

**FOLATE AND VITAMIN B-12 STATUS OF ADULT VEGETARIANS
AND NON-VEGETARIANS IN A CHRISTIAN COMMUNITY IN
MAYRA, POKUASE**

BY

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DECLARATION

I, SOMAH AGYEMAN-NKANSAH declare that I wholly undertook the study reported under the supervision of Dr. George Asare (Maj. Rtd.) and Dr. Matilda Asante of the School of Biomedical and Allied Health Sciences, University of Ghana and that except portions where references have been duly cited, this dissertation is the outcome of my research.



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ABSTRACT

Background: Vegetarianism is the restriction of diet to disallow some or all foods of animal origin and consuming mainly foods of plant origin. There is paucity of data on dietary intakes of vegetarians in Ghana. Vegetarian diets are typically low in certain micronutrients particularly iron, zinc and vitamin B₁₂ but high in dietary fibre, vegetables, whole grains and legumes. The low intake of these micronutrients may affect the serum micronutrient profile of vegetarians. Folate and vitamin B₁₂ status of the Ghanaian is unknown.

Aim: To assess dietary intakes and measure serum concentrations of folate and vitamin B₁₂ status among adult vegetarians and non-vegetarians.

Methods: A case-control study design was employed. Ninety (90) participants comprising 45 vegetarians and 45 non-vegetarians were purposively selected from a Seventh Day Adventist Church in Mayra, Pokuase. Structured questionnaires were administered to obtain information on socio-demographic characteristics of participants. Anthropometric measurements and blood pressure measurements were taken. Dietary intake was assessed using 24-hour recalls and a food frequency questionnaire. Fasting blood samples were collected for measurement of vitamin B₁₂ and folate concentrations.

Results: The mean ages of the vegetarians and non-vegetarians were 37.1 ± 12.5 years and 30.8 ± 7.7 years respectively. Majority (93%) of the vegetarians were lacto-vegetarians. No significant differences were found in weight, height, BMI, folate intakes and serum folate concentrations between the two groups. Mean visceral and body fats were significantly higher in vegetarians than non-vegetarians ($p = 0.001$ and 0.031 respectively). Dietary and serum concentrations of vitamin B₁₂ were significantly lower in vegetarians compared to non-vegetarians ($p = 0.0001$ and 0.05 , respectively). Serum vitamin B₁₂ positively correlated

with dietary vitamin B₁₂ in vegetarians ($r = 0.424$, $\rho = <0.001$) as well as non-vegetarian ($r = 0.315$, $\rho = 0.020$). Serum folate concentrations had a positive correlation with dietary folate levels in both vegetarians ($r = 0.417$, $\rho = <0.001$) and non-vegetarians ($r = 0.214$, $\rho = 0.021$).

Conclusion: Findings of this study showed a positive relationship between dietary intakes of vitamin B₁₂ and folate and serum concentrations of vegetarians and non-vegetarians. Low serum vitamin B₁₂ and folate concentrations and low folate intake among vegetarians and non-vegetarians still remain a concern and require careful and in-depth analysis into more acceptable and balanced dietary intake of these nutrients.



DEDICATION

This work is dedicated as a token of sincere regard to my husband, Ransford for supporting me all the way and having faith in me, and to Michaela, my treasure for being my inspiration.



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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Although human beings are alike in terms of their genome, several significant physiological differences including height, eye colour, skin colour, response to drug treatments and dietary intake exist among them. The particular diet a person takes contributes largely to the occurrence or absence of major diseases. Restricting or excluding a particular food or foods from the diet without careful and appropriate substitution or supplementation may result in loss of essential nutrients, consequently posing various health risks and deficiencies in an individual (Laskowska-Klita *et al.*, 2011). One group of people who exclude certain foods from their diets are vegetarians.

Vegetarianism is the restriction of diet to disallow some or all foods of animal origin and consuming mainly foods of plant source (Dorland's Medical Dictionary, 2007). Vegetarianism is not a new concept and can be dated back to several years (Weinsier, 2000). The vegetarian usually consumes a diet consisting mostly of plant-based foods including fruits, vegetables, legumes, nuts, seeds, and grains (Marsh *et al.*, 2012). A Vegan doesn't eat any animal products including meat, poultry, seafood, eggs and dairy foods. Most vegans also won't use honey or other animal products (Marsh *et al.*, 2012). There is a myriad of reasons why people choose to be vegetarians, or avoid some or all animal products. These include economic, geographic, ethical and ecological (including animal rights and welfare), health concerns, different taste and sensory preferences, philosophical reasons (example religious teachings), family influences and food safety scares (Leitzmann *et al.*, 2008).

There are many differences in the diets of vegetarians and non-vegetarians, the most significant difference being the absence of red meat intake and animal flesh. Although the vegetarian diet is considered a healthy one, these health benefits may be counteracted by the deprivations of certain micronutrients, especially the ones obtained from animal products (Mezzano *et al.*, 2000). Red meat, for example, is a good source of iron, zinc, preformed vitamin A and vitamin B₁₂; milk and dairy products are rich in bio-available calcium and provide useful amounts of a diverse range of other minerals and vitamins and oily fish is rich in vitamin D. Vegetarian diets have been found to be high in copper, too high in carbohydrates, low in zinc, low in proteins and usually lack high quality proteins, extremely low in Vitamin D, omega-3 fatty acids, B-complex vitamins and very low in the important sulphur compounds like taurine, cysteine, carnitine and methionine. These compounds are important for liver detoxification (Laskowska-Klita *et al.*, 2011).

Several studies have been conducted worldwide about vegetarians with differing results. A study conducted to determine selected nutrition-related health aspects including certain vitamin levels in adult vegetarian Seventh-day Adventists reported lower vitamin B₁₂ dietary intakes among vegetarians but similar serum vitamin B₁₂ levels among both vegetarians and non-vegetarians (Harman and Parnell, 1998). Another study conducted in Korea to compare folate and vitamin B₁₂ intakes of vegetarians and non-vegetarians showed that the serum vitamin B₁₂ levels of all 54 Buddhist vegetarian except one and the serum folate levels of all 62 non-vegetarians except one fell within a normal range (Lee, 2011). Serum vitamin B₁₂ levels and haemoglobin concentrations have been reported to be significantly lower in vegetarians than in non-vegetarians with significantly higher serum folate concentrations in vegans compared to lacto-ovo-vegetarians and non-vegetarians. Lacto-ovo-vegetarians had

significantly higher intakes of folate and significantly lower intakes of vitamin B₁₂ and lower serum vitamin B₁₂ levels compared with the controls (Faber *et al.*, 1986). A study conducted in some selected communities in Ghana to compare the dietary intakes of vegetarian and non-vegetarian children showed that the vegetarian diets were devoid of vitamin B₁₂ (Osei-Boadi, *et al.*, 2012).

There are indications that vegetarianism is increasing in Ghana and this is evidenced in the springing up of vegetarian restaurants and associations. The Vegetarian Association of Ghana (VegGhana) is a non-profit, non-religious society which aims to promote the benefits of vegetarianism. It has a membership of about three hundred (300) people and is open to both vegetarians and individuals who have the intention of becoming vegetarians. To our knowledge, no study has been conducted in Ghana to assess the folate and Vitamin B₁₂ status of vegetarians and hence the need to conduct this current study.

1.2 PROBLEM STATEMENT

When a food or a group of foods are omitted from the diet on a regular basis, a person may be at risk of some dietary deficiencies. Such a diet should be balanced and nutritionally complete to ensure optimal nutrition and prevent consequent nutritional deficiencies. Possible nutritional deficiencies, particularly those from animal sources with vegetarian diets raise concerns. One of this is vitamin B₁₂ deficiency.

There is paucity of information on dietary composition of vegetarians in Ghana. The situation as it is, makes it difficult for dieticians to take a position on vegetarian diets as one approved for all individuals who choose it. Furthermore, the decision to modify the

vegetarian diet should be based on evidence-based information but this is unavailable in the Ghanaian community.

1.3 SIGNIFICANCE OF THE STUDY

Findings of the study may provide useful information on the quality of diet of vegetarians in Ghana. The results of this study will reveal whether the absence of animal and/or dairy products will affect certain micronutrient status of vegetarians in Ghana. This information will be useful to dieticians, nutrition-related professionals, vegetarians and the general public. It will also serve as a reference material and the basis for nutrition education and counselling.

1.4 HYPOTHESES

1. Folate and vitamin B₁₂ intakes of vegetarians and non-vegetarians are not significantly different.
2. There are no significant differences in vitamin B₁₂ and folate serum concentrations of vegetarians and non-vegetarians

1.5 AIM AND OBJECTIVES

1.5.1 AIM

The aim of the study was to assess folate and vitamin B₁₂ status of vegetarians and non-vegetarians in a Seventh Day Adventist church in Mayra, Pokuase.

1.5.2 SPECIFIC OBJECTIVES

- i. To estimate and compare nutrient intake of vegetarians and non-vegetarians.

- ii. To measure serum folate and vitamin B₁₂ concentrations in vegetarians and non-vegetarians.
- iii. To determine the relationship between dietary intakes of vitamin B₁₂ and folate and serum folate and vitamin B₁₂ concentrations of vegetarians and non-vegetarians.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TRENDS OF VEGETARIANISM

Highly restrictive diets are those which are usually very low in calories and often severely limit the intake of some food sources of important nutrients or may involve high-risk eating patterns. As a result, these diets may interfere with growth as well as lead to certain physiological effects (Laskowska-Klita *et al.*, 2011). Few studies have been conducted to compare the nutritional quality of restrictive diet. One of these studies which compared the quality and contributing components of vegan, vegetarian, semi-vegetarian and omnivorous diets revealed that the vegan diet had the lowest total energy content, better fat intake profile, lowest protein and highest dietary fibre compared to the omnivorous diet. The diets of vegetarians, semi-vegetarians and pesco-vegetarians were mostly better in terms of quality of nutrients than the diet of omnivores (Clarys *et al.*, 2014; Laskowska-Klita *et al.*, 2011). A study undertaken in India to ascertain the relationship between subclinical health complaints in lacto-vegetarian adults revealed the need to increase the micronutrient intakes of vegetarian populations (Chiplonkar and Agte, 2007).

Vegetarianism is not a diet; it is a lifestyle change (Kovacs, 2009). With vegetarianism, there is a complete change in dietary lifestyle with a strict adherence to plant-based diet. Avoiding some, or all, foods of animal origin is not a contemporary thing or a new concept. The term vegetarian was not coined until the mid-19th century in the United Kingdom (Spencer, 1994).

Many anthropologists and paleontologists in East Africa have reportedly unearthed remains of early hominids whose dentition suggests that they were primarily vegetarian, as they had broad, flat teeth that would not be suitable for an omnivore (Wilson and Ball, 1999).

The early Africans were nomads and the food they ate depicted a vegetarian lifestyle while most Europeans embraced a more omnivorous diet. Since the early times, many Africans have fruits, grains and vegetables as part of their meals and many survived on roots, berries and leaves from their farms. In some parts of Africa, food is scarce and many Africans have developed the ability to create meals from ingredients available to them (Puskar-Pasewicz, 2010). History has revealed that African foods are centered on consumption of vegetables and grains. Most traditional African meals are vegetable and grain-based (Puskar-Pasewicz, 2010).

Vegetarianism is an old and respectable practice, and its popularity seems to be growing. This is a complex philosophy which can be misleading to many people. The vegetarian lifestyle in Africa may not be as common as in other continents and may be found among just a small number of people (Puskar-Pasewicz, 2010).

One of the biggest dietary problems for vegetarians is overindulgence of carbohydrates (Coleman and Fiveash, 2008). It is common for vegetarians to eat the suggested amount of servings of fruits and vegetables and fill the remaining calories with carbohydrates.

2.3 REASONS FOR VEGETARIANISM

Apart from improving health and promoting well-being, there are several reasons why people would choose to be vegetarians, or avoid some or all animal products. These include economic, geographic, ethical and ecological (including animal rights and welfare), health concerns, different taste and sensory preferences, philosophical reasons (example religious teachings), family influences and food safety scares. (Cathro 1994; Sabate *et al.*, 2001; Leitzmann, 2008).

2.3.1 Health Concerns

Vegetarianism may be due to personal health reasons including diabetes control, cancer prevention and lowering blood pressure (FAO/WHO, 2002). Some studies suggest that some vegetarian diets can prevent certain nutritional deficiencies as well as diet-related chronic diseases (Sanders, 1999; Nestle, 1999). Recent advances in scientific studies have shown that diets such as well-balanced vegetarian diets are seen to improve health than cause diseases as compared to meat-based diets (Sabate, 2003). Also, evidence suggests there are positive effects of a vegetarian diet on blood pressure, heart disease as well as body composition variables (Nivedita *et al.*, 2012).

2.3.2 Religious Principles and Beliefs

Another reason why people choose to be vegetarians is to uphold religious principles and beliefs. There have been different kinds and groups of people who choose not to eat meat, for various reasons, and some of these motives, mostly religious, are still apparent (Weinsier, 2000). Various religions exist among Africa's population and some of these choose diets and practices similar to those of vegetarians as a result of their religious beliefs. Some of these

religions are Hinduism, Buddhism, Jainism, Hare Krishna and some Christian groups like the Seventh-Day Adventist Church (Melina and Davies, 2003).

2.3.3 Economic Reasons

In certain parts of the world, particularly Africa many people choose a vegetarian diet as a result of financial constraints they face. In certain parts of Africa, food is scarce and many Africans have developed the ability to create meals from ingredients available to them and survive on roots, berries and leaves they gather on their farms. Poultry, fish and meat as well as other animal products are less available and more expensive than the fruits and vegetables (Puskar-Pasewicz, 2010).

2.3.4 Ecological Protection

Some vegetarians are often of the view that choosing a vegetarian diet may benefit the environment in a positive way. Choosing a vegetarian diet may help preserve the natural habitat of animals as well as preserve the animals themselves. It can also help protect the environment against land degradation, global warming as well as preserve natural resources (Marlow *et al.*, 2009).

2.4 TYPES OF VEGETARIANS

A vegetarian may only eliminate red meat from their diet or avoid foods of animal origin. More than 50% of those who consider themselves vegetarian do not eat meat or fish, but will consume dairy and egg products (California Department of Public Health, 2000). There are different varieties of vegetarians, who exclude or include certain foods. Robinson and Hackett, (1995) classify vegetarian diets as demi-vegetarians, pesco-vegetarians, lacto-ovo-

vegetarians, ovo-vegetarians, lacto-vegetarians, vegans, macrobiotic and fruitarian. The common types of vegetarians by definition are;

- i. *Lacto-Ovo-Vegetarian*: A vegetarian whose diet contains eggs and dairy products, but no meat, poultry, and fish.
- ii. *Lacto-Vegetarian*: A vegetarian whose diet contains dairy products, but no meat, poultry, fish and eggs.
- iii. *Pollo-Vegetarian*: A vegetarian whose diet contains eggs and dairy products, as well as poultry. Meat and seafood are not eaten.
- iv. *Pesco-Vegetarian*: A vegetarian whose diet contains fish and seafood.
- v. *Semi-Vegetarian*: The least restrictive vegetarian, whose diet is similar to the lacto-ovo vegetarian but occasionally contains meat, poultry fish and seafood.
- vi. *Vegan or Strict Vegetarian*: The most restrictive vegetarian, whose diet contains no animal products: meat, fish, seafood, poultry, milk and other dairy products, or eggs.

2.5 DIETARY RECOMMENDATIONS FOR VEGETARIANS

A wide spectrum of dietary patterns make up the vegetarian diet, some obviously more restrictive than others. Nutrient intake will, therefore, vary considerably even among vegetarians, especially where different subgroups exist (American Dietetics Association, 2003).

Nutritional status, would consequently be at risk if for any reason whatsoever, a group of foods is omitted from the diet on regular basis. Therefore, in order to ensure optimal nutrition and also prevent various nutritional deficiencies, it would be imperative to ensure that one's diet is balanced and nutritionally complete all the time (Hunt and Roughead, 1999). For example, when meat and meat products are excluded from the diet, it is important

to replace the nutrients which are provided by these foods in one way or the other to restore complete nutrition in an individual. There are concerns about possible nutritional deficiencies with restricted vegetarian diets, particularly those obtained from animal sources (Henderson *et al.*, 2003a, 2003b).

A position paper of the American Dietetic Association and Dietitians of Canada on the vegetarian diet concluded that the diet of a vegetarian, even a vegan can meet current recommendations for nutrients including protein, iron, zinc, calcium and Vitamin B₁₂. All types of well-planned vegetarian diets are appropriate for all stages of the life cycle, including pregnancy, lactation, infancy, childhood, and adolescence.

This can be done by incorporating fortified foods or supplements into the dietary plan, proper meal planning and education on food purchase and preparation methods, local food sources of specific nutrients as well as individual dietary modifications. Food guides which specify portion sizes and food groups may also be helpful (American Dietetic Association, 2003).

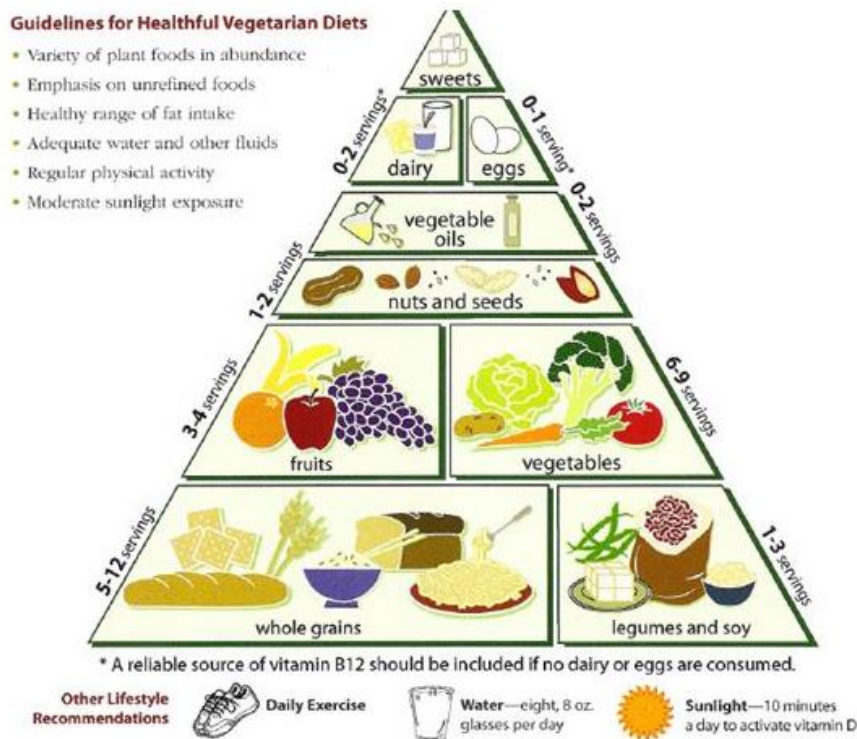


Figure 2.0 Vegetarian Food Guide Pyramid

Source: (Loma Linda University, School of Public Health, Department of Nutrition)

The recommendations provided by the food guide pyramid for vegetarians (fig 2.0) require that 5-12 servings of whole grains made up of breads, cereals, pasta and rice as well as 1-3 servings of legumes and soy be consumed every day or eaten liberally. Brown, whole-wheat and whole-grain versions instead of refined grains should be chosen for greater benefit. In addition to these, fortified cereals, especially those fortified with Vitamin B₁₂ and iron should be chosen for vegans (Haddad *et al.*, 1999).

Serving portions of 3-4 fruits and 6-9 vegetables are recommended every day. These foods should be consumed generously and should be varied and colourful (Haddad *et al.*, 1999).

Low fat options of milk, yoghurt, cheese and fortified alternatives should be consumed moderately with 0-2 servings each day. Soy milk and soy products consumed by vegans should be fortified with calcium to help meet daily requirements (Haddad *et al.*, 1999).

Vegetable fats and oils as well as sweets and salt should be consumed sparingly. It is important to eliminate saturated fats and replace them with monounsaturated and polyunsaturated fats which tend to lower blood cholesterol (Haddad *et al.*, 1999)

A modified vegetarian food guide pyramid for lacto vegetarians and vegans by Venti and Johnston, (2002) similar to the one shown above encouraged the intake of green leafy vegetables, dried fruit, and nuts and seeds. These foods were added to the traditional food group, increasing the protein, calcium, iron, and zinc contents of the meal plans by 15–20%. In Ghana, there is no special meal guide for vegetarians which includes local and indigenous Ghanaian foods considering seasonal variations. This makes it difficult for vegetarians to plan meals using local Ghanaian foods.

2.6 MACRONUTRIENT INTAKE OF VEGETARIANS

2.6.1 Energy

According to Henderson *et al.* (2003a), while meat and meat products provide a substantial amount of energy in the typical diet (15%), there is no reason to suggest that energy intakes are compromised by adults following a vegetarian or vegan diet. Both vegetarian and vegan diets may contain foods that are energy-dense such as vegetable oils, nuts, seeds and foods prepared with vegetable oils such as pastries, cakes and biscuits. Studies by Leblanc *et al.*

(2000) and Nathan *et al.* (1996) found that vegetarians had similar, or slightly lower, energy intakes than meat-eaters in the same population, for both adults and children.

Davey *et al.*, (2003) also reported that vegan diets were generally lower in energy than lacto- and lacto-ovo- vegetarian diets.

2.6.2 Protein

Some studies have reported that even though the quality of plant protein varies, a vegetarian or vegan can easily meet protein requirements with adequate calories and a variety of plant-based foods. Individual plant foods, with a few noteworthy exceptions, are not considered complete protein sources, as they do not have adequate levels of all essential amino acids (Herens *et al.*, 1992; Sanders and Manning 1992). For example, most grains are low in lysine. However, regular inclusion of legumes such as soya beans, kidney beans, pinto beans and lentils, makes up for the low level of lysine in grains. Complementary protein sources do not, however, need to be eaten at the same meal, as long as a variety of plant foods are consumed daily (Young and Pellet, 1994).

2.6.3 Fat

A study by Waldmann *et al.* (2003) comparing vegetarians with meat-eaters reported that semi-vegetarian and lacto-ovo-vegetarian diets contain similar or only slightly lower amounts of fat, because vegetarian diets can include fat from other food sources. The average proportion of energy provided by fat was similar for meat-eaters, fish-eaters and lacto-ovo-vegetarian women, but lacto- ovo-vegetarian men and all vegans obtained a considerably lower proportion of dietary energy from total fat and from saturates compared with the former groups.

2.7 MICRONUTRIENT INTAKES AND STATUS

Meat and meat products, and other foods obtained from animal sources, are very good sources of certain vitamins and minerals. Red meat, for example, is a good source of iron, zinc, preformed vitamin A and vitamin B₁₂. Milk and dairy products are rich in bio-available calcium and provide useful amounts of several other minerals and vitamins and oily fish is rich in vitamin D (Burgess *et al.*, 2001). However, certain components in plant foods affect the absorption and metabolism of some micronutrients. Phytates are antioxidant compounds found in whole grains, legumes, nuts and seeds. These for instance, impair the absorption of minerals such as zinc and iron (Hunt and Roughead, 1999). In view of all these, the question remains whether diets that exclude meat provide adequate amounts of micronutrients.

Micronutrient malnutrition has long-ranging effects on health, learning ability and productivity, leading to high social and public costs, reduced work capacity in populations due to high rates of illness and disability and tragic loss of human potential (FAO/WHO, 1992). Therefore, overcoming micronutrient deficiencies is a prerequisite for ensuring rapid and appropriate development. Although the most severe problems of micronutrient malnutrition are found in developing countries, people of all population groups in all regions of the world can be affected by micronutrient deficiencies. Approximately two billion people, about a third of the world's population are today deficient in one or more micronutrients (Thompson and Amoroso, 2014). The Food and Agricultural Organization (FAO, 2014) strongly emphasizes that food-based approaches, which include food production, dietary diversification and food fortification, are sustainable strategies for improving the micronutrient status of populations. Vegetarian women and teenagers are

advised to consume adequate intakes of iron as they do not consume meat. Vitamin C should also be adequate in the diet to facilitate the absorption of iron.

2.7.1 Folate

Folate is a term which describes a family of B-group vitamins. It comprises an aromatic pteridine ring linked to p-amino benzoic acid and one or more glutamate residues (Gregory, 1997). It includes naturally occurring folates obtained from foods and folic acid, a synthetic form used in supplements and food fortification. The bioavailability of natural folates obtained from food is approximately 50% lower than that of folic acid (Gregory, 1997). Eating folate-rich natural food sources has been seen to result in a smaller increase in red blood cell folate concentration as compared to folic acid supplements or cereals which are fortified with folic acid (Cuskelly *et al.*, 1996).

Folate, also known as folic acid acts as coenzymes in a single-carbon transfer in the metabolism of nucleic and amino acids. Deoxyribonucleic acid and methylation cycles both regenerate tetrahydrofolate (a form of folate). Folate can be lost through excretion from the urine, skin, and bile. As a result, the body needs to replenish its folate content by adequate intakes from the diet (Whitney and Rolfes, 1999). When dietary folate is inadequate, the activity of both the DNA and the methylation cycles are reduced. This reduces cell division (particularly the red blood cell) resulting in anaemia (Fedosov *et al.*, 1996).

Processes which involve a reduction in cell division also occur which lead to an increased susceptibility to infection as well as poor absorption and a decrease in blood coagulation (Institute of Medicine, 1998). The decrease in the methylation cycle causes a rise in plasma homocysteine which has been associated with the aetiology of cardiovascular disease

(CVD). Two vitamins, B₁₂ and B₆, are also required for the methylation cycle. Interrupting the methylation cycle from impaired folate status or decreased vitamins B₁₂ or B₆ may pose risks like neuropathy (Institute of Medicine, 1998).

Folate deficiency may present with symptoms including weakness, fatigue, difficulty in concentration, irritability, headache, palpitations, shortness of breath and atrophic glossitis (Whitney and Rolfes, 1999). This deficiency is usually seen in people who consume an inadequate diet. Conditions such as coeliac disease, tropical sprue and various malabsorption conditions may worsen it (Whitney and Rolfes, 1999). Pregnancy significantly increases requirements of folate and puts pregnant women at risk. The result of folate deficiency during pregnancy is neural tube defects (NTD's), such as spina bifida as well as other birth defects (O' Keefe *et al.*, 1995). Folate deficiency may also lead to macrocytic anaemia, a condition which is usually characterized by the production of fewer but larger red blood cells which impairs their oxygen-carrying ability. During lactation there is loss of folate in milk which increases requirements for the lactating mother (O' Keefe *et al.*, 1995).

A decrease in serum folate concentration as a result of inadequate intakes leads to a decrease in erythrocyte folate concentration. This then leads to a rise in homocysteine concentrations and megaloblastic changes in tissues with rapidly dividing cells like the bone marrow and eventually results to macrocytic anaemia (Fedosov *et al.*, 1996). Moderate elevation of plasma homocysteine concentration is in itself a risk factor for CVD, stroke, poor cognitive function and osteoporosis. Populations which consume diets which are adequate in folate still present a range of elevated plasma homocysteine which can be lowered by increasing daily intakes of folic acid by 100 or 200µg/day (Sauberlich *et al.*, 1987).

Folate may be found in a wide variety of foods but in relatively low concentrations. It is most often abundant in legumes, while green leafy vegetables are outstanding sources. Diets which contain adequate portions of fresh green vegetables (>3 servings per day) are good sources of folate. Fortified grain and cereal products also contribute to folate intake. Folate may be lost during harvesting, storage, distribution and as a result of certain cooking methods (Cuskelly *et al.*, 1996).

The adult Recommended Daily Allowance (RDA) is 400µg/day (Smolin and Grosvenor, 2000). A greater content of folate is reflected in a vegetarian diet due to the abundance of fruits and vegetables and hence an expected higher serum folate levels among these individuals. Some studies have reported higher serum folate concentrations among vegetarians compared to non-vegetarians (Armstrong *et al.*, 1974; Bar-Sella *et al.*, 1990; Gilsing and Crowe, 2010).

2.7.2 Vitamin B₁₂

Vitamin B₁₂ is a relatively large and complex vitamin. According to Elmadfa and Leitzmann (2004), vitamin B₁₂ is an essential micronutrient that plays a fundamental role in cell division and in one-carbon metabolism. Its absorption occurs by both an active and a passive mechanism. Vitamin B₁₂ in food is protein bound and liberated from food protein by an active mechanism in the stomach where it binds to a salivary R-binder (a family of haptocorrins). It is released again in the upper small intestine and attaches to the intrinsic factor (IF). The vitamin B₁₂-IF complex proceeds to the lower end of the small intestine, where it is absorbed by specific ileal receptors (Green and Miller, 2007). The two main vitamin B₁₂ transport proteins in human plasma are haptocorrin and transcobalamin (Green

and Miller, 2007). There is a complex process involved in gastrointestinal absorption of dietary vitamin B₁₂ that exists in humans. First, vitamin B₁₂ released from food is bound to the salivary vitamin B₁₂ binding protein (haptocorrin) in the stomach. After breakdown of the protein complex by pancreatic proteases in the duodenum, vitamin B₁₂ is released and binds to the gastric vitamin B₁₂- binding protein (intrinsic factor) in the proximal ileum. The protein complex is then able to enter mucosal cells in the distal ileum through a process of receptor-mediated endocytosis. This gastrointestinal absorption is what ensures bioavailability of dietary vitamin B₁₂ (Russell-Jones and Alpers, 1999).

The passive mechanism of vitamin B₁₂ absorption occurs equally across the absorptive surface of the gastrointestinal tract (Elmadfa and Leitzmann, 2004). This is an inefficient process and only 1–2% of an oral dose can be absorbed this way. An important component of cobalamin absorption and conservation is the partial reabsorption of biliary vitamin B₁₂ by the enterohepatic circulation (Elmadfa and Leitzmann, 2004).

Vitamin B₁₂ participates as cofactor in two important intracellular metabolic reactions. The first of these is the mitochondrial reaction in which the enzyme methylmalonyl-CoA mutase requires cobalamin in the form of 5-deoxyadenosylcobalamin (converting methylmalonyl-CoA to succinyl-CoA). The second is the cytosolic reaction that requires cobalamin in the form of methylcobalamin for folate-dependent methylation of the sulfur amino acid homocysteine to form methionine, catalyzed by the enzyme methionine synthase. Methionine metabolism is regulated by vitamin B₁₂, folate, and vitamin B₆. The methionine synthase reaction is also necessary for normal DNA synthesis (Elmadfa and Leitzmann, 2004).

Vitamin B₁₂ is the only vitamin that is synthesized exclusively by microorganisms (Taga, 2007). Dietary sources are primarily of animal origin such as meats, dairy products, and eggs. It is not supplied by plant foods unless they have been exposed to specific bacterial action. There is no evidence to suggest that vitamin B₁₂ – producing bacteria in the human large bowel can contribute substantially to the vitamin B₁₂ needs of the individual (Green and Miller, 2007). Humans in rural areas may obtain some vitamin B₁₂ through ingestion of cobalamin contained in bacteria-contaminated plant foods (Green and Miller, 2007). Thus, based on current knowledge and understanding, vegans and to a lesser degree other vegetarians are advised to use vitamin B₁₂ – fortified foods or supplements. There are studies which show that serum concentrations of Vitamin B₁₂ are generally seen to be lower among vegetarians, particularly vegans and this has been found to decrease as the duration of following the vegetarian diet increases (Allen, 2009; Gilsing and Crowe, 2010).

According to Tonstad (2009) the association of vitamin B₁₂ with animal foods such as fish, meat, poultry, eggs, milk and dairy products has created the myth that this vitamin can only be obtained from these foods and that a vegetarian or vegan diet provides a substandard amount. Consequently, B₁₂ has become a contentious issue. Concerns that vegetarians, and especially vegans, are at risk of B₁₂ deficiency prevail even though the evidence suggests that the meat-eating elderly are by far the group most likely to be deficient in B₁₂ (Whitney and Rolfes, 1999).

Various metabolic reactions in the body like amino acid and odd-chain fatty acid metabolism as well as the biosynthesis of methionine and methylmalonyl-CoA mutase have various

compounds of Vitamin B₁₂ which function as coenzymes. Some of these compounds are methylcobalamin and 59-deoxyadenosylcobalamin (Chen *et al.*, 1994).

Vitamin B₁₂ deficiency usually occurs in the general population and is due to a lack of intrinsic factor which prevents the absorption of the vitamin. This type of deficiency leads to pernicious anaemia (Scott, 1997). Vitamin B₁₂ deficiency presents with signs of megaloblastic anaemia and neuropathy (Institute of Medicine, 1998). Vegetarians, particularly vegans have a greater risk of developing vitamin B₁₂ deficiency as compared to non-vegetarians (Millet *et al.*, 1989). They require a daily consumption of Vitamin B₁₂-fortified foods or vitamin B₁₂-containing dietary supplements to prevent vitamin B₁₂ deficiency. A considerable number of elderly people with low serum Vitamin B₁₂ concentrations without pernicious anaemia were seen to have poor absorption of dietary vitamin B₁₂ (Baik and Russell, 1999). This malabsorption is usually seen in people with some gastric malfunctioning, such as atrophic gastritis with reduced secretion of stomach acid (Park and Johnson, 2006).

The Institute of Medicine reports that the recommended daily allowance of Vitamin B₁₂ for adults who are 51 years and above should be obtained through consuming foods fortified with vitamin B₁₂ in crystalline or supplement form (Institute of Medicine, 1998). The recommended dietary intakes of Vitamin B₁₂ are 2.4 micrograms daily for ages 14 years and older, 2.6 micrograms daily for pregnant females and 2.8 micrograms daily for breastfeeding females (Institute of Medicine, 1998).

According to Russel *et al.* (2001), vegan diets which are well-planned may provide adequate amounts of vitamin B₁₂ for all ages; infants, children and adults with very little probability of

problems occurring and many children have been brought up as very healthy vegans (Russell *et al.*, 2001). Where a lactating mother has a low intake of this vitamin, deficiency may start after a few months in exclusively breast-fed babies because amounts for the baby to build up stores in the liver may be woefully inadequate (Grattan-Smith *et al.*, 1997).

Plant sources such as grains, nuts, pulses, vegetables do not contain B₁₂. However, when these sources are contaminated with bacteria which produce B₁₂, they may obtain helpful amounts from the soil. Plants grown at home may provide useful amounts. A study done on a group of Iranian vegans people who did not develop a deficiency in vitamin B₁₂ reported that these people grew their vegetables in human manure and apart from that, did not exhibit good hygienic practices of careful washing before use (Herbert, 1998).

2.8 HEALTH IMPLICATIONS OF VEGETARIAN DIETS

Vegetarian diets are often associated with a number of health advantages, including lower blood cholesterol levels, lower risk of heart disease, lower blood pressure levels, and lower risk of hypertension and type 2 diabetes. Vegetarians tend to have a lower body mass index (BMI) and lower overall cancer rates (Craig, 2009). A study conducted to determine the effects of a non-vegetarian diet compared with a lacto-ovo-vegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in men reported that the consumption of a diet containing meat contributed to greater gains in fat-free mass and skeletal muscle with resistance –training than a lacto-ovo-vegetarian diet (Campbell, 1999).

Vegetarian diets tend to be lower in saturated fat and cholesterol, and have higher levels of dietary fibre, magnesium and potassium, vitamins C and E, folate, carotenoids, flavonoids,

and other phytochemicals. In the Adventist Health Study on diet and health of vegetarians 43 associations were made between plant foods and chronic diseases and all the protective effects were observed for foods of plant origin whereas all the harmful effects were related with the intake of meat (Fraser, 1999; Willet, 1999).

Plant foods contain antioxidants which have been suggested to prevent cardiovascular disease and some types of cancer (Wiseman *et al.*, 2000). Various substances present in fruits, vegetables and other plant foods have been shown to possess anticarcinogenic properties (American Institute for Cancer Research, 1996). In addition to these, whole grains, fruits, vegetables, legumes, nuts and other plant foods provide active substances which have been labelled as 'essential nutrients'. Not only are fruits and vegetables rich sources of carotenoids, ascorbic acid, tocopherols and folic acid, they are also rich sources of fibre, indoles, thiocyanates, coumarins, phenols, flavonoids, terpenes, protease inhibitors, plant sterols and several other unknown and unnamed phytochemicals and non-nutrient compounds which are cancer-protective (American Institute for Cancer Research, 1996).

Vegans, and to a lesser extent vegetarians, have low serum concentrations of vitamin B₁₂. A study conducted to investigate differences in serum Vitamin B₁₂ concentrations between omnivores and vegetarians revealed lower Vitamin B₁₂ concentrations and higher folate concentrations in vegans as compared to omnivores. Half of the vegans were seen to be Vitamin B₁₂ deficient and were expected to have a higher risk of developing symptoms related to Vitamin B₁₂ deficiencies (Gilling and Crowe, 2010). When serum levels of vitamin B₁₂ and folate were investigated in 132 Thai vegetarians, it was observed that serum vitamin B₁₂ decreased and serum folic acid levels increased according to the duration of

vegetarianism in the vegetarians. The longer the duration in female participants, the lower the vitamin B₁₂ levels (Prayurahong *et al.*, 1993). Another study conducted in selected communities in Accra and Cape Coast to determine dietary status of vegetarian children recommended the need for vitamin B₁₂ supplementation for all vegetarian children as the diets of the vegetarian children lacked Vitamin B₁₂ (Osei-Boadi *et al.*, 2012). Due to the fact that vegetarian diets consist mainly of plant foods, it is important that special attention is given to the intake of certain nutrients such as vitamin B₁₂, because they may be in a less available form or in lower concentrations in plant foods.

CHAPTER THREE

3.0 METHODS

3.1 STUDY DESIGN

A case-control study was carried out. This design was adopted because the study sought to compare the micronutrient status and dietary intake of both vegetarians and non-vegetarians in a Christian community.

3.2 STUDY SITE

This study was carried out at the premises of the New Historic Seventh Day Adventist Church at Mayra, Pokuase. Pokuase is a community located near the Accra-Kumasi Highway in the Amasaman District located North-West of Accra, the country's capital. It is about 45 minutes' drive from Accra. It is a mixed community which serves both residential and other purposes. It is located a few kilometers away from Achimota and surrounded by other suburbs, including Ofankor, Amasaman, Ablekuma, Medie and Asofan. Pokuase is a peri-urban area which can be attributed to the construction of the Accra-Nsawam road, which has reduced the total traveling time for most residents (Lamudi, 2015).

3.3 STUDY PARTICIPANTS

The population for this study comprised vegetarians (vegans and lacto-vegetarians) and non-vegetarians. The vegetarians (cases) were all members of the New Historic Seventh Day Adventist church. Seventh Day Adventists do not smoke tobacco or drink alcohol and are advised not to eat meat and foods containing animal flesh. This, however, is not a condition

to be a member of the church. The non-vegetarians were members of the same church as well as individuals living around the church premises.

3.4 SAMPLE SIZE DETERMINATION

A sample size of 92 was determined for the study. 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.

$$N = \left[\frac{Z^2(P)(1 - P)}{d^2} \right] \quad \text{Mohamad } et \text{ al., (2013).}$$

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

$$N=92$$

Where ‘N’ is estimated sample size, Z = Z statistic for a level of confidence, d = precision, P = expected prevalence or proportion of vegetarians in Accra. Although minimum sample size was 92, 45 vegetarians and 45 non-vegetarians were considered because of difficulty in recruitment.

3.5 SAMPLING TECHNIQUE

Purposive sampling was used to select the vegetarians (cases) and non-vegetarians (controls).

3.6 PARTICIPANTS

3.6.1 Inclusion Criteria

All consenting individuals who were aged 17 to 60 years were recruited into the study. The cases included subjects who had been vegetarians for more than 2 years and the control group was made up of apparently healthy non-vegetarians.

3.6.2 Exclusion Criteria

Individuals who had been vegetarians for less than two years were excluded (Kumar *et al.*, 2012). Subjects outside the age range of 17 to 60 years were excluded from the study as well as subjects who refused to consent. The age limit of sixty (60) years was because Vitamin B₁₂ level decreases as age increases (Bernard *et al.*, 1998). Pregnant women were also excluded. This was because situations whereby stress is imposed on the vegetarian, like pregnancy have been seen to adversely affect Vitamin B₁₂ levels (Grattan-Smith *et al.*, 1997).

3.7 PROCEDURE FOR DATA COLLECTION

3.7.1 Questionnaire Administration

Structured questionnaires (Appendix II) were administered to assess socio-demographic information, lifestyle behaviours and dietary intake of the participants. Participants were interviewed in a private room using the language each participant was comfortable with.

3.7.1.1 Dietary Assessment

3.7.1.1.1 24-Hour Recall

A three day 24-hour recall which comprised two weekdays and one weekend was used to assess nutrient intake of the participants. Each participant was interviewed to provide information through personal contact about food and fluid consumed over a 24-hour period with quantities consumed as well as cooking methods employed. To make it easy for the respondents to recall accurately the previous day's food intake, food models were used to estimate portion sizes. Food records were reviewed by the researcher together with the participant to ensure that the information had been properly described. Handy measures were used for the analysis. The data was then used to determine differences in nutrient intakes between vegetarians and non-vegetarians.

3.7.1.1.2 Food Frequency Questionnaire

A food frequency questionnaire was administered to assess dietary intake of participants on habitual basis (appendix III) (Adapted from Asare, 2011). This questionnaire included specific food groups which were consumed over a reference period. The food consumption patterns of the participants were assessed by multiple responses where participants were asked how often a particular food or beverage was consumed. Categories which ranged from 'never' (where there was no consumption at all) to 'once a month' were included in the questionnaire and participants chose one of the options which applied to them. The questionnaire was pre-tested among twenty vegetarians during one of the regular meetings of the Vegetarians Association of Ghana (VAG).

3.7.2 Anthropometric Measurements

3.7.2.1 Weight and body fat

Body weight was measured to the nearest 0.1 kg with subjects in an upright position wearing light clothing and without shoes. The body mass index (BMI) of the individuals was computed as the ratio of weight in kilogram (kg) divided by height in meter squared (m²). This was used to classify obesity based on the World Health Organization (WHO, 2012) criteria. Body weight, visceral and body fat were measured using the Omron Bio impedance analyzer (Omron Healthcare, Incorporated, model HBF-514C) while participants had no footwear on.

3.7.2.2 Height measurement

Height was measured to the nearest 0.1cm with the subjects in an upright position wearing light clothing and without shoes. Height was measured with a portable Seca Stadiometre (Model, SEC-213).

3.7.3 Sample Collection and Laboratory Assay of Blood Samples

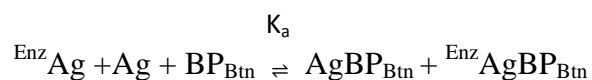
After an overnight fast (8 – 12 hrs), 3 ml of venous blood samples were collected from the antecubital space of the forearm by a trained phlebotomist into gel separator tubes. The blood samples were drawn between the hours of 6am-9am. The tubes were placed on ice and transported to the laboratory for separation. Samples were centrifuged at 5000 rpm for 5 minutes. The serum was separated into eppendorf tubes and stored at -20°C till analysis was due.

3.7.4 Biochemical Analysis

All the blood samples collected were analyzed for their vitamin B₁₂ and folate content at the Chemical Pathology Unit of the School of Biomedical and Allied Health Sciences, Korle-Bu.

3.7.4.1 Folate Analysis

The principle behind the analysis is the competitive binding protein assay (type 8). Competition occurs between the native antigen and the conjugate for a number of binding sites and this is illustrated as:



BP_{Btn} = Biotinylated Binding Protein (Constant K_b Quantity)

Ag = Native Antigen (Variable Quantity)

${}^{\text{Enz}}\text{Ag}$ = Enzyme-antigen Conjugate (Constant Quantity)

BP_{Btn} = Antigen-Binding Protein Complex

${}^{\text{Enz}}\text{AgBP}_{\text{Btn}}$ = Enzyme-Antigen-Binding Protein Complex

k_a = Rate Constant of Association

k_b = Rate Constant of Disassociation

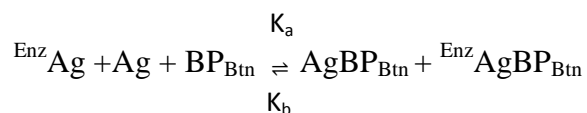
$K = k_a / k_b$ = Equilibrium Constant

The test was performed according to the manufacturer's instructions (Monobind Inc. Product Code: 7525-300). Fifty microlitres of the folate calibrator control or samples were pipetted into the assigned wells. The same amount of folate enzyme reagent was added to each well. Fifty microlitres (50 μl) of the Folate Biotin Reagent was then added and the microplate

mixed gently for 20-30 seconds. The microplate was covered and incubated for 45 minutes at 25°C. The contents of the microplate were discarded by decantation. Three hundred and fifty microlitres (350µl) of wash buffer was added and decanted. A total of three washes were done. One hundred microlitres (100µl) of substrate reagent was then added to all the wells. Contents of each well were then incubated at room temperature for 20 minutes. Fifty microlitres (50µl) of stop solution was added to each well for 15-20 seconds. The absorbance was read in each well at 450 nm (using a reference wavelength of 620-630 nm).

3.7.4.2 Vitamin B₁₂ analysis

The principle behind this analysis is Delayed Competitive Enzyme Immunoassay (Type 9) Reagents (biotinylated antibody and serum containing the antigen) were mixed to create a reaction between the antigen and antibody as shown in the reaction below:



BP_{Btn} = Biotinylated Binding Protein (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

EnzAg = Enzyme-antigen Conjugate (Constant Quantity)

BP_{Btn} = Antigen- Binding Protein Complex

EnzAgBP_{Btn} = Enzyme-Antigen-Binding Protein Complex

k_a = Rate Constant of Association

k_b = Rate Constant of Disassociation

K = k_a / k_b = Equilibrium Constant

The test was performed according to the manufacturer's instructions (Monobind Inc. Product Code: 7625-300). Fifty microlitres (50 μ l) of the appropriate extracted vitamin B₁₂ calibrator control or specimen was pipetted into the assigned well and the same amount of vitamin B₁₂ Enzyme Reagent was added to all wells. Fifty microlitres (50 μ l) of the vitamin B₁₂ Biotin Reagent was added to each well and the mixed gently by swirling plate for 20-30 seconds. The microplate was covered and incubated for 45 minutes at 25°C.

The contents of the microplate were discarded by decantation. Three hundred and fifty microlitres (350 μ l) of wash buffer was added and decanted. A total of three washes were done. Hundred microlitres (100 μ l) of substrate reagent was added to all the wells. It was then incubated at room temperature for 20 minutes and 50 μ l of stop solution was added to each well for 15-20 seconds. The absorbance was read at 450 nm (using a reference wavelength of 620-630 nm). A dose response curve was used to ascertain the concentrations of folate and vitamin B₁₂.

3.5 DATA ANALYSIS

3.5.1 Statistical Analysis

Data was transferred into SPSS (version 20.0) and analyzed. Both the descriptive and inferential statistical tools were used to analyze the data gathered. The ESHA-FPRO nutrient analysis software (version 6.02) was used to estimate nutrient intake. Qualitative data were summarized as proportions, percentages (gender) and quantitative as mean, standard deviation and ranges (height, weight and ages). Data was tested for normality.

Chi square test was used to determine associations between categorical variables. The independent *t*-test was used to compare mean levels of micronutrient status among

vegetarians and non-vegetarians. Regression was used to determine the strength of relationship between dependent and independent variables. All tests were computed as two-tailed and p-values less than 0.05 were considered statistically significant.

3.6 ETHICS

Ethical approval for the study was obtained from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences. [SAHS-ET./10395380/AA/9A/2013-2014] (Appendix 1). Permission was also sought from the church authorities, particularly the pastor and elders to carry out the study. Participants who were recruited for this study were provided with information about the purpose of the study and consent was sought from each of them by appending their signatures and thumbprint marks where necessary. Confidentiality and anonymity of the subjects were ensured.

CHAPTER FOUR

4.0 RESULTS

4.1 SOCIO- DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

A total of 90 participants were used for the study. These consisted of 45 vegetarians and 45 non-vegetarians. The mean age of the overall population was 34.0 ± 10.8 years. No significant differences in age were seen between vegetarians and non-vegetarians. Only a few (7.7%) of the participants did not have formal education. Majority of the vegetarians (60%) compared to non-vegetarians (40%) were married. Table 4.1 shows the socio-demographic characteristics of the participants. A greater proportion (93%) of participants were lacto-vegetarians while a few were vegans (Figure 4.2). More than half of the cases reported that they became vegetarians for religious purposes; a few reported that they did so to protect the environment, while about a third stated that their reason for becoming vegetarian was to promote health (Figure 4.2).

Table 4.1 Socio-demographic characteristics of participants

Characteristic	Vegetarians (V) (n=45)	Non-vegetarians (NV) (n=45)	Total (n=90)	ρ –Value
Age (yr)	37.0 ± 12.1	30.8 ± 7.7	33.9± 10.6	0.430
Gender				
Male	26 (57.8)	21 (46.7)	47 (52.2)	0.291
Female	19 (42.2)	24 (53.3)	43 (47.8)	
Marital Status				
Single	14 (31.1)	25 (55.6)	39 (43.3)	0.041*
Married	27 (60.0)	18 (40.0)	45 (50.0)	
Divorced/Widowed	4 (8.9)	2 (4.4)	6 (6.6)	
Educational Background				
No formal education	6 (13.3)	1 (2.2)	7 (7.8)	0.264
Basic education	16 (35.6)	18 (40.0)	34 (37.8)	
HND	14 (31.1)	17 (37.8)	31 (34.4)	
Degree	9 (20.0)	9 (20.0)	18 (20.0)	
Employment Status				
Unemployed	6 (13.3)	3 (6.7)	9 (10.0)	0.014*
Employed	24 (53.3)	32 (71.1)	56 (62.2)	
Retired	8 (17.8)	0 (0.0)	8 (8.9)	
Student	7 (15.6)	10 (22.2)	17 (18.9)	
Length of Vegetarianism				
2 to 10 years	30 (33.3)	0	0	
11 to 20 years	8 (8.9)	0	0	
21 to 30 years	6 (6.7)	0	0	
31 to 40 years	0	0	0	
	1 (1.1)	0	0	

*Significant at $p < 0.05$

4.2 VEGETARIANISM

4.2.1 Types and Reasons for becoming a Vegetarian

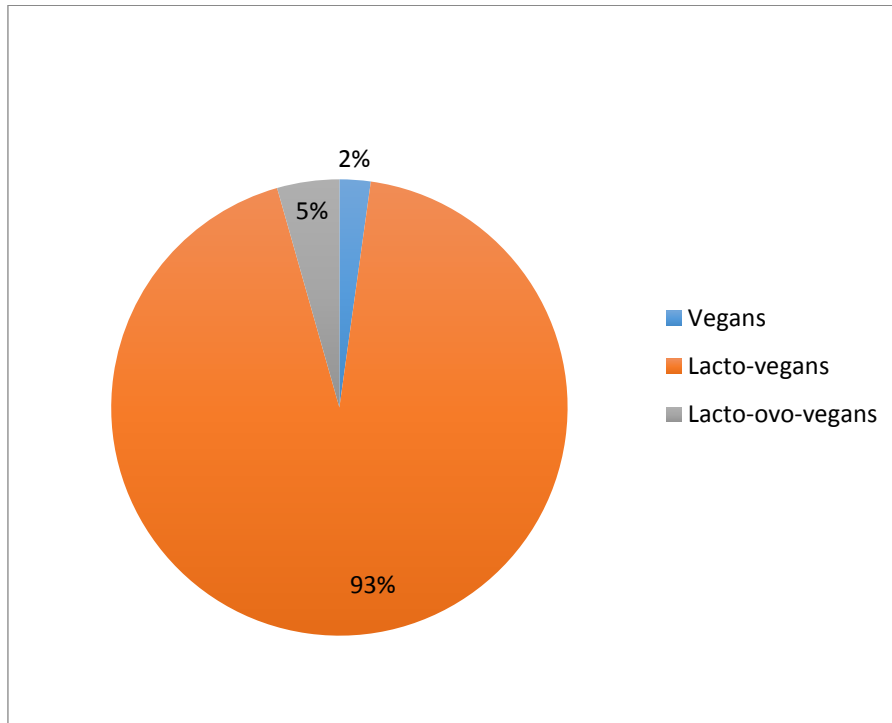


Figure 4.1 Types of vegetarians.

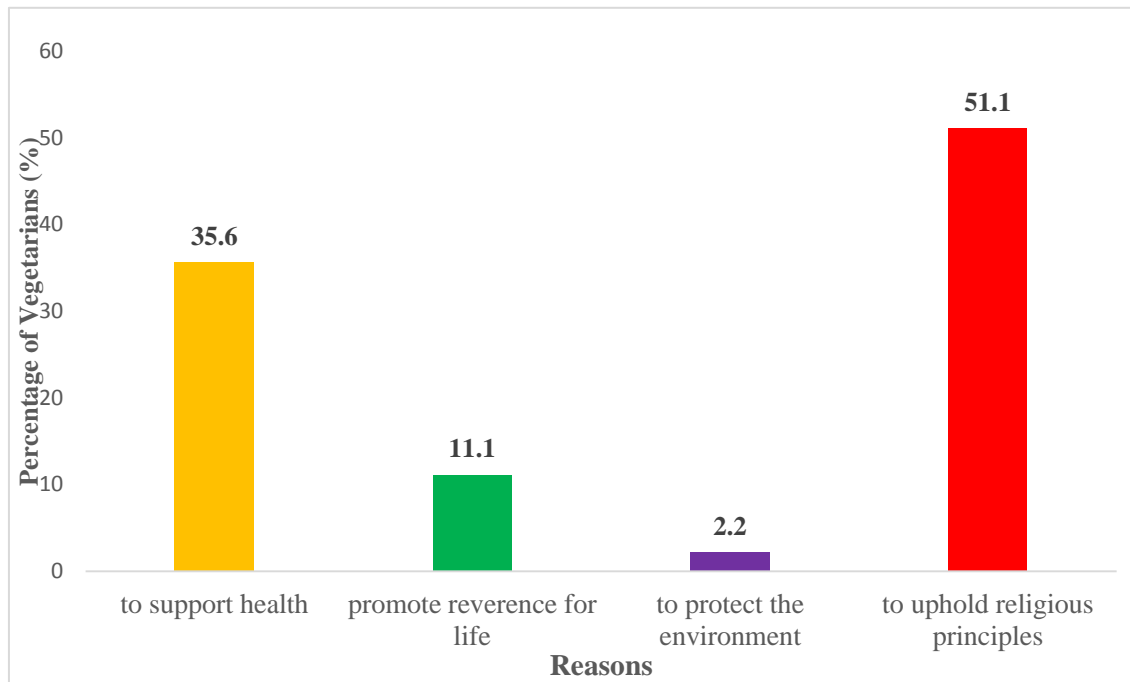


Figure 4.2 Reasons for becoming a vegetarian

4.3 MEAL PATTERN AND SNACKING HABITS OF PARTICIPANTS

Table 4.2 shows the meal pattern and food habits of vegetarians and non-vegetarians. There was no significant difference ($p = 0.276$) between the number of meals eaten in a day in both groups. However, it was observed that more than half of the vegetarians stated that they ate two meals a day. Majority of the vegetarians (51.1%) skipped breakfast whilst non-vegetarians (64.4%) reported that they did not skip breakfast, though this was not significantly different ($p = 0.136$). More than half of the non-vegetarians (68.9%) reported that they ate snacks and 51.1% of the vegetarians reported that they did not eat snacks. There was a significant difference ($p = 0.001$) among the various snacks taken, between the vegetarians and non-vegetarians. Fruits and vegetables were the main snacks consumed by the vegetarians (51.1%) while most of the non-vegetarians (31.1%) reportedly snacked on

soft drinks. There was no significant difference ($\rho = 0.093$) in the frequency of fruits and vegetables consumption between both groups.

Table 4.2 Meal pattern and food habits of participants

Characteristic	Vegetarians (V) (n=45)		Non-vegetarians (NV) (n=45)		ρ -Value
	Frequency	%	Frequency	%	
Patterns					
Number of main meals					
One	4	8.9	2	4.4	0.276
Two	27	60.0	22	48.9	
Three	14	31.1	21	46.7	
Skip Breakfast					
Yes	23	51.1	16	35.6	0.136
No	22	48.9	29	64.4	
Snacks in between meals					
Yes	22	48.9	31	68.9	0.05*
No	23	51.1	14	31.1	
Types of snacks					
Fruits and vegetables	15	33.3	6	13.3	0.001*
Soft drinks	3	6.7	14	31.1	
Pastries	4	8.9	11	24.4	
Frequency of fruit intake					
Daily	20	44.4	9	20.0	0.093
Once a week	18	40.0	21	46.7	
Once a month	4	8.9	9	20.0	
Occasionally	3	6.7	4	8.9	
Never	0	0	2	4.4	

*Significant at $p < 0.05$

4.4 LIFE STYLE BEHAVIOURS

Table 4.3 shows the lifestyle behaviours of participants in the study. Smoking was uncommon in both groups. With regards to alcohol intake, less than 5% of the vegetarians reported that they consumed alcohol compared to 20.0% of non-vegetarians. The most frequently consumed alcohol for the non-vegetarians was beer 5%, which was consumed occasionally.

Table 4.3 Life style behaviours of participants

Characteristic	Vegetarians (V) (n=45)		Non-vegetarians (NV) (n=45)		ρ -Value
	Frequency	%	Frequency	%	
Current smokers	0	0	1	2.2	0.315
Alcohol drinkers	2	4.4	9	20.0	0.024*
Type of alcohol					
Wine (~12% -OH)	1	2.2	2	4.4	
Beer (~3% -OH)	0	0	5	11.1	0.019*
Hard liquor (~40% -OH)	1	2.2	2	4.4	
Frequency of alcohol intake					
Daily	0	0	1	2.2	
Once a week	1	2.2	5	11.1	0.644
Once a month	0	0	1	2.2	
Occasionally	1	2.2	1	2.2	
Quantity of intake					
½ medium glass wine (100ml)	1	2.2	1	2.2	
1 mini Guinness/beer (350ml)	0	0.0	5	11.1	0.732
1 tot of spirits/whisky (20ml)	1	2.2	1	2.2	
Other	0	0.0	1	2.2	

4.5 COMPARISON OF ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENTS

There were no significant differences in weight, height, BMI and diastolic blood pressure of vegetarians and non- vegetarians ($\rho > 0.05$). However, visceral fats, body fat and systolic blood pressure were significantly higher in vegetarians compared to non-vegetarians ($\rho < 0.05$).

Table 4.4 Comparison of Anthropometric and Blood Pressure Measurement between Vegetarians (n = 45) and Non-Vegetarians (n = 45)

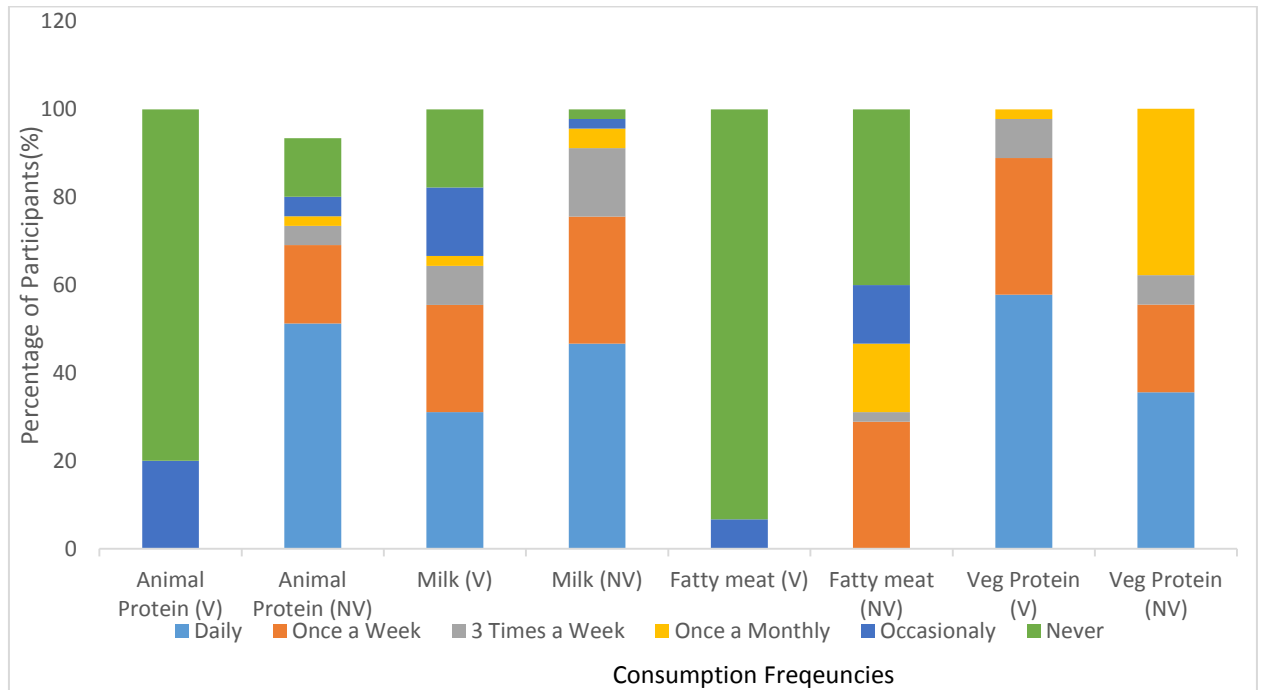
Variable	Anthropometric Measurement (mean \pm SD)		ρ -value
	Vegetarians	Non-Vegetarians	
Weight (kg)	71.03 \pm 12.62	67.65 \pm 8.09	0.098
Height (m)	1.66 \pm 0.08	1.66 \pm 0.73	0.143
BMI (kgm ⁻²)	25.80 \pm 4.53	24.72 \pm 2.80	0.115
Visceral fats (%)	8.77 \pm 4.32	6.42 \pm 3.29	*0.001
Body fats (%)	28.64 \pm 12.13	23.99 \pm 8.75	*0.031
Systolic BP (mmHg)	128.49 \pm 15.50	123.51 \pm 7.11	*0.041
Diastolic BP (mmHg)	79.87 \pm 10.33	80.04 \pm 5.26	0.919

*significantly different between vegetarians and non-vegetarians

4.6 FREQUENCY OF CONSUMPTION OF FOOD

4.6.1 Animal Protein and Vegetable Protein

A higher proportion of non-vegetarians consumed milk daily compared to the vegetarians (46.7% vs. 31.1%) as shown in figure 4.3. Consumption of vegetable proteins was higher in vegetarians (57.8%) compared to non-vegetarians (35.6%).



NV= Non-vegetarians V=Vegetarians

Figure 4.3 Frequency of consumption of animal and vegetable protein of vegetarians and non-vegetarians.

4.6.2 Fruits, Vegetables, Fruit Juices and hot and cold drinks

A significantly higher percentage ($\rho = 0.001$) of the vegetarians (66.7%) consumed vegetables daily than non-vegetarians (28.9%). Similarly, a significantly higher proportion ($\rho = 0.001$) of vegetarians (71.1%) consumed fruits daily compared non-vegetarians (22.2%). Again daily consumption of fruit juice intake was significantly higher ($\rho = 0.001$) among the vegetarians (38.7%) than the non-vegetarians (8.9%).

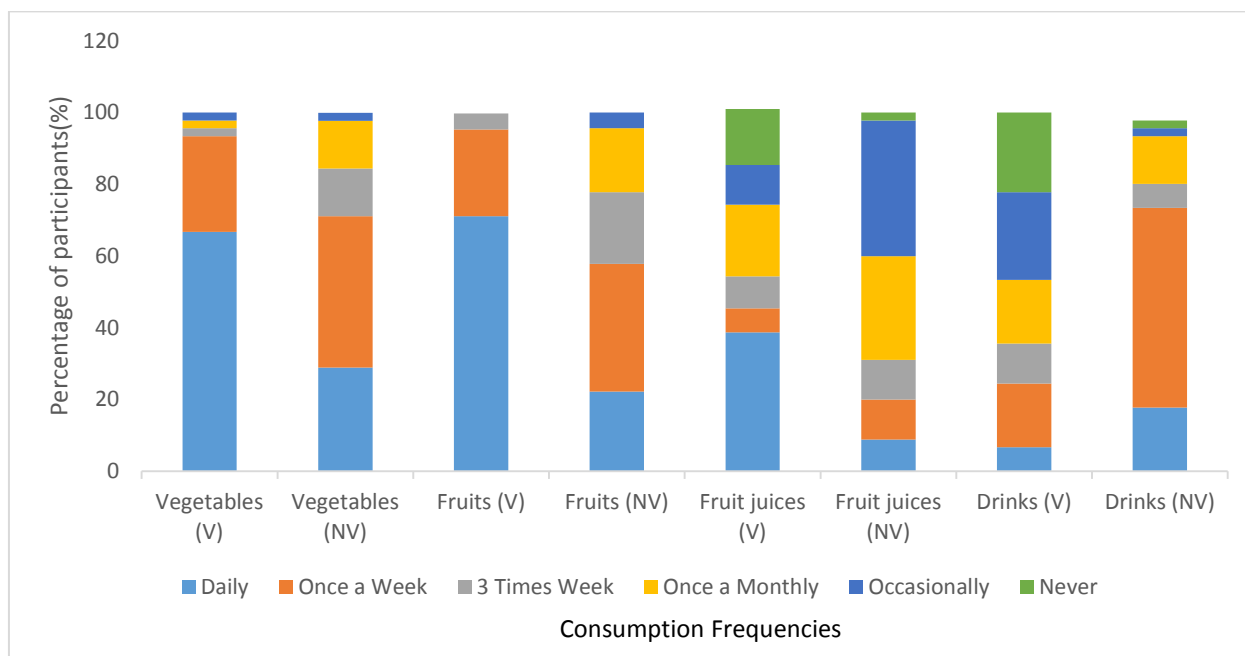


Figure 4.4 Frequency of consumption of fresh fruits and vegetables, fruit juices and drinks between vegetarians and non-vegetarians.

4.6.3 Tubers, Cereals and Grains

There were no significant differences ($\rho = 0.461$ and $\rho = 0.115$ respectively) in intakes between groups. However, consumption of cereals and grains was lower in the vegetarian group.

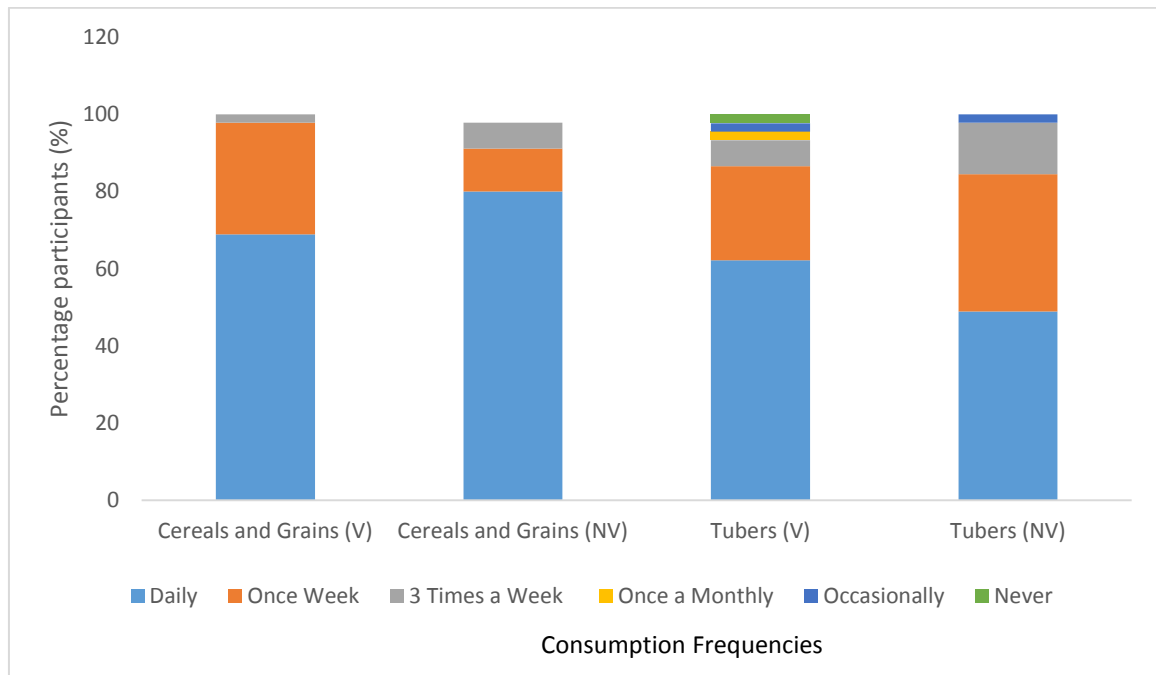


Figure 4.5 Frequency of consumption of cereals, grains and tubers between vegetarians and non-vegetarians.

NV= Non-vegetarians N=Vegetarians

4.6.4 Deep Fried Foods, Fast Foods and Pastries

The proportion of participants who consumed deep fried foods (e.g. fried yam and plantain) once a week was significantly lower ($\rho = 0.001$) among vegetarians (26.7%) than non-vegetarians (37.8%). Although the consumption of fast foods was not common in both groups, a significantly lower proportion ($\rho = 0.03$) of the vegetarians (22.2%) compared to non-vegetarians (53.3%) reported that they ate it occasionally. However, vegetarians who never consumed pastries of any kind were significantly higher ($\rho = 0.001$) (33.3%) than non-vegetarians (6.7%).

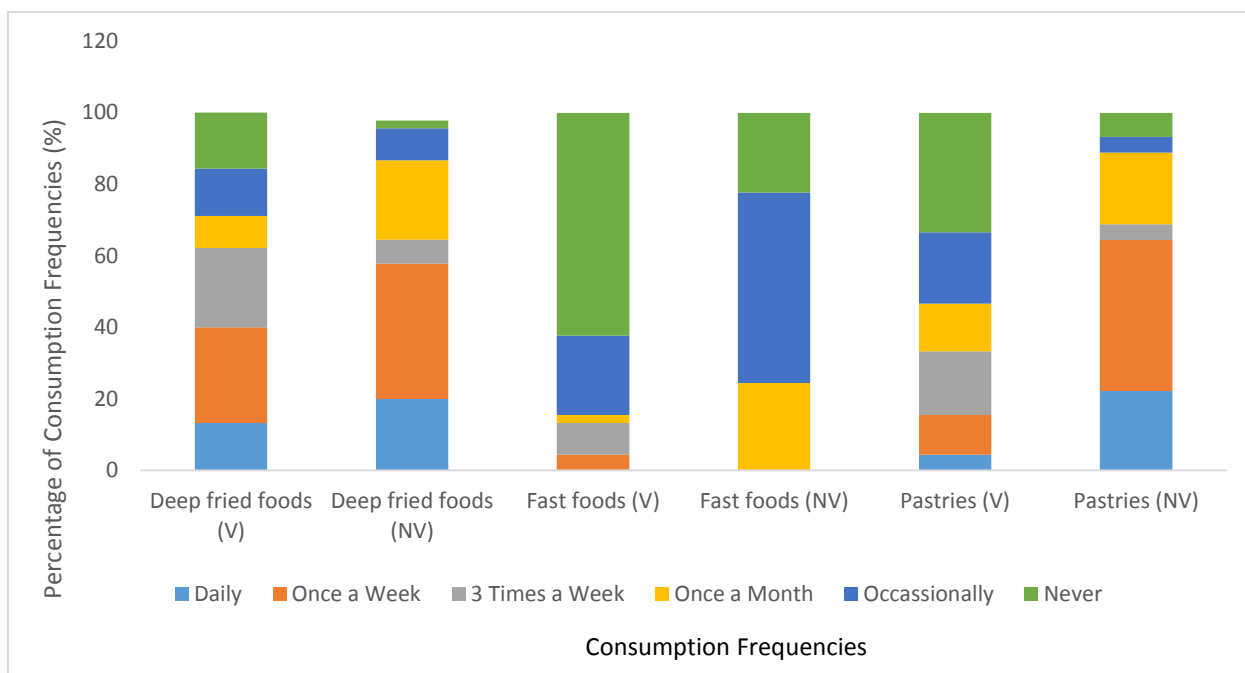


Figure 4.6 Frequency of consumption of deep fried foods, fast foods and pastries between vegetarians and non-vegetarians.

NV = Non-vegetarians **V** = Vegetarians

4.7 ENERGY AND NUTRIENT INTAKES

The daily energy, macro- and micronutrient intakes of vegetarians and non-vegetarians are shown in Tables 4.5 and 4.6 respectively. The total energy intakes of both vegetarians and non-vegetarians did not differ significantly between groups. Non-vegetarians derived a significantly higher proportion ($p = 0.001$) of their energy from proteins, compared to vegetarians. The intakes of saturated fats, cholesterol, riboflavin, vitamin B₁₂, selenium, Vitamin D and calcium were also significantly higher ($p < 0.05$) in non-vegetarians with vegetarians having higher intakes of polyunsaturated fats and dietary fibre. No significant differences were found in folate intakes of vegetarians.

Table 4.5 Mean daily energy and macronutrient intake

Nutrient	Vegetarians (V) (n = 45)	Non-vegetarians (NV) (n = 45)	P - Value
Energy/kcal	2003 ± 534	2152 ± 347	
Protein/g*	62.6 ± 19.9	77.1 ± 21.0	0.001*
(% contribution to energy intake)	(12.5%)	(14.3%)	
Carbohydrate/g	369.6 ± 119.0	383.0 ± 136.8	
(% contribution to energy intake)	(73.8%)	(71.2%)	
Fat/g	58.0 ± 35.2	53.6 ± 29.2	
(% contribution to energy intake)	(26.0%)	(22.4%)	
SFA/g*	4.6 ± 3.9	11.5 ± 4.6	0.0001*
(% contribution to fat intake)	(7.9%)	(21.4%)	
MUFA/g	5.9 ± 4.9	7.6 ± 5.5	
(% contribution to fat intake)	(10.2%)	(14.2%)	
PUFA/g*	15.8 ± 6.9	5.7 ± 5.0	0.0001*
(% contribution to fat intake)	(27.2%)	(10.7%)	
Cholesterol/mg*	38.9 ± 54.6	178.8 ± 100.9	0.0001*
Dietary fibre/g*	36.2 ± 11.6	23.1 ± 6.8	0.0001*

Data is presented as (Mean ± SD)

*Significantly different between vegetarians and non-vegetarians

Table 4.6 Intake of micronutrients

Micronutrient	Vegetarians (V) (n = 45)	Non-vegetarians (NV) (n = 45)	P- Value
Riboflavin/mg*	0.8 (0.11 – 3.22)	1.3 (0.40 – 14.20)	0.001*
Vitamin B ₁₂ /m g*	0.6 (0.00 – 3.65)	2.6 (0.00 – 7.60)	0.0001*
Selenium/μg*	0.1(0.00 – 4.00)	13.2 (0.00 – 24.00)	0.0001*
Vitamin D/μg*	2.1 (0.00 – 129.70)	10.3 (0.78 – 23.20)	0.0001*
Folate/μg	158.8 ± 361.9	194.9 ± 436.6	
Calcium/mg*	398.0 ± 197.6	888.1 ± 294.3	0.0001*

Data is presented as median (range) for all micronutrients except for folate and calcium which are presented as mean ± SD.

*Significantly lower in vegetarian compared to non-vegetarians

4.8 BIOCHEMICAL ANALYSIS

Mean serum folate concentrations of both vegetarians and non-vegetarians were below the normal range (2-20 ng/ml) (Fischbach and Dunning, 2008). Mean serum folate level was relatively lower among the vegetarians, compared to non-vegetarians. Mean serum Vitamin B₁₂ levels of both vegetarians and non-vegetarians were also below the normal range (200-900 pg/ml) (Salwen, 2011). There was no significant difference between the mean folate concentrations of vegetarians and non-vegetarians ($p = 0.8232$). However, mean serum vitamin B₁₂ concentrations was significantly lower in vegetarians ($p < 0.05$) than non-vegetarians.

4.7 Mean serum folate and vitamin B₁₂ levels in vegetarians and non-vegetarians

	Vegetarian	Non -vegetarian
Serum folate/ng/ml	1.20 ± 0.45	1.19 ± 0.23
Serum B ₁₂ /pg/ml	0.22 ± 0.05	0.31 ± 0.12

4.9 CORRELATION BETWEEN SERUM AND DIETARY VITAMIN B₁₂ AND FOLATE LEVELS IN RELATION TO BOTH VEGETARIANS AND NON-VEGETARIANS

Serum vitamin B₁₂ was strongly positively correlated with dietary levels in vegetarians ($r=0.424$, $\rho < 0.001$) as well as in non-vegetarian ($r=0.315$, $\rho = 0.020$). Similarly, serum folate concentrations had a strong positive correlation with the dietary levels in both vegetarians ($r=0.417$, $\rho < 0.001$) and non-vegetarians ($r=0.214$, $\rho = 0.021$).

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSIONS

5.1 DISCUSSION

The socio-demographic characteristics were compiled for 45 vegetarians and 45 non-vegetarians. The mean ages of vegetarians and non-vegetarians were not significantly different and this indicated a younger age group similar to findings by Laysson *et al.*, (2002) and Baines *et al.*, (2007) who reported that more women and younger individuals chose vegetarianism. Similarly, in this present study mean age of vegetarians was higher than that of non-vegetarians (Davey *et al.*, 2003). However, contrary to the finding of Laysson *et al.*, (2002), female vegetarians were less.

Very few of the participants had no formal education. Most participants had education up to the secondary level. Vegetarians have been shown to be more educated than non-vegetarians (Davey *et al.*, 2003). This study showed similar levels of education among both groups of participants and there was no significant difference in educational levels among the two groups of participants contrary to findings of a study conducted in the Netherlands (Hoek *et al.*, 2004). The authors compared socio-demographic characteristics and attitudes to food and health of vegetarians and non-vegetarians. Their findings revealed that vegetarians had higher education levels, higher socio-economic status and lived in urbanized residential areas compared to non-vegetarians (Hoek *et al.*, 2004). Participants in this study were recruited from the same community and much more importantly, most were members of the same church hence similar beliefs, background and ecology.

Smoking among participants in this study was uncommon. This is consistent with findings of other studies in vegetarian populations. Smoking has been shown to have teratogenic effects on an individual. It has been shown to have effects on reproduction as it is a reproductive toxicant (Sobinoff *et al.*, 2014). Vegetarians normally have regular physical activity and usually abstain from smoking and alcohol consumption than non-vegetarians (Davey *et al.*, 2003). A number of studies have shown that vegetarians usually tend to drink less alcohol and less exercise than non-vegetarians (Rottka , 1990; Thorogood , 2009; Bergan and Brown , 1980). Apart from the above, most of the participants as a result of their religious beliefs and affiliation with the church were not likely to indulge in smoking. Seventh Day Adventists do not smoke tobacco and drink alcohol although this is not a condition of membership in the church. The results of this study generally showed a healthy lifestyle from majority of the participants as well as low alcohol consumption.

Majority of the participants ate two main meals and this may be a result of their work pattern and lifestyle. Since most of the participants were employed, they could afford two or three main meals daily and hence the minority consuming only one main meal daily. Also, most of the breakfast meals were made up of cereals and bread or tea or chocolate drink and bread. Snacks were also consumed by majority of the participants from both groups even though more non-vegetarians (68.9%) ate snacks. This may be as a result of lack of variety of vegetarian snacks to choose from.

Milk was consumed more frequently by the non-vegetarians than the vegetarians. In addition to the above, majority of the vegetarians compared to the non-vegetarians consumed

vegetable proteins more frequently. Consumption of animal products especially milk and meat increases with income and urbanization (Oniang *et al.*, 2003). Since most of the participants were employed with an income and vegetarians were able to consume milk frequently. In addition to this, the community was a peri-urban one and hence milk and milk products were easily accessible. Frequent milk consumption by the non-vegetarians was quite low and this can be attributed to the fact that the low consumption levels are due to low production, lack of preservation technology and also the high prevalence of lactose intolerance among African populations (Oniang *et al.*, 2003).

Vegetables including tomatoes, garden eggs and okro were consumed daily by 66.7% of the vegetarians. This was quite interesting because a higher percentage was expected from vegetarians as vegetables form the basis of the vegetarian diet. Fruits and vegetables are important in the diet because they provide vitamins A, C and folate and minerals including iron and magnesium as well as dietary fibre. Dietary fibre is important as it helps the digestive system and is also necessary in preventing a lot of diseases, particularly cardiovascular diseases (Oniang *et al.*, 2003). Usually, vegetarians tend to consume fewer overall calories; a lower proportion of calories from fat (particularly saturated fat) and cholesterol; and higher intakes of fruits, vegetables, whole grains, nuts, soy products, fibre, and phytochemicals than non-vegetarians. Usually, fruits and vegetables form the bulk of the diet (Key *et al.*, 2009). In this study, however, daily vegetable intake was 66.7% which was quite low. This may be attributed to the fact that most of the vegetarians were lacto-vegetarians hence the inclusion of certain animal products in their diet.

Generally, deep fried foods, fast foods and pastries were not consumed usually by both vegetarians and non-vegetarians. These results indicate a certain level of health

consciousness among participants which enables them make healthier choices of foods and snacks which may be as a result of teachings on health in that particular denomination. Another reason may also be that fast food and pastry joints are very uncommon eating places in the area. Restaurants and big supermarkets are also quite uncommon.

Findings of this study did not show significant differences in mean energy intake between the two groups but the contribution of protein to daily energy intakes did differ although that of carbohydrates and fats were not significantly different. This study showed that total calories were lower among vegetarians than non-vegetarians and supports the fact that vegetarians are known to have lower energy intakes than non-vegetarians (Key *et al.*, 2009). Some of these dietary intakes are different among many populations and although studies by Leblanc *et al.* (2000) Nathan *et al.* (1996) and Davey *et al.* (2003) have consistently found that vegetarians have similar, or slightly lower energy intakes than non-vegetarians in the same population for both adults and children. Another study by Levin *et al.*, (1986) differs where it was reported that the vegetarian diet supplied a significantly greater amount of energy than the non-vegetarian diet (3031 kcal/day vs. 2627 kcal/day).

However, the fact that a lower proportion of this lower energy intake usually comes from fat in vegetarians, particularly saturated fat and cholesterol, than non-vegetarians (Key *et al.*, 2009) was not clearly established in the results of that study. To the contrary, a higher proportion of fat was seen among vegetarians than non-vegetarians in this study. This was, however, not significantly different. The proportion of fat from saturated and polyunsaturated fats also showed significant differences among the two groups; saturated fatty acids were significantly lower in vegetarians than non-vegetarians and polyunsaturated

fatty acids were higher among vegetarians than in non-vegetarians. Meat and meat products usually contribute about 22% to saturated fat intake so it is reasonable to expect that when these are omitted or reduced from the diet, the saturated fat would be reduced substantially (Carlson *et al.*, 2013; Colin-Ramirez *et al.*, 2014). The health benefits of polyunsaturated fats cannot be overemphasized as consuming adequate amounts has been seen to be critical in all stages of the life cycle (Carlson *et al.*, 2013; Colin-Ramirez *et al.*, 2014).

Protein intake was significantly different among the two different groups, vegetarians showing a lower contribution to energy intake than non-vegetarians. Similarly, among Flemish and British populations, a lower protein intake among vegetarians than non-vegetarians was reported (Davey *et al.*, 2003; Deriemaeker *et al.*, 2010). These differences in nutrient intake may be unfavourable or not and the impact on health of the relatively lower intakes of protein among vegetarians is unclear (Antony, 2003). Although the quality of plant protein is varied, a vegetarian can have adequate protein intake with adequate calories from a variety of plant based foods. Most plant foods, however, are not considered complete where protein is concerned due to the fact that they do not possess all essential amino acids (Herens *et al.*, 1992; Sanders and Manning 1992). Most grains, for example, are low in lysine even though a recent study reports an increase in plant sources of protein which results in a higher overall protein intake among vegetarians than non-vegetarians (Lee, 2009). When these grains are consumed with legumes like beans and lentils, these low levels of lysine are usually catered for (Young and Pellet, 1994). It is important that foods are nutritionally balanced and adequately planned with all these in mind for both vegetarians and non-vegetarians, particularly vegetarians and other restrictive meal plans.

A significantly higher dietary fibre reported by vegetarians was expected as the vegetarian diet is high in vegetables, legumes, nuts, whole grains, fruits which apart from providing vitamins, minerals and phytochemicals, are also rich sources of dietary fibre (Key *et al.*, 2009).

With the exception of folate, significantly lower intakes of calcium, vitamin B₁₂, vitamin D and selenium were reported among vegetarians. Meat and meat products and other foods obtained from animal sources are very good sources of these vitamin and minerals. Red meat is one of these and apart from being a good source of iron, provides useful amounts of zinc, vitamin D, calcium, and several other minerals and vitamins. Fish is also a good source of vitamins and minerals which include vitamin D and calcium (Burgess *et al.*, 2001). Eliminating or excluding these from the diet would imply lower amounts of these minerals and vitamins which may even pose risks for deficiencies. The vegetarian diet usually excludes these and hence lower amounts of these minerals are expected. The inadequacy of vitamin B₁₂ in vegetarians has been consistently established by various studies (Obersby *et al.*, 2013; Gammon *et al.*, 2012; Yajnik *et al.*, 2006). This has been associated with the exclusion of animal products such as meat, dairy products and eggs from the diet (Green and Miller, 2007). Antony (2003) also reported that compared to non-vegetarians, vegetarians are more likely to develop Vitamin B₁₂ deficiencies as a result of reduced bioavailability from plant sources. There was however, no significant difference between folate intakes of vegetarians and non-vegetarians. Low folate intake among vegetarians, albeit not significantly different, was not expected as generally, vegetarian diets tend to be high in folate of which sources include breads, cereals, legumes, fruits and vegetables. In addition,

mean folate dietary intakes of both groups fell below recommended daily intake of 400 μ g (Institute of Medicine, 1998).

Results of the study showed no significant differences between blood serum folate of vegetarians and non-vegetarians. However, serum vitamin B₁₂ concentrations were significantly lower in vegetarians. Several studies have slightly varying results. One such study conducted to determine selected nutrition-related health aspects in adult vegetarians who were Seventh-day Adventists reported lower vitamin B₁₂ dietary intakes among vegetarians than non-vegetarians but similar serum vitamin B₁₂ levels among both vegetarians and non-vegetarians (Harman, 1998). Another study among adult Seventh Day Adventists showed higher serum vitamin B₁₂ levels among vegetarians compared to non-vegetarians (Armstrong *et al.*, 1974). The results of this study, however, confirm reports that non-vegetarians have a significantly higher serum vitamin B₁₂ level than vegetarians. Age may be a factor which relates to vitamin B₁₂ concentrations, though unclear. A research conducted to investigate differences in serum vitamin B₁₂ concentrations between omnivores and vegetarians revealed lower vitamin B₁₂ concentrations and higher folate concentrations in vegetarians as compared to omnivores (Gilsing and Crowe, 2010). The results of this study, however, reflect no significant difference in serum folate levels between vegetarian and non-vegetarians although a relatively lower mean folate level was recorded among the vegetarians. Failure to find significant differences between the two groups may be explained by the fact that vegetarian subjects recruited for the study were mostly lacto-vegetarians and as a result, may have had dietary intakes of fruits and vegetables comparable or no different from that of the non-vegetarians. According to Gregory (1997), the bioavailability of natural folates obtained from food is approximately 50% lower than that of folic acid and this could

have been another reason for the low serum levels of folate. Eating folate-rich natural food sources have been seen to result in a smaller increase in red blood cell folate concentration as compared to folic acid supplements or cereals which are fortified with folic acid (Cuskelly *et al.*, 1996).

The positive correlation between dietary intakes of vitamin B₁₂ and folate and serum levels of these nutrients was expected as natural sources of vitamin B₁₂ in human diets have been known to be restricted to animal-origin diets; it is believed that those people with low animal food diets are more susceptible to cobalamin deficiency (Park and Johnson, 2006). High folate intakes have been associated with high serum folate levels and rare cases of vitamin B₁₂ deficiency (Dickinson, 1995).

5.2 CONCLUSIONS

Overall, the study was able to show a positive relationship between vitamin B₁₂ and folate dietary intake and serum levels of vegetarians and non-vegetarians. Vitamin B₁₂ intakes of vegetarians were significantly lower than that of non-vegetarians ($\rho = 0.0001$). There was no significant difference between the mean serum folate concentrations of vegetarians and non-vegetarians ($\rho = 0.8232$). However, mean serum vitamin B₁₂ concentrations was significantly lower in vegetarians ($\rho = 0.05$) than non-vegetarians. Low serum vitamin B₁₂ and folate levels among vegetarians and non-vegetarians still remain a concern and require careful and in-depth analysis into more acceptable and balanced dietary intake of these nutrients.

5.3 LIMITATIONS OF THE STUDY

- i. Methods of assessing dietary intake may be inaccurate as there may be a possible over or underestimation of certain foods eaten. It was also difficult to measure exact amounts of foods eaten hence approximations were made by both participants and researcher.
- ii. This study was unable to capture the seasonal variation of fruit and vegetable intake which could have influenced the results reported.
- iii. This study comprised different types of vegetarians, mainly lacto vegetarians and vegans. Due to the small numbers of vegetarians and the difficulty accessing them in general, analysis was done for both types of vegetarians.

5.4 RECOMMENDATIONS

It is recommended that:

- i. Another study of this nature could be carried out in a typical urban community where educational levels, income levels, religious beliefs and principles as well as work patterns and lifestyle practices may differ and may influence dietary intakes and consequently blood serum levels.
- ii. Another study could also be carried out to assess dietary intake and trace element status of adult vegetarians.
- iii. It would also be important for dietitians during dietary counselling sessions for vegetarians to replace nutrients which are provided by foods which are excluded from the diet with other foods which provide the same or equal amounts of those nutrients to restore complete nutrition.

- iv. Vegetarian diets should also include foods which are fortified with vitamin B₁₂ and folate on a regular basis to prevent or reduce risks of deficiencies in an individual.
- v. Nutrition education could be intensified among vegetarian populations on how to vary their diets so as to prevent deficiencies in these populations.

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APPENDIXES

APPENDIX I

UNIVERSITY OF GHANA, SCHOOL OF ALLIED HEALTH SCIENCES

INFORMED CONSENT FORM

I, **Somah Agyeman-Nkansah**, am conducting a research on Vitamin B₁₂ and folate status 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, folate, Vitamin B₁₂ and iron status among vegetarians and non-vegetarians. About 3ml of venous blood will be drawn from you to measure the micronutrients in the blood. Body weight and height of the participant 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 micronutrient status, specifically vitamin B₁₂ and folate.

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

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:

NAME OF PARTICIPANT:

SIGNATURE/THUMBPRINT : TEL :

APPENDIX II**QUESTIONNAIRE**

Participant's ID

Date.....

Socio –Demographic Status

1. Age of participant (years): 20-29 [] 30-39 [] 40-49 [] 50 -59 [] 60 and above []
2. Gender: Male [] Female []
3. Marital Status: Single [] Married [] Divorced []
Widowed [] Separated []
4. Religion: Christian [] Muslim [] Buddhists [] Hindu []
others, please specify
5. Educational Background: No formal education [] Basic education (middle/JHS)
[] SHS/O –Level [] HND/Diploma Certificate [] Bachelor Degree []
Post Degree []
6. Employment Status: Employed [] Unemployed [] Retired [] Student []
7. 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 []
8. What is your main reason for becoming a vegetarian?
 - i. To support personal health and healing []
 - ii. To promote reverence for life []
 - iii. To protect the environment. []

- iv. To uphold religious and philosophical principles. []

B. LIFE STYLE BEHAVIOUR

1. Do you smoke? YES [] NO []

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

2. Do you drink alcohol? YES [] NO []

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

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

285ml of beer (½ large beer bottle, one full mini Guinness)

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

60ml of (brandy, vermouth, aperitif)

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

C. ANTHROPOMETRIC AND PHYSICAL MEASUREMENTS

15. Weightkg 16.Height..... meters 17.BMI.....Kg/m²

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

Hg

APPENDIX III**D. 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						

APPENDIX IV**E. 24-HR RECALL (CURRENT FOOD INTAKE)**

Time		Meal	Quantity
Breakfast	Weekend 1	1.	1.
		2.	2.
	Weekday 1	1.	1.
		2.	2.
	Weekday 2	1.	1.
		2.	2.
Mid-morning Snack			
Lunch	Weekend 1	1.	1.
		2.	2.
	Weekday 1	1.	1.
		2.	2.

	Weekday 2	1.	1.
		2.	2.
Mid-afternoon snack			
Supper	Weekend 1	1.	1.
		2.	2.
	Weekday 1	1.	1.
		2.	2.
	Weekday 2	1.	1.
		2.	2.
Late-evening snack			

APPENDIX V

F. LABORATORY RESULTS

Micronutrients	Level
Vitamin B₁₂ (µmol/L)	
Folate (nmol/L)	

APPENDIX VI**ETHICAL CLEARANCE FORM**

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

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

My Ref. No. SAHS/ 10395380
Your Ref. No.



P. O .Box KB 143
Korle Bu
Accra
Ghana

7th July, 2014.

Ms. Somah Agyeman-Nkansah,
Dept. of Dietetics,
SAHS,
Korle Bu.

Dear Ms. Agyeman-Nkansah,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET. /10395380/AA/9A/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: “Dietary Intake and Selected Micronutrients Status of Vegetarians in Accra”

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