vegetarians. Blood pressure and anthropometric measurement (weight, height, visceral fat and body fat) was measured for all the participants. A structured questionnaire was used to obtain socio- demographic data of the participants. Dietary intake was assessed using a 24-hour recall and food frequency questionnaire. Venous blood samples were collected to assess serum and plasma levels of the endogenous antioxidants, catalase and superoxide dismutase.

Results: The mean age of vegetarians and non-vegetarians was 36.1± 8.2 and 30.7± 7.6 years, respectively. The mean age between both groups was significantly different (p=0.007). Systolic blood pressure was significantly higher in vegetarians compared to non-vegetarians (*p* =0.049). There was a significant difference in the mean serum level of superoxide dismutase between vegetarians and non-vegetarians (p=0.044). However, there was no significant difference in the mean plasma level of catalase between both groups (p=0.075). Mean intake of protein was significantly different between both groups (p=0.001), whilst significant difference was found in the intake of the antioxidant micronutrient Vitamin E (p=0.006). Mean dietary zinc intake was significantly lower in the vegetarian group (p=0.000), compared to the non-vegetarian group. Dietary vitamin C intake in vegetarians showed a significant moderate positive correlation with SOD levels (p=0.04).

Conclusion: Significantly high intake of dietary vitamin E in vegetarians could account for the significantly higher mean level of superoxide dismutase in this group. Comparatively higher intakes of Vitamins A and C in vegetarians, though not significant, could account for the higher levels of endogenous antioxidants in blood. There is therefore the need to examine more enzymatic antioxidants as well as to quantify dietary antioxidants.

DEDICATION

This work is dedicated to my Heavenly Father, Jehovah the Almighty God, to my parents and to my grandmother as appreciation for their love, protection, spiritual and financial support throughout my life.

ACKNOWLEDGEMENTS

I am very grateful to God for the knowledge, skill and strength with which He graced upon me to carry out this research project. The journey has not been easy but His Word has always been my encouragement and peace.

I am also most grateful to my Supervisors, firstly, Prof. George Asare (Maj. Rtd.) for his enthusiasm, dedication and motivation towards my research work, and secondly to Dr. Matilda Asante for her admirable knowledge and insightful contribution to this work.

My sincerest gratitude goes to my parents and grandmother, for their unwavering love, care, prayers and immense financial support throughout my life. To my siblings, Kwadwo Boateng, Amma Serwaa and Kofi Amoako, and my Uncle, Dr. Eric Adjei of CSIR, I would like to say a big thank you for your love, support and encouragement.

Special thanks go to Michael Ansah-Nyarko and my sister Amma Serwaa for their dedication during the analysis of data, to the staff and research assistants of the Chemical Pathology Laboratory of the School of Biomedical and Allied Health sciences for their contribution to this work, and to Pastor Class-Peters of the New Life Historic Seventh-Day Adventist church and members of the congregation for agreeing to be part of this research.

Finally, my heartfelt gratitude goes to Mr. David Amoako-Kusi for his love, emotional and spiritual support that encouraged me during the final part of this research project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AND	-	Academy of Nutrition and Dietetics
BMI	-	Body Mass Index
CAT	-	Catalase
DNA	-	Deoxyribonucleic acid
EPIC	-	European Prospective Investigation into Cancer and Nutrition
MUFA	-	Mono unsaturated fatty acid
NV	-	Non-Vegetarians
PUFA	-	Poly unsaturated fatty acid
ROS	-	Reactive Oxygen Species
SFA	-	Saturated Fatty Acid
SOD	-	Superoxide Dismutase
V	-	Vegetarians
WHO	-	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

According to Dorland's Medical Dictionary for health consumers (2007), vegetarianism is a dietary lifestyle that involves the restriction of diet, disallowing some or all foods of animal origin, thereby consuming mainly foods of plant origin. People choose to adopt a vegetarian lifestyle due to reasons such as concern about the environment, animal welfare, health concerns and religious beliefs (British Dietetic Association, 2011).

Vegetarians' main source of protein and other important micronutrients such as vitamin B_{12}, zinc and iron are from plant products, mostly fruits, legumes and vegetables. Although the vegetarian's diet can provide enough protein if a variety of the protein-rich plant products are consumed, the protein quality from plant sources is low compared to that from animal sources (Messina and Messina, 1996). Research also shows that vegans (strict vegetarians) usually have low intakes of calcium and vitamin B_{12} (Davey *et al.*, 2003). As a result, assumptions have been made about the adequacy of a vegetarian diet in providing the recommended daily allowance of protein, calcium and vitamin B_{12}. However, the Academy of Nutrition and Dietetics (2009) has stated in its position paper that; "appropriately planned vegetarian diets, including total vegetarian or vegan diets, are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases.

Studies in the area of nutritional assessment and the function of dietary components of food in the body have found that plant foods are a rich natural source of antioxidants, as well as vitamins and minerals (Hamid *et al.*, 2010).

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals which are produced as a result of oxidation reactions (Hamid *et al.*, 2010).

Free radicals are formed in the body as a result of conditions such as exposure to cigarette smoke, stress, pollution, illness and even exercise (Hamid *et al.*, 2010). Also, utilization of oxygen by the body results in protein, fat and carbohydrate metabolism (Kumar *et al.*, 2012).

Free radicals cause cell damage and have been found to be an important contributory factor in the occurrence of diseases such as brain dysfunction, cancer, cardiovascular diseases, as well as in the process of ageing. The function of antioxidants in the body is to deactivate free radicals before they attack cells (Somannavar and Kodliwadmath, 2012).

Total vegetarian diets are composed of only plant products which include fruits and vegetables, as well as grains, legumes, nuts and seeds. The plant products the consumed are rich sources of vitamins such as vitamins A, C and E which act directly as antioxidants, as well as protein and minerals such as zinc, iron, magnesium and selenium, which function as co-factors for antioxidant enzymes synthesized in the body.

Thorough studies into antioxidants, such as vitamin C and E, beta carotene and other carotenoids, polyphenols, as well as endogenous antioxidants such as glutathione peroxidase and superoxide dismutase, have shown that their actions in the body contribute greatly to the prevention of degenerative processes and diseases such as ageing, cardiovascular diseases, and cancer, which come about as a result of tissue damage (Gupta and Sharma, 2006).

Stanner *et al.* (2004) found that a lower risk of heart disease and some neurological diseases was recorded in individuals who consumed fruits and vegetables.

1.2 Statement of the problem

Most daily occurrences and processes such as exercise, stress, illness, drug intake and pollution result in an increase in free radical exposure. Free radicals have been shown to be a part of the pathogenesis of at least 50 diseases (Halliwell, 1994). Even beneficial processes such as the oxidation of fats, proteins and carbohydrates, which make use of oxygen, result in the exposure to free radicals. This exposure is believed to be of very great significance when it comes to the process of ageing, as well as in the pathogenesis of diseases such as cancer and cardiovascular disease, both of which have gradually become the leading causes of death, in recent years (WHO, 2007). As a result of this exposure, antioxidants, which serve as the first line of defense against the action of free radicals, and which can be obtained through the foods we eat, become very necessary for the body in order to maintain the integrity of cells and prevent tissue damage.

Due to the dietary habits of vegetarians, it is assumed that they consume more fruits and vegetables in their diet and are most likely to have a better antioxidant status. However there is minimum literature on the dietary intake of vegetarians in Ghana. There is also minimum information to ascertain whether vegetarians in Ghana consume adequate amount of fruits, nuts, legumes, grains, cereals and vegetables to show distinct difference in their antioxidant levels as compared to non-vegetarians.

1.3 Significance of the Study

This study will help to determine whether a vegetarian diet, through the provision of antioxidants, has the ability to provide the best protection and action against free radical formation and their destructive action in cells and tissues. The results obtained will assist in making recommendations to individuals who generally want to consume a healthy diet, as part of a healthy lifestyle, or to individuals suffering from disease conditions in which free radicals play an important role.

1.4 Hypotheses

1. Vegetarians have significantly higher blood levels of superoxide dismutase than non-vegetarians.
2. Vegetarians have significantly higher plasma levels of catalase than non-vegetarians.
3. There is a significant relationship between intake of antioxidant vitamins and levels of superoxide dismutase in serum and catalase in plasma among vegetarians and non-vegetarians.

1.5 Aim of the Study

The aim of the study was to compare dietary intake and levels of two common endogenous antioxidants, superoxide dismutase and catalase in 7th Day Adventist vegetarians and non-vegetarians in Mayera and Dansoman in the Greater Accra region.

1.6 Objectives

The specific objectives of the study were:

1. To assess and compare the nutrient intakes of the vegetarians and non-vegetarians.
2. To compare frequency of consumption of food among the vegetarians and non-vegetarians.
3. To determine intakes of selected dietary antioxidants (vitamin A, C and E) and their relationship with levels of the endogenous antioxidants, superoxide dismutase and catalase among the vegetarians and non-vegetarians.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Vegetarianism before the 19th century, (though the term was coined during the period), was a decision, justified on the basis of metaphysical and moral arguments (Whorton, 1994). According to Whorton (1994), vegetarianism is a lifestyle that began in the early 1800s, usually among some philosophers. The idea of vegetarianism, being a lifestyle that promoted non-violence against animals (Kerry *et al.*, 2001) was earlier recorded among a number of people in ancient India and ancient Greek civilizations (Spencer, 1993). According to Passmore (1975) although vegetarianism almost disappeared from Europe in the 4th to 6th centuries, the renaissance in Europe saw a re-emergence of the practice (Spencer, 1993), leading to its practice among a wider group of people in recent years. Vegetarianism was even justified with a bible reference from Genesis 1:29, showing that the vegetarian diet had its origination from God, and that was the diet expected of humans for consumption (Smith, 1983).

2.2 Defining Vegetarianism

According to the Dorland's Medical Dictionary (2007), a vegetarian is an individual who does not eat meat fish, poultry, game, any form of shellfish, or products derived from any of these sources. The society also gave a second definition which describes a vegetarian as an individual who lives on plant-based diets of grains, vegetables, fruits, pulses, nuts and seeds, either or not, exempting dairy product and eggs. Stahler's study (2005) on the number of adolescent vegetarians in the United States (U.S.) showed 3% of 8-18 year olds being vegetarians, with close to 1% following a strict vegan diet. A nationwide poll carried out in the United States in 2006 revealed that approximately 2.3% of the adult population never consumed meat, fish or poultry, emphasizing their consistency in following a vegetarian diet (Stahler, 2006).

Due to the dietary habit of excluding flesh meat in meals, as well as dairy products and eggs, research has shown that vegans (strict vegetarians) usually have low intakes of calcium and vitamin B_{12} (Davey *et al*., 2003). Also, although the vegetarian's diet could provide enough protein if a variety of the protein-rich plant products are consumed, the protein quality from plant source is low, compared to that from animal sources (Messina and Messina, 1996). As a result, assumptions were made about adequacy of a vegetarian diet in providing the recommended daily allowance of protein, calcium and vitamin B_{12}. However, the Academy of Nutrition and Dietetics (2009) has stated in its position paper that "appropriately planned vegetarian diets, including total vegetarian or vegan diets, are nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases.

If an adult vegetarian diet is well planned, involving choices from a variety of nutritionally adequate foods, it is possible for the individual to receive sufficient nutrients and calories necessary to support a healthy life (Academy of Nutrition and Dietetics, and Dietitians of Canada, 2003). On the other hand, studies by Ambroszkiewicz *et al.* (2006;

2007) suggests that because of the inability of children to consume sufficient food at a time and throughout the day, it is difficult for them to meet their nutritional needs from a vegetarian or vegan diet.

2.3 Types of Vegetarians

Although generally, vegetarians have a lifestyle of abstaining from anything associated with the "exploitation" of animal, there are variations in the lifestyle, ranging from flexible to extremely strict vegetarians. According to Robinson and Hackett (1995), vegetarian diets can be classified into the following:

Vegan: Avoids intake of every food derived from animals.

Lacto-ovo-vegetarian: Avoids all animal flesh but consumes milk and eggs.

Ovo-vegetarian: Avoids consumption of all animal flesh and milk, but consumes eggs.

Lacto-vegetarian: Includes dairy products in diet, avoids all flesh foods and eggs.

Pesco-vegetarian: Includes fish (and possibly other seafood). May include dairy products and eggs, but totally avoids meat and poultry.

Demi-vegetarian (semi-vegetarian): Occasionally consumes meat/poultry/fish.

Fruitarian: This individual goes an extra mile to be concerned about the life of the plants from which meals are obtained. As a result, the diet generally consists only of foods that do not kill the plant of origin. Foods consumed include fresh and dried fruits, nuts, seeds and a few vegetables. Additionally, an individual can fall under the category of a vegetarian if meat, fish or poultry is consumed less than once a week (Sabate *et al.*, 2001).

2.4 Reasons for Vegetarianism

Studies have shown that for a majority of people around the world, mostly from developing countries, economic and geographic reasons account for their dependence on plant-based diets alone (Rottka, 1990). According to Whorton (1994) recent practice of vegetarianism is usually attributed to moral and other non-scientific reasons as opposed to the earlier practice by philosophers, whose reasons were much more scientific. Sabate *et al*. (2001) pointed out many reasons why people choose to adapt vegetarianism which include cost, family influences, sensory and taste preferences, philosophical reasons (*e.g.* religious teachings such as Buddhism or membership of the Seventh-Day Adventists) or as a reaction to food safety scares, such as salmonella, bovine spongiform encephalopathy (BSE), *Escherichia. coli*, or the use of antibiotics or growth hormones in meat production. People also choose to adopt a vegetarian lifestyle due to reasons such as concern about the environment, animal welfare, health concerns and religious beliefs (British Dietetic Association, 2011).

2.5 Health Benefits of a Vegetarian Diet

2.5.1 High Intakes of Certain Micronutrients

Vegetarians have been reported to have high intakes of iron, magnesium, potassium and copper; thus the presence of adequate amounts of these nutrients in their diets could be attributed to the wide variety of fruits, vegetables and plant-based diets consumed (Waldmann *et al.,* 2003). According to Perry and his colleagues (Perry *et al.*, 2002), iron intakes of vegans and vegetarians are almost equal to that of omnivores or sometimes even higher. It may be argued that since meat provides very high amounts of iron in their most readily available form (the heme form), vegetarians could be deficient in adequate iron stores, because their source of iron is non-heme, which is sensitive to absorption inhibitors like phytates. However, research has shown that non-haeme iron is equally sensitive to absorption enhancers like vitamin C, of which vegetarians obtain adequate amounts from their consumption of fruits and therefore, iron intake and absorption may not be a problem for vegetarians if they consume plant foods from very iron-rich sources (Davey *et al*., 2003).

2.5.2 Favourable Anthropometry and Reduced Risk of Associated Diseases

Vegetarian and vegan adults have been shown to have lower body mass indices (BMI), usually 1-2 kg/m^2 less than comparable non-vegetarians, with vegans even having a lesser BMI as compared to vegetarians (Appleby *et al*., 2002). In a study by Philips *et al.* (2004), it was observed that a change in an omnivorous diet to a vegetarian diet resulted in a change in anthropometric measurement towards values that favoured a lower risk of development of coronary heart disease (CHD).

2.5.3 Favourable Results in Lipid Profile

A study carried out by Robinson *et al.* (2002) has confirmed that transitioning from an omnivorous diet to that of a vegetarian diet produces favourable results in the lipid profile of an individual. Results from another study conducted in Korea to compare the lipid profile of vegetarians and non-vegetarians, showed a significant lower level of lipids in vegetarians than in non-vegetarians (Kyung *et al*., 2012). A similar study was carried out by Béderová *et al.* (2000) to compare nutrient intake and corresponding biochemical parameters in adolescent vegetarians and non-vegetarians. Results obtained indicated a more favourable lipid profile in the vegetarian participants due to the consumption of less fat, with a higher intake of fat obtained rather from plants. The study observed not only lower risk parameters of artherosclerosis, but also comparatively higher values of antisclerotic factors in the vegetarian group.

2.5.4 Reduction in the Risk of Cancers and Other Degenerative Diseases

A significant reduction in the risk of cancers of the lungs, stomach oesophagus and colorectum have been reported to occur with increase in consumption of fruits and vegetables, according to a research carried out by Riboli and Norat (2003). In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, evidence showed that consumption of dietary fibre, which can be obtained mostly from whole grains, fruits and vegetables, was inversely related to the development of large bowel cancer (Bingham *et al.*, 2003). Allen *et al.* (2000) in their study to determine levels of the hormone, insulin-like growth factor-1 in vegan men, observed that the hormone, which is thought to be associated with the aetiology of several cancers, was low in vegans compared with lacto-ovo vegetarians and non-vegetarians, thereby conferring on them the advantage of a reduced risk of colorectal cancer.

Not all research studies have supported these claims. Earlier research by Fraser (1999), who sought to establish a relationship between diet and certain degenerative diseases, his results showed that when gender, age and smoking was adjusted, there was no difference in the risk of development of breast, prostate, lung and uterine cancers. This means that in the development of cancers, diet is not the only determinant, but smoking and age also play important roles.

Furthermore, various studies on the relationship between vegetarianism and cancer have not succeeded in establishing clear differences in cancer rates between vegetarians and omnivores (Key *et al.,* 2006). Also, diets that are usually soy-based may be adequate for individuals suffering from chronic kidney disease and could slow progression of the disease (Bernstein *et al*., 2007).

2.5.5 Extension of Life Expectancy Due To Beneficial Lifestyle Behaviours

Again, from the EPIC cohort study, a conclusion was reached that certain lifestyle behaviours which included not smoking, moderate alcohol intake, being physically active, and the consumption of at least five fruit and vegetable servings a day, all lifestyle behaviours of vegetarians (Key, 1999), were estimated to amount to an additional 14 years of life (Khaw *et al.,* 2008). In the same study however, regular intake of processed meats increased the risk of cardiovascular diseases and deaths from cancer, which was actually observed among the non-vegetarians.

However, these results also conflict with some studies done on a very large scale, involving large populations in other parts of the world. For example, in a typical study the Icelanders, Swiss, Norwegians, and the Swedes who are considered the healthiest people in the European continent (and with very high life expectancy), were found to consume large amounts of fish, poultry and dairy products, which fall under animal products (Ginter, 2008).

2.5.6 Maintenance of Normal Blood Pressure

Studies have linked the consumption of vegetarian diets to favourable results for systolic and diastolic blood pressures. Results from the Adventist Health Study-2 (AHS-2) on the relationship between vegetarian diets and blood pressure showed than vegan participants had lower systolic blood pressure than omnivorous subjects and were less likely to be taking antihypertensive medications (Pettersen *et al.*, 2012). Brathwaite *et al.,* in 2003 also proved this point. In their study carried out to determine the prevalence rate of diabetes, obesity and hypertension in vegetarian and non-vegetarian participants, they observed a comparatively lower prevalence rate of hypertension in long-term vegetarians than in non-vegetarians, with emphasis on “long term vegetarians”.

2.6 Health Problems Associated With a Vegetarian Diet

2.6.1 Vitamin B_{12} Deficiency, High Homocysteine Levels and Risk of Some Non-communicable diseases

In a study carried out by Mezzano *et al*. (1999) to determine the comparative levels of homocysteine in vegetarians and meat-eaters, it was observed that plasma homocysteine was 41% higher in vegetarians than comparable meat-eaters. This was attributable to their low serum vitamin B_{12} concentrations, which was reported in 21 out of 26 vegetarians. Among vegetarians and non-vegetarians, vegans have been found to have the highest concentration of homocysteine in serum (Obeid *et al.,* 2002). Research has shown homocysteine to be an independent risk factor in the development of cardiovascular disease (Taylor *et al.*, 2000). This shows that a vegetarian diet may not always be protective against the development of cardiovascular disease and therefore adequate supplementation and use of vitamin B_{12} fortified foods is required to prevent these high homocysteine levels from occurring in vegetarians (Herrmann, 2003). Vegetarians must also be advised to plan their diet very well and monitor their plasma vitamin B_{12} status regularly in order to detect early, low cobalamin status (Elmadfa and Singer, 2009).

Vitamin B_{12} deficiency could also result in megaloblastic anaemia with symptoms which include abnormal sensations in the limbs, weakness or demyelination in peripheral nerves and the central nervous system, leading to a range of psychiatric disorders (Craig & Pinyan, 2001). A point worth noting however is that the visible expression of these symptoms may be suppressed for a while when folic acid intakes are high, and this is the situation observed in both vegetarians and non-vegetarians since both groups have been found to have adequate intakes in folate (Thomas and Bishop, 2007).

2.6.2 Zinc deficiency

A research has shown vegetarians to have low intakes of the micronutrient zinc (Li *et al.*, 2000). Strategies to employ in reducing zinc deficiency or improving zinc status in vegetarian include consumption of zinc-fortified food, zinc supplementation and dietary practices that increase the bioavailability of dietary zinc especially from the plant sources (Foster *et al.*, 2013). These strategies, according to Foster *et al.* (2013) are very important especially in vegetarians whose serum zinc concentrations are at the lower end or below that of the reference range, or in those who generally have consistent low zinc intakes relative to their diet.

2.7 Macronutrient Intake of Vegetarians

2.7.1 Energy Intake

In a study that sought to analyze the dietary patterns of vegetarians and omnivores, the mean total energy intake was found to be comparable between the two groups at a p-value of >0.05 (Clarys *et al.*, 2013). This showed that energy intake between vegetarian and non-vegetarian subjects were almost equal. Evidence from a cohort study has shown that an increase in weight gain, as a result of increase in calorie intake in breast cancer diagnosed women, increased the mortality in the group by 13%, for each 5 kg gain in weight (Nichols *et al.*, 2009). Calorie restriction has been found to result in inhibited accumulation of oxidatively damaged proteins in laboratory rats (Youngman *et al.*, 1992). This may imply that there is a strong relationship between the amount of calories consumed and the production of reactive oxygen species. Sleep deprivation has been associated with increased consumption of calories from snacks (Nedeltcheva *et al.*, 2009).

2.7.2 Carbohydrate

This macronutrient provides the most readily available form of energy. Each gram of carbohydrate provides 4 kcal of energy to the body (Mahan *et al.*, 2012). Main carbohydrate sources include cereals, grains and tubers, which both vegetarians and non-vegetarians have no restriction on their intake. As a result, most studies (Nadimi *et al.*, 2013; Shridhar *et al.,* 2014; Clarys *et al.,* 2013) but a few, have found no significant difference in the intake of carbohydrates between the two groups. In certain studies, intake of carbohydrates between vegetarians and non-vegetarians was not significantly different (Nadimi *et al*., 2013). However, in another study conducted to compare dietary pattern between vegetarians and non- vegetarians, carbohydrate and fibre intake was significantly higher in vegetarians than in the omnivorous subjects (Shridhar *et al.,* 2014, Clarys *et al.,* 2013). This was attributed to the fact that the vegetarians obtained their carbohydrates from more unrefined sources (Clarys *et al.,* 2013). Whole grain, total dietary fibre and cereal fibre have shown strong inverse association with incidence of diabetes (Meyer *et al.,* 2000) showing that unrefined carbohydrates are more protective against diabetes as compared to refined carbohydrates.

2.7.3 Protein

Biochemically, proteins are macromolecules which are made up of one or more long chains of amino acids. Nutritionally, proteins are one of the three important macronutrients needed daily by the body in specific amounts, as part of a balance. To a molecular biologist or biochemist, proteins are important to every human because of the various roles they play in various biochemical processes in the body which include replication of DNA, acting as transporters that move molecules from one point in the body to another, responding to stimuli as well as acting as enzymes that speed up metabolic reactions (Lodish *et al.,* 2004). Protein is known as a building block because it is the main nutrient that is responsible for maintaining and growing lean muscle, through the action of amino acids. Another reason why proteins are so important involves the fact that they repair worn-out tissues or wounds. Requirements for proteins are not the same for each life cycle. The Recommended Daily Allowance (RDA) for protein either at each stage of life of a healthy individual (whether toddler, adolescent, adult, during pregnancy or during lactation and old-age) or within a specific age group has been obtained as 0.8 to 1.0 g/kg body weight/ day (Mahan *et al.,* 2012).

Protein is obtained from two sources, namely animal and plant sources. Examples of animal sources of proteins include meat (beef, mutton, and venison), egg, poultry, fish and milk. Plant protein can be obtained from legumes (beans and lentils), pulses and nuts (peanuts, cashew nuts, almonds).

Since vegetarians avoid consumption of meat and or animal products they depend on plant sources of protein to meet their daily requirement whilst non-vegetarians obtain protein from animal sources as well as from plants. According to the Academy of Nutrition and Dietetics (2009), in their position paper on vegetarian diets, there have been arguments about the quality of protein from plant sources and the adequacy of protein obtained from a single plant source per meal. However, it has been established that careful planning of a vegetarian diet may provide enough protein even from a single plant source and may not require combinations from different plant protein sources per meal.

Some studies have reported a lower relative intake of proteins in vegetarians as compared to non-vegetarians. A research by Clarys *et al.* (2013) showed that the absolute and relative protein intake by vegetarians was significantly lower than in non-vegetarians. In another study by Nadimi *et al.* (2013) showed that the mean intake of protein (40.45 ±

19.41g) by vegetarians was significantly lower (p=0.04) compared to intakes in non-vegetarians (56.96 ± 11.94 g).

2.7.4 Lipids (Fats)

Four main types of fat make up the fats found in the food we eat, and these are grouped into healthy and unhealthy fats. These types are monounsaturated fats, polyunsaturated fats (omega 6 and omega 3 fats) referred to as healthy fats, and the unhealthy fats namely saturated fats and trans fat (Mayo Clinic, 2014). Examples of monounsaturated fats are olive oil, canola oil, avocados and oils found in nuts and seeds. Examples of polyunsaturated fats include safflower oils, sesame and sunflower oils, which fall under omega 6 fats and oils from fatty fish, flaxseed and soybean, which fall under omega 3 oils (Heart and Stroke Foundation, 2010). Saturated fats on the other hand are obtained from animal sources of food, whilst trans fat are obtained from oils that go through partial hydrogenation, which makes cooking with them easier. Trans fats are found in snacks and fast foods.

A study was carried to examine the change in total fat intake and plasma lipid levels when saturated fat was replaced with polyunsaturated and monounsaturated fat. The results showed that fat intake decreased by 5.1% energy and 2.9% energy when saturated fat was replaced with monounsaturated and polyunsaturated fat, respectively (Hodson *et al.*, 2001). The same study recorded lower plasma total cholesterol, low-density lipoprotein and high-density lipoprotein by 19%, 22% and 14%, respectively, when saturated fat was replaced by polyunsaturated fat. A study has also shown that replacing saturated fat by polyunsaturated or monounsaturated fat lowers both low density lipoproteins (LDL) and high density lipoproteins (HDL) (Siri-Tarino *et al.*, 2010).

In a study to determine nutrient intake of vegetarian and non-vegetarian women, vegetarian participants recorded a higher ratio of polyunsaturated fat to saturated fat intake than their non-vegetarian counterparts. (Janelle and Barr, 1995). Another study however has recorded similar intake of polyunsaturated fat between vegetarian and non-vegetarian groups (Dourado *et al.,* 2011). Nadimi *et al.* (2013) in a study to determine the association of vegan diet with body composition and oxidative stress found no significant difference in fat intake between the two groups. A study carried out in the United States by Haddad and Tanzman (2003) found that the vegetarian participants had lower intakes of total fat, saturated fat and cholesterol, compared with the non-vegetarian participants.

2.8 Micronutrient intake of vegetarians

2.8.1 Iron

Iron is a very important micronutrient that is obtained naturally in food. There are two forms, from which iron is obtained, namely haeme iron, obtained from animal sources and non- heme iron, obtained from plant sources. Iron forms part of haemoglobin and myoglobin. These are proteins that help in the transport and transfer of oxygen from the lungs, where exchange of oxygen takes place, to tissues and muscles, respectively (Agget, 2012). Associations have been recorded between serum homocysteine and markers of iron deficiency, in which significantly higher serum homocysteine levels have been reported in individuals with iron deficiency anaemia (Sirdah *et al.*, 2014). Children who consume iron-deficient diet and those with iron deficiency anaemia have been found to show poor cognitive development. Supplementation with iron tends to produce remarkable improvement in motor and language capabilities in children (Stoltzfus *et al.*, 2001). Effects of a vegetarian diet on iron status depend on the bioavailabilty of iron from plant foods rather than the amount of total iron in the diet (Venderley and Campbell, 2006).

Iron intakes were found to be equal among vegetarians and non-vegetarians in a study conducted by Ball and Bartlett (1999), although vegetarians recorded slightly higher intake of iron. The highest percentage of the iron consumed in vegetarian participants, were obtained from cereals and cereal products, followed by vegetables, then breakfast cereals, and the lowest percentage from fruits. Recent studies however have produced different outcomes. For example, in the EPIC-Oxford study by Davey *et al.* (2003) that examined the lifestyle characteristics and nutrient intakes between the two groups, the highest intake of iron was recorded among the vegan group. Li *et al.* (2000) as well recorded higher iron intakes in vegetarians than in non-vegetarians in their study that sought to determine micronutrient intake in vegetarian and vegan men, as well as in men with different meat intakes. However, serum ferritin levels in vegetarians have been found to be significantly lower than in non-vegetarians, the result being attributed to the reduced bioavailability of the iron derived from plant sources (Ball and Bartlett, 1999; Li *et al.*, 2000).

2.8.2 Zinc

Zinc is one micronutrient that has generated lots of questions about its availability and adequacy in vegetarian diets. Plant sources of zinc include nuts, legumes and whole grains. These are very good sources of the micronutrient. However zinc found in plants has reduced bioavailability, as a result of the binding of phytates to it, and hereby, making absorption difficult (Cena *et al*., 2008). The RDA for zinc for adult men and women is 11 mg and 8 mg respectively (Academy of Nutrition and Dietetics, 2009). According to the Academy of Nutrition and Dietetics, whole grain products usually have higher zinc content as compared to refined grains and so even in the presence of phytate, relatively more zinc is absorbed from whole grain products than refined products. Requirements for zinc in vegetarians whose diets most of the time consists of phytate-rich sources may exceed the recommended dietary intake.

Recommendations on meeting zinc needs for individuals on plant-based diets include increasing zinc bioavailability by soaking dried beans in water and discarding the water prior to cooking, using fermented soy products, selecting whole grain cereals instead of refined alternatives and putting emphasis on zinc-rich foods such as nuts legumes, whole grains and avoiding the intake of calcium supplements before or after consuming foods that are rich in zinc (Messina *et al*., 2004).

In a review paper that sought to bring together information on results of various studies carried out on zinc status of vegetarians, the summary revealed that out of the 34 research works reviewed, 26 assessed and compared male and female vegetarians with non-vegetarian subjects and recorded that dietary zinc intakes and zinc concentration in serum was significantly lower in individuals that habitually followed vegetarian diets than omnivorous diets (Foster *et al*., 2013). Vegans have been found to have the lowest intake of zinc in a comparative study that assessed dietary intake among meat-eaters, fish-eaters, lacto-ovo vegetarians and vegans (Davey *et al.*, 2003). Meat-eaters in a different study recorded higher intakes of zinc than did their vegetarian and vegan counterparts (Li *et al.*, 2000). Others however have observed that dietary zinc intake between both groups are comparable (Rauma and Mykkanen, 2000).

2.8.3 Vitamin C

One indispensable micronutrient and antioxidant vitamin is Vitamin C, found mostly in fruits and vegetables. Water-soluble vitamin C is one antioxidant vitamin capable of neutralizing hydrogen peroxide, superoxide radical and hydroxyl radical, which are all reactive oxygen species (ROS) (Percival, 1998). A very important role played by Vitamin C in a vegetarian diet is its ability to act as an efficient iron-absorption enhancer. Vitamin C increases and facilitates the absorption of non-haeme iron obtained from plant sources, whose absorption is usually inhibited by absorption inhibitors like phytates (Davey *et al.*, 2003).

Vegetarians reported higher intake of vitamin C than their non-vegetarian counterparts in earlier studies by Janelle and Barr 1995 (in a study carried out in a Metropolitan area in Canada) and Harmann and Parnell, 1998 (New Zealand). In the EPIC-Oxford study, vegetarians again recorded the highest intake of vitamin C (Davey *et al.*, 2003). A more recent study on vegetarian dietary intake also ended up with similar results. In the study conducted by Shridhar *et al*. (2014), although non-vegetarians reported higher intake of fruits, vegetarians still showed higher intake of dietary Vitamin C with an observed higher intake of vegetables. In a study conducted to determine the protective plasma value of vitamin C in an adult population of vegetarians and non-vegetarians from Slovakia, it was observed that 88% of the vegetarian participants had optimum protective value as opposed to the non-vegetarian participants who had only 46% of their participants having an optimum protective value (Krajčovičová-Kudláčková *et al.*, 2007). Therefore, taking into consideration most research work on vegetarian versus non-vegetarian diet, as well as an article that discussed antioxidant status in vegetarian and omnivorous diet, it will be efficient to conclude that a vegetarian diet helps in the maintenance of an optimum antioxidant vitamin status.

2.8.4 Vitamin E

Vitamin E is another important antioxidant vitamin responsible for neutralizing the effect of free radicals, mostly lipid peroxides and hydrogen peroxides, in the body (Percival, 1998). Rich sources of fat-soluble vitamin E include whole grains, nuts, seeds, plant oils, avocado and green leafy vegetables. According to Pryor (2000), vitamin E protects the body's system against the production of malondialdehyde.

Studies conducted by Orlich *et al.* (2014) and Haddad and Tanzman (2003) have shown that vegetarians tend to consume more legumes, nuts and seeds, as these serve as main sources of protein in their diet. In the famous EPIC-Oxford study on vegetarian diet and lifestyle, vegans were observed to have the highest intakes of dietary vitamin E, compared with lacto-ovo vegetarians and non-vegetarians, probably indicating that the stricter the vegetarian diet, the higher the chance of obtaining high amounts of vitamin E (Davey *et al.*, 2003). A study has even revealed that the level of vitamin E in the blood of lacto-vegetarians and lacto-ovo-vegetarians is comparatively higher than that of non-vegetarians (Somannavar and Kodliwadmath, 2012). In this study, it was observed that the decreased level of vitamin A and E in the blood of non-vegetarians was associated with decreased levels of the enzymatic antioxidant glutathione peroxidase, as opposed to that of vegetarians who recorded comparatively high levels of both the enzymatic antioxidants and non-enzymatic antioxidant vitamins.

2.9 Reactive Oxygen Species (ROS) and Oxidative Stress

Reactive oxygen species (ROS) are chemically reactive molecules such as peroxides and oxygen ions (Glade, 2003) and are produced naturally, as by-products of the normal metabolism of oxygen (Virginie *et al.*, 2012). They are generated and released during normal processes of body cells, such as the degradation of haemoglobin, as well as generated by the human immune system during the inflammatory response to injury by immune cells such as activated macrophages and neutrophils (Conforti *et al.*, 2007). They are considered toxic to the body. Examples of ROS include hydroxyl radical, singlet oxygen, peroxyl radical, semiquinone radical and alkoxyl radical (Caballero, 2006). The mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation are all endogenous sources of ROS (Inoue *et al.*, 2003).

According to Maxwell (1995), about 5%, or even more, of oxygen breathed in during inhalation is converted to ROS. In the normal processes of cells, bonds that split usually result in molecules with paired electrons. However, in situations where weak bonds split, there is the formation of free radicals which are extremely unstable, and in a bid to gain some form of stability, react quickly with other compounds (Hamid *et al.*, 2010).

According to Virginie *et al.*, (2012), ROS is therefore a collective term for several oxidizing compounds such as hydrogen peroxides, superoxide anions, hydroxyl radicals that exhibit a characteristic high reactivity due to the presence of unpaired valence shell electrons.

The formation of free radicals also occur in processes such as exposure to some environmental conditions, for example sunlight, radiation, high oxygen levels, microbial lysis during immune function, and oxidation of substrates with high affinity for oxygen, for example fatty acids (Caballero, 2006). Glade (2003) established that ROS can be both harmful and beneficial in biological systems depending on the environment. ROS benefit the body by taking part in the physiological roles of cellular responses such as cell signaling and defense against infectious agents (Glade 2003). However, at high concentrations, ROS causes “oxidative stress”, characterized by a result in damage to cell structures, including lipids and membranes, proteins and nucleic acids (Poli *et al.*, 2004). Diseases such as degenerative diseases of aging, cancer, diabetes mellitus, protein energy malnutrition (PEM), cardiovascular diseases and atherosclerosis have been shown to result from an overwhelming of the body's system with ROS (Omoregie and Osagie, 2011).

A study confirmed that ROS levels increase drastically during conditions such as exposure to ultraviolet radiation and heat exposure (Devasagayam *et al.*, 2004). ROS cause harmful results such as oxidation of polyunsaturated fatty acids (lipid peroxidation; whose product malondialdehyde is an indicator of oxidative stress), damage of DNA (leading to possible mutation), oxidation of amino acids in proteins and inactivation of specific enzymes by oxidation of co-factors (Brooker, 2011). Evidence from a study conducted by Turpeinen *et al.* (1998) therefore shows that oxidative stress could increase as a result of large intake of polyunsaturated fats, leading to endothelial malfunction. Also, a study by Vijayakumar *et al*. (2004) to determine the antioxidant effect of black pepper observed that rats that were fed a high fat diet exhibited significantly lowered levels of the antioxidant enzymes, glutathione peroxidase, superoxide dismutase and catalase as well as an increased level of thiobarbituric acid reactive species (TBARS) in the heart, aorta, liver and kidney. This further explains the relationship between ROS and diseases associated with very vital organs in the human body. Oxygen free radicals have been found to distort the structure and function of tissues, resulting in oxidative stress leading to so many diseases (Omoregie and Osagie, 2011; Kumar *et al.*, 2012).

Nutritional oxidative stress therefore is said to occur when there is a disturbance in the redox state caused as a result of either inadequate supply of nutrients that favour proxidant reactions or from excess oxidative load, resulting from excess free radical formation and the inability of the antioxidant system to keep it under control (Sies *et al*., 2005).

In another study that investigated the risk factors of ischaemic heart disease in rural populations of Nilgiris, south India, with focus on oxidative stress, researchers discovered that individuals with the disease had lowered levels of the antioxidants SOD, catalase and ascorbic acid in plasma, and an increased level of TBARS, which is a predictor of lipid peroxidation (Kumar *et al.*, 2012). Conclusion was therefore drawn that the major causes of cardiovascular diseases amongst the rural populations of the Nilgiris, south India, were preventable causes such as smoking and high fat intake (thereby encouraging an increased rate of lipid peroxidation), all of which cause oxidative stress (Kumar *et al*., 2012).

A study on antioxidants, oxidant stress and essential fatty acids in vegetarians and non-vegetarians revealed lower levels of plasma lipid peroxides in the former (Manjari *et al*., 2001), suggesting that vegetarians have lower oxidative damage, compared with their non-vegetarian counterparts. A study carried out on the ability or otherwise of a vegetarian diet to provide genomic stability recorded significantly higher levels of DNA damage in the non-vegetarians compared with the vegetarians, although total antioxidant capacity between both groups were similar (Kazimírová *et al.*, 2004). It was therefore concluded in the study that a vegetarian diet could provide some level of protection against oxidative stress. Results from a study by Somannavar and Kodliwadmath (2012) on the correlation between oxidative stress and anti-oxidant defense in South Indian urban vegetarians and non-vegetarians revealed a significantly higher malondialdehyde (MDA) level in the non-vegetarian group, indicating increased lipid peroxidation in the non-vegetarian group, compared with the vegetarian group. These studies have proven that vegetarians are at a lowered risk on oxidative stress and damage.

2.10 Diet and Antioxidant Levels

Guidelines on how to get enough antioxidants through food include eating a rainbow of fruits and vegetables, choosing a colourful fruit and vegetable, choosing whole grain products, adding nuts to salad and soups and snacking on vegetables (Academy of Nutrition and Dietetics, 2009). In a study to determine oxidative DNA damage in relation to nutrition, it was concluded that an incorrect nutritional habit and lifestyle determines the extent of DNA damage in the body (Dusinská and Krajcovicová-Kudlácková, 2004). In this study, DNA strand breaks with apurinic/apyrimidinic sites, oxidized purines and oxidized pyrimidines were assessed and compared in 24 subjectively healthy vegetarians and 24 non-vegetarians. The results showed a significantly reduction in DNA strand breaks and oxidized purines in vegetarians. In the same study, a significantly higher intake of protective food commodities among the vegetarian subjects was observed.

The antioxidant network is unable to function properly when intake of dietary antioxidants such as vitamins C and E, carotenoids, polyphenols and other micronutrients such as selenium is low or their availability to the body is impaired (Sies *et al*., 2005). When adequate dietary polyphenols are ingested, it helps lower the susceptibility of low density lipoproteins (LDL) to oxidation, and improves endothelial dysfunction (Sies *et al*., 2005).

Due to the fact that vegetarians consume many plant foods, such as cereals, pulses, fruits and vegetables, their diet contains more sources of antioxidant vitamins, such as vitamin C and E, than non-vegetarians, since they are likely to replace animal protein source with plant protein sources (Rauma *et al.*, 2000). Levels of antioxidant trace elements like zinc, iron and copper have however been recorded as low in vegetarians as compared to non-vegetarians, resulting from a higher intake of plant trace element absorption inhibitors, like phytates, found in most plants, in the former group (Krajčovičová-Kudláčková *et al.*, 2003).

A study by Krajčovičová-Kudláčková *et al.* (2003) measured the levels of the antioxidant vitamins, vitamin C and vitamin E in vegetarians and non-vegetarians. Findings were that, an average plasma value of antioxidant vitamins, C and E as well as the ratios, vitamin C/vitamin E, vitamin E/ cholesterol (LDL protection), vitamin E/triacylglycerols (polyunsaturated fatty acid protection) were higher than the normal threshold values in the vegetarian group. This indicates overthreshold plasma values of essential exogenous antioxidants in vegetarians, which means a reduction in the risk of occurrence of diseases caused by free radicals (Krajčovičová-Kudláčková *et al.*, 2003).

2.11 Antioxidant Protection

Fortunately, oxidation caused by free radicals can be minimized by a range of antioxidants (Bjelakovic *et al.*, 2007). The role of antioxidants in the body is to deactivate free radicals or slow down their action before they attack and cause damage to cells (Gupta and Sharma, 2006). Antioxidants are therefore the body's first line of defense against damage by free radicals. However, since ROS partake in certain important cellular activities and functions such as redox signaling, the duty of antioxidants is to keep the amount of ROS at a level that prevents destruction of the body's cells and not to totally remove them (Rhee, 2006). Earlier studies into antioxidants involved the role of antioxidants in the prevention of rancidity in saturated fats, where the rate of oxygen consumption was monitored in a setup involving fat and oxygen in a closed container (German, 1999).

The antioxidant protection system consists of endogenous antioxidants, dietary antioxidants and metal binding proteins (Percival, 1998). Dietary antioxidants include vitamin C, vitamin E, beta carotene and polyphenols, among others (Hamid *et al.*, 2010). Examples of endogenous antioxidants include the enzymes copper/zinc and manganese-dependent SOD, iron-dependent catalase (CAT), selenium-dependent glutathione peroxidase (GPx) as well as uric acid and bilirubin (Hamid *et al.*, 2010). This combination of protective ability is referred to as antioxidant capacity. Antioxidant capacity defines the ability of cells to protect themselves against oxidative stress through the different actions of the various intrinsic factors such as CAT, SOD, GPx and extrinsic factors obtained from foods such as vitamin A, C, E and phytochemicals (Kim *et al.*, 2012). The antioxidant enzymes carry out their catalytic activities effectively in the presence of trace minerals such as manganese, iron zinc, copper and selenium, which act as co-factors (Hamid *et al.*, 2010). It is therefore possible that the ability of these antioxidant mechanisms to function properly may be affected and compromised through inadequate dietary intake of the above mentioned trace elements (Duthie and Brown, 1994).

In humans, vitamin E is a very powerful dietary antioxidant that exist as membrane bound in the cell and acts in its most active form, α-tocopherol (Hensley *et al*., 2004). The main function of vitamin E is to protect against lipid peroxidation and subsequent production of the harmful product, MDA, which is an indicator of oxidative stress (Pryor, 2000). Oxidative damage is reduced by an overall improved antioxidant status above the threshold plasma values of the various essential antioxidants (Krajčovičová-Kudláčková *et al*., 2003).

2.11.1 Superoxide Dismutase (SOD)

Superoxide dismutase is an antioxidant enzyme considered as part of the first line antioxidant defence amongst others such as catalase (CAT), glutathione reductase (GSH), glutathione peroxidase and minerals, and works in accordance with them, performing its duty by quenching superoxide ions formed in the body (Gupta and Sharma, 2006). The co-factors of the superoxide dismutase enzymes are zinc, manganese and copper (Noori, 2012). SOD catalyses the dismutation of the superoxide anion to an oxygen molecule (O_2) and hydrogen peroxide (H_2O_2), which are less reactive species (Mates *et al.*, 1999).

A study carried out on transgenic mice showed the inability of the mice to survive for more than two weeks after birth, and a series of biochemical defects ascribed to oxidative stress, when SOD-2 (the superoxide dismutase gene) was knocked out. (Melov *et al*., 1999).This shows the significance of the presence of SOD in living systems. Diet has been found to affect the epigenetic regulation of the MnSOD gene (Thaler *et al*., 2009).

Some studies have found that SOD concentrations are higher in vegetarians compared with non-vegetarians (Manjari *et al.,* 2001, Rauma *et al.,* 1995). Other studies however have found no significant difference in the SOD levels in vegetarians and non-vegetarians (Haldar *et al.,* 2007), especially in situations where the individuals were suffering from chronic diseases like cancer (Kim *et al.*, 2012).

2.11.2 Catalase (CAT)

Catalase plays its antioxidant role by reacting with hydrogen peroxide, produced from dismutation of the superoxide anion, to form H_2O and O_2, thereby, detoxifying hydrogen peroxide and protecting cells from any H_2O_2 produced within them (Mates *et al.,* 1999). Catalase is an iron-dependent antioxidant (Hamid *et al.*, 2010) and its activity is influenced by the amount of exogenous antioxidants consumed. For example, the activity of catalase was found to increase significantly when nephrotic rats were administered with soy protein and genistein (the major isoflavone of soybean) (Javanbakht *et al.*, 2014). Similarly, the activity of catalase has been found to be higher in workers chronically exposed to lead, after administration of beta carotene (Kasperczyk *et al.*, 2014).

2.12 Fruits and Vegetables

Antioxidants are found in a variety of foods such as vegetables, fruits, grains, cereals, eggs, meat, legumes, nuts and even tea (Rietveld and Wiseman, 2003). Research has shown that the risk of developing most types of cancers is doubled by low dietary intake of fruits and vegetables (Ames, 1994). According to Amitom (2001), several health benefits such as prevention of age-related degenerative diseases, cancer and stroke, are associated with consumption of berries, due to the presence of certain phytochemicals. These phytochemicals when consumed enhance the production and activity of the endogenous antioxidant properties in the body (Amitom, 2001).

Phenolic compounds which include phenolic acids, flavonoids and flavonolignans in certain herbs have been found to act as antioxidants at the molecular level (Craig, 1999) as well as help in the prevention of development of coronary artery disease (Hertog *et al.*, 1993). The study by Craig (1999) is supported with another study in which phenolic-rich plant extracts of *Silybum marianum* and *Prunella vulgaris* were administered to rats with high-sucrose diet induced oxidative stress. The results showed an overall increase in protective components against oxidative stress, including glutathione peroxidase and SOD (Škottová *et al.*, 2003). All these related studies suggest that in a bid to increase one's overall antioxidant status, a variety of plant foods is needed.

2.13 Cereals and grains

Other antioxidant compounds such as the polyphenolic antioxidants in foods, such as whole-wheat cereals are more stable (Baublis *et al*., 2000). Phenolic compounds or acids are present in all cereals and are derived from benzoic and cinnamic acids (Dykes and Rooney, 2007).The unique phytochemical composition of whole grains is part of the reasons why they are known to provide many health benefits. In a study that investigated the complete phytochemical profiles of free, soluble conjugated and insoluble bound forms as well as antioxidant activities in the uncooked whole grains, corn, rice, wheat and oats, corn was found to have the highest total phenolic content. Corn recorded 15.55 ± 0.60 μmol of gallic acid equiv/g of grain, followed by wheat, oats and rice, all of which recorded 7.99 ± 0.39, 6.53 ± 0.19 and 5.56 ± 0.17 μmol of gallic acid equiv/g of grain respectively (Adom and Liu, 2002). In the same study, the same trend was observed for grains that had the highest antioxidant activities. Corn had the highest antioxidant activity, followed by wheat, then oats and finally rice. Whole grain breakfast cereals have been found to have even higher antioxidant status than most fruits and vegetables, recording 2200-3500 trolox equivalents (TE)/100 g as compared to fruits and vegetables which recorded 600-1700 TE and 450 TE respectively (Miller *et al.*, 2000).

Studies have shown that due to the omission of animal protein from their diet, vegetarians consume more grain and cereal based diet compared to non-vegetarians and are therefore conferred all the benefits provided by this class of foods (Rauma *et al.*, 2000 ; Orlich *et al.*, 2014; Haddad and Tanzman, 2003).

2.14 Legumes, Seeds and Nuts

Legumes are rich sources of folate, dietary fibre calcium, iron potassium, zinc and most importantly antioxidants. Nuts have been found to be among various dietary plants with the highest content of total antioxidants and out of them, chestnut, walnuts and pecans have the highest content of antioxidants (Blomhoff *et al.*, 2006). Consumption of legumes, precisely, soybeans have been inversely associated with the risk of type-two diabetes (Villegas *et al.*, 2008). It has been found that the best way to cook legumes and still preserve their antioxidant capability is through pressure boiling over a shortened processing time (Xu and Chang, 2008).

Vegetarians have been found to consume more legumes, nuts and seeds, compared with non-vegetarians, since these food commodities serve as their main source of protein, as a result of omission of animal protein from their diet. (Rauma *et al.*, 2000; Orlich *et al.*, 2014; Haddad and Tanzman, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

The research was a case-control study. The Purposive and convenience sampling techniques were used in recruiting participants for both the case and control groups.

3.2 Study Sites

The case group was recruited from the New Life Historic Seventh-Day Adventist church in Mayera, a suburb of Accra. The control group was obtained from a branch of the church in Dansoman. The congregation gathered together on Saturdays between the hours of 10:00 and 12:30 GMT for their religious fellowship.

3.3 Subject Recruitment

Prior to recruitment of participants, the nature of the study was explained to the authorities of the churches. The authorities then gave permission so that the rationale behind the study and the procedure of obtaining data was explained to the entire congregation. Ethical issues concerning the study were also clearly stated to the individuals and only vegetarian and non-vegetarian participants who willingly accepted to take part in the study were recruited. The subjects for the study were non-vegetarians between the ages of 18-50 and vegetarian adults, 18-50 years, who had lived a vegetarian lifestyle for not less than 2 years.

3.4 Sample Size Determination

A sample size of 50 was determined based on the population of the Vegetarians' Association of Ghana, Accra (about 300 members). An allowance of 10% and a non-response rate of 20% were given. An absolute precision of 5% and a confidence interval of 95% were used in accordance with the strategic function of Epi Info Statistical Software were used. .

$$N = [\frac{Z^2(P)(1-p)}{\epsilon^2}]$$

$$N = [\frac{1.96^2(0.05)(0.95)}{0.06^2}]$$

$$N=50$$

Where 'N' is estimated sample size

'E' is desired margin of error

'Z' is the critical Z score on the desired level of confidence (95%)

'P' is the prevalence of vegetarians in Accra.

However, due to the difficulty in obtaining vegetarian participants, attributed to the small number of vegetarians in the Greater Accra Region, 39 cases and 30 controls were used in this study.

3.5 Subject Selection

3.5.1 Inclusion Criteria:

Participants included in the study were lacto-vegetarians, lacto-ovo vegetarians and vegans between the ages of 18-50 years. The control group made was made up of non-vegetarian individuals.

3.5.2 Exclusion criteria

Individuals who had been vegetarians for less than twenty-four months, and vegetarian and non-vegetarian participants below the age of 18 years were excluded from the study.

3.6 Ethical Approval

Ethical approval for the study was obtained from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (Ethical Identification Number: **SAHS-ET./10396481/AA/10A/2013-2014.**

3.7 Pre-Testing of Questionnaire

Questionnaire to be used for the study was pre-tested among a representative group of vegetarians in Sakumono, in the Greater Accra region, prior to the main study. Data obtained was reviewed and the questionnaire fine-tuned to suit the desired study population. Questions about dietary information such as intake of herbal preparations and the composition of the herbal preparations and frequency of consumption of fruits and vegetables were added.

3.8 Data Collection

Collection of data was done in two phases. One phase involved the administration of the pre-tested questionnaires. Questionnaires were used to collect information which included socio-economic status, food frequency, previous medical history, demographic characteristics, anthropometry (height, weight and body mass index (BMI)) and other lifestyle risk factors (smoking and alcohol consumption). Blood pressure measurement was also taken from the participants.

The second phase involved drawing of blood samples, by qualified laboratory scientists, from participants who had given their consent.

3.9 Anthropometric Measurement

Anthropometric data collected in the study included weight, height, body mass index (BMI), visceral fat and body fat. The BMI was obtained by dividing the weight (in kilograms) of each participant by the square of the height (in metres). All measurements were compared with various standards and normal ranges.

3.9.1 Weight

Weight was measured to the nearest 0.1 kg, using the BF= 506 Omron Healthcare Omron Body Composition Monitor (IL, USA). Participants were requested to empty pockets of all objects and remove shoes, socks and any extra clothing before standing on the scale for measurement to be taken. Participants were also requested to stand erect and still.

3.9.2 Height

Height of participants was measured to the nearest 0.1 cm. The instrument used was a portable Seca stadiometer (Hamburg, Germany). Participants were requested to take off shoes and socks and stand on the base plate of the stadiometer, with their back and head straight. Their feet were placed together, with their heels touching the back of the plate of the stadiometer, and the head plate, lowered to touch the top of the head, noting the height of the participant.

3.9.3 Visceral Fat and Body Fat Percentages

Visceral fat and body fat percentages were also measured using the BF= 506 Omron Healthcare Omron Body Composition Monitor (IL, USA).

The ranges for body fat and visceral were adapted from the Tanita Scale and were therefore classified as follows:

Rating from 1 to 12 indicated healthy visceral fat

Rating from 13-59% indicated excess level of visceral fat

Body Fat:

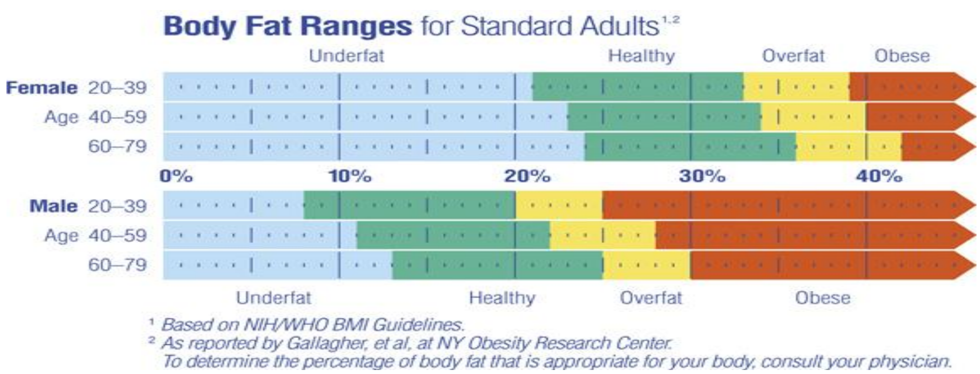


Figure 3.1 Body fat ranges for adults

3.10 Blood Pressure Measurement

Blood pressure was measured in a calm environment. Subjects were made to sit upright, feet placed flat on a smooth ground and with the left arm relaxed on a hold-up, at a height approximate to the level of the heart. Participants were made to sit and rest for a minimum of 5 minutes before blood pressure was measured. The Omron HEM-742-E2 (Hoofddorp, The Netherlands) was used to measure the blood pressure of the participants, two times at an interval of one minute, and the average taken as the blood pressure reading.

3.11 Dietary Intake Assessment

Food intake was assessed by obtaining dietary history of the participants through an interview. This was done using a 24 hour dietary recall (two week days and one weekend day), to assess current intake of food, and a validated food frequency questionnaire (Asare, 2011) to assess usual food intake. Information on portion sizes of food consumed was also obtained and participants were assisted in estimating portion sizes, using food models.

3.12 Blood Sample Collection and Handling

Three millilitres of blood sample was drawn from the antecubital vein of participants into EDTA tubes. Blood samples were collected before 9:00 hours in the morning. Samples were kept in a cool box and transported to the Chemical Pathology Laboratory of the School of Biomedical and Allied Health Sciences, University of Ghana, Korle-bu campus.

3.13 Sample Preparation and Storage

Prior to storage, the blood samples were divided into two parts, one part for analysis of SOD and the other for catalase. For SOD analysis, 500 µl of whole blood was centrifuged using Heal Force Neofuge 23R (Shanghai, China) at 3000rpm for 10 minutes. The supernatant was discarded and red blood cells washed four times with 3.0 mls of 0.9% saline solution, with the supernatant being discarded after each centrifuge. The rest of the original blood samples were centrifuged at 5000 rpm for 5 min and plasma aliquoted into sample collection tubes. Washed red blood cells samples were stored at 4^{o}C to preserve the cells, while plasma samples were stored at -20^{o}C.

3.14 Biochemical Analysis

3.14.1 Superoxide Dismutase (SOD)

The role of SOD is to accelerate the dismutation of the toxic superoxide ($^{-}O_2$) radicals that are produced during oxidative energy processes to hydrogen peroxide and molecular oxygen.

$$(^{-}O_2)_2 \quad + \quad 2H^{+} \xrightarrow{SOD} H_2O_2 \quad + \quad O_2$$

The method employs xanthine and the enzyme, xanthine oxidase (XOD) to generate $^{-}O_2$ radicals that react with 2-(-4-Iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. SOD activity is measured by the degree of inhibition of this reaction.

3.14.2 Reagents

Randox Laboratories Ltd. SOD kit was used according to the manufacturer's protocol. The kit consisted of the following:

1. Buffer
2. Mixed substrate
3. Ransod diluents (0.01 M phosphate buffer, pH 7.0)
4. Standard
5. Xanthine Oxidase

3.14.3 Preparation of Standards

The lyophilized standard was reconstituted with 10 ml of redistilled water and serial dilutions prepared as follows:

	Volume of standard solution	Volume of sample diluent
S6	Undiluted Standard	-
S5	5 ml of S6	5 ml
S4	5 ml of S5	5 ml
S3	5 ml of S4	5 ml
S2	5 ml of S3	6 ml
S1	-----------	5 ml

3.14.4 Sample Preparation

Two milliliters (2ml) of cold redistilled water was added to the previously washed cells mixed and left to stand at 4^{o}C for 15 minutes. The lysate was then diluted with 2.4 ml phosphate buffer, to achieve a 200-fold dilution factor (df) with percentage inhibition between 30% and 60%.

3.14.5 Assay Procedure

The procedure was followed according to the manufacturer's instruction. The reaction was carried out at 37^{o}C and absorbance read against air blank at 505 nm. The initial absorbance was read after 30 seconds and final absorbance, read after 3 mins.

Calculation:

$$\frac{A2 - A1}{3} = \delta\mathrm{A/min}\ (standard/sample)$$

Sample diluents rate (S1 rate) = rate of uninhibited reaction =100%

Rates of all standard and diluted samples were converted into percentages of the sample diluent rate and subtracted from 100% to give the percentage inhibition.

$$100 - \frac{(100 \times \delta\mathrm{A}_{\mathrm{std/min}})}{(\delta\mathrm{A}_{\mathrm{s1/min}})} = \%\ inhibition$$

A curve of percentage inhibition for each standard against Log_{10} standard concentration of SOD U/ml was plotted. Using the percentage inhibition of the sample from the equation, SOD concentration was obtained from the standard curve.

SOD units/ml of whole blood = SOD units/ml from standard curve x 200 (df)

SOD units/g haemoglobin was then calculated using the equation:

$$\frac{\text{SOD units/ml}}{\text{g haemoglobin/ml}} = \text{SOD units/g haemoglobin}$$

3.14.6 Catalase

The role of catalase is to accelerate the dismutation of toxic hydrogen peroxide radicals that are produced during oxidative energy processes, to water (H_2O) and molecular oxygen.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

3.14.7 Reagents

The analysis was carried out using the following reagents

Buffer

Potassium dichromate ($K_2Cr_2O_7$)

Hydrogen peroxide solution

Distilled water (H_2O)

3.14.8 Preparation of Standards

Using six different concentrations of H_2O_2 as shown below, a standard curve was obtained.

Concentration	Absorbance
0	0.311
0.1	0.667
0.2	0.881
0.3	0.905
0.4	0.917
0.5	0.987

3.14.9 Assay Procedure

Into a clean test tube, 0.2 ml of H_2O_2 was aliquoted and 0.5 ml of the buffer was added. To this, 0.05 ml of the sample was added and the flask swirled gently. Potassium dichromate (0.2 ml) was then added and the test tube heated in a boiling bath for 10 minutes to decompose the blue precipitation and produce a green solution. The solution was then transferred into cuvettes and the absorbance read at 570 nm in the spectrophotometer. Using the standard curve, the amount of H_2O_2 (units/ml) remaining in the solution, when the enzyme was stopped with the potassium dichromate was determined.

3.15 Data Processing and Analysis

Dietary data was analyzed using the nutritional analysis software ESHA-FPRO version 5.

Statistical analysis was done using Statistical Package for Social Scientists (SPSS) version 17.0. Results of endogenous antioxidant levels were computed as one-tail whilst all other results were computed as two-tail and statistical significance was considered at a P-Value of ≤ 0.05. The independent *t*-test was used to compare mean levels of antioxidants among case and the controls. Qualitative data were summarized as proportions and percentages, whilst quantitative (height, weight and ages) was summarized as mean, standard deviation and ranges. Pearson's correlation was used to assess relationship between intake of dietary antioxidants and serum and plasma levels of endogenous antioxidants.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio- Demographic Characteristics of Participants.

A total of 69 participants were sampled for this study. Out of the 69 participants, 56.5% were vegetarians and 42.3% were non-vegetarians. Lacto-vegetarians constituted the majority of vegetarians sampled for the study. The mean age of all participants was 33.9±8.8 years with vegetarian participants recording a mean age of 36.1±8.2 years, and 30.7±7.6 years for the non-vegetarian participants. A greater proportion of vegetarians (33.3%) had education up to both the basic and higher national diploma (HND) level.

More than half of the participants were employed (vegetarians= 71.8% and non-vegetarians=76.7%), with vegetarians constituting majority of participants who were employed (54.9%). In relation to the marital status of the individuals, a greater number of both vegetarians and non-vegetarians were married (Table 4.1).

Table 4.1 Socio-demographic characteristics of participants

Variables	V (n=39) Frequency	%	NV (n=30) Frequency	%
Gender				
Male	23	59.0	15	50
Female	16	41.0	15	50
Marital Status				
Single	14	35.9	13	43.3
Married	21	53.8	15	50
Divorced	1	2.6	0	0
Widowed	3	7.7	2	6.7
Educational Background				
No formal education	6	15.4	3	10
Basic Education	13	33.3	10	33.3
HND	13	33.3	10	33.3
Tertiary	7	18.0	7	23.4
Employment Status				
Employed	28	71.8	23	76.7
Unemployed	5	12.8	1	3.3
Student	3	7.7	6	20
Retired	3	7.7	0.0	0.0

4.2 Vegetarianism

4.2.1 Types and reasons for choice of vegetarianism

Lacto-vegetarians constituted 89.7% of the total number of vegetarians sampled, whilst lacto-ovo vegetarians and vegans constituted 7.7% and 2.6% respectively.

About 64.1% of vegetarians reported they became vegetarians because of religious reasons. Others reported they did so to support health (25.6%), whilst a few (2.6%)

reported they did so to protect the environment (Fig 4.1). About 69.2% of vegetarians reported they had been involved in the lifestyle for a period of between 2-10 years whilst 10.3% had been vegetarians for a period of between 21-30 years (Fig 4.2).

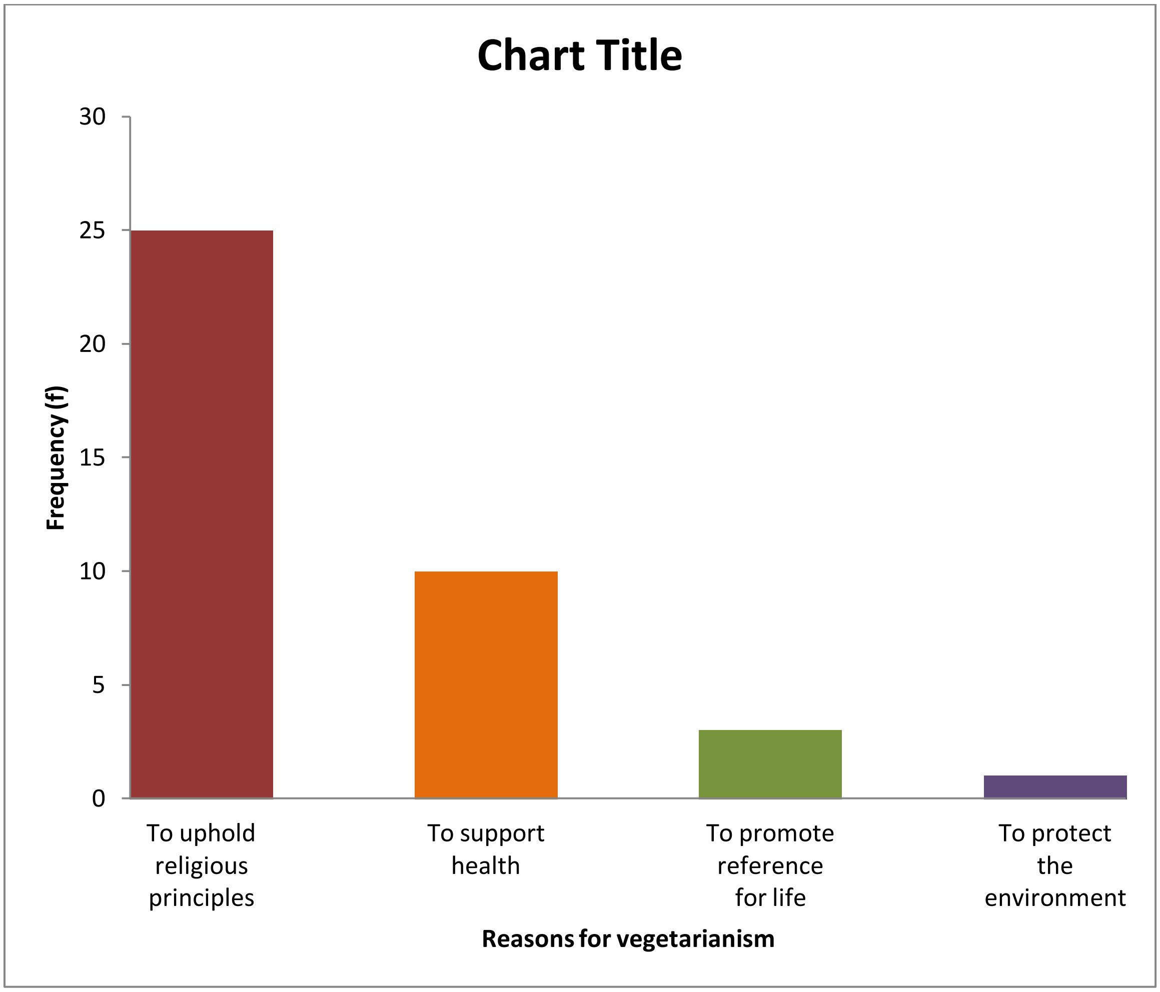


Figure 4.1 Reasons for becoming a vegetarian

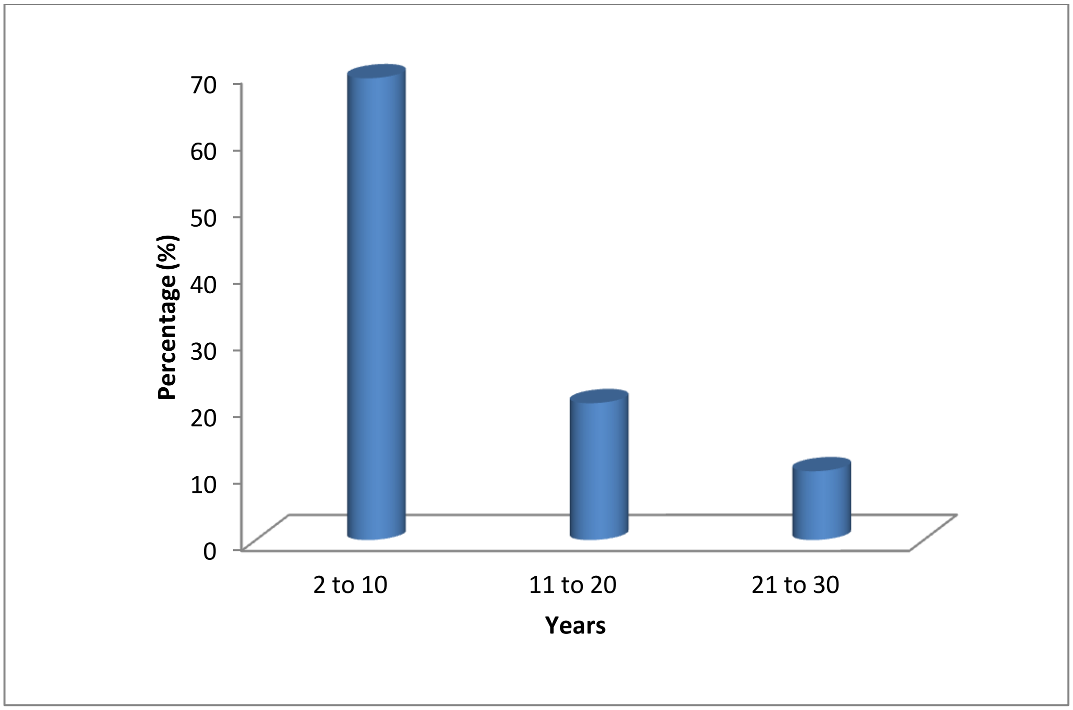


Figure 4.2 Years of vegetarianism

4.3 Dietary Habits and Meal Patterns of Participants

A high percentage of vegetarians (56.4%) stated that they consumed two meals a day whilst non-vegetarians who ate three meals a day constituted 40.0%. Frequency of number of meals consumed in a day was similar among both groups. Majority of vegetarians (53.9%) and non-vegetarians (70.0%) reported that they did not skip breakfast. About 51.3% of vegetarians and 66.7% of non-vegetarians reported intake of snacks in between meals. Fruits and vegetables constituted the main snack item consumed by majority of vegetarians (65.0%), whilst pastries were consumed by most non-vegetarians (45.0%) as snack (Table 4.2).

Table 4.2 Dietary habits and meal patterns of vegetarians (V) and non-vegetarians (NV)

Characteristic	Vegetarians (V) (n=39)		Non-vegetarians (NV) (n=30)	
	Frequency	**%**	**Frequency**	**%**
Number of main meals/day				
One	2	5.1	1	3.3
Two	22	56.4	17	56.7
Three	15	38.5	12	40.0
Skip Breakfast				
Yes	18	46.1	9	30.0
No	21	53.9	21	70.0
Snacks in between meals				
Yes	20	51.3	20	66.7
No	19	48.7	10	33.3
Types of snacks				
Fruits and vegetables	13	65.0	4	20.0
Soft drinks	3	15.0	7	35.0
Pastries	4	20.0	9	45.0
Frequency of fruit intake				
Daily	19	48.7	5	16.7
Once a week	14	35.9	16	53.3
Once a month	3	7.7	8	26.7
Occasionally	3	7.7	1	3.3
Never	0.0	0.0	0.0	0.0

4.4 Lifestyle Behaviours

There was significant difference (p=0.02) between the consumption of alcohol in vegetarians and non-vegetarians. Alcohol consumption was reported by less than three percent (2.6%) of vegetarians whilst 20.0% of non-vegetarians reported consumption of alcohol. The most frequently consumed alcoholic beverage among non-vegetarians was beer (10.0%). This was mostly consumed weekly. Smoking was however not common among individuals of both study groups and the difference between smokers was not significant (p=0.20) (Table 4.3).

Table 4.3 Lifestyle behaviour of participants Vegetarians (V) and non-vegetarians (NV)

Characteristic	Vegetarians (V) (n=39)		Non-vegetarians (NV) (n=30)	
	Frequency	**%**	**Frequency**	**%**
Current smokers	1	2.6	3	15.0
Alcohol drinkers	1	2.6	6	20.0
Type of alcohol				
Wine	0	0.0	1	3.3
Beer	0	0.0	3	10.0
Spirits	0	0.0	2	6.7
Brandy	1	2.6	0	0.0
Frequency intake of alcohol				
Daily	0	0.0	1	3.3
Once a week	0	0.0	3	10.0
Once a month	0	0.0	2	6.7
Occasionally	1	2.6	0	0.0
Quantity of intake of alcohol				
½ medium glass wine	0	0.0	1	3.3
1 mini Guinness/beer	0	0.0	3	10.0
1 tot of spirits/whisky	1	2.6	2	6.7
Other	0	0.0	0.0	0.0

4.5 Anthropometric and Blood Pressure Parametres of Participants

Table 4.4 shows there were significant differences in the visceral fat percentage and systolic blood pressure between vegetarians and non-vegetarians (p=0.039 and 0.049 respectively). However, there were no significant differences in weight, height, BMI, body fat and diastolic blood pressure between of the vegetarians and non- vegetarians (all p's >0.05).

Table 4.4 Comparison of anthropometric and blood pressure measurement between Vegetarians (V) and Non-vegetarians (NV)

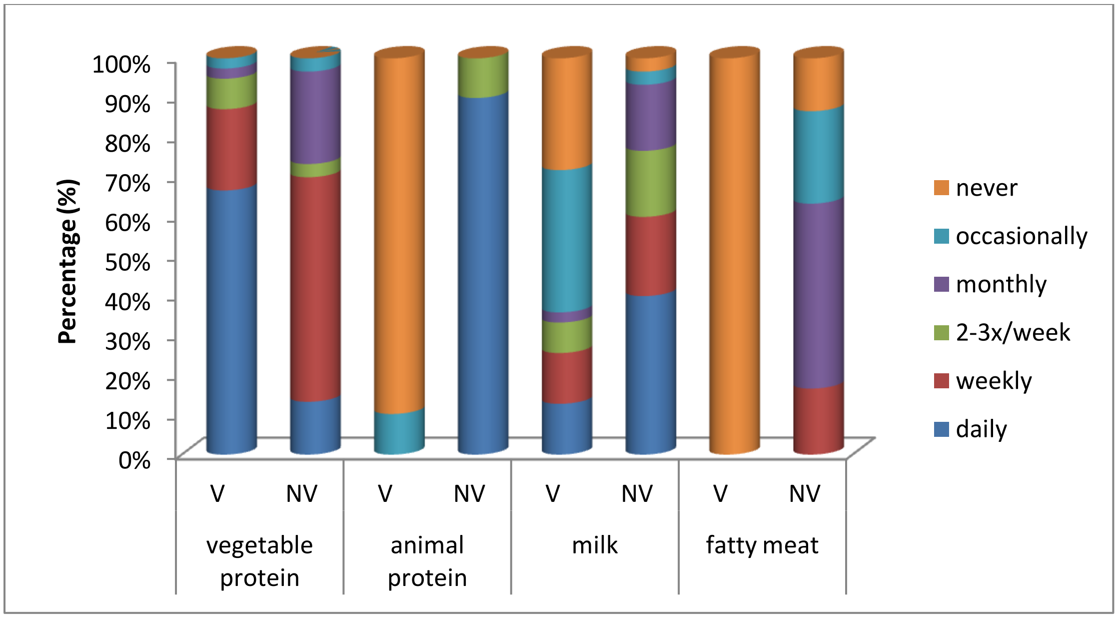
	Anthropometric and blood pressure measurement (mean ± SD)		
Variable	Vegetarians (n=39)	Non-vegetarians (n=30)	p-value
Weight (kg)	69.19 ± 13.13	69.62 ± 11.21	0.444
Height (m)	1.66±0.08	1.65 ± 0.07	0.359
BMI (kg/m^2	25.23 ± 4.63	25.37 ± 2.87	0.445
Visceral fat (%)	7.95 ± 4.49	6.27 ± 2.66	**0.039***
Body fat (%)	26..88 ± 11.00	23.80 ± 8.66	0.109
Systolic BP (mmHg)	127.29 ± 17.39	122.03 ± 5.02	**0.049***
Diastolic BP (mmHg)	79.74 ± 10.19	79.87 ± 3.92	0.475

***Significant at p≤ 0.05**

4.6 Frequency of Food Consumption

4.6.1 Animal protein and vegetable protein

Majority of vegetarians (66.7%) consumed vegetable protein on daily basis (everyday), whilst consumption of vegetable protein by non-vegetarians (56.7%) was observed to be mostly on weekly basis (once a week). Their sources of vegetable protein included "agushie", soybeans and peanuts, consumed in the form of soups and stews, as well as non-dairy creamers. Only 12.8% of vegetarians consumed milk on daily basis compared to 40.0% of non-vegetarians. About thirty-six percent (35.9%) of vegetarians however consumed milk about 2-3x/week (Figure 4.3).

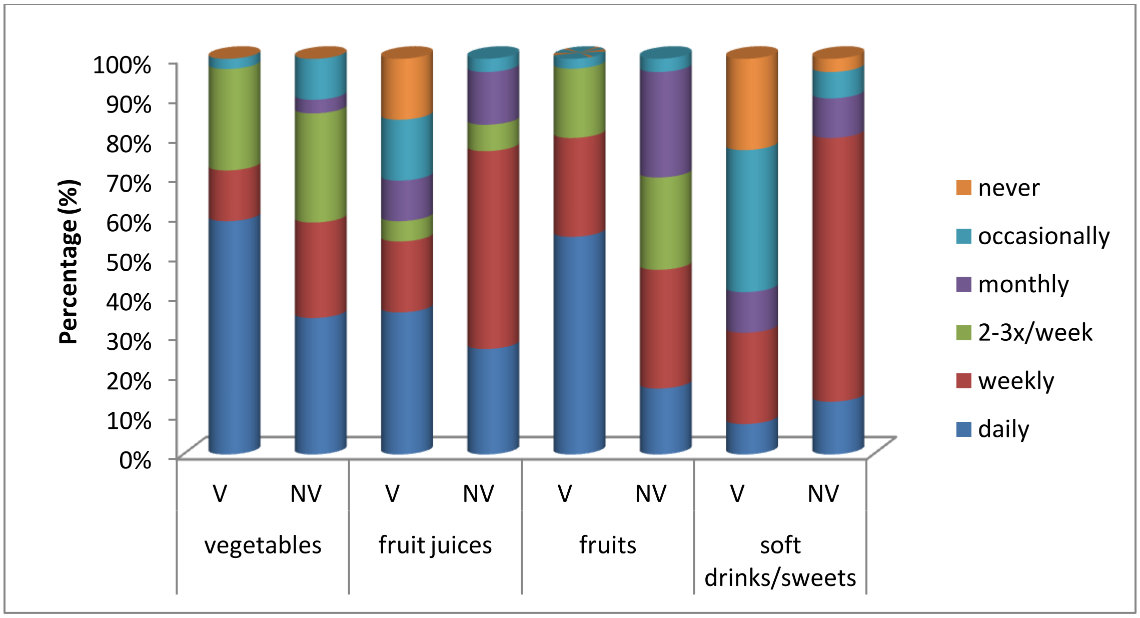


V=Vegetarians NV=Non-vegetarians

Figure 4.3 Comparison of intake of animal protein and vegetable protein in vegetarians and non-vegetarians

4.6.2 Fruits, Vegetables, Fruit juices and Soft Drinks

A greater proportion of vegetarians (58.9%) than non-vegetarians (33.3%) consumed vegetables on daily basis. Vegetarians reported higher daily consumption of fruits (56.4%) than did non-vegetarians (16.7%). Frequency of daily consumption of fruit juice was higher among vegetarians (35.9%) than non-vegetarians (26.6%). Frequency of consumption of soft drinks was low in both groups (Figure 4.4).

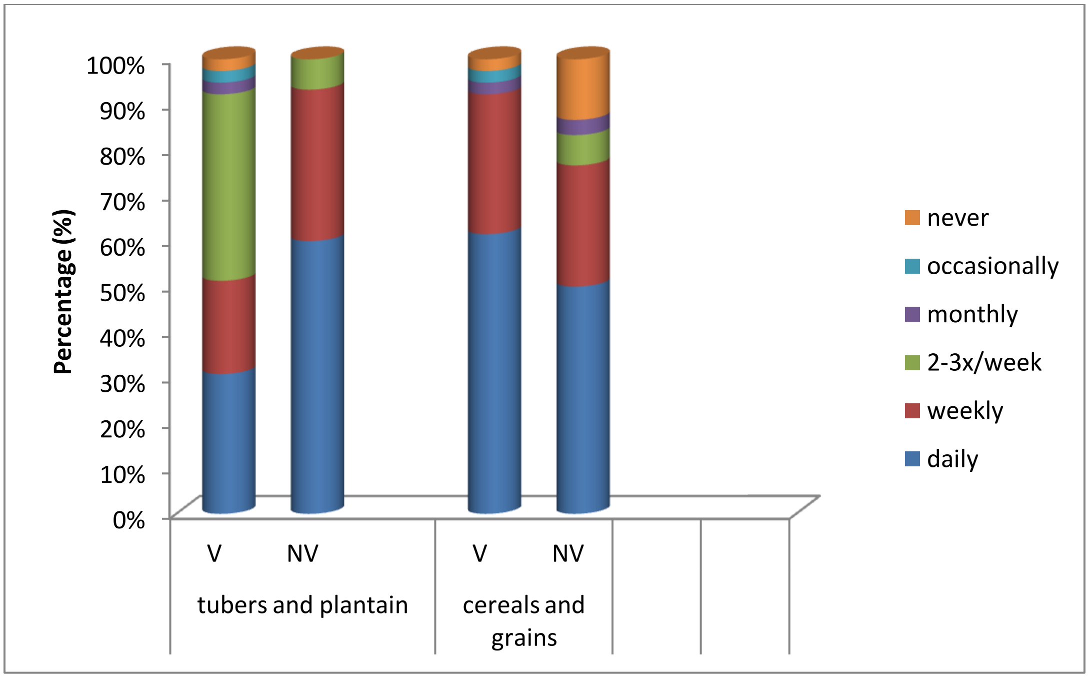


V=Vegetarians NV=Non-vegetarians

Figure 4.4 Comparison of intake of vegetables, fruits, fruit juices and soft drinks/sweets in vegetarians and non-vegetarians

4.6.3 Tubers, Cereals and Grains

Figure 4.5 shows that higher proportions of non-vegetarians (33.3% and 60.0%) had respectively higher weekly and daily consumption of tubers and plantain than did vegetarians (20.5% and 30.8%). On the other hand, vegetarians reported a higher daily consumption of cereals and grains (61.5%) than did non-vegetarians (50.0%).

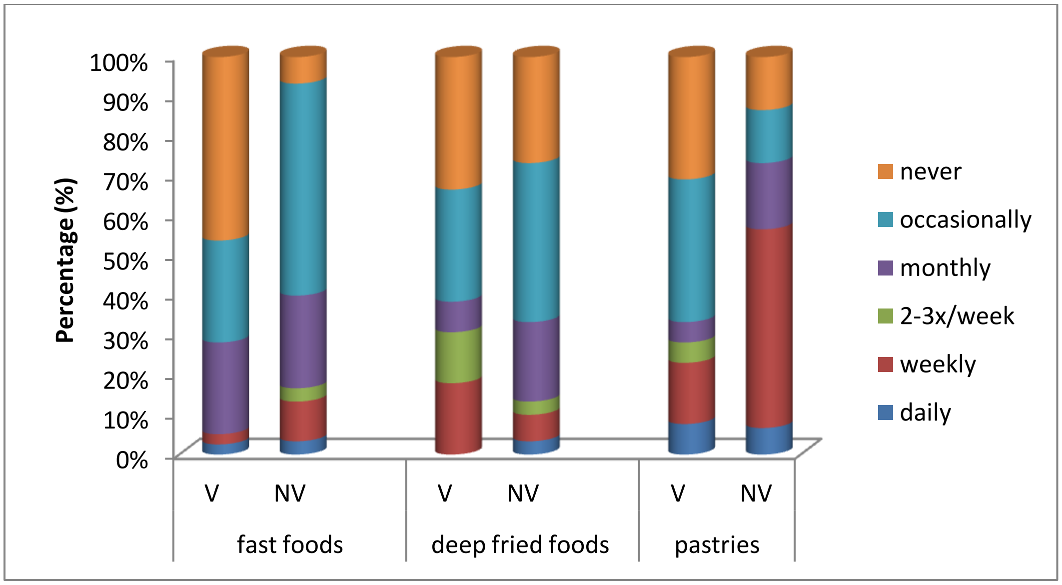


V=Vegetarians NV=Non-vegetarians

Figure 4.5 Comparison of intake of tubers, cereals and grains

4.6.4 Fast foods, deep fried foods and pastries

A greater proportion of vegetarians reported never consuming deep fried foods (33.3%) whilst 28.2% occasionally consumed deep fried foods. On the other hand, 6.7% of non-vegetarians consumed deep fried foods weekly. The highest proportion of non-vegetarians (40.0%) consumed deep fried foods occasionally. Vegetarians who never consumed fast foods (46.2%) were more than non-vegetarians (6.7%) who also reported never consuming fast foods. The proportion of vegetarians who never consumed pastries (30.8%) was greater than that of non-vegetarians (13.3%) who did not (Figure 4.6).



V=Vegetarians NV=Non-vegetarians

Figure 4.6 Comparison of intake of fast foods, pastries and deep fried foods

4.7 Energy and Nutrient Intakes

4.7.1 Macronutrient intake in vegetarians and non-vegetarians

Total energy intake was not different between both groups. Protein intake was different between vegetarians and non-vegetarians, with vegetarian mean protein intake being lower than non-vegetarians (Table 4.5).

Table 4.5 Macronutrients intake in vegetarians (V) and non-vegetarians (V)

Nutrient	Vegetarians (V) n=39	Non-vegetarians (NV) n=30	p-value
Energy/kcal	1871±243	1972±264	0.323
Protein/g (% of energy intake)	38.92±7.03 (8.32%)	62.99±14.87 (12.78%)	**0.001***
Carbohydrate/g (% of energy intake)	288.82±60.86 (61.75%)	289.08±62.70 (58.64%)	0.212
Fat/g (% of energy intake)	57.26±15.60 (27.54%)	60.69±16.18 (26.70%)	0.554
SFA/g	8.8 ±4.9	12.6±6.4	**0.003***
MUFA/g	8.3±4.1	9.4±4.6	0.27
PUFA/g	9.5 ±5.00	3.4±1.7	**0.000***

***Significant at p ≤0.05**

4.7.2 Mean daily intake of antioxidant vitamins and selected micronutrients

There was a significant higher intake of vitamin E in vegetarians than non-vegetarians (p=0.013). Vegetarians recorded the highest mean intake of vitamin C although the difference was statistically not significant (p=0.12). There was no significant difference between the intakes of iron (p=0.41) and Vitamin A (p=0.16) between both groups. Low intakes of zinc among vegetarians were however observed (Table 4.6).

Table 4.6 Micronutrients and dietary antioxidant intake in vegetarians (V) and non-vegetarians (NV).

Micronutrient	**Vegetarians (V) (n = 39)**	**Non-vegetarians (NV) (n = 30)**	**p-value**
Vitamin C/mg	80.46±61.38	61.99±24.06	0.12
Vitamin E/mg	6.3±4.0	4.3±1.5	**0.013***
Vitamin A/ RE	1462±977	1187±457	0.16
Iron/mg	20.6±12.6	22.8±6.58	0.41
Zinc/mg	6.5±1.2	9.6±2.1	**0.000***

***Significant at p ≤0.05**

4.8 Biochemical Analysis

Figure 4.7 shows that vegetarians in this study had significant higher mean levels (p=0.044) of serum superoxide dismutase (3437±1633 units/g haemoglobin) compared to non-vegetarians (2837±1106 units/g haemoglobin). There was no significant difference (p=0.075) between mean plasma levels of catalase in vegetarians and non-vegetarians (11.11±16.00 units/ml and 9.76±18.61 units/ml) respectively. However, vegetarians recorded comparatively higher mean levels of catalase (Figure 4.8).

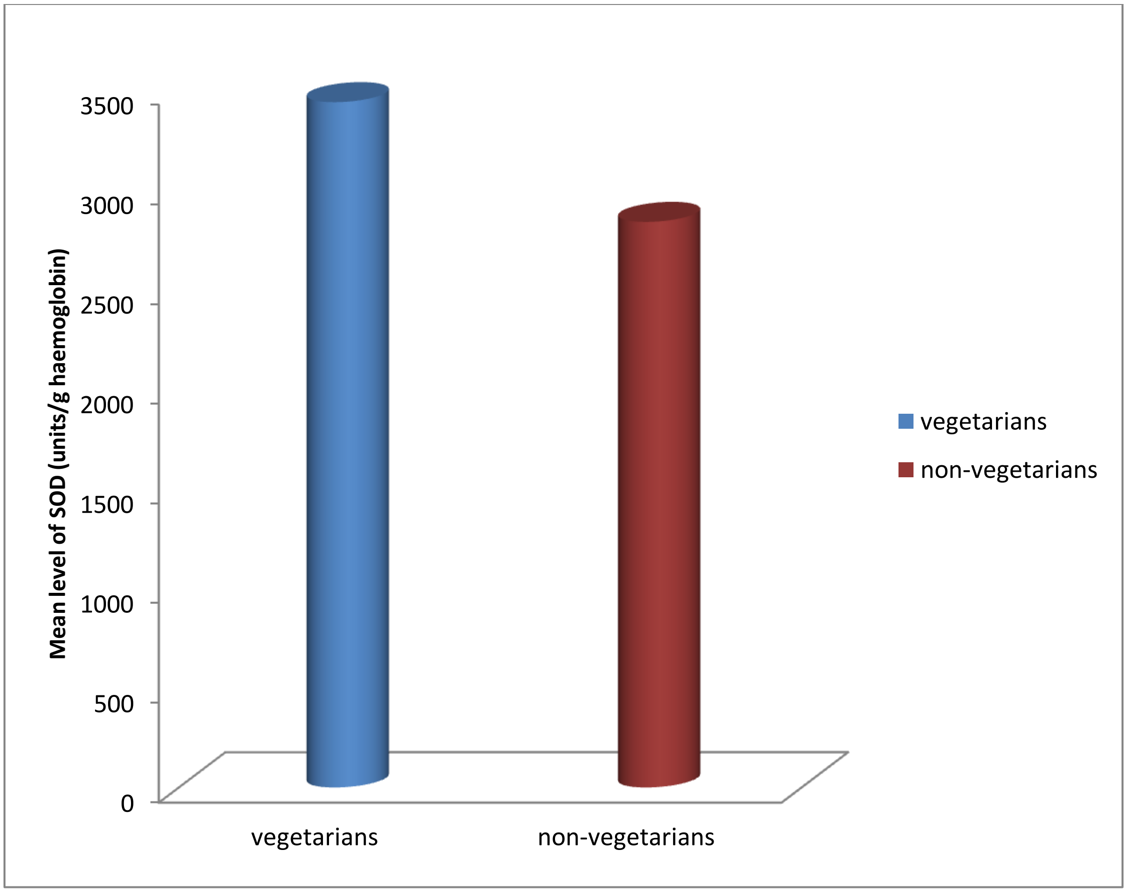


Figure 4.7 Mean serum levels of superoxide dismutase in vegetarians and non-vegetarians

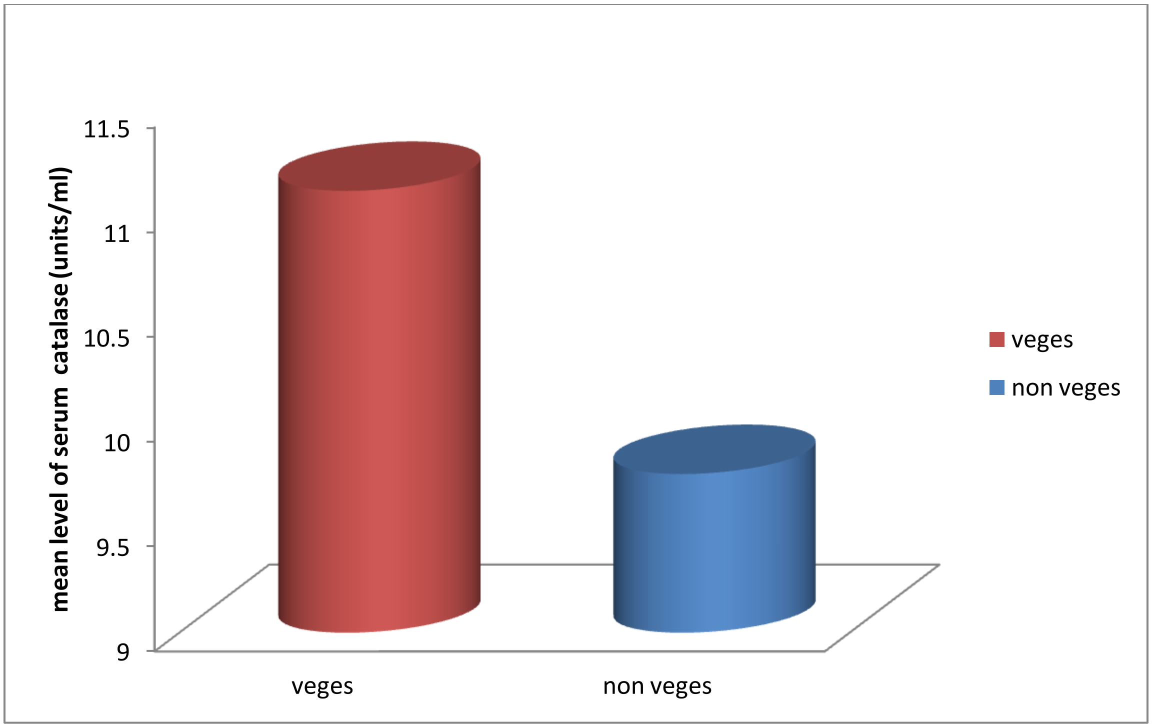


Figure 4.8 Mean plasma levels of catalase in vegetarians and non-vegetarians

Table 4.7 Correlation between intake of dietary antioxidants and serum (SOD) and plasma (catalase) enzymatic antioxidant levels

	Superoxide Dismutase		**Catalase**	
	r-value	**p-value**	**r-value**	**p-value**
Vegetarians N=39				
Vitamin A	-0.22	0.17	-0.07	0.67
Vitamin C	0.45	**0.04***	-0.06	0.72
Vitamin E	-0.29	0.08	0.46	**0.03***
Non-Vegetarians N=30				
Vitamin A	-0.07	0.72	-0.27	0.15
Vitamin C	-0.30	0.11	-0.22	0.25
Vitamin E	-0.11	0.55	0.02	0.90

Significant at $p \leq 0.05$

Vitamin C showed a significant moderate positive relationship with superoxide dismutase in vegetarians (p=0.04), whilst Vitamin E also showed a moderate positive relationship with catalase, though that of vitamin E was not significant (p= 0.08). The observed relationship between vitamin E and catalase among the non-vegetarian group was however weak. In non-vegetarians, Vitamin C showed a negative relationship with catalase (Table 4.8).

4.9 Relationship between years of vegetarianism and serum and plasma levels of endogenous antioxidants

The number of years of vegetarianism correlated positively but weakly with superoxide dismutase. However, this relationship was significant (p=0.02). A weak negative correlation was observed between the years of vegetarianism and plasma catalase levels. (Table 4.9).

Table 4.8 Correlation between years of vegetarianism and serum and plasma levels of endogenous antioxidants

Superoxide dismutase		Catalase	
r-value	**p-value**	**r-value**	**p-value**
0.372	**0.02***	-0.077	0.64

Significant at p ≤0.05

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study was performed to help determine and compare the influence of diet on the serum and plasma antioxidant levels of vegetarians and non-vegetarians. The antioxidant enzymes Superoxide dismutase and catalase were chosen as the main antioxidants for the study. The increasing knowledge of the protective ability of antioxidants in the human body has resulted in extensive research work being carried out frequently.

Studies on vegetarianism as well have become more frequent due to the ever growing population of people who as a result of personal, cultural or religious reasons decide to adopt vegetarianism. Earlier studies on vegetarians sought to determine the adequacy of the diet in meeting nutritional needs since the lifestyle was meant to omit certain foods that provide very indispensable macronutrient and micronutrients.

Evidence that vegetarians tend to be older than non-vegetarians (Davey *et al*., 2003) supports the results from this study in which the mean age of vegetarians was 36.1±8.2 whilst that of non-vegetarians was 30.7±7.6. Also, according to Davey *et al.* (2003), due to concerns about body size, weight and shape, more females than males opted for vegetarian and vegan diets. However, results from this study showed a reverse occurrence. More vegetarian males (59%) than female vegetarians (41%) were sampled from the study, whilst an equal number of males and females were sampled in the non-vegetarian group. This however could be due to sampling bias.

Again, contrary to results by Davey *et al.* (2003) that showed vegetarians to be more educated than non-vegetarians, results of this study showed otherwise. Judging from the fact that about 64.1% chose to be vegetarians to uphold religious principles, this result is possible especially in a setting where not only knowledge, but to a large extent, religious doctrines influence the need to adopt vegetarian dietary lifestyle. Also, educational level of individuals could be dependent on their geological location.

A Study on vegetarianism has reported fewer percentages of vegans, compared with lacto-vegetarians and lacto-ovo vegetarians (Toohey *et al.*, 1998). This study was no exception. Results showed that vegans contributed the least percentage (2.6%) to the total vegetarian population. This shows that fewer vegetarians can adhere to a strict vegan diet. According to Smith *et al.* (2000), vegetarians are more likely to adhere to this lifestyle for a longer period than individuals who adapt weight-reducing diets. Long period of adherence to a vegetarian diet was observed in this study. About sixty-nine percent (69.2%) had been vegetarians for between 2-10 years whilst 10.3% had been vegetarians for between 20-30 years.

Results from this study showed that participants were conscious of disease-causing and life threatening behaviours. This is evident in the small percentages of participants who smoked or consumed alcohol from both groups. Only few vegetarians smoked or consumed alcohol. These results are supported by the EPIC cohort study, in which a conclusion was reached that certain lifestyle behaviours which included not smoking and moderate alcohol intake are the lifestyle behaviours of vegetarians (Key, 1999).

No significant difference was observed in the BMI of the vegetarians and non-vegetarians in this study, although one study has recorded differences with non-vegetarians having a higher BMI than vegetarians (Appleby *et al.*, 2002). Results however are in accordance with studies conducted by Kumar *et al*. (2012) (in rural Bangladesh), and Dourado *et al.* (2011).

Vegetarian participants recorded a mean visceral fat percentage that was significantly higher than that of non-vegetarians. This could be attributed to the fact that the sample size in this study was very small. However, also considering the significant difference in the mean age between both groups and the fact that even intake of energy was not different between both groups, the evidence of increased visceral fat accumulation with increasing age, as well as decrease in the body's metabolism with increasing age could account for the significantly high visceral fat percentage in the vegetarian group

Another interesting observation was the result of no significant difference in the body fat percentage, though most studies have recorded a lower body fat percentage in vegetarians (Kumar *et al.*, 2012, Turner-McGrievy *et al.*, 2014, Delgado *et al.*, 2000). However, results obtained are in accordance with that of Brathwaite *et al.*, (2003). In that study, a possibility of a reverse occurrence is seen when results of the study showed a significant association between a vegetarian diet and obesity.

It is also known that diet is not the only factor that affects body fat deposition and distribution. Other factors that affect the body fat occurrence include genetics, old age, physical activity and gender. Older adults are known to deposit fat easily compared with younger adults. This could contribute to the result of the no significant difference in body fat since most vegetarians were older than non-vegetarians. Also, women tend to have more body fat deposition than men.

The significant difference observed in the systolic blood pressure between both groups is in accordance with results from the Adventist Health Study-2 (AHS-2) on a study to determine the relationship between vegetarian diets and blood pressure which showed that vegan participants had lower systolic blood pressure than omnivorous participants (Key, 1999).

Food intake by vegetarians and non-vegetarians differ in the source from which protein is obtained. However with the omission of animal protein, vegetarians need to incorporate more plant-based protein diet into their meals. Main sources of protein in a vegetarian diet are legumes, seeds and nuts. Results from this study showed that majority of vegetarians (66.7%) consumed vegetable protein more frequently than non-vegetarians (13.3%). As expected, not one vegetarian consumed any meat or animal flesh. This showed that vegetarian participants had an understanding of what the lifestyle was about.

Regular intake of processed meat has been shown to be associated with a high rate of deaths from cancer, of which free radicals play a role. This observation was seen more among the non-vegetarians who obviously consumed these products than the vegetarians (Khaw *et al.*, 2008).

Nuts are great sources of dietary vitamin E in diet and have been found to be among various dietary plants with the highest content of total antioxidants. Of the nuts, chestnut, walnuts and pecans have the highest content of antioxidants (Blomhoff *et al.*, 2006). Legumes are rich sources of dietary fibre, iron, potassium, zinc and most importantly antioxidants. Consumption of legumes, precisely, soybeans have been inversely associated with the risk of type two diabetes (Villegas *et al*., 2008). It has been established that the best way to cook legumes and still preserve their antioxidant capability is through pressure boiling over a shortened processing time (Xu and Chang, 2008).

With reference to the food frequency questionnaire used in the study, the highest frequency for consumption of fruits (orange, pawpaw, pineapple, banana, apple, avocado) and vegetables (carrots, cabbage, tomatoes, okro and garden eggs) was found to be on daily basis (consumed daily) for vegetarian participants (59.0%), whilst that of non-vegetarians was 33.3%. Frequent consumption of local vegetables like cocoyam leaves (locally called "kontomire"), okro, turkey berries, garden eggs and tomatoes consumed in the form of soups or stews was recorded. Consumption of other exotic vegetables such as cabbage, carrots, green pepper, lettuce, cauliflower, was not a common practice observed among this group. This may be due to fact that most of these vegetables are relatively expensive compared to local vegetables. Judging from the level of education of the majority, jobs available may not pay enough to grant them the benefit of consuming these expensive food commodities. For some however, consumption of these exotic vegetables may not be a common practice.

Guidelines on how to get enough antioxidants through food include eating a rainbow of fruits and vegetables and choosing a colourful fruit and vegetables (Academy of Nutrition and Dietetics, 2009). Consumption of a wide variety of fruits and vegetables leads to adequate intake of iron, magnesium, potassium and copper (Waldmann *et al.*, 2003). These micronutrients play important roles in the formation and efficiency in functions of superoxide dismutase (zinc, magnesium and copper dependent) and catalase (iron-dependent) in the body. Also, frequent consumption of fruits and vegetables in vegetarians is very important because they are the main sources of vitamin C which is largely needed to facilitate the absorption of non-haeme iron in the body (Davey *et al.*, 2003). Adequate iron absorption is necessary because this micro nutrient serves as a co-factor for the formation of the antioxidant catalase.

From analysis of the data obtained in the study from the food frequency questionnaire, it was observed that the frequency of intake of cereals and grains such as rice, maize and wheat was higher among the vegetarian group (61.5%) than among the non-vegetarian group (50.0%). This is in accordance with a study conducted by Rauma and Mykkänen

(2000) in which more intakes of cereals and grains were recorded to be higher in vegetarians than in non-vegetarians.

Protective food commodities including oil seeds, dark grain products and grain sprouts recorded in a study accounted for the maintenance in the integrity of the DNA strands, due to the role they play in the reduction of the action of reactive oxygen species (Dusinská and Krajcovicová-Kudlácková , 2004). When adequate dietary polyphenols, obtained from plant diets are ingested, it helps lower the susceptibility of Low Density lipoproteins (LDL) to oxidation, and improves endothelial dysfunction (Sies *et al*., 2005).

Frequent consumption of fast foods and common deep fried foods such as "kelewele", fried yam and fried sweet potatoes was not observed among both vegetarian and non-vegetarian groups. However, vegetarians consumed less of these high trans-fatty acids containing foods frequently, compared to non-vegetarians**.** These results indicate that vegetarianism is viewed and understood as a health conscious lifestyle by the participants who practiced it, and this is evidenced by their record of having the lowest intake of fast foods and sweets, which are high in trans fat, saturated fat and refined sugars. This observation is supported by a study that recorded strong correlations and levels of agreement ($p<0.001$) between the 'health conscious' mentality and vegetarian dietary and lifestyle patterns in men and women (Northstone and Emmet, 2010). Frequent consumption of foods containing plant oils as well as their over-processing, results in high-fat intake, which could lead to an increased rate of lipid peroxidation (Kumar *et al.*, 2012).

Most studies conducted on the levels of endogenous antioxidants have found the levels of SOD and catalase to be higher in vegetarians than in non-vegetarians (Manjari et al., 2001; Krajčovičová-Kudláčková *et al.*, 1995). Results obtained from this study also showed a significant difference in the SOD levels between both groups. However, an insignificant difference was observed in the CAT levels between both groups.

Superoxide dismutase is a zinc-dependent antioxidant. It has been established that inadequate intake of protein could affect the availability of zinc in the body and its ability to efficiently act as co-factor for SOD (Mohanty *et al.*, 2002). However, it has as well been proven that high protein diet promotes increased homocysteine levels, which lead to exhibition of superoxide anions, and stimulation of the generation of ROS in cells (Gurujeyalakshmi *et al.*, 2000). Production of superoxide anions means more scavenging task for SOD, thereby leading to a reduction of its levels in the body. On the other hand, a study has shown the activity of zinc-dependent SOD to be independent of the influence of dietary zinc and therefore deficiencies in the dietary component will not immediately reduce the activity of the enzyme (Aggett and Favier, 1993). It is possible therefore that the comparatively low levels of zinc in vegetarians did not affect the levels of SOD. On the other hand, the relatively higher intake of protein in the non-vegetarian group could account for the relatively low levels of plasma SOD in the non-vegetarian participants.

Vitamin C has proven to be a very powerful antioxidant. The superoxide radical anion, hydroxyl radical, singlet oxygen and hydrogen peroxide are some ROS scavenged by vitamin C (Rose, 1989; Weber *et al.*, 1996). Main sources of vitamin C are fruits and vegetables. Although the result of the analysis of the current dietary vitamin C intake (from 24-hour recall) in this study did not show a significant difference between the vegetarian and non-vegetarian participants, vitamin C intake was higher in vegetarians.

The result obtained from the food frequency questionnaire as well, showed a higher frequent consumption of vitamin C containing foods (fruits) among vegetarians. Plasma ascorbate levels are maintained through recycling, by certain mechanisms in the body. Although the processes involved are affected by the reducing environment that exists in metabolically active cells, this could imply that frequent and adequate dietary intake of vitamin C is also necessary to maintain the conservation of this essential nutrient, thereby, making it accessible for scavenging of ROS.

Proper maintenance of plasma catalase level and activity, though iron-dependent, also to a large extent is dependent on SOD. A necessary synergistic relationship has been proven by an earlier study that showed the inhibition of catalase by superoxide radical (Kono and Fridovch, 1982). This therefore supports results of this study in which comparatively higher plasma SOD levels are associated with high catalase levels in vegetarians compared with non-vegetarians. This as well shows the importance of vitamin C in determining catalase levels, as this dietary antioxidant scavenges superoxide radical anion which has been shown to inhibit catalase. However, the difference in the plasma catalase levels between both groups was not significant. This could be attributed to the fact that vegetarian participants were older than non-vegetarians. Research has established that increase in age leads to a reduction in catalase levels (Sohal and Arnold

1990; Sullivan-Gunn and Lewandowski, 2013). In this study, mean age was significantly different between both groups (p=0.007), with vegetarians recording a higher mean age than the non-vegetarians.

Results showed that vegetarians consumed significantly higher levels of polyunsaturated fatty acids (PUFA), compared to the non-vegetarian participants. Research has proven polyunsaturated fatty acids to be easily susceptible to lipid peroxidation (Brooker, 2011). However, research also has shown that the main role played by dietary vitamin E as a dietary antioxidant is to provide protection against oxidation of PUFAs (Percival, 1998; Pryor, 2000; Wolf, 2005). Comparatively higher dietary intake of vitamin E by vegetarians could therefore explain the maintenance of higher levels of plasma endogenous antioxidants due to a possible reduction in lipid peroxidation, compared to non-vegetarians.

5.2 Limitations of the Study

I. Due to the small sample size, findings may not be representative of the total vegetarian population in Ghana.

II. The tool for nutrient analysis did not make provision for the assessment of intake of beta-carotene, which happens to be the main component of Vitamin A that plays a very large antioxidant role.

III. The dietary intake analysis tool also did not have some Ghanaian foods and products on the list and this made it difficult to effectively assess nutrient intake. On the other hand, some Ghanaian food commodities that were listed in the tool did not have values for some nutrient components like vitamin E, saturated fat, MUFA and PUFA, and so similar foreign alternatives were employed.

IV. Possible underestimation or overestimation of dietary intake on the part of both groups of participants and researcher may have occurred due to the methods of dietary assessment.

5.3 Conclusions

Dietary vitamin C intake in vegetarians, showed a moderate positive correlation with SOD level. Other dietary antioxidants (Vitamins A and E) showed either a weak positive correlation or weak negative correlation with the levels of the enzymatic antioxidants, SOD and catalase. Significantly higher intake of dietary vitamin E was observed among participants in the vegetarian group. This could account for the significantly higher mean level of superoxide dismutase in this group. Comparatively higher intakes of vitamins A and C in vegetarians, though not significant, could account for the higher levels of endogenous antioxidants in their blood.

Also, frequency of intake of fruits, vegetables, legumes, nuts and seeds, as well as cereals and grains were reportedly higher in vegetarians than in non-vegetarians, though not significantly different. With the exception of protein, of which vegetarians were found to have significant lower intakes compared to non-vegetarians, macronutrient intake between both groups were found to be similar. Zinc intake was found to be higher in non-vegetarians.

A similar study into antioxidant levels in vegetarians and non-vegetarians in Ghana could include assessment of serum levels of vitamins A, C and E, as well as take into consideration other factors like age, physical activity and disease conditions, which could positively or negatively affect antioxidant levels.

There is the need to examine more enzymatic antioxidants as well as to quantify dietary antioxidants.

REFERENCES

Academy of Nutrition and Dietetics (2009), Zinc in Vegetarian Diets, Vegetarian Nutrition.

Academy of Nutrition and Dietetics and Dietitians of Canada (2003), Position of the Academy of Nutrition and Dietetics and Dietitians of Canada, Vegetarian Diets. J Am Diet Assoc, 103:748-765.

Academy of Nutrition and Dietetics. (2009) "Position of the Academy of Nutrition and Dietetics: Vegetarian Diets".

Adom K.K. and Liu R.H. (2002) Antioxidant activity of grains. *Journal of Agriculture and Food Chemistry*, 50:6182-6187.

Aggett P.J. (2012) Iron, In: Erdmann J.W, Macdonald I.A, Ziesel S.H, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell: 506-520.

Aggett P.J. and Favier A. (1993) Zinc. *International Journal of Vitamin and Nutrition Research*, 63:301–307.

Allen N., Appleby P. and Davey G. (2000) Hormones and Diet: Low Insulin-Like Growth Factor-1 but Normal Bioavailable Androgens in Vegan Men. *British Journal of Cancer*, 83: 95–97.

Ambroszkiewicz J., Klemarczyk W., Chelchowska M., Gajewska J. and Laskowska-Klita T. (2006). Serum homocysteine, folate, vitamin B12 and total antioxidant status in vegetarian children. *Advanced Medical Science,* 51:265-268.

Ambroszkiewicz J., Klemarczyk W., Chelchowska M., Gajewska J., and Laskowska-Klita T. (2007). Serum concentration of biochemical bone turnover markers in vegetarian children. *Advanced Medical Science*, 52:279-282.

American Dietetic Association (2010) Factsheet, “Antioxidants”.

Ames B. (1994). Natural antioxidants in human health and disease, Ed. Frei, B. Academic Press: San Diego.

Amitom. (2001). The White book on antioxidants in tomatoes products and their health benefits Ed, R. Bilton, M.P. Bilton, M. Gerber, P. Glolier, And C. Leoni. CMTI Publication, Avignon, Issn 1145-9565.

Appleby P., Davey G. and Key T. (2002). Hypertension and blood pressure among meat eaters, fish eaters, vegetarians and vegans in Epicoxford. *Public Health Nutrition,* 5:645-654.

Asare J. (2011). Obesity and its determinants among junior high school children in the Accra metropolis: MPhil thesis, University of Ghana, Legon.

Ball M.J. and Bartlett M.A. (1999). Dietary intake and iron status of Australian vegetarian women. *American Journal of Clinical Nutrition*, 70:353-358.

Baublis A., Lu C., Clydesdale F. and Decker E. (2000). Potential of wheat-based breakfast cereals as a source of dietary antioxidants. *American journal of College Nutrition,* 19:308–311.

Béderová A., Kudlácková M., Simoncic R., Magálová T., Grancicová E., Klvanová J., Brtková A. and Barteková S. (2000). Comparison of nutrient intake and corresponding biochemical parameters in adolescent vegetarians and non-vegetarians. *Casopis Lekaru Cesychk*, 139:396-400.

Bernstein A.M., Treyzon L. and Li Z. (2007). Are high protein, vegetable-based diets safe for kidney function? A review of the literature. *Journal of American Dietetic Association*, 107: 644-650.

Bingham S., Day N. and Luben R. (2003) Dietary fibre in food and protection against colorectal cancer in The European Prospective Investigation into Cancer and Nutrition (Epic): An Observational Study. *Lancet,* 361: 1496–1501.

Bjelakovic G., Nikolova D., Gluud L.I., Simonetti R.G. and Gluud C., (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention:

systematic review and meta-analysis. *Journal of the American Medical Association,* 297: 842–857.

Blomhoff R., Carlsen M.H., Andersen L.F. and Jacobs D.R. (2006). Health benefits of nuts: potential role of antioxidants. *British Journal of Nutrition*, 96:52-60.

Brathwaite N., Fraser H.S., Modeste N., Broome H., King R. (2003). Obesity, diabetes, hypertension, and vegetarian status among Seventh-Day Adventists in Barbados: preliminary results. *Ethnicity and Disease*, 13:34-39.

British Dietetic Association (2011). Vegetarian Diet; Keeping a healthy balance, food fact sheet.

Brooker R.J. (2011). Genetics: Analysis and Principles (4th Ed.). Mcgraw-Hill Science.

Caballero B. (2006). Antioxidant Nutrients. John Hopkin's Bloomberg School of Public Health, John Hopkins University, copyright 2006.

Cena E.R., Heneman K. and Zidenberg-Cherr S. (2008) Vegetarian Diets, nutrition and health info sheet, Publication 8373.

Centres for Disease Control and Prevention (2014). Body Mass Index: Considerations for Practitioners.

Chan J., Jaceldo-Siegel K. and Fraser G.E. (2009). Serum 25-Hydroxyvitamin D status of vegetarians, partial vegetarians and non-vegetarians: The Advenist Health Study-2. *American Journal of Clinical Nutrition*, 89: 1686-1692.

Clarys P., Deriemaeker P., Huybrechts I., Hebbelinck M. and Mullie P. (2013). Dietary Pattern Analysis: A comparison between matched vegetarian and omnivorous subjects. *Nutrition Journal*. 12:82.

Conforti F., Sosa S., Marrelli M., Menichini F., Statti G. A., Uzunov D., Tubaro A., Menichini F. and Loggia R.D. (2007). *In vivo* anti-inflammatory and in-vitro antioxidant level activities of Meditteranean dietary plants. *Journal of Ethnopharmacology*, 116:144-151.

Craig W. and Pinyan L. (2001). Nutrients of concern in vegetarian diets. In: *Vegetarian Nutrition*, (J Sabate Ed.), Pp. 299–332. Crc Press, Boca Raton.

Craig W.J. (1999). Health-promoting properties of common herbs. *American Journal of Clinical Nutrition, 70:491–499.*

Davey G.K., Spencer E.A., Appleby P.N., Allen NE., Knox K.H. and Key T.J. (2003). Epic Oxford: Lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutrition, 6: 259–269.*

Delgado M., Gonzalez B. and Castillo, M. J. (2000). Physical exercise reverse diet induced increase in LDL – cholesterol and APO B levels in healthy ovo –lacto vegetarian subjects. *Nutrition Research,* 45:1707 – 1714.

Devasagayam T.P.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S.S. and Lele (2004). "Free radicals and antioxidants in human health: Current status and future prospects". *Journal of Association of Physicians of India,* 52: 796.

Dorland's Medical Dictionary for Health Consumers, 2007.

Dusinská M. and Krajcovicová-Kudlácková M. (2004) Oxidative DNA damage in relation to nutrition. *Neoplasma,* 51:30-33.

Duthie G.G. and Brown K.M. (1994). Reducing .the risk of cardiovascular disease, Chapter 2, P. 19-38, In: Functional Foods, Ed. Goldberg, I. Chapman and Hall: New York.

Dykes L. and Rooney L.W. (2007). Phenolic compounds in cereal grains and their health benefits. *American Association of Cereal Chemists International, Incoporated,* 52, No. 3.

Elmadfa I. and Singer I. (2009). Vitamin B-12 and homocysteine status among vegetarians: A Global Perspective, *The American Journal of Clinical Nutrition*, 89:1693-1698.

Dourado K.F., Cámara E.A., Campos F.S. and Shinohara N.K.S. (2011). Relation between dietary and circulating lipids in lacto-ovo vegetarians. *Nutricion Hospitalaria*, 26:959-964.

Foster M., Chu A., Petocz P. and Samman S. (2013). Effect of vegetarian diets on zinc status: A systematic review and meta-analysis of studies in humans. *Journal of Food Science and Agriculture,* 93: 2362–2371.

Fraser G. (1999). Associations between diet and cancer, ischemic heart disease and all-cause mortality in non-hispanic white California Seventh-Day Adventists. *American Journal of Clinical Nutrition*, 70:532-538.

German, J.B. (1999). "Food Processing and Lipid Oxidation".Advances In Experimental Medicine and Biology. *Advances in Experimental Medicine and Biology*, 459: 23–50.

Ginter E. (2008). Vegetarian diets, chronic diseases and longevity. *Bratislavske Lekarske Listy*, 109:463-466.

Glade M.J. (2003). The role of reactive oxygen species in health and disease. Northeast Regional Environmental Public Health Centre University of Massachusetts. *Amerst Nutrition*, 19:401-403.

Gupta V.K. and Sharma S.K. (2006). Plants as natural antioxidants. Review article, Rajendra Institute of Technology And Sciences, 5:326-334.

Gurujeyalakshmi G., Wang Y. and Giri S.N. (2000). Suppression of bleomycin-induced nitric oxide production in mice by taurine and niacin. *Nitric Oxide* 4:399-411.

Haddad E.H. and Tanzman J.S. (2003) what do vegetarians in the United States eat? *American Journal of Clinical Nutrition*, 78:626-632.

Haldar S., Rowland I.R., Barnett Y.A., Bradbury I., Robson P.J., Powell J. and Fletcher J. (2007). Influence of habitual diet on antioxidant status: a study in a population of vegetarians and omnivores. *European Journal of Clinical Nutrition*, 61:1011–1022.

Halliwell b. (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet*, 344:721-724.

Hamid O. O., Aiyelaagbe L. A., Usman O., Ameen M. and Lawal A. (2010). Antioxidants: Its medicinal and pharmacological applications, *African Journal of Pure and Applied Chemistry*, 4:142-151.

Harman S.K. and Parnell WR. (1998). The nutritional health of New Zealand vegetarian and non-vegetarian Seventh-day Adventists: selected vitamin, mineral and lipid levels. *New Zealand Medical Journal*, 111:91-94.

Heart and Stroke Foundation (2010). Types of fats. Health check program. Heart and stroke foundation of Canada. Retrieved On July 20th 2014.

Hensley K., Benakass E.J., Bolli R., Comp P., Grammas P., Hamdheydari L., Mou S., Pye Q.N., Stoddard M.F., Wallis G., Williamson K.S., West M., Wechter W.J. and Floyd R.A. (2004). New perspectives on vitamin E: gamma-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine. *Free Radical Biological Medicine*, 36:1-15.

Herrmann W., Schorr H. and Obeid R. (2003) Vitamin B-12 Status, Particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia In Vegetarians. *American Journal of Clinical Nutrition* 78: 131–136.

Hertog M.G.L., Feskens E.J.M., Hollman P.C.H., Katan M.B. and Kromhout D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, 342:1007–1011.

Hodson L., Skeaff C M. and Chisholm W A. (2001). The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *European Journal of Clinical Nutrition*, 55:908-915.

Inoue M., Sato E.F. and Nishikawa M. (2003). Mitochondrial generation of reactive oxygen species and its role in aerobic life. *Current Medicinal Chemistry*, 10:2495-2505.

Janelle K.C. and Barr S.I. (1995) Nutrient intakes and eating behavior scores of vegetarian and nonvegetarian women. *Journal of American Dietetic Association*. 95:180-186, 189.

Javanbakht M.H., Sadria R., Djalali M., Derakhshanian H., Hosseinzadeh P., Zarei M., Azizi G., Sedaghat R., Mirshafiey A. (2014). Soy Protein and Genistein Improves Renal Antioxidant Status In Experimental Nephrotic Syndrome. *Nefrologia*, 34:483-490.

Jose´ M., Mate´ S., Mez C. and Castro I. (1999) Antioxidant Enzymes and human diseases. Department of molecular biology and biochemistry, Faculty of Sciences, University of Ma´Laga, *Clinical Biochemistry*, 32: 595–603.

Kasperczyk S., Dabrowski M., Kasperczyk J., Ostalowska A., Zalejska-Fiolka J., and Birkner (2014). Beta-carotene reduces oxidative stress, improves glutathione metabolism and modifies antioxidant defense systems in led-exposed workers. *Toxicology applied Pharmacology*, 280:36-41.

Kazimírová A., Barancoková M., Volkovová K., Staruchová M., Krajcovicová-Kudlácková M., Wsólová L., Collins A.R. and Dusinská M. (2004). Does a vegetarian diet influence genomic stability? *European Journal of Nutrition*, 43:32-38.

Kerry E.D., Walters S., Portmess L. (2001) Religious vegetarianism from Hesiod to the Dalai Lama. *Albany*, Pp 13-46.

Key T. J. (1999). Mortality in vegetarians and non - vegetarians detailed findings from a collaborative analysis of 5 prospective studies. *American Journal of Clinical Nutrition*, 70: 88-93.

Key T.J., Appleby P.N. and Rosell M.S. (2006). Health effects of vegetarian and vegan diets. *Proceedings of Nutrition Society, 65: 35 – 41.*

Khaw K.T., Wareham N., Bingham S., Welch A., Luben R. and Day N. (2008). "combined impact of health behaviours and mortality in men and women: the Epic-Norfolk prospective population study". *Public Library of Science and Medicine,* 5:12.

Kim M.K., Cho S.W., Park Y.K. (2012). Long-term vegetarians have low oxidative stress, body fat, and cholesterol levels. *Nutrition Research and Practice,* 6: 155–161.

Kono Y. and Fridovich I. (1982). Superoxide radical inhibits catalase. *Journal of Biological Chemistry,* 257: 5751-5754.

Krajčovičová-Kudláčková M., Šimončič R., Babinská K. and Béderová A. (1995). Levels of lipid peroxidation and antioxidants in vegetarians. *Bratislavske Lekarske Listy*, 11:207-211.

Krajčovičová-Kudláčková M., Ursinyova M., Blazicek P., Spustova V., Ginter E., Hladikova V. and Klvanova J. (2003). Free radical disease prevention and nutrition. *Bratislavske Lekarske Listy*, 104:64-68.

Krajčovičová-Kudláčková M., Babinska K., Valachovicova M., Paukova V., Dusinská M. and Blazicek P. (2007). Vitamin C protective plasma value. *Bratislavske Lekarske Listy*, 108:265-268.

Kumar E.P., Mukherjee R., Senthil R., Parasuraman S. and Suresh B. (2012). "Evaluation of oxidative stress and antioxidant status in patients with cardiovascular disease in rural populations of the Nilgiris, South India". Department of Pharmacology, JSS College of Pharmacy, Ootacamund 643001, India.

Kyung K., Woon S.C. and Kyoung P.Y. (2012). Long-term vegetarians have low oxidative stress, body fat and cholesterol levels. *Nutrition Research Practice*, 62 155:161.

Langseth L., From The Editor: Antioxidants And Diseases of The Brain. *Antioxidant Vitamins Newsletter*, 4:3.

Li D., Sinclair A.J.B., Mann N.J., Turner A. and Ball M.J. (2000). Selected micronutrient intake and status in men with differing meat intakes; vegetarians and vegans. *Asia Pacific Journal of Clinical Nutrition*, 9:18-23.

Lodish H., Berk A., Matsudaira P., Kaisser C.A., Rieger M., Scott M.P., Zipurksy S.L. and Darnell J. (2004). Molecular Cell Biology (5^{th} edition). New York, New York: WH Freeman and Company.

Mahan K.L., Escott-Stump S. and Raymond J. (2012) .Krause's Food and the Nutrition Care Process. 13^{th} Edition.

Manjari V., Suresh Y., Sailaja Devi M.M. and Das U.N. (2001). Oxidant stress, anti-oxidants and essential fatty acids in South Indian vegetarians and non-vegetarians. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 64:53-59.

Mates J. M., Perez-Gomez C. and De Castro I. N. (1999). Antioxidant enzymes and human diseases. *Clinical Biochemistry, 32*:595–603.

Maxwell S.R.J., (1995). Prospects for the use of antioxidant therapies. *Drugs,* 49:345-361.

Mayo Clinic (2014) Dietary Fats: Know Which Types To Choose.

Melov S., Coskun P., Patel M., Tuinstra M., Cottrell B., Jun A.S., Zastawny T.H., Dizdaroglu M., Goodman S.I., Huang T.T., Miziorko H., Epstein C.J. and Wallace D.C. (1999). Mitochondrial disease in superoxide dismutase 2 mutant mice. *National Academy of Science*, 96: 846–851.

Messina M., Mangels R. and Messina V. (2004). The dietitian's guide to vegetarian diets: Issues and applications., 2nd Ed. Sudbury, Ma: Jones And Bartlett Publishers.

Messina M.J. and Messina V.I. (1996). The dietitian's guide to vegetarian diets: Issues and applications. Gaithersburg, Md: Aspen Publishers.

Meyer K.A., Kushi L.H., Jacobs D.R., Slavin J., Sellers T.A. and Folsom A.R. (2000). Carbohydrates, dietary fibre and incident of type 2 diabetes in older women. *American Society for Clinical Nutrition*, 71:921-930.

Mezzano D., Munoz X. and Martinez C. (1999) Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. *Thrombosis and Haemostasis* 81: 913–917.

Miller H.E., Rigelhof F., Marquart L., Prakash A. and Kanter M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition*, 19:3.

Mohanty P., Ghanim H., Hamouda W., Aljada A. and Garg R. (2002). Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. *American Journal of Clinical Nutrition*, 75:767-772.

Nadimi H., Yousefi N.A., Djazayery A., Hosseini M. and Hosseini S. (2013). Association of vegan diet with RMR, body composition and oxidative stress. *Acta Scientiarum Polonorum Technologia Aliment*, 12:311-318.

Nedeltcheva A.V., Kilkus J.M., Imperial J., Kasza K., Schoeller D.A. and Penev P.D. (2009). Sleep curtailment is accompanied by increased intake of calories from snacks. *American Journal of Clinical Nutrition*, 89:126-133.

Nichols H.B., Trentham-Dietz A. and Newcomb P.A. (2009). Body mass index before and after breast cancer diagnosis: Associations with all-cause, breast cancer, and cardiovascular disease mortality. *Public medical centre*, 18:1403-1409.

Noori S. (2012). An overview of oxidative stress and antioxidant defensive system. *Open Access Scientific Report*, 1:413.

Northstone K. and Emmett P.M. (2010). Dietary patterns of men in ALSPAC: associations with socio-demographic and lifestyle characteristics, nutrient intake and comparison with women's dietary patterns. *European Journal of Clinical Nutrition*, 64:978-986.

Obeid R., Geisel J. and Schorr H. (2002). The impact of vegetarianism on some haematological parameters. *European Journal of Haematology*, 69: 275–279.

Omoregie E.S. and Osagie A.U. (2011). Effect of *Jatropha Tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats. *Journal of Food Biochemistry*. 35: 409-424.

Orlich M.J., Jaceldo-Siegl K., Sabaté J., Fan J., Singh P.N. and Fraser G.E. (2014). Patterns of food consumption among vegetarians and non-vegetarians. *British Journal of Nutrition*, 112:1644-1653.

Outila T.A., Karkkainen M.A., Seppanen R.H. and Lmberg-Allardt C.J. (2000). Dietary intake of vitamin D in premenopausal, healthy vegans was insufficient to maintain concentrations of serum 25-hydroxyvitamin d and intact parathyroid hormone within normal ranges during the winter in Finland. *Journal of the American Dietetic Association,* 100:434-441.

Passmore J. (1975). The treatment of animals. *Journal of the History of Ideas,* 36:196-201.

Percival M. (1998). Antioxidants. Advanced nutrition Publications, Inc.

Perry C., Mcguire M. and Neumark-Sztainer D. (2002). Adolescent vegetarians: How well do their dietary patterns meet the healthy people? *Archives of Pediatrics and Adolescent Medicine,* 156:431–437.

Pettersen B.J., Anousheh R., Fan J., Jaceldo-Siegl K., Fraser G.E. (2012) Vegetarian diets and blood pressure among white subjects: Results from the Adventist Study-2 (AHS-2). *Public Health Nutrition,* 1909-1916.

Philips F., Hackett A.F. and Stratton G. (2004). Effects of changing to a self-selected vegetarian diet on anthropometric measurements in UK adults. *Journal of Human Nutrition and Dietetics,* 17:249-255.

Poli G., Leonarduzzi G., Biasi F. and Chiarpotto E. (2004). Oxidative stress and cell signaling. *Current Medicinal Chemistry,* 11:1163-1182.

Pryor W.A. (2000). "Vitamin E and heart disease: basic science to clinical intervention trials". *Free Radical Biology and Medicine,* 28: 141–164.

Pujari K.N., Jadkar S.P., Kulkarni A., Patil C.G. and Tuljapurkar V.B. (2011) Superoxide Dismutase Levels In Leukemias. *International journal of Basic Medical Sciences*, 2:96-100

Rauma A.L., Törrönen R., Hänninen O., Verhagen H., Mykkänen H., (1995). Antioxidant status in long-term adherents to a strict uncooked vegan diet. *American journal of Clinical Nutrition,* 62:1221-1227.

Rauma A.L. and Mykkänen H. (2000). Antioxidant status in vegetarians versus omnivores. *Nutrition,* 16:111-119.

Rhee S.G. (2006). Cell signaling: H_2O_2, A necessary evil for cell signaling. *Science*, 312:1882-1883.

Riboli E. and Norat T. (2003). Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition,* 78:559–569.

Rietveld A. and Wiseman S. (2003). Antioxidant effects of tea: Evidence from human clinical trials. *Journal of Nutrition*, 133: 3285-3292.

Robinson F. and Hackett A. (1995) Vegetarianism: What's in a name? Vegetarianism and health: A discussion of current ideas. Liverpool John Moores University, Liverpool.

Robinson F., Hackett A. and Billington D. (2002). Changing from a mixed to self-selected vegetarian diet influence on blood lipids. *Journal of Human Nutrition and Dietetics*, 15: 323–329.

Rose R.C. (1989). The ascorbate redox potential of tissues: a determinant or indicator of disease? *News in Physiological Sciences*, 4:190–195.

Rosqvist F., Iggman D., Kullberg J., Cedernaes J.J. and Johansson H. (2014). Overfeeding Polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Winter*, 13:34-39.

Rottka H. (1990). Health and vegetarian lifestyles. *Nutritional Adaption to New Lifestyles*, (J Somogyi, K Kostin Eds). Oxford University Press, Oxford. Pp. 176–194.

Sabate J., Ratzin-Turner R. and Brown J. (2001). Vegetarian Diets: Descriptions and Trends. In: *Vegetarian Nutrition*, (J Sabate Ed.), CRC Press, Boca Raton. Pp.3–18.

Shridhar K., Dhillon P.K., Bowen L., Kinra S., Bharathi A.V., Prabhakaran D., Reddy K.S. and Ebrahim S. (2014). Nutritional profile of Indian vegetarian diets--the Indian Migration Study (IMS). *Nutrition Journal*, 13:55.

Sies H., Stahl W. and Sevanian A. (2005). Nutritional, dietary and postprandial oxidative stress. *Journal of Nutrition*, 969-972.

Sirdah M.M., Yassin M.M., El Shekhi S. and Lubbad A.M. (2014). Homocysteine and vitamin B12 status and iron deficiency anaemia in female university students from Gaza Strip, Palestine. *Revista Brasileira Hematologia Hemoterapia*, 36:208-212.

Siri-Tarino P.W., Sun Q., Hu F.B. and Krauss R.M. (2010). Saturated fat, carbohydrate, and cardiovascular disease. *American Journal of Clinical Nutrition*, 91: 502-509.

Škottová N., Kazdová L., Oliyarnyk O., Vecera R., Sobolová L. and Ulrichová J. (2003). Phenolics-rich extracts from *Silybum Marianum* and *Prunella Vulgaris* reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. *Pharmacological Research*, 50: 123–130.

Smith C.F., Burke L.E. and Wing R.R. (2000). Vegetarian and weight-loss diets among young adults. *Obesity Research*, 8:123-129.

Smith T.G. (1983). Tyron's regimen for women sectarian health in the seventeenth century.

Sohal R.S., Arnold L. and Orr W.C. (1990). Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in Drosophila melanogaster. *Mechanisms of Ageing and Development*, 56:223-235.

Somannavar M.S. and Kodliwadmath M.V. (2012). Correlation between oxidative stress and antioxidant defence in South Indian urban vegetarians and non-vegetarians. *European Review of Medical and Pharmacology Science*, 16:351-354.

Spencer C. (1993). The heretic's feast. A history of vegetarianism, P. 69-84.

Spencer E.A., Appleby P.N. Davey G.K. and Key T.J. (2003). Diet and body mass index in 38000 Epic-Oxford meat-eaters, fish-eaters, vegetarians and vegans. *International Journal of Obesity Related Metabolic Disorder*, 6:728-734.

Stahler C. (2005) How many youth are vegetarian? The vegetarian resource group web site. http://www.vrg.org/journal/vj2005issue4/vj2005issue4youth.htm. Retrieved on 4th July 2014.

Stahler C. (2006) How many adults are vegetarian? The vegetarian resource group web *site. http://www.vrg.org/journal/vj2006issue4/vj2006issue4poll.htm.* Retrieved on 4th July 2014.

Stanner S.A., Hughes J., Kelly C.N. and Buttriss J. (2004). A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition,* 7:407-422.

Stoltzfus R.J., Kvalsvig J.D., Chwaya H.M. (2001). Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: Double blind, placebo controlled study. *British Medical Journal,* 323:1389–1393.

Sullivan-Gunn M.J. and Lewandowski P.A. (2013). Elevated hydrogen peroxide and decreased catalase and glutathione peroxidase protection are associated with ageing sarcopenia. *BioMed Central Geriatrics* 13:104.

Taylor B.V., Oudit G.Y. and Evans M. (2000). Homocysteine, vitamins and coronary artery disease. Comprehensive review of the literature. *Canadian Family Physician,* 46:2236-2245.

Thaler R., Karlic H., Rust P. and Haslberger A.G. (2009). Epigenetic regulation of human buccal mucosa mitochondrial superoxide dismutase gene expression by diet. *British Journal of Nutrition*, 101:743-749.

Thomas B. and Bishop J. (2007). Manual of dietetics practice, 4^{th} ed. Oxford: Blackwell Publishing Limited.

Toohey M.L., Harris M., Foster G. and Melby, L. (1998). Cardiovascular disease risk factors are lower in African- American vegans compared to lactoovo-vegetarians. *Journal of the American College of Nutrition*, 5:425-434.

Turner-McGrievy G. and Harris M. (2014) Key elements of plant-based diets associated with reduced risk of metabolic syndrome. *Current Diabetes Report*, 14:524.

Turpeinen A.M., Basu S. and Mutanen M. (1998). A high linoleic acid diet increases oxidative stress in vivo and affects nitric oxide metabolism in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 59:229–233.

Tyron T. (1683). The way to health, long life and happiness. London, Sowle.

Venderley A.M. and Campbell W.W. (2006). Vegetarian diets. *Sports medicine*, 34:293-305.

Vijayakumar R.S., SuryaD. and Nalini N. (2004). Antioxidant efficacy of black pepper (*Piper Nigrum* L.) and piperine in rats with high fat diet induced oxidative stress. *Redox Report* 9:105-110.

Villegas R., Gao Y., Yang G., Li H., Elasy T.A., Zheng W. and Shu X.O. (2008). Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai women's health study. *American Journal of Clinical Nutrition*, 87:162-167.

Virginie B., Sayan C., Bala C. (2012). Reactive oxygen species are induced by kaposi`s sarcoma–associated herpesvirus early during primary infections of endothelial cells to promote virus entry. *Journal of Virology*, 87: 1733-1740.

Waldmann A., Koschizke J. W., Leitzmann C. and Hahn A. (2003): Homocysteine and cobalamin status in German vegans. *Public Health Nutrition*, 7:467-472.

Weber P., Bendich A. and Schalch W. (1996). Vitamin C and human health—a review of recent data relevant to human requirements. *International Journal for Vitamin and Nutrition Research*, 66:19–30.

WHO (2007) Fact Sheet, "The top ten causes of death". Fact Sheet N° 310.

Whorton J. (1994). Historical development of vegetarianism. *American Journal of Clinical Nutrition*, 59:1103-1109.

Wolf G. (2005). The discovery of the antioxidant function of vitamin E: The contribution of Henry A. Mattill. *The Journal of Nutrition*, 135:363–366.

Xu B. and Chang S.K.C. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Science direct*, 110:1-13.

Youngman L.D., Park J.Y. and Ames B.N. (1992). Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. *Proceedings of the national academy of sciences,* 89.

APPENDIX I

INFORMATION SHEET

I, Boadiwaa Ofori-Amanfo, am conducting a research on oxidative stress levels of vegetarians and non-vegetarians. I am a final year student offering MSc. Dietetics with the school of Allied Health Sciences, College of Health Sciences, University of Ghana, Korle Bu.

The main focus of this proposed study is to determine the relationship between dietary intake and oxidative stress levels in vegetarians and non vegetarians.

About 3ml of venous blood will be drawn from you. Body weight and height will be measured in upright position wearing light clothing and without foot wear using Seca weighing scale and a stadiometer respectively. The weighing scale will be sterilized with menthylated spirit after each use to prevent cross-contamination of participants. The researcher will sterilize her hand using a hand sanitizer after each section. You will answer a few questions about yourself.

The information provided will not be harmful to you in any way. The participants will be assured of confidentiality by the researcher, that is, your data will only be known to the researcher and you, the participant, and the information will be kept under an encrypted sticks or password-protected computers. If the information is published in any scientific journal, you will not be identified by name. This study may contribute to the existing knowledge of vegetarian diet and oxidative stress levels.

There is no risk involved in the study except little discomfort and bruises the participants might experience at the sight of the blood drawn. The laboratory technologists will be extra careful when drawing the blood sample to minimize bruises and/ or discomfort. In case the participants experience bruises or discomfort at the sight of blood drawn, first aid will be provided.

Participation in this study is voluntary, without any cost and you are free to withdraw at any point in time without losing any medical treatment.

The researcher will be available and willing to answer any further questions about the research, now or during the course of the project.

CONSENT FORM

I agree that the research project named above has been explained to my satisfaction and I agree to take part in this study. I understand that I am agreeing by my signature/thumbprint on form to take part in this research project and I understand I will receive a signed copy of this consent form for my records.

NAME OF RESEARCHER: ………………………………………………………………………

DATE: …………………………………… SIGNATURE: ……………………………..............

TELEPHONE NUMBER: ……………………………..

NAME OF PARTICIPANTS: ……………………………………………………………………….

SIGNATURE/THUMBPRINT: ………………………. DATE: ……………………………..

MOBILE NUMBER: …………………………………

APPENDIX II

QUESTIONNAIRE

Participant's ID ………………………..

Date…………………………………………

Socio –Demographic Status

Age of participant (years): 20-29 [ ] 30-39 [ ] 40-49[ ] 50 -59 [ ] 60 and above [ ]

Gender: Male [ ] Female [ ]

Marital Status: Single [ ] Married [ ] Divorced [ ] Widowed [ ] Separated [ ]

Religion: Christian [ ] Muslim [ ] Buddhists [ ] Hindu [ ] others, please specify …………………………

Educational Background: No formal education [ ] Basic education (middle/JHS) [ ] SHS/O – Level [ ] HND/Diploma Certificate [ ] Bachelor Degree [ ] Post Degree [ ]

Employment Status: Employed [ ] Unemployed [ ] Retired [ ] Student [ ]

a. Are you a vegetarian? YES [ ] NO [ ]

b .If yes, how long have you been a vegetarian? ………………………………

c. Please indicate the type: Vegans [ ] Lacto-vegetarians [ ] Ovo-lacto vegetarian [ ] Non vegetarian [ ]

What is your main reason for becoming a vegetarian?

To support personal health and healing [ ]

To promote reverence for life [ ]

To protect the environment. [ ]

To uphold religious and philosophical principles. [ ]

B. LIFE STYLE BEHAVIOUR

Do you smoke? YES [ ] NO [ ]

a. If yes how often? Daily [ ] Weekly [ ] monthly [ ] occasionally [ ]

Do you drink alcohol? YES [ ] NO [ ]

If yes how often Daily [ ] Weekly [ ] Monthly [ ] Occasionally [ ]

One serving of alcohol: 120 ml of wine (½ medium glass of dry wine)

285 ml of beer ($^{1}/_{2}$ large beer bottle, one full mini Guinness)

30 ml (1 tot) of spirit, whisky gin, akpeteshi and alcoholic bitters)

60 ml of (brandy, vermouth, aperitif)

c. On average, how many servings of alcohol do you take daily? ....

C. ANTHROPOMETRIC AND PHYSICAL MEASUREMENTS

15. Weight ………kg 16. Height……….meters 17. BMI……………….Kg/m^2

18. Body Fat………. % 19. Visceral Fat………% 20. Blood Pressure……… mm Hg

FOOD FREQUENCY QUESTIONNAIRE (Adapted from Asare J, 2011)

Instruction: Please tick how often you consume any of the food items listed in the box

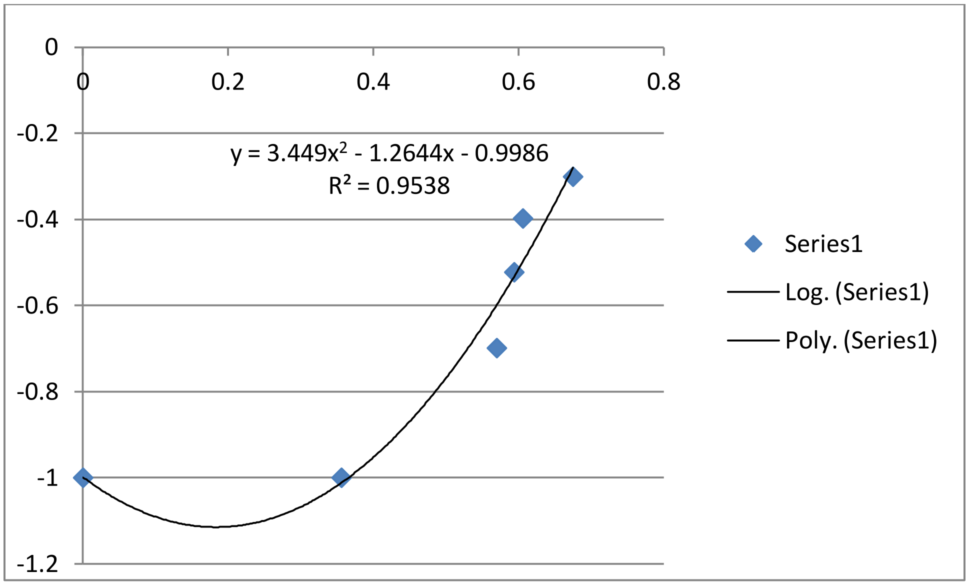
FOOD GROUP	**Not often**	**1-2x**	**3-4x**	**5-6x**	**Daily**	**Never**
Starchy roots, plantain						
Cassava						
Plantain						
Cocoyam						
Yam						
Cereals and cereal products						
Maize						
Rice						
Bread						
Animal Products						
Fish						
Meat						
Poultry						
Eggs						
Milk						
Cheese						
Legumes, nuts and oil seeds						
Beans						
Groundnuts						
Bambara						

Soybean						
Cowpea						
Melon seeds[Agushie]						
Fruits						
Orange						
Mango						
Pineapple						
Watermelon						
Banana						
Avocado Pear						
Vegetables						
Tomatoes						
Garden eggs						
Green leaves						
Okro						
Fats and oils						
Palm oil						
Refined oil eg coconut oil						
Margarine						

24-HR RECALL (CURRENT FOOD INTAKE)

Time		Meal	Quantity
Breakfast	Weekend 1	1. 2.	1. 2.
	Weekday 1	1. 2.	1. 2.
	Weekday 2	1. 2.	1. 2.
Mid-morning Snack			
Lunch	Weekend 1	1. 2.	1. 2.
	Weekday 1	1. 2.	1. 2.
	Weekday 2	1. 2.	1. 2.
Mid-afternoon snack			
Supper	Weekend 1	1. 2.	1. 2.
	Weekday 1	1. 2.	1 2.
	Weekday 2	1. 2.	1. 2.
Late-evening snack			

Catalase standard curve



APPENDIX III

SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA
ACADEMIC AFFAIRS

Phone: +233-0302-687974/5
Fax: +233-0302-688291

P. O .Box KB 143
Korle Bu
Accra
Ghana

My Ref. No. SAHS/ 10396481
Your Ref. No.

7th July, 2014.

Ms. Boadiwaa Ofori-Amanfo,
Dept. of Dietetics,
SAHS,
Korle Bu.

Dear Ms. Ofori-Amanfo,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET. /10396481/AA/10A/2013-2014.

Following a meeting of the Ethics and Protocol Review Committee of the School of Allied Health Sciences held on Monday 24th March, 2014, I write on behalf of the Committee to approve your research proposal as follows:

TITLE OF RESEARCH PROPOSAL: **"Influence of Diet on Oxidative Stress Level of Vegetarians in a Ghanaian Community"**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Committee on completion of the research. The Committee may observe the procedures and records of the research during and after implementation.

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

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

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

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

Thank you.

Yours sincerely,

Dr. Michael Mark Addae
(Chairman, Ethics and Protocol Review Committee)

cc Dean
Co-ordinator/HoD, Dept. of Dietetics
Senior Assistant Registrar