

**LEVELS OF ENDOGENOUS SEX HORMONES IN MALE
VEGETARIANS AND NON-VEGETARIANS**

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DECLARATION

I, Yauniuck Yao Dogbe, hereby declare that this dissertation is the result of my own research work under the supervision of Dr. George Asare and Dr. Matilda Asante. There has not been any previous submission of this work for a Master of Science degree here or elsewhere. References to works of other authors cited have been duly acknowledged.

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ABSTRACT

Background: Wholly plant-based diets have been reported to have many beneficial outcomes. In some studies, higher circulating levels of free androgens have been suggested to increase the risk of developing prostate cancer. Men are reported to have lower levels of the sex hormone-binding globulin which may increase in response to oestrogen and decrease in response to androgens. The vegetarian diet consisting of vegetables and legumes and particularly soy products, are rich in phytoestrogens, which compete with other oestrogens for receptor binding. For the male vegetarian, increased serum concentration of oestrogens may influence the levels of testosterone and sex hormone-binding globulin. A study to examine the levels of endogenous sex hormones in vegetarian and non-vegetarian male has not yet been conducted in Ghana.

Aim: The aim of the study was to examine the levels of endogenous sex hormones in vegetarian and non-vegetarian males.

Methods: This was a case-control study of 52 healthy males (22 vegetarians and 30 non-vegetarians) recruited from four locations in the Greater-Accra Region. A structured questionnaire was used to obtain background and anthropometric data of all participants. Current and usual dietary intake was assessed using a 24-hour recall and a modified food frequency questionnaire respectively. Blood samples were collected after an overnight fast and serum hormone levels assessed by radioimmunoassay.

Results: Vegetarians were relatively older than non-vegetarians. The mean age of the participants was 49.4 ± 12.7 years. Motivation for choosing the dietary lifestyle was more religious than health inclined. Half of the vegetarians were lacto-ovo vegetarians.

All the participants were non-smokers, with alcohol consumption of one or two standard drink servings in 57% (n=17) of non-vegetarians. Vegetarians weighed less than non-vegetarians but BMI was insignificantly different. Mean diastolic blood pressure was significantly lower ($p=0.025$) in vegetarians (77.1 mmHg) than non-vegetarians (84.4 mmHg). There were significant differences in mean intake of protein ($p=0.001$), calcium ($p=0.003$), iron ($p=0.001$) and zinc ($p=0.043$). With the exception of calcium, mean intakes of the above mentioned micronutrients were all lower in vegetarians. The mean serum testosterone levels of vegetarians (20.0 nmol/L) was significantly lower ($p=0.022$) than in non-vegetarians (24.6 nmol/L) but mean serum oestradiol levels was significantly higher ($p=0.037$) in vegetarians (339 pmol/L) than in non-vegetarians (279 pmol/L). Significant differences ($p=0.001$) were found comparing age and type of protein (animal vs. plant) consumed with hormone levels. Besides oestradiol in non-vegetarians, there were inverse but insignificant associations in age and type of protein consumed with hormone levels. Mean serum oestradiol of non-vegetarians who did not consume soy compared to mean protein intake was directly associated although insignificant.

Conclusion: There were significant differences in mean weight, diastolic blood pressure and visceral fat of vegetarians and non-vegetarians but mean BMI, systolic blood pressures and body fat were not significantly different. Mean serum testosterone was significantly lower in vegetarians but mean serum oestradiol was significantly higher in vegetarians. No significant associations were found between types of protein frequently consumed and endogenous levels of serum sex hormones testosterone and oestradiol.

DEDICATION

I dedicate this dissertation to GOD. I also dedicate this work to Madame Beatrice Ami Eviam, my family and friends for their encouragement and to the struggle for scientific discoveries.



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ABBREVIATIONS

ADA	American Dietetic Association
BMI	Body Mass Index
BP	Blood Pressure
CHD	Coronary Heart Disease
DHEA	Dehydroepiandrosterone
DHEAs	Dehydroepiandrosterone-Sulphate
DHT	Dihydrotestosterone
IMFNB	Institute of Medicine, Food and Nutrition Board
MUFA	Monounsaturated Fatty Acid
NHANES	National Health and Nutrition Examination Survey
PUFA	Polyunsaturated Fatty Acid
SDA	Seventh Day Adventist
SFA	Saturated Fatty Acid
SHBG	Sex Hormone-Binding Globulin
UG	University of Ghana
UVB	Ultraviolet B
WHO	World Health Organisation

GLOSSARY

Koose	Bean cake
Kontomire	Cocoyam (<i>Xanthosoma mafafa</i>) leaves
Waagashi	Curdled milk fried in oil

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

An optimal diet is one that maximizes health and longevity and therefore prevents nutrient deficiencies and is composed of foods that are available, safe and palatable. According to the Academy of Nutrition and Dietetics (Cullum-Dugan and Pawlak, 2015) a well-planned vegetarian diet is adequate for all stages of the life cycle including infancy, childhood and adolescence and provides multiple beneficial nutrients including mono- and polyunsaturated fatty acids, antioxidant vitamins, phytochemicals and fibre (Segasothy and Phillips, 1999).

Vegetarianism describes a diet that excludes all meat and fish and some dairy products. Thus a vegetarian is one who avoids meat, fish, poultry and dairy products in his diet. However, there still persists inconsistency in describing what vegetarianism entails and how people identify themselves in this dietary behavioural choice. Consequently, vegetarians can be grouped into lacto-ovo vegetarians who consume dairy products and/or eggs and the more extreme vegans who consume only fruits and vegetables, excluding all foods made from or containing meat, fish and poultry. Other sub-classification do exist in the categories of vegetarians reported elsewhere (Meister, 1997; Russo, 2008). They include the *freegans* (Gross, 2009) who choose a dietary lifestyle based on personal philosophies, *raw foodists* (Russo, 2008; Cullum-Dugan and Pawlak, 2015) who choose a strict diet consisting of fresh and uncooked foods like fruits, nuts, seeds and vegetables.

The *semi-vegetarian* chooses a dietary lifestyle based on plant foods and the occasional consumption of animal foods like beef, pork, poultry or fish, once or twice weekly (Cullum-Dugan and Pawlak, 2015). Others choose a strict diet based on whole-foods and plant based diet that includes fish but no other flesh foods, referred to as the *macrobiotic* diet (Cullum-Dugan and Pawlak, 2015). Motivations for choosing a vegetarian meal vary, often stemming from philosophical or spiritual ideologies (Fox and Ward, 2008) or resulting from religious conversions or seasonal availability of foods (Boyle, 2007) conveniently consumed by vegetarians. It is important to identify that all these choices to some extent influence diet and nutrition and the resulting consequences may prove a challenge to deal with.

Evidence suggests that people who consume vegetarian diets have lower risk of coronary heart disease (Thorogood *et al.*, 1994; Szeto *et al.*, 2004), diabetes (vanDam *et al.*, 2002), cancer (Thorogood *et al.*, 1994) and other chronic diseases than meat-eaters. The protective effects of these foods are probably mediated through the presence of multiple beneficial nutrients including mono- and polyunsaturated fatty acids, antioxidant vitamins, phytochemicals, fibre, and soya protein (Segasothy and Phillips, 1999).

Vegetables and legumes, particularly soy products, are rich in phytoestrogens, which compete with other oestrogens for receptor binding (Adlercreutz, 1990) and also possess weak oestrogenic effects (Shutt and Cox, 1972). Some studies have found that vegetarian diets – specifically soya and vegetable proteins – decrease insulin and glucagon levels (McCarty, 1999; Kuo *et al.*, 2004). Soya beans and processed soya bean products contain isoflavones such as genistein and diadzein (Coward *et al.*, 1993).

The phytoestrogens and isoflavones such as genistein and daidzein predominantly obtained from soybeans and soybean products may contribute to healthy bones, brain and immune function (Mahan *et al.*, 2012). Decreased intake of fat (Hämäläinen *et al.*, 1984; Dorgan *et al.*, 1996), increased dietary fibre (Dorgan *et al.*, 1996), and changes to the vegetarian diet (Raben *et al.*, 1992) have reportedly resulted in lower plasma androgen levels in some intervention studies.

The vegetarian diet has previously been associated with increased circulating levels of sex-hormone binding globulin (SHBG) (Belanger *et al.*, 1989; Key *et al.*, 1990). Also, any effects of dietary constituents on levels of SHBG are likely to affect sex hormone metabolism through influence on hormone binding and clearance. Most steroid hormones are bound to specific proteins, example, SHBG, a carrier protein that regulates the amount of bioavailable oestradiol and testosterone in the blood (Fendrick *et al.*, 1998). The biological activity and metabolic clearance of oestrogens and androgens are mentioned to be strongly influenced by the binding protein which reversibly binds the steroid hormones with different affinities (Burke and Anderson, 1972; Anderson, 1974; Dunn *et al.*, 1981). It has been reported that oestradiol is not bound to SHBG and that is thought to be bioavailable (Siiteri *et al.*, 1981).

Circulating steroids of importance in men are testosterone (T), dihydrotestosterone (DHT) and oestradiol (E). Testosterone is mainly produced by the testicles and DHT is produced in the prostate, testes, hair follicles, and adrenal glands. Androgens and oestrogens circulate either free (1–2% of T) or bound to the serum proteins, albumin (binds 40–60% of T) and SHBG (binds 40–80% of T, which is biologically inactive) (Klee and Heser, 2000).

The interrelationships between oestrogens, androgens and SHBG are complex. Sex hormone-binding globulin regulates the proportions of the sex steroids which are bound and free, but the sex steroids themselves can also affect the concentration of SHBG. Averagely, men have lower serum concentrations of SHBG than women, and the concentration of SHBG can increase in response to oestrogens and decrease in response to androgens. However, the effects of steroids on SHBG are in most circumstances less obvious than the effects of SHBG on sex steroids. Factors such as body mass index (BMI), which alter SHBG, can cause changes in the total serum concentration of sex steroids because the control of sex hormone production by negative feedback is regulated by the concentration of free steroid. If SHBG decreases, the proportion of free steroid increases and therefore steroid production is reduced in order to maintain a constant concentration of free steroid (Key *et al.*, 2001).

The beneficial and adverse effects of a vegetarian or plant-based diet on human health have been studied extensively. However the mechanism through which the vegetarian diet achieves its purpose influencing blood cholesterol, glucose and hormonal balance remains unclear. One theory proposes that vegetarian diets may induce alteration in hormone secretion, which in turn regulates lipid and carbohydrate metabolism and change blood lipid and glucose levels. The interrelationship among diets, hormone levels and blood lipid and glucose concentrations have been studied, but are less clear and consistent in man than in animals (Forsythe, 1995; Persky *et al.*, 2002; Kuo *et al.*, 2004).

Presently, no study has reported hormone levels in vegetarians and/or meat-eating individuals in Ghana. With a Vegetarian Association and the presence of some restaurants serving vegetarian dishes just like elsewhere in other countries, an interesting

opportunity arises to understand how the dietary lifestyle influences the mechanism of hormonal (sex) action in the Ghanaian.

1.2 PROBLEM STATEMENT

Wholly plant-based diets have been reported to have many beneficial outcomes. However, some studies suggest that higher circulating levels of free androgens may increase the risk of developing prostate cancer (Gann *et al.*, 1996, Eaton *et al.*, 1999). Men are reported to have lower levels of the protein SHBG which may increase in response to oestrogen and decrease in response to androgens (Key *et al.*, 2001). Vegetables and legumes, particularly soy products, are rich in phytoestrogens, which compete with other oestrogens for receptor binding (Adlercreutz, 1990). For the male vegetarian, increased serum concentration of oestrogens may influence the levels of testosterone and sex hormone-binding globulin. The mechanism with which this interplay affects serum concentration of sex hormones in the Ghanaian male vegetarian remains an interesting area of scientific study and concern on male reproductive health. Also, there is paucity of data on dietary habits and the influence of vegetarianism on health in Ghana.

1.3 SIGNIFICANCE OF THE STUDY

It is hoped that this study may provide a reference for counseling on alternate or complementary use of other sources of protein by male vegetarians. It may also provide information on the relationship between a vegetarian or wholly plant-based diet and endogenous sex hormones in males. Finally, this study would add to existing knowledge on vegetarian diet and its influence on sex hormone levels in males.

1.4 AIM OF THE STUDY

The aim of this study was to examine the levels of testosterone and oestradiol in vegetarian and non-vegetarian males.

1.5 OBJECTIVES OF THE STUDY

The specific objectives of this study were to;

1. Compare the serum levels of testosterone and oestradiol in vegetarian and non-vegetarian males.
2. Test the association between age, protein and soy intake on levels of testosterone and oestradiol.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY OF VEGETARIANISM

The formation of the Vegetarian Society in the United Kingdom in 1847 represented the emergence of the modern idea of vegetarianism (Amato and Partridge, 1989; Gregory, 2007; Preece, 2008). Although the term *Vegetarian* was not coined by the Vegetarian Society UK until the mid-19th century, the practice of vegetarianism would seem to date back to early man (Spencer, 1994). Palaeontologists in East Africa have unearthed remains of early hominids whose dentition suggests that they were primarily vegetarian, as they had broad, flat teeth that would be unsuited to an omnivorous diet (Wilson and Ball, 1999). According to *The Oxford English Dictionary*, the general use of the term “vegetarian” appears to have been largely due to the formation of the Vegetarian Society, first used in late 1830’s (Preece, 2008).

The Vegetarian Association of Ghana which is registered as a non-profit, non-religious society of individuals with the sole desire of educating Ghanaians about healthy alternatives of a meat- or fish-based diet. They can boast of a membership of more than one thousand nationwide and about two hundred in the Greater-Accra region. It has among its core mandate, the desire to promote vegetarianism and plants based products by bringing all vegetarians in Ghana under one umbrella as well as create the opportunity for disseminating information through print, broadcast and internet media by professionals, vegetarians to raise the level of awareness on the benefits of a plant based diet (<http://www.vegghana.org/about.html>).

2.2 TYPES AND CATEGORIZATION OF VEGETARIANISM

The two most common subclasses of vegetarians are the lacto-ovo or vegan (Melina and Davies, 2003).

2.2.1 *Lacto-ovo vegetarian*

Lacto-ovo vegetarians avoid all animal flesh but do use eggs (ovo) and dairy products (lacto). Some lacto-vegetarians use only dairy products but not eggs and others are ovo-vegetarians using eggs but not dairy products (Melina and Davies, 2003).

2.2.2 *Vegan*

An individual who avoids all products of animal origin including eggs, dairy products, gelatin (made from bones and connective tissues of animals) and honey is known as a vegan. Vegans avoid animal products not only in their diet but in every aspect of their lives. They may shun leather goods, wool and silk, tallow soaps, and other products made with animal ingredients (Melina and Davies, 2003).

2.2.3 *Other sub-classifications*

Other types of vegetarians based on personal philosophies are *freegans* (Gross, 2009), *raw foodists* (Russo, 2008), and the separation of total vegans (uses plant foods only) from dietary vegans (does not necessarily try to exclude non-food uses of animals) (International Vegetarian Union, 2008). Freegans practice a vegan lifestyle but make exceptions in situations where food may go waste. Similarly, raw foodists prefer consumption of foods in their raw, uncooked form. The semi-vegetarian avoids red meat such as beef and pork, eating dairy, poultry and seafoods (Meister, 1997).

Different classifications described by the American Council on Science and Health (Meister, 1997) include the *pollo-* and *pesco-vegetarian*. Both eliminate red meat, but the *pollo-* and *pesco-vegetarians* avoid seafood and poultry respectively. The *fruitarian* is considered the strictest consuming only the fruit portion of the plant. Any food that would injure the plant such as root and leaf vegetables are avoided (Meister, 1997).

2.3 MOTIVATIONS AND REASONS FOR CHOOSING A VEGETARIAN DIET

People decide to vary dietary practices for several reasons, often shifting from a meat diet to a meatless diet or vice versa. This is often based on medical recommendations, religious conversion and seasonal availability of food and vegetarians (Boyle, 2007) are no different.

Motivations for being vegetarian are not static, and can be added, dropped, or modified over time (Beardsworth and Keil, 1992). For instance, Hamilton (2006) reported that among a sample of vegetarian adults in the UK, 74% of participants reported having changed their motives for being vegetarian; 34% had added a motive, 13% had dropped a motive and 23% had both added new motives and dropped original motives. Also, the types of foods one eats changes over time.

In a survey of current and former vegetarian women in Vancouver, majority (63%) of current vegetarians consumed a smaller array of animal products than when they first became vegetarian (Barr and Chapman, 2002), many attributing the change to having learned more about vegetarian nutrition and factory farming making them decrease consumption of dairy products and eggs.

Additionally, 42% of the sample reported intentions to eat animal foods less frequently. Twenty-seven percent (27%) had not changed the number of animal products they consumed, and the remaining 10% had increased the number of animal products in their diet (Barr and Chapman, 2002).

It is somewhat difficult to maintain the vegetarian diet (Barr and Chapman, 2002), with people making transitions from vegetarianism and meat-eating. In a study of ex-vegetarian women in Vancouver, 29% cited health concerns (e.g. fatigue, anaemia) as causing them to resume an omnivorous diet, 23% resumed because of missing the taste of meat, 20% because of a change in living situation (e.g. moving in with a meat-eating family), and 17% because of the perception that being a vegetarian was too time consuming (Barr and Chapman, 2002). In another study of vegetarians recruited primarily from the US, Canada, and the UK, health vegetarians focused on the effects of a vegetarian diet on personal health, fitness, and energy, whereas “ethical vegetarians often cast their motivations within a philosophical, ideological, or spiritual framework” (Fox and Ward, 2008).

Several thematic issues arise from people’s motivation for adopting a vegetarian lifestyle. They may be categorized into personal health concerns, adherence to religious beliefs and practices as well as moral concern and respect for animal health and welfare.

2.3.1 Personal health concerns

Before the advent of modern vegetarianism, people’s abstention from flesh-eating was a form of “self-denial”. People avoided eating meat because they believed abstinence was good for the body (Davis, 2006).

Health concerns are also the major reason motivating individuals who are ‘partial vegetarians’, who choose not to eat red meat, limit their consumption of flesh to fish, or select only organic products (American Dietetic Association, 2003; Bedford and Barr, 2005; Hoek *et al.*, 2004).

It has been proposed that two separate models for the adoption of a vegetarian diet exist: health and ethical. Health vegetarians avoided red meat from their diets focusing essentially on the benefits and barriers to changing their diet out of concern for potential diseases (Jabs *et al.*, 1998).

2.3.2 Adherence to religious beliefs and practices

The belief system that supports the barrier between meat consumption and a plant based diet in Western civilization can be traced back to the teachings of Pythagoras (Stuart, 2006; Spencer, 1995). Abstinence from the consumption of meat and animal products is an element of some religious practices including Buddhism and Seventh Day Adventism (Fraser, 2003). Others choose a secular vegetarianism, grounded in non-religious motivations (Whorton, 1994). The religious belief of followers led them not only to the idea that “eating patterns as manifesting a deeper moral relationship” but also faith that “meat-eating as symbolic of man’s fall” (Twigg, 1981).

2.3.3 Moral concern and respect for animal health and welfare

Vegetarianism permits the practitioner to avoid being an accomplice in the killing of animals (Hamilton, 2006). This question of the treatment of animals is contradictory (Loughnan *et al.*, 2010). On one hand people eat animals and participate in their wholesale slaughter whereas on the other, they love animals and consider them part of the

family introducing a ‘meat paradox’, thus people both love animals and love eating them as well.

Ethical vegetarians adopted their vegetarian diet for reasons of animal welfare, focusing primarily on moral considerations. They tended to adopt their diets abruptly associating meat with disgust and emotional distress, and reducing this distress by creating consistency between their diet and their beliefs about animal welfare (Jabs *et al.*, 1998).

2.4 NUTRIENT CONSIDERATIONS OF THE VEGETARIAN DIET

2.4.1 *Macronutrients*

Comparisons of the food intakes of vegetarians and non-vegetarians show that vegetarian diets generally provide relatively large amounts of cereals, pulses, nuts, fruits and vegetables. Together with the differences in intakes of animal foods, these differences in food intake result in several characteristic differences in nutrient intake. Vegetarian diets are usually rich in carbohydrates, n-6 fatty acids, dietary fibre, carotenoids, folic acid, vitamin C, vitamin E and magnesium, and relatively low in protein, saturated fat, long-chain n-3 fatty acids, retinol, vitamin B₁₂ and zinc; vegans can have particularly low intakes of vitamin B₁₂ and low intakes of calcium (Davey *et al.*, 2003).

2.4.1.1 *Energy*

As reported by Taber and Cook (1980), carbohydrate and fat contributed 54% and 33% of energy respectively in meals of vegetarian males living in Penobscot County, Maine, whose intake approximated to those of the USA Dietary Goal. Vegetarians tend to have a similar energy intake to non-vegetarians (Draper *et al.*, 1993).

In general, energy intake of vegans and vegetarians ranged from 5% to 22% lower than that of non-vegetarians (Kennedy *et al.*, 2001; Spencer *et al.*, 2003), similar to findings by Davey *et al.*, 2003, who reported lower energy intake in male vegans. Population-based studies have shown energy intakes of vegetarians that were as much as 464 kcal lower than non-vegetarians (Kennedy *et al.*, 2001; Barr and Chapman, 2002; Newby *et al.*, 2005). In a recent study, Farmer *et al.* (2011) also reported the mean energy intake of vegetarians to be slightly lower than that of non-vegetarians in the National Health and Nutrition Examination Survey (NHANES 1999-2004). In order to prevent the catabolism of dietary protein to provide energy, it is important to ensure sufficient energy intake for growth by including more of energy dense foods such as nuts, dried fruits, soya, cheese, vegetable oils and margarines.

2.4.1.2 *Carbohydrates*

In a study of Chinese Buddhist vegetarians and non-vegetarians, Pan *et al.* (1993) found significant differences in carbohydrate intake with vegetarians obtaining $\approx 57\%$ of energy from the nutrient; protein supplied $\approx 12\%$ and fat 24.75%. Intake of carbohydrate and other nutrients (total and saturated fat, vegetables and dietary fibre) were noticed to be more in vegetarians than non-vegetarians (Appleby *et al.*, 1999). Vegetarian diets can be high in carbohydrate and vegans have the highest carbohydrates intakes providing 50-65% of total energy intake (Davey *et al.*, 2003). Generally vegetarians use more energy from carbohydrates (Bedford and Barr, 2005).

2.4.1.3 Protein

Messina and Burke (1997) mentioned in the American Dietetic Association's position paper that even though all essential and nonessential amino acids can be supplied by plant sources, the total amount of protein provided in a vegetarian diet, although adequate, is less than that provided in a non-vegetarian diet. The main nutritional differences between plant and animal protein source diets is the higher, more bioavailable, micronutrient content of animal protein than plant protein. Although the adequacy of proteins in vegetarian diet is sometimes questionable, Craig and Mangel (2009) reported that vegetarian diets usually exceed protein requirements, but may provide less protein than a non-vegetarian diet. Strict protein selection is not necessary, but adequate energy food intake including a variety of plant foods such as legumes, whole grains, nuts, seeds, soy products and vegetables are essential in providing dietary protein (Young and Pellet, 1994; America Dietetics Association, 2003).

2.4.1.4 Fats and Oils

Vegetarian diets have been shown to be low in poly-unsaturated fat and lower in vegan diets than in diets containing meat (Rosell *et al.*, 2005). The diet of semi vegetarian and lacto-ovo vegetarians have been found to contain similar quantities of total fat (Draper *et al.*, 1993) and essential fatty acids (Davis and Kris-Etherton, 2003) to those of omnivores. Vegetarian diets have lower levels of atherogenic lipoproteins, and vegetarians in a study by Djousse *et al.* (2004) have been reported to have 32% and 44% lower level of total and low density lipoprotein (LDL) cholesterol respectively compared to omnivores.

The European Prospective Investigation into Cancer (EPIC)-Oxford Study also found that total fat intake compared with omnivores was significantly lower in vegans (Davey *et al.*, 2003). Vegetarians obtain n-3 fats predominantly from the omega-3 fatty acids α -linolenic acid (ALA) but there is debate over the efficiency of conversion of ALA to the longer-chain docosahexaenoic acid (DHA) and eicosapentaenoic acid: EPA (Davis and Kris-Etherton, 2003).

It has been shown that plasma levels of EPA and DHA in vegans are not related to the duration of adherence to the diet over periods of ≤ 20 years, suggesting that the endogenous production of these fatty acids in vegetarians and vegans may result in low but stable plasma concentrations (Rosell *et al.*, 2005). There is some evidence to suggest that the requirement for n-3 fatty acids in vegetarians is higher because of the inefficient conversion of ALA to EPA and DHA and the lack of direct sources of these fatty acids (Key *et al.*, 2006).

Also, a recent study showed a smaller difference in plasma n-3 polyunsaturated fatty acid status between fish eaters and non-fish eaters than would be expected from dietary intakes, which could be explained by a greater conversion of ALA to EPA and DHA in the non-fish eaters (Welch *et al.*, 2010). Walnuts and soy products provide a good source of ALA, with smaller amount presents in green leafy vegetables (Marsh *et al.*, 2012). Omega-3 fatty acids play an important role in health and disease, particularly with respect to cardiovascular health and also inflammatory diseases (Yashodhora *et al.*, 2009) and for a vegetarian, it has been suggested that a ratio range 2:1 to 4:1 of n-6 to n-3 would maximise conversion (Davis and Kris-Etherton, 2003).

2.4.2 Micronutrients

2.4.2.1 Vitamins

Vitamins constitute a group of organic compounds which are essential in small quantities for the normal metabolism of other nutrients and maintenance of physiological well-being. These compounds cannot be synthesized by the body and must be obtained from the diet (Yeung and Laquatra, 2003).

2.4.2.1.1 Vitamin D

Plasma concentrations of 25-hydroxyvitamin D [25(OH) D], the major circulating form of vitamin D, vary throughout the year owing to the cutaneous biosynthesis of vitamin D when skin is exposed to sufficient quantities of UVB radiation (usually sunlight) (DeLuca, 2004). The Adventist Health Study found no relationship between serum vitamin D concentration and vegetarian status, suggesting that factors other than diet have a greater influence on vitamin D levels (Chan *et al.*, 2009). Foods providing the highest amount of vitamin D per gram naturally are all from animal sources such as cod liver oil, finfish, and shellfish (National Institute of Health, 2008). The only naturally occurring plant sources of vitamin D are certain types of mushrooms in which it is present in small amounts (National Institute of Health, 2008).

2.4.2.1.2 Vitamin B₁₂

By total elimination of food of animal origin, vegetarians decrease their intake of some essential nutrients, including vitamin B₁₂ which typically is found only in foods of animal origin. Thus, the avoidance of animal products in association with a strict vegetarian diet

may lead to a deficiency of vitamin B₁₂ (Herbert, 1988; Dwyer, 1991). Clinical evidence of vitamin B₁₂ deficiency has been reported in some vegans but is apparently uncommon (Antony, 2003). Dietary vitamin B₁₂ deficiency is also now recognized to be a serious problem in non-vegetarian populations with a low intake of meat because of poverty (Stabler and Allen, 2004). However, high liver stores combined with effective enterohepatic recirculation have long been known to prevent healthy adult vegan vegetarians from developing vitamin B₁₂ deficiency (Herbert, 1987).

2.4.2.2 *Minerals*

Minerals are essential for body functions. They are classified as macrominerals or microminerals (trace elements) depending on their dietary requirement and may function as cofactors of enzymes, as components of organic compounds and as structural components of bones and teeth and are catalysts for many biological processes (Yeung and Laquatra, 2003).

2.4.2.2.1 *Iron*

The iron content of vegetarian diets is typically quite similar to that of non-vegetarian diets, but the bioavailability of the iron is lower because of the absence of haeme-iron. Vegan diets are usually higher in iron than lacto-vegetarian diets because dairy products are low in iron. For example, in the EPIC-Oxford study, estimated iron intakes among 43,582 women were 12.6, 12.8, 12.6 and 14.1 mg/d for meat-eaters, fish-eaters, lacto-vegetarians and vegans, respectively (Davey *et al.*, 2003). Studies of iron status (Waldmann *et al.*, 2004) have consistently shown that serum ferritin is lower in vegetarians than in non-vegetarians and that haemoglobin (Hb) levels are similar or

slightly lower in vegetarians than in non-vegetarians. Low iron status is not common in men, but is moderately common among premenopausal women throughout the world (Key *et al.*, 2006). A healthy selection of iron containing foods with sufficient vitamin C aiding in absorption of non-haeme sources of iron is helpful (Hallberg, 1981).

2.4.2.2.2 *Calcium*

Calcium intakes of lacto-ovo vegetarians are similar to or higher than those of non-vegetarians whereas intakes of vegans tend to be lower than both groups and may fall below recommended intakes (Messina *et al.*, 2004). Many vegans may find it easier to meet their calcium needs if calcium-fortified foods or dietary supplements are utilized (Weaver *et al.*, 1999). The bioavailability of calcium from soy milk fortified with calcium carbonate is equivalent to cow's milk although limited research has shown that calcium availability is substantially less when tricalcium phosphate is used to fortify the soy beverage (Zhao *et al.*, 2005). Fortified foods such as fruit juices, soy milk, and rice milk, breakfast cereals can contribute significant amounts of dietary calcium for the vegan (Messina *et al.*, 2003).

2.4.2.2.3 *Zinc*

Zinc intakes of vegetarians vary with some research showing zinc intakes near recommended intakes (Davey *et al.*, 2003) whereas others found zinc intakes of vegetarians significantly below recommended intakes (Ball and Bartlett, 1999; Janelle and Barr, 1995). The bioavailability of zinc from vegetarian diets is lower than from non-vegetarian diets, mainly due to the higher phytic acid content of vegetarian diets (Hunt, 2003). Thus, zinc requirements for some vegetarians whose diets consist mainly of phytate-rich unrefined grains and legumes may exceed the recommended dietary

allowance (IMFNB, 2001). Zinc sources include soy products, legumes, grains, cheese, and nuts. The bioavailability of zinc may be improved with preparation methods such as soaking and sprouting of beans, grains, and seeds as well as leavening bread, this reduces binding of zinc by phytic acid. Organic acids, such as citric acid, can also enhance zinc absorption to some extent (Lönnerdal, 2000).

2.5 SEX HORMONE SYNTHESIS

Steroid hormones regulate a wide variety of developmental and physiological processes from foetal life to adulthood. Steroid hormones are all synthesized from cholesterol and hence have closely related structures based on the classic cyclopentanophenanthrene 4-ring structure (Steiger and Reichstein, 1937; Kendall *et al.*, 1934). The first step in steroidogenesis takes place within mitochondria by the action of steroidogenic acute regulatory protein (StAR) partially facilitating the movement of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane. Free cholesterol is nearly insoluble (critical micellar concentration, 25–40 nM) (Haberland and Reynolds, 1973). Some cholesterol may be incorporated into vesicular membranes that fuse with other membranes, thus delivering cholesterol from one intracellular compartment to another, but this appears to be a minor pathway (Soccio and Breslow, 2004). The human adrenal can synthesize cholesterol *de novo* from acetate (Mason and Rainey, 1987), but most of its supply of cholesterol comes from plasma low-density lipoproteins (LDLs) derived from dietary cholesterol (Gwynne and Strauss, 1982). Conversion of cholesterol to pregnenolone in the mitochondrion is regulated by the P450_{scc} as the initial step in the steroid hormone biosynthesis (Miller, 1988).

Once cholesterol has been converted to pregnenolone, it may undergo 17 α -hydroxylation by P450c17 to yield 17-hydroxypregnenolone or converted to progesterone (the first biological steroid hormone). Human P450c17 catalyzes the 17 α -hydroxylation of Δ^5 pregnenolone and Δ^4 progesterone with equal efficiency but catalyses the 17, 20 lyase conversion of 17 α -hydroxyprogesterone (17-OHP) to Δ^4 androstenedione very poorly. Thus most sex steroid synthesis proceeds through DHEA with little proceeding through 17-OHP evidenced by the large amounts of DHEA produced by both foetal and adult adrenal glands (Miller, 1988).

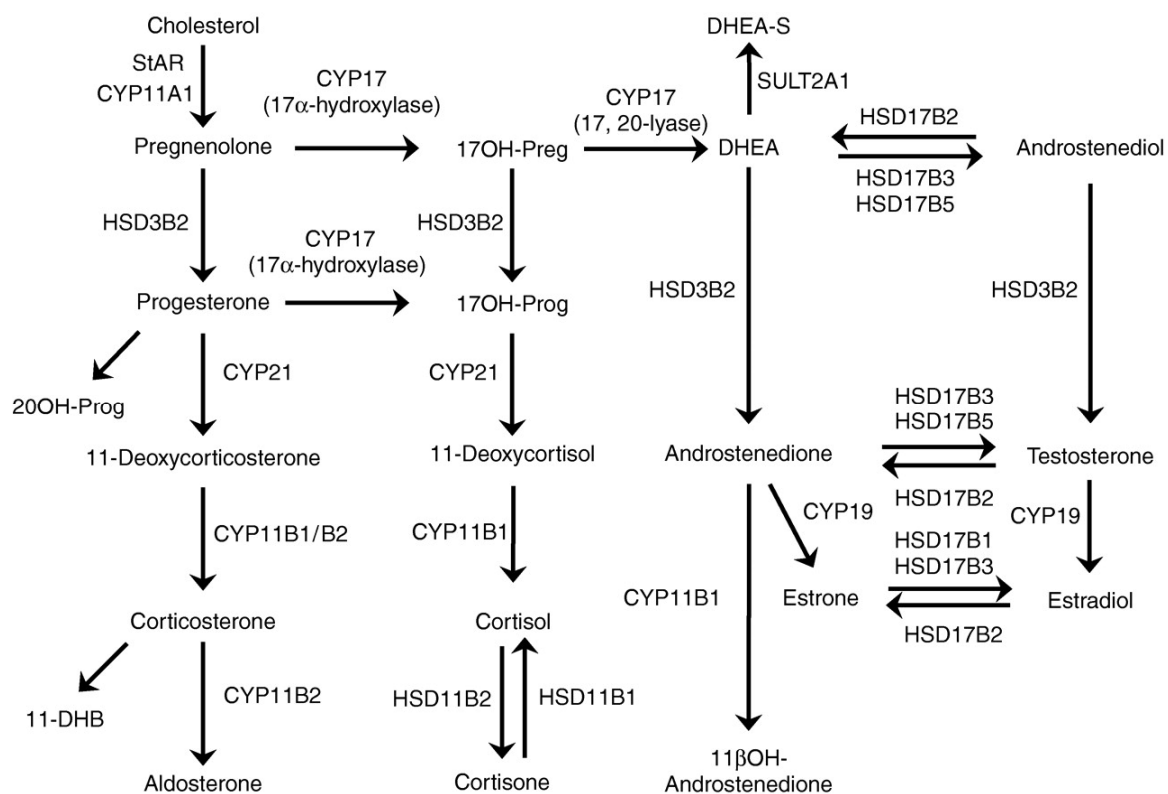


Figure 2.1 *Steroid biosynthesis pathways. Summary of the steroidogenic pathways leading to synthesis of glucocorticoids, mineralocorticoids, androgens, and oestrogens (Xing et al., 2011).*

Dehydroepiandrosterone is the most abundant steroid in the circulation of adults of reproductive age (Orentreich *et al.*, 1984). Dehydroepiandrosterone, DHEAs, and androstenedione are produced almost exclusively by the adrenal zona reticularis catalysed by 3 α -Hydroxysteroid Dehydrogenase (3 α HSD). Altogether (DHEA, DHEAs and androstenedione) they are termed “weak androgens” having a much lower affinity for the androgen receptor than testosterone. They are converted peripherally to the more active testosterone by the enzyme 17 β -Hydroxysteroid Dehydrogenase (17 β HSD) (Nussey and Whitehead, 2001).

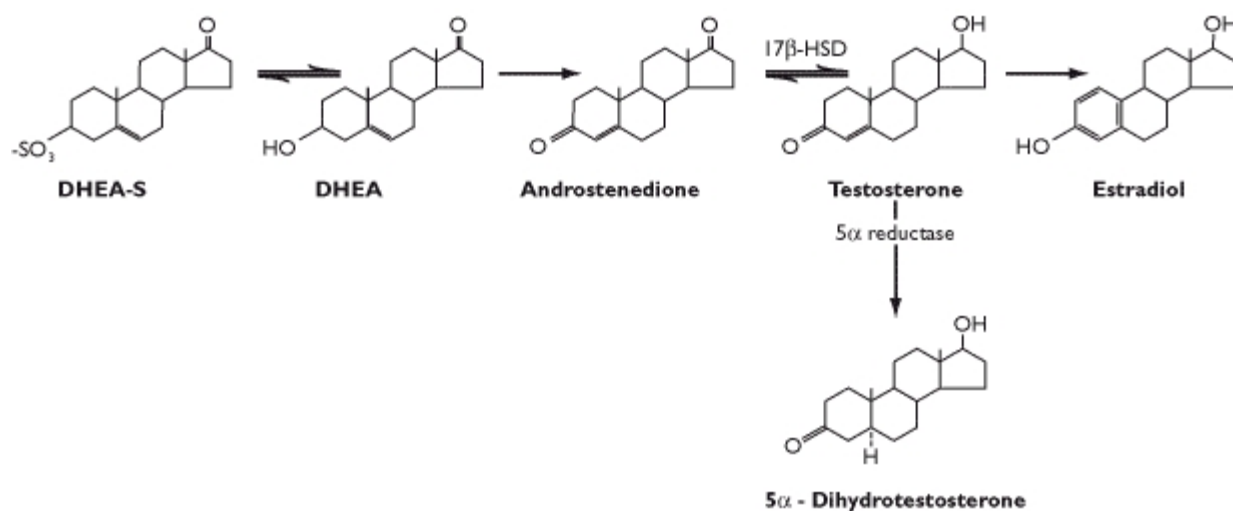


Figure 2.2 *Structural pathway of hormone synthesis (Nussey and Whitehead, 2001).*

In males the amount released from the adrenal glands and converted to testosterone is physiologically insignificant compared to the amount secreted by the testes. Oestrogens are produced from androgens by a complex series of reactions catalyzed by a single aromatase enzyme P450aro (Simpson *et al.*, 1994), in peripheral tissues especially fat, can convert substantial portions of circulating androstenedione and testosterone in

women to oestrone and oestradiol. In some target tissues, testosterone is 5 α -hydroxylated to the more potent 5 α -dihydrotestosterone or oestradiol (Nussey and Whitehead, 2001).

2.6 MALE SEX HORMONES

2.6.1 Sex Hormone-Binding Globulin (SHBG)

Sex hormone-binding globulin (SHBG) is a major carrier of androgens and oestrogens in human plasma. It is a glycoprotein with high binding affinity for testosterone (T) and dihydrotestosterone (DHT) and lower affinity for oestradiol (E2). Sex hormone-binding globulin is produced in the liver, and its plasma levels are important in the regulation of plasma free and albumin-bound androgens and oestrogens (Rosner, 1990; Bond and Davis, 1987)

2.6.2 Testosterone

Testosterone, a steroid hormone derived from cholesterol, occurs more abundantly in circulation among men, than women (Guyton, 1991). It is synthesized in the testes among males, the ovaries among females, and possibly the adrenal glands in both sexes (Guyton 1991; Davis and Tran 2001). Testosterone regulates many physiological processes, including muscle protein metabolism, some aspects of sexual and cognitive functions, secondary sex characteristics, erythropoiesis, plasma lipids, and bone metabolism (Bhasin and Bremmer 1997; Wilson, 1988).

It has been postulated that in hypogonadal states there is a preferential deposition of abdominal adipose tissue. Increased accumulation of adipose tissue leads to an increase in aromatase activity and hence a higher conversion of testosterone to oestradiol, which

results in a further depression of testosterone concentrations and an increased deposition of abdominal fat (Seidell *et al.*, 1990).

2.6.3 Oestrogen

Oestrogens are synthesized from androgens by the aromatase complex, which contains the cytochrome P450 enzyme encoded by the *CYP19* gene (Carreau *et al.*, 2002). Oestrogens play key roles in the development and maintenance of reproductive function and fertility (Nilsson *et al.*, 2001; O'Donnell *et al.*, 2001; Hess and Carnes, 2004; Carreau *et al.*, 2008). The hormone also has an important role in pathological processes observed in tissues of the reproductive system (Prins and Korach, 2008; Ellem and Risbridger, 2009). In addition, they exert a vast range of biological effects in the cardiovascular, musculoskeletal, immune, and central nervous systems (Nilsson *et al.*, 2001). The most potent oestrogen produced in the body is 17 β -oestradiol (E2).

2.7 HORMONE LEVELS IN VEGETARIANS

Strong associations recently reported between plasma levels of sex hormones and the risk of type II diabetes also show associations of similar magnitude for free sex hormones and total sex hormones (Ding *et al.*, 2007). This further indicates the importance of the bioactivity of both free and bound fractions. It has been suggested that insulin stimulates testosterone production and suppresses SHBG production in men (Pasquali *et al.*, 1995).

On the other hand, results of prospective studies show that low levels of SHBG and testosterone play a role in the development of insulin resistance and subsequently the development of type II diabetes (Stellato *et al.*, 2000; Simon *et al.*, 1997; Oh *et al.*, 2002).

A decrease in endogenous testosterone is associated with an increase in triglycerides, also smaller dense LDL-cholesterol particles were found to be associated with a low total testosterone level and SHBG (Haffner, 1996).

Although some studies of hormone levels in vegetarians suggest that plasma oestrogen levels are not different from those of non-vegetarians (Thomas *et al.*, 1999), such studies have shown that vegans have lower plasma levels of insulin-like growth factor-I than either meat-eaters or lacto-vegetarians (Allen *et al.*, 2000). Oestradiol was found to be associated with apolipoprotein E (Van Pottelbergh *et al.*, 2003). Vegetarians, and especially vegans, often consume substantial amounts of soya beans or foods made from soya beans; soya foods are rich in phyto-oestrogens, which have been hypothesized to reduce breast cancer risk but research on this topic has so far been inconclusive (Peeters *et al.*, 2003).

Clinical studies have associated low circulating levels of SHBG with impaired glucose control, (Ding *et al.*, 2007; Lindstedt, 1991; Golden *et al.*, 2007; Sutton-Tyrrell *et al.*, 2005) implicating the globulin in the maintenance of glucose homeostasis. Reed *et al.* (1987) noted that normal men fed a high fat diet had a decrease in SHBG levels, whereas a diet low in fat resulted in an increase in SHBG levels. Vermuelen *et al.* (1996) noted that a high protein diet increased SHBG levels. However, rabbits fed a diet low in protein showed a marked increase in SHBG levels (Longcope *et al.*, 1987). Hill *et al.* (1980) had long found a decline in the testosterone concentration among men who were briefly switched from a western higher fat diet to vegetarian diets.

Comparing men on western diet and vegetarian diet has shown vegetarians to have lower SHBG but similar testosterone concentrations (Belanger *et al.*, 1989; Adlercreutz 1990; Pusateri *et al.*, 1990). Most studies on the relationship between diet and hormones have compared hormone concentrations between groups on special diets, such as those eating vegetarian diets, low fat-high fibre and typical western diets (Field *et al.*, 1994).

Findings may be difficult to interpret since vegetarians and non-vegetarians differ in many ways and factors like aging, smoking, alcohol use and general dietary intakes may need further investigations to obtain a better understanding of hormone levels in these two groups.

For instance, animal studies had long discovered that alcohol administration resulted in reduced plasma concentration of testosterone (Badr and Bartke, 1974; Van Thiel *et al.*, 1975). Chronic alcohol consumption also suggests lowering plasma testosterone in normal men. Vegetarians are not known to consume alcohol but comparing hormone concentration in vegetarians and non-vegetarians without taking other factors into consideration may isolate findings from generalization.

2.8 INFLUENCES ON LEVELS OF SEX HORMONES

2.8.1 Age

In men, aging is associated with a gradual decline in testosterone (Feldman *et al.*, 2002; Harman *et al.*, 2001). This decrease accompanies changes in body composition, including increases in fat mass and decreases in lean body mass (van den Beld *et al.*, 2000), disorders of insulin and glucose metabolism (Tsai *et al.*, 2004; Stellato *et al.*, 2000; Haffner *et al.*, 1996), and dyslipidaemia (Tsai *et al.*, 2004; Zmuda *et al.*, 1997).

Testosterone may have more direct effects on vascular reactivity and cardiac muscle (Sader *et al.*, 2003, Ong *et al.*, 2000). More recently, low serum testosterone and SHBG levels have been directly associated with the metabolic syndrome, both longitudinally (Laaksonen *et al.*, 2004) and cross-sectionally (Muller *et al.*, 2005).

2.8.2 Body Mass Index

In a cross-sectional study of 60,903 men and women in the Adventist Health Study-2 conducted between 2002 and 2006, the authors (Tonstad *et al.*, 2009) found vegans to have an average body mass index (BMI) of 23.6 kg/m² compared to non-vegans who had a mean BMI of 28.8 kg/m². The lower BMI of vegetarians puts them at a lower risk of death from ischaemic heart disease when compared with non-vegetarians (Key *et al.*, 1998). Considerations from research shows individuals following a vegetarian diet tend to be leaner than their omnivore counterparts (Alewaeters *et al.*, 2005) potentially accomplished by avoiding meat (Sabaté and Wein, 2010) and focusing instead on a low-energy, high nutrient-density diet. The vegetarian diet pattern rich in whole grains, legumes, vegetables, and dietary fibre (Barnard *et al.*, 2005) contributes bulk while reducing the number of calories per unit volume (Rolls *et al.*, 2005).

In addition to decreasing energy intake, dietary fibre increases satiety and reduces hunger (Howarth *et al.*, 2001). The higher whole grain profile slows digestion, reduces rates of glucose absorption and plasma insulin (McIntosh and Miller, 2001). Thus, the well planned vegetarian diets may have certain advantages when used as a possible dietary strategy to treat or prevent obesity (Tonstad *et al.*, 2009). In normal healthy males, inverse relationships have been found between BMI and testosterone as well as positive

associations with SHBG (Gray *et al.*, 1991). The binding globulin was more correlated with BMI and age than with serum testosterone concentration (Longcope *et al.*, 1990).

Similarly, Field *et al.* (1994) discovered androstenedione, testosterone and SHBG to be inversely associated with BMI, even after controlling for age and smoking in men between ages 38 and 70 years, just as Key *et al.* (1990) had previously found in a vegetarian study that BMI was inversely correlated with testosterone and SHBG, but was not significantly correlated with free testosterone.

2.8.3 Soy

Soy contains considerable amounts of isoflavones which provide antioestrogenic effects and has been suggested to prevent some cancers (Messina, 1999). The isoflavones are similar in structure to mammalian oestrogen. On the basis of structure alone, these isoflavones bind to oestrogen receptors acting more as partial oestrogen agonist and antagonists (Setchell and Adlercreutz, 1988). Oestrogens can have non classical actions distinct from their classical genomic actions (Brann *et al.*, 1995) and these include effects on plasma membranes and on cell signaling pathways (Kim *et al.*, 1998). This implies that at certain concentrations, which may depend on many factors including receptor numbers, occupancy and competing oestrogen concentration, rather than acting as oestrogen mimics and initiating oestrogen-like actions, they may antagonize and inhibit oestrogen action. These effects would also be tissue specific (Setchell and Cassidy, 1999).

The isoflavones genistein and diadzein, found in soy beans have numerous biological functions and their potentially multifaceted health-promoting effects include cholesterol

reduction, improved vascular health, preserved bone mineral density, and reduction of menopausal symptoms in women (Anderson *et al.*, 1999). Allen *et al.* (2001) suggested that soy milk intake, as a marker of isoflavone intake, is not associated with serum sex hormone concentrations among free-living Western men. Similarly, high levels of the isoflavones genistein and daidzein and their glycosides found in soy foods have been implicated in the prevention of prostate cancer through their effects on hormone metabolism.

2.8.4 Type of Diet

Dietary factors have been implicated in the levels of steroid sex hormones (Bishop *et al.*, 1988; Anderson *et al.*, 1987; Raben *et al.*, 1992) and the replacements of certain food nutrients have been suggested to be the cause. For instance, the replacement of dietary carbohydrate with protein has been shown to decrease testosterone concentration (Bishop *et al.*, 1988). In a randomized crossover dietary intervention study to evaluate the effects of replacing meat protein with a soy product, the authors (Habito *et al.*, 2000) found blood concentrations of sex hormones not too different after the two diets. They concluded that the replacement may have minor effect on biologically active sex hormones which may influence prostate cancer risk, not excluding other dietary factors like BMI, physical activities and lifestyle characteristics that may influence sex hormones (Gates *et al.*, 1996). Several other studies show individuals consuming a diet containing 20% fat compared with a diet containing 40% fat (Goldin *et al.*, 1994; Hämäläinen *et al.*, 1984; Ingram *et al.*, 1987; Reed *et al.*, 1987) have significantly lower concentrations of testosterone.

2.8.5 Physical Activity

Some observational studies and a few small trials (Nieman, 1999; Barr and Rideout, 2004) have questioned and investigated whether vegetarianism is associated with beneficial or detrimental effects on athletic performance. There is little evidence though, that athletic performance is different between vegetarians and non-vegetarians and as long as the diet is nutritionally adequate, more research is needed in this area. Particular care needs to be taken to ensure adequate iron status. Also, Barr and Rideout (2004) provide some evidence that the lower muscle creatine concentration in vegetarians may reduce supramaximal exercise performance. Raben *et al.* (1992) reported a significant decrease in resting testosterone concentrations and an attenuation in the exercise-induced increase in testosterone in male endurance athletes who switched from a meat-rich diet to a lacto-ovo vegetarian diet.

2.9 IMPLICATIONS OF THE VEGETARIAN DIET

A well-planned vegetarian diet is considered to be adequate for all stages of the life cycle including infancy, childhood and adolescence (Cullum-Dugan and Pawlak, 2015). This agrees with the assertion that it is likely that the benefits of the vegetarian diet result from both a reduced consumption of potentially harmful dietary components (Segasothy and Phillips, 1999). These include saturated fat, cholesterol, animal protein, red meat, and haeme iron, and an increased consumption of beneficial dietary components, including fruit, vegetables, whole grains, legumes, and nuts, which are rich in dietary fiber, antioxidants, and phytochemicals (Sabaté, 2003).

Foods of plant origin are significant sources of antioxidant vitamins, minerals, unsaturated fats, complex carbohydrates, non-essential amino acids and are unique or principal sources of vitamin C, folate, fibre and various non-nutrients. The nutrient content of foods consumed together with a healthy lifestyle is strongly associated with prevention of cancer and other chronic diseases (Block *et al.*, 1992; Key *et al.*, 1998; Fraser, 1999; Krajcovicova-Kudlackova *et al.*, 2003).

There is evidence suggesting vegetarian diets to be beneficial in lowering the risk of coronary heart disease (Thorogood *et al.*, 1994; Szeto *et al.*, 2004), diabetes (vanDam *et al.*, 2002), cancer (Thorogood *et al.*, 1994) and other chronic disease when compared with meat-diets.

A lower BMI, blood pressure, fasting plasma triacylglycerol, total and LDL-cholesterol level and LDL oxidizability in vegans or lacto-vegetarians than matched omnivores has been reported (Lu *et al.*, 2000; Hung *et al.*, 2002). The effect of a vegetarian diet on plasma cholesterol is dependent on the exact composition of the diet, particularly in relation to saturated and unsaturated fatty acids. Furthermore a vegetarian diet including a portfolio of cholesterol-lowering foods such as soya bean and nuts is said to have reduced serum LDL-cholesterol substantially more than a control vegetarian diet (Jenkins *et al.*, 2003).

Thus, a healthy combination of food sources for the vegetarian marks the beginning of a healthy dietary lifestyle. Supplementation for nutrients of concern in the vegetarian diet may be required to ensure non-deficiency states in the vegan vegetarian.

CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

The research was a case-control study. The convenience sampling technique was used to recruit participants into the study.

3.2 STUDY SITES

Four study locations were used in the present study, the premises of the Rosicrucian Fellowship and Science of Spirituality societies located at Ablah-Adjei, Madina in the Ga East District and Banana-Inn respectively. Controls were recruited at the premises of the Mamprobi Evangelical Presbyterian Church of Ghana and the University of Ghana College of Health Sciences located at Korle-Bu, Accra.

3.3 STUDY PARTICIPANTS

Vegetarians (lacto-ovo and vegans) and non-vegetarians were included in the study. Vegetarians were recruited from the Rosicrucian Fellowship and Science of Spirituality. Non-vegetarians were worshippers from the Mamprobi Evangelical Presbyterian Church of Ghana and workers from the University of Ghana College of Health Sciences.

3.4 SAMPLE SIZE DETERMINATION

An absolute precision of 5% and a confidence interval of 95%, in accordance with the strategic function of Epiinfo Statistical Software were used. Allowance was given for a non-participation rate of 10% and non-response rate of 20%.

$$\begin{aligned}
 N &= \frac{(z)^2 (P) (1-p)}{E^2} \\
 &= \frac{1.96^2 (0.05 \times 0.95)}{0.06} \\
 &= 50
 \end{aligned}$$

Where N is estimated sample size

E is desired margin of error

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

P is the prevalence of vegetarians in Ghana. A sample of 50 for cases and 50 for controls making a total of hundred (100) was to be obtained. However, due to time and difficulty in obtaining consenting participants, fifty-two individuals were recruited in this study.

3.5 INCLUSION AND EXCLUSION CRITERIA

3.5.1 Inclusion Criteria

To be included in the study, participants (i) had to have been vegetarians for more than two years, (ii) were aged between 18 and 75 years, (iii) had no history of hormone related medical conditions (diabetes mellitus, prostate cancer), (iv) had no history of steroid use and (v) had no history of smoking and alcohol use.

3.5.2 Exclusion Criteria

Participants who had been vegetarians for less than two years were not involved in the study. Any participant with a history of steroid use, alcohol intake exceeding recommended intake for males (> two or three standard units per day) and smoking were excluded from the study.

3.6 ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (**EIN:SAHS-ET./10231437/AA/3A/2013-2014; Appendix I**). Permission was sought from the leadership of the Vegetarian Associations at their various locations, the Mamprobi Evangelical Presbyterian Church and the College of Health Sciences, UG. Participants' information sheet (Appendix II) detailing the purpose of the research were attached to consent forms signed by the researcher and the participants and a copy each retained by both parties. Participants' confidentiality was ensured at all times and participation was clearly understood to be voluntary.

3.7 RESEARCH TOOL

A structured questionnaire (Appendix III) comprising open- and close-ended questions was used to obtain participants information on demography and social backgrounds. A modified food frequency questionnaire (Appendix III) and two 24-hr recalls (one weekend and one weekday) was used to obtain participants usual and current food intake respectively. Anthropometric data were obtained using a Seca stadiometer (SEC-213), a bioimpedance analyzer (Omron HBF-514C) and an electronic blood pressure monitor (Omron MIT Elite Plus). Blood samples were obtained by a qualified laboratory scientist.

3.8 SAMPLING TECHNIQUE

Convenience sampling was used to obtain the twenty-two (22) healthy male vegetarians and thirty (30) male non-vegetarians recruited into this study.

All participants were those who consented to the study after the purpose of the study had been explained to their full understanding, after which a consent form was signed by the researcher and the participant.

3.9 PRE TESTING

A pre-test of the study protocol (questionnaire) was conducted on ten (10) non-vegetarians at the premises of the Martyrs of Uganda parish church at Mamprobi to exclude all ambiguity and increase efficiency in the data collection process.

3.10 DATA COLLECTION

Data was collected between February and May, 2015 between the hours of 7:00 am and 11:00 am after participants had been on an overnight fast. Anthropometric data and blood samples were obtained after multiple visits to study sites. Participants' information were obtained in a private area to ensure participants' comfort. Interviews were administered in English and any other local language spoken and understood by the participant.

3.11 ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENTS

Participants were made to stand in an upright position wearing minimal clothing without footwear in order to obtain weight and height measurements. Height was measured with a Seca stadiometer (SEC-213, Hamberg, Germany) with the participant in an upright position, to the nearest 0.5 cm. Bodyweight (measured to the nearest 0.1 kg), visceral fat and percentage body fat of the participants were measured with the use of an Omron HBF-514C bioimpedance analyzer. Blood pressure (BP) was measured with an Omron MIT Elite Plus (Illinois, USA) upper arm blood pressure monitor for three consecutive

times (five minutes apart) with patient seated in an upright position and the mean calculated to the nearest whole number. The body mass index (BMI) was computed as the ratio of weight (kg) divided by height in meters squared (m^2). Classification was based on the World Health Organizations' (2010) criteria.

3.12 DIETARY ASSESSMENT

3.12.1 24-Hour Recall

Two 24- hour recalls, one weekend and one weekday were used to assess nutrient intake of the participants. The second recall was obtained through a telephone interview for some participants and a face-to-face interview for others. Participants were interviewed to describe all food and snacks consumed over the previous 24-hour period.

3.12.2 Food Frequency Questionnaire

A modified food frequency questionnaire adapted from a study by Aggrey-Aboagyeh (2011) (Appendix III) was used to assess the frequency of consumption of foods from the Ghanaian six food groups. Starchy roots and plantain, cereals and animal products, legumes, nuts and oil seeds, fruits and vegetables as well as fats and oils were foods included in the questionnaire. Participants' consumption patterns were assessed by multiple responses in which participants were to respond to how often foods and snacks were consumed within the week with likely responses including 'not often', 'one-to-two', 'three-to-four', 'five-to-six times', 'daily' and 'never' at all.

3.13 SAMPLE COLLECTION

After an overnight fast, between 8 – 12 hrs, 3 mls of venous blood samples were drawn into serum separator tubes (from the antecubital space of the forearm) by a qualified

laboratory scientist. Blood samples were collected between the hours of 7 am and 11 am. The tubes containing the blood samples were placed on ice in a cool box and quickly transported to the laboratory where they were centrifuged at 3000 rpm for 5 minutes. The remaining serum was separated and emptied into eppendorf tubes and stored at -20 °C until ready for analyses.

3.14 BIOCHEMICAL ANALYSES

All blood samples were analysed at the Chemical Pathology Unit of the School of Biomedical and Allied Health Sciences, Korle-Bu, Accra.

3.15 HORMONE ASSAY PROCEDURE

The procedure for hormonal assay used was taken according the manufacturer's instruction. The content of the kits are displayed in tables 3.1 and 3.2.

Table 3.1 Reagents used for assay procedure

Vial	Content	Testosterone	Oestradiol
A	Enzyme Reagent (ml)	1.0	1.0
B	Conjugate Buffer (ml)	7.0	6.0
C	Biotin Reagent (ml)	6.0	6.0
D	Wash Solution (ml)	20.0	20.0
E	Substrate (TMB ₉)	7.0 (A)	12.0¶
F	Substrate (H ₂ O ₂)	7.0 (B)	N/A
G	Stop Solution (ml)	8.0†	8.0‡

TMB = tetramethylbenzidine, ¶ = (TMB, H₂O₂), †=1N HCl, ‡ = H₂SO₄,
N/A=Not applicable

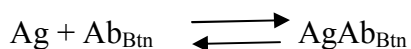
Table 3.2 Concentrations of hormone calibrators in vials used for assay procedure

Hormone Calibrators		
Vial	Testosterone (ng/ml)	Oestradiol (pg/ml)
A	0	0
B	0.1	20
C	0.5	100
D	1.0	250
E	2.5	500
F	5.0	1500
G	12.0	3000

3.15.1 Assay Principle

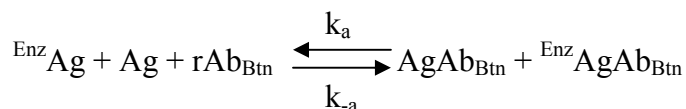
3.15.1.1 Oestradiol

Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:



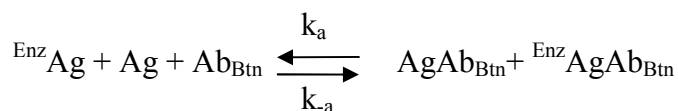
Ab_{Btn} =Biotinylated antibody, Ag =Antigen (Variable Quantity), AgAb_{Btn} =Immune Complex

After a short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites not consumed in the first incubation.



3.15.1.2 *Testosterone*

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:



Ab_{Btn} = Biotinylated Antibody (Constant Quantity), Ag = Native Antigen (Variable Quantity)

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

AgAb_{Btn} = Antigen-Antibody Complex

${}^{\text{Enz}}\text{AgAb}_{\text{Btn}}$ = Enzyme-antigen Conjugate -Antibody Complex

k_a = Rate Constant of Association, k_{-a} = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$\text{AgAb}_{\text{Btn}} + {}^{\text{Enz}}\text{AgAb}_{\text{Btn}} + \text{Streptavidin}_{\text{CW}} \Rightarrow \text{immobilized complex}$

$\text{Streptavidin}_{\text{CW}}$ = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references (Table 3.3) of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown is ascertained (T- Product Code 3725-300; E2- Product Code: 4925-300).

Table 3.3 Normal reference intervals for hormone levels in the EIA test system

Category	Males
Testosterone (nmol/L)	8.7 – 34.7
Oestradiol (pmol/L)	14.6 – 345.0

Cumming and Wali (1985)

3.15.2 Reagent Preparation

Some amount (0.7 ml) of the testosterone enzyme reagent (A) was added to the vial containing steroid conjugate buffer (B). The wash solution (D) was diluted to 1000 ml with distilled water in a suitable storage container. Substrate A was mixed with substrate B and mixed thoroughly by shaking the vial gently. For oestradiol, only the wash solution was diluted to 1000 ml with distilled water in a suitable storage container.

3.15.3 Test Procedure

The microplates' wells were formatted and labeled for each calibrated serum reference, case and control to be assayed.

For testosterone, 10 µL of the calibrated references, cases and controls sera were introduced into the assigned wells by pipetting. Fifty microlitres (50 µL) of the testosterone enzyme reagent (A) previously prepared was added to all wells and swirled gently for 20-30 seconds to mix. Fifty microlitres (50 µL) of the biotin reagent (C) was

added to all wells, swirled gently to mix for 20-30 more seconds and covered to incubate for an hour at room temperature.

With regards to oestradiol, 25 μL of the appropriate calibrated reference, cases and controls sera were added to the assigned well. Fifty microlitres (50 μL) of the oestradiol biotin reagent (C) was added to all wells and swirled for 20-30 seconds, covered and incubated for half an hour at room temperature. Fifty microlitres (50 μL) of oestradiol enzyme reagent (A) was added to all wells, swirled gently for 20-30 seconds and incubated for 90 minutes.

The contents of the microplates were discarded by decanting and blotted with dry absorbent paper. Three hundred and fifty microlitres (350 μL) of wash buffer (D) previously prepared was used to wash the microplate wells for three cycles. Hundred microlitres (100 μL) of stop solution (G) was added to all wells in the same order exercising caution not to shake the plate. The plate was incubated for fifteen minutes (testosterone) and twenty minutes (oestradiol) respectively. Fifty microlitres (50 μL) of the stop solution was added and gently mixed for 15-20 seconds. Absorbance was read in each well for each hormone at a wavelength of 450 nm, using a reference wavelength of 620-630 nm in a microplate reader (Labsystems Multiskan MS, Amersham Biosciences UK, Ltd.)

3.16 DATA ANALYSES

Results were presented as frequencies, percentages, mean $\pm$ standard deviation (SD) with tables and figures provided where needed. The Anderson-Darling test was performed to test for normal distribution of the study population.

Two sample t-tests were used to examine differences in socio-demographic characteristics as well as hormone levels between vegetarians and their meat-eating counterparts. Correlation tests were performed to test associations between age, protein and soy intake on levels of hormones. All tests were computed at confidence interval of 95% and p values less than or equal to 0.05 considered to be significant. Data was analysed using the Statistical Package for Social Sciences (SPSS version 16.0), Minitab (version 15), Microdiet (version 3), as well as Microsoft Excel.

CHAPTER FOUR

4.0 RESULTS

4.1 SOCIO-DEMOGRAPHIC AND LIFESTYLE CHARACTERISTICS OF VEGETARIANS AND NON-VEGETARIANS

A total of 52 males comprising 42% (n=22) vegetarians and 58% (n=30) non-vegetarians were sampled in the current study. The mean age of the total population was 49.4 ± 12.7 years. Most participants were between the ages of 30-69 years. Nearly half (41%) of the participants from the vegetarian group were between the ages of 50-59 years, closely followed by 32% who were within the ages of 60-69 years. The mean age of the vegetarians (57.6 ± 10.9 yrs) was slightly higher than non-vegetarians (43.3 ± 10.4 yrs) but the difference was not significant. Most of the vegetarians (77%) and non-vegetarians (67%) were married. Majority of participants from both groups reported that they were Christians. All participants had some formal education (Table 4.1).

Table 4.1 Socio-Demographic and lifestyle characteristics of vegetarians and non-vegetarians

Variable	Vegetarian n=22		Non-vegetarian n=30	
	N	%	N	%
Marital status				
Single	4	18	7	23
Married	17	77	20	67
Divorced, widowed	1	5	3	10
Religion				
Christian	22	100	25	83
Muslim	0	0	5	17
Education				
Basic	3	14	6	20
Secondary	7	32	13	43
Tertiary	12	54	11	37
Employment				
Employed	13	59	26	87
Unemployed	0	0	2	7
Student	8	36	1	3
Retired	1	5	1	3
Alcohol intake				
Yes	0	100	17	57
No	22	100	13	43
Frequency of Alcohol consumption				
Occasional	0	0	14	82
Weekly	0	0	3	18

4.1.1 Length of vegetarian status, types of vegetarians and main reason for choice

All vegetarians had been lacto-ovo and vegan for more than two years. About a third (36%) had been practicing vegetarianism for between eleven to twenty years (Figure 4.1). Half (50%) of the vegetarians reported that they occasionally consumed egg, dairy and

their products (Figure 4.2). Nearly half (46%) of the vegetarians mentioned that their practice of vegetarianism was a derived benefit from the adherence to religious practices and beliefs other than personal choice concerning healthy eating practices. Twenty-seven percent (27%) of vegetarians (n=6) reportedly decided to be vegetarians because of personal health concerns, adherence to religious beliefs and moral concern for animal health and welfare. Only 9% (n=2) of vegetarians sampled in this study based their decision on personal health concerns and moral concern for animal health and welfare (Figure 4.3).

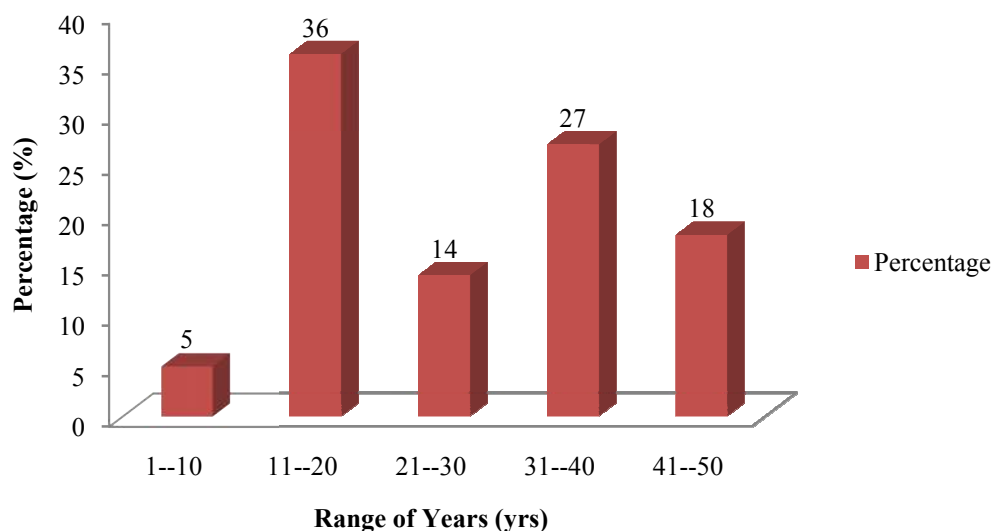


Figure 4.1 *Length of vegetarian status*

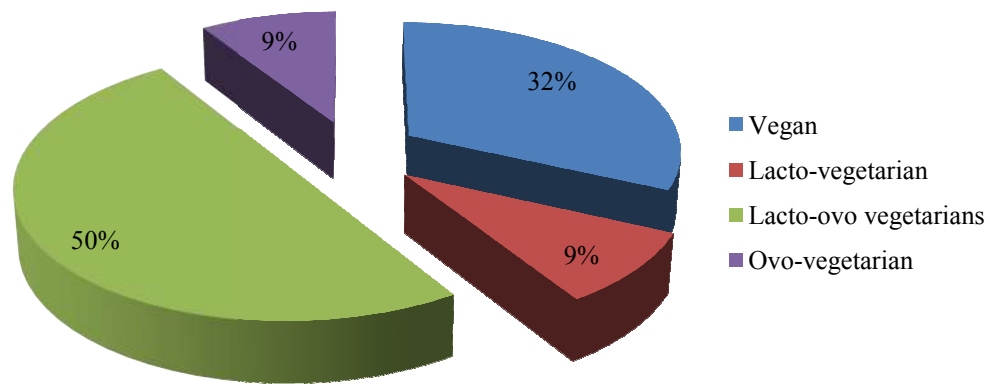


Figure 4.2 *Types of Vegetarians*

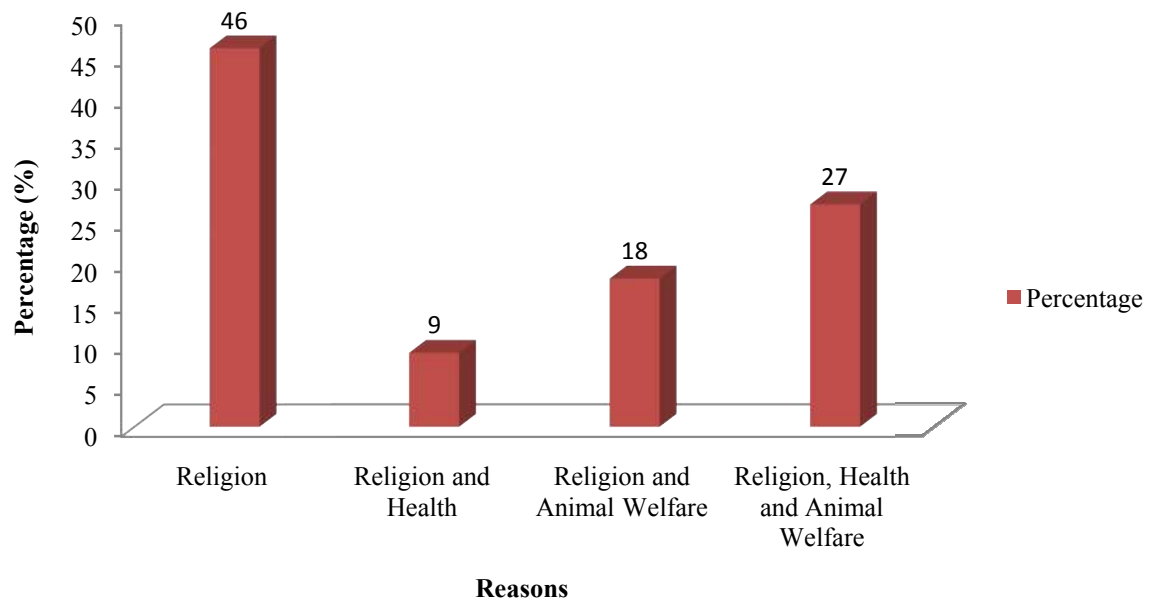


Figure 4.3 *Reasons for choosing to be vegetarian*

4.1.2 Smoking and Alcohol Consumption

There was no history of smoking among participants; however, there were 57% (n=17) of non-vegetarians who reported that they consumed alcohol. Out of this, 82% (n=14) consumed one or two servings of alcoholic drinks and beverages occasionally whereas 18% (n=3) consumed between two and four servings of alcohol once or three times weekly. Also, no vegetarian reported alcohol use (Table 4.1).

4.2 ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENTS

Tables 4.2, 4.3 and 4.4 show anthropometric and blood pressure measurements of vegetarians and non-vegetarians. There were no significant differences in mean BMI, body fat and systolic blood pressure between vegetarians and non-vegetarians. Conversely, weight, visceral fat and diastolic blood pressure were significantly lower in vegetarians compared to non-vegetarians.

Table 4.2 Mean anthropometric and blood pressure measurements of vegetarians and non-vegetarians

	Vegetarian n=22	Non-Vegetarian n =30	
Parameters	Mean $\pm$ SD	Mean $\pm$ SD	<i>p-value</i>
Weight (Kg)	69.0 $\pm$ 10.1	76.2 $\pm$ 13.1	0.018*
BMI (Kg/m²)	23.6 $\pm$ 3.2	24.8 $\pm$ 3.8	0.100
Blood Pressure (mmHg)			
Systolic BP	130.5 $\pm$ 19.1	130.6 $\pm$ 18.0	0.983
Diastolic BP	77.1 $\pm$ 12.1	84.5 $\pm$ 9.9	0.025*
Body Fat (%)	18.3 $\pm$ 6.6	20.1 $\pm$ 7.1	0.178
Visceral Fat (%)	7.3 $\pm$ 3.9	8.5 $\pm$ 4.7	0.005*

Table 4.3 Body Mass Indices (BMI) classification of vegetarians and non-vegetarians

BMI classification*	BMI (kg/m ²)	Participants	
		Vegetarian (%)	Non-vegetarian (%)
Underweight	<18.5	0	1 (3.3)
Normal	18.5 - 24.9	16 (72.7)	15 (50.0)
Overweight	25.0 - 29.9	5 (22.7)	11 (36.7)
Obese Class 1	30.0 - 34.9	1 (4.5)	3 (10.0)
Total		22	30

*WHO (2010), BMI classification

Table 4.4 Blood Pressure of vegetarians and non-vegetarians

BLOOD PRESSURE*	Participants		
	Vegetarian n=22	Non-Vegetarians n=30	<i>p-value</i>
	N (%)	N (%)	
Systolic BP (mmHg)			<i>0.225</i>
Normal < 120	9 (40.9)	5 (16.7)	
Pre-HPT 120-139	8 (36.4)	16 (60.0)	
Stage I 140-159	3 (13.6)	5 (16.7)	
Stage II >160	2 (9.1)	2 (6.7)	
Diastolic BP (mmHg)			<i>0.055</i>
Normal < 80	14 (63.6)	8 (26.7)	
Pre-HPT 80-89	4 (18.2)	11 (36.7)	
Stage I 90-99	3 (13.6)	10 (33.3)	
Stage II >100	1 (4.5)	1 (3.3)	

*Chobanian *et al.*, (2003) BP Classification

4.3 FOOD CONSUMPTION PATTERN

4.3.1 *Starchy Root, Plantain, Cereal and Cereal Products*

With the exception of maize, there were no significant differences in the frequency of consumption of foods in this group. Maize consumption was significantly higher ($p=0.002$) in vegetarians. The food item was consumed daily by 54.5% of vegetarians compared to 43.3% of non-vegetarians.

More than half (59.1%) of vegetarians consumed cassava or meals containing cassava compared to 36.7% ($n=11$) of non-vegetarians. Response to frequency of plantain consumption showed similarity (54.5% vs. 50%) between the groups who consumed the food item once or twice weekly. However more non-vegetarians consumed plantain occasionally compared to vegetarians (13.6% vs. 26.7%). Cocoyam (*Xanthosoma mafafa*) was occasionally consumed by both groups. More non-vegetarians ($n=12$, 40%) consumed yam once or twice weekly than vegetarians ($n=10$, 45.5%).

Consumption of rice and bread showed no significance between groups. More vegetarians however consumed more of rice and bread than non-vegetarians. (Figure 4.4)

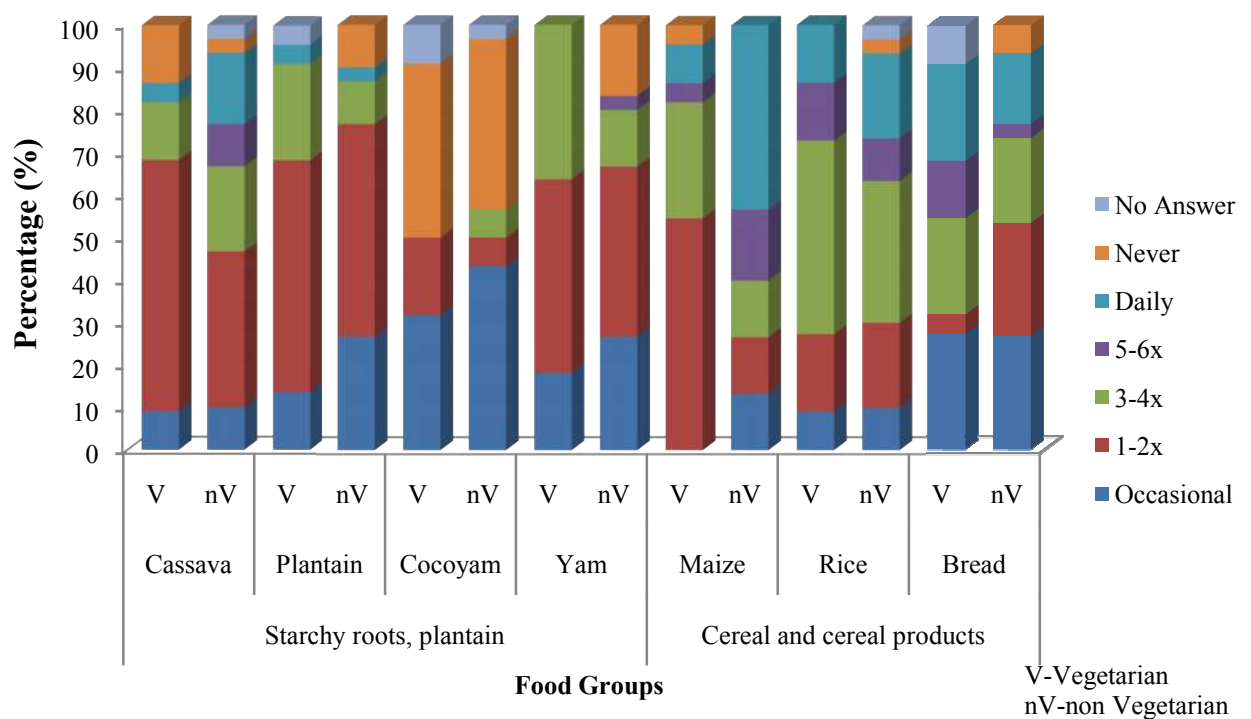


Figure 4.4 *Frequency of consumption of starchy roots, plantain, cereals and cereal products by vegetarians and non-vegetarians*

4.3.2 Animal and Animal Products

As expected, consumption pattern showed significant differences for fish, meat and poultry products between both groups with non-vegetarians having significantly higher intakes ($p=0.001$) of the animal products. Most non-vegetarians (60%) consumed fish daily followed by those (26.7%) who consumed it three to four times weekly.

Frequency of consumption of milk was not significantly different ($p=0.269$) between groups. Only 13.5% ($n=3$) of vegetarians consumed eggs at least once or three times weekly whereas the remaining 86.4% ($n=19$) of vegetarians did not consume egg (Figure 4.2, Figure 4.5). Egg consumption as well as cheese / 'waagashi' showed significant differences in intakes between groups ($p=0.001$ and $p=0.040$ respectively) (Figure 4.5).

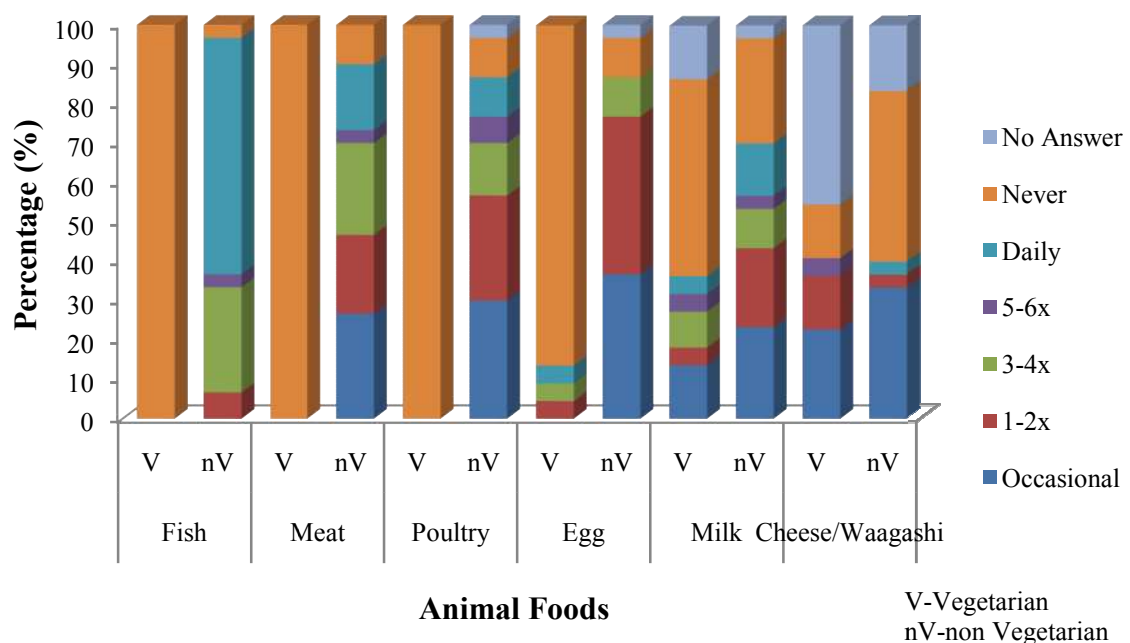


Figure 4.5 *Frequency of consumption of animal and animal products by vegetarians and non-vegetarians*

4.3.3 Legumes

With the exception of soy bean, there were no significant differences in the consumption of leguminous foods. More than half (54.5%) of vegetarians consumed cowpea one to four times weekly compared to half (50%) of non-vegetarians who consumed the legume one to four times within the week. Similar patterns were observed for consumption of groundnuts. It was consumed once or twice weekly by 32.7% (n=17) of participants from both groups. Other types of beans such as bambara beans (*Vigna subterranean*) were not consumed frequently. Consumption of soy bean was significantly higher ($p=0.001$) in vegetarians.

Although it was consumed occasionally, a higher proportion of non-vegetarians (26.7%, n=8) than vegetarians (13.6%, n=3) reported that they consumed it. For vegetarians who consumed soy bean, the pattern of consumption was between one and five times throughout the week (Figure 4.6).

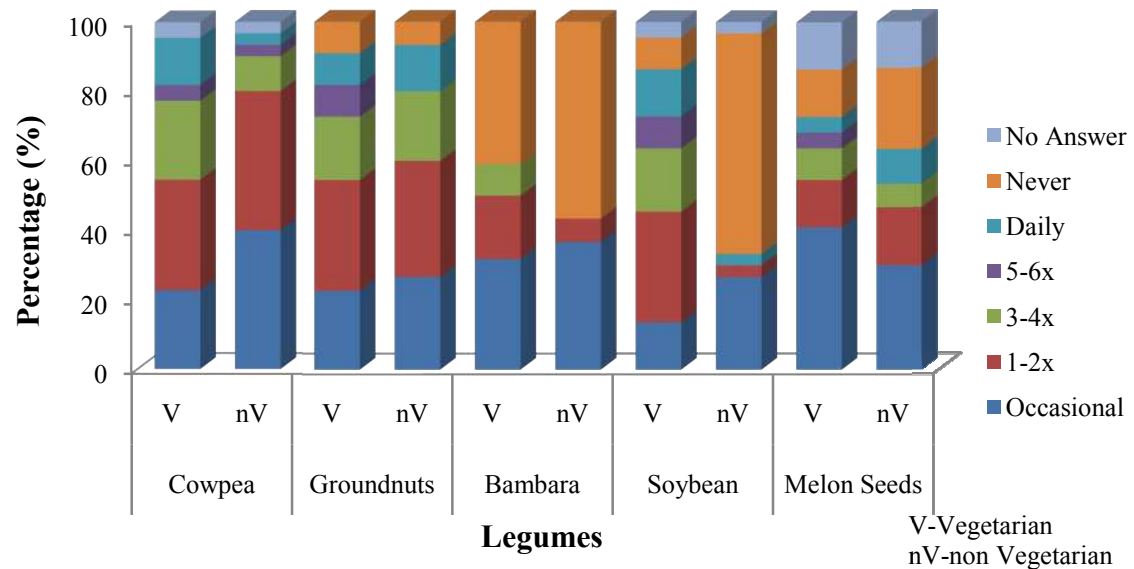


Figure 4.6 *Frequency of consumption of legumes and nuts by vegetarians and non-vegetarians*

4.3.4 Fruit Intake

There was no significant difference in orange consumption by participants in both groups. Mango, watermelon, pineapple and banana were not frequently consumed by both groups. There were no significant differences in the frequency of consumption of these fruits. Frequency of consumption of pawpaw was significantly lower ($p=0.003$) in vegetarians even though it was occasionally consumed by participants from both groups (Figure 4.7).

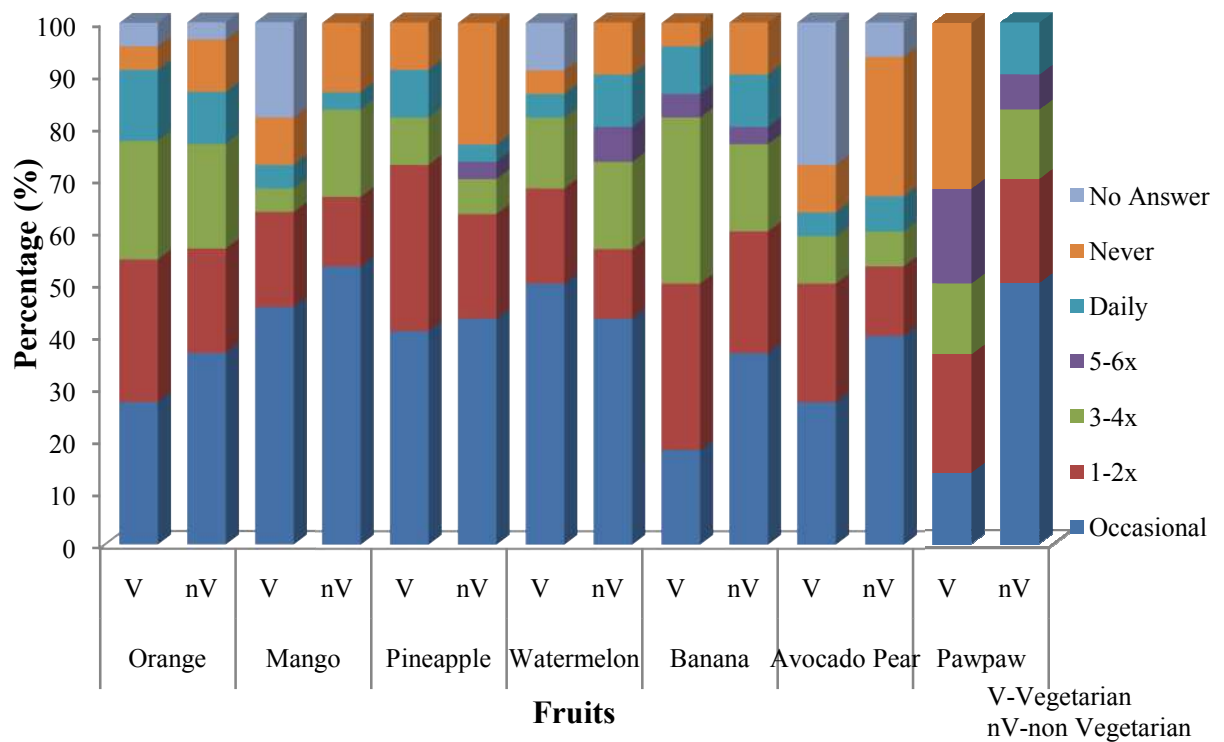


Figure 4.7 Frequency of consumption of fruits by vegetarians and non-vegetarians

4.3.5 Vegetable Intake

Most respondents consumed tomatoes daily and significantly higher proportion ($p=0.046$) of vegetarians (54.5%) consumed green leafy vegetables daily compared to non-vegetarians (23.3%) (Figure 4.8).

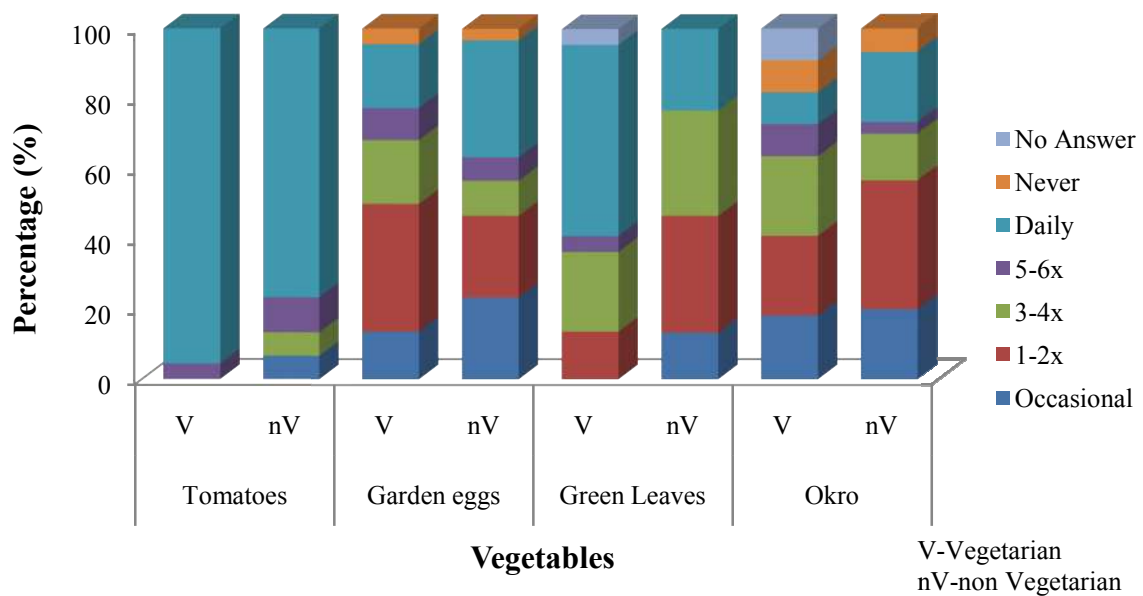


Figure 4.8 *Frequency of vegetable consumption by vegetarians and non-vegetarians*

4.3.6 Fats and Oil Intake

There was no significant difference in the frequency of consumption of palm oil, refined oils and margarine (Figure 4.9).

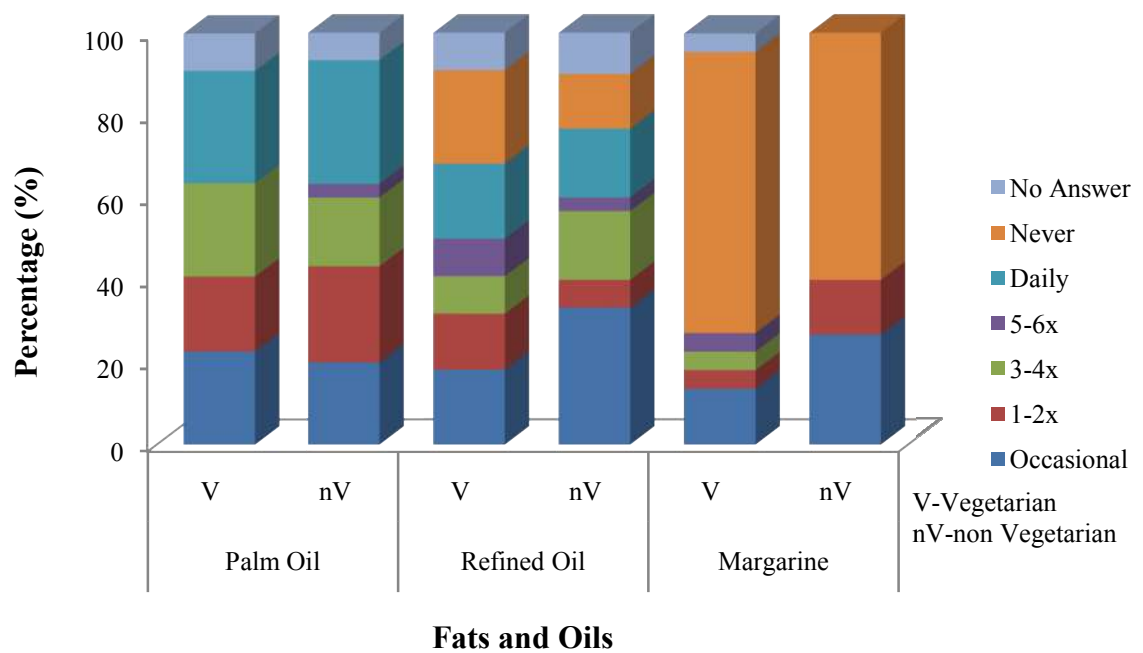


Figure 4.9 *Frequency of fats and oil consumption by vegetarians and non-vegetarians*

4.4 NUTRIENT INTAKE ANALYSES

The mean energy, carbohydrate and fat intakes were all higher in non-vegetarians than vegetarians but were not significantly different. The mean protein intake was significantly lower in vegetarians than non-vegetarians. Dietary cholesterol was also significantly lower in vegetarians. With regards to the selected micronutrients analysed in this study, mean intakes of calcium was significantly higher in vegetarians than non-vegetarians. Contrary to this intakes of iron and zinc were significantly lower in vegetarians.

Table 4.5 Mean nutrient intakes of vegetarians and non-vegetarians

	Vegetarian n=22	Non-Vegetarian n=30	
Nutrients	Mean $\pm$ SD	Mean $\pm$ SD	<i>p-value</i>
Energy (kcal)	1320 $\pm$ 669	1459 $\pm$ 419	0.396
Carbohydrate (g)	221 $\pm$ 141	235 $\pm$ 84	0.690
(% contribution)	62.2 $\pm$ 9.8	59.7 $\pm$ 8.0	0.332
Protein (g)	37.6 $\pm$ 14.6	54.6 $\pm$ 13.3	0.001*
(% contribution)	11.9 $\pm$ 2.8	58.5 $\pm$ 11.4	0.001*
Fat (g)	37.3 $\pm$ 19.3	40.4 $\pm$ 18.3	0.557
(% contribution)	28.8 $\pm$ 18.7	24.9 $\pm$ 8.8	0.331
Dietary fibre (g)	13.7 $\pm$ 7.5	12.9 $\pm$ 8.2	0.730
Cholesterol (mg)	7.8 $\pm$ 25.7	62 $\pm$ 120	0.023*
Calcium (mg)	717 $\pm$ 375	432 $\pm$ 159	0.003*
Iron (mg)	13.6 $\pm$ 6.3	19.9 $\pm$ 7.1	0.001*
Zinc (mg)	5.4 $\pm$ 3.9	7.4 $\pm$ 2.6	0.043*

Table 4.6 shows major foods sources of protein, dietary cholesterol and some selected micronutrient intakes of vegetarians and non-vegetarians.

Table 4.6 Major foods contributing to differences in nutrient intake by vegetarians and non-vegetarians

Nutrient	Vegetarian	Non-Vegetarian
Protein	Soy bean Mushroom Cowpea Peanuts	Fish Meat Poultry Cowpea
Cholesterol	Eggs Vegetable soups	Fish Meat Poultry Vegetable soups
Calcium	Soy bean Cowpea Mushroom Whole wheat meal Palm soup Kontomire stew	Fish Meat Egg Peanuts Bread Palm soup
Iron	Cowpea Plantain Kontomire 'Koose'	Fish Meat Poultry Cowpea
Zinc	Soy bean Mushroom Cowpea	Poultry Egg

Vegetable soups included kontomire soup and light soup.

4.5 HORMONE LEVELS IN PARTICIPANTS

4.5.1 Testosterone levels in participants

All vegetarians had testosterone levels in the normal range (Cumming and Wali, 1985). Three quarters (77%) of the non-vegetarian group also had normal testosterone levels and 23% (n=7) non-vegetarians had above normal testosterone levels. Altogether, 87% of participants had normal testosterone whereas 13% had levels above the normal range (Table 4.7).

4.5.2 Oestradiol levels in participants

Overall, three quarters (87%) of participants had oestradiol levels within the normal reference intervals (Cumming and Wali, 1985). Fifty-nine percent (59%) of vegetarians (n=13) had oestradiol levels within the normal range compared to eighty-seven percent (87%) of non-vegetarians (n=26). Forty-one percent (41%) of vegetarians (n=9) and 13% non-vegetarians (n=4) had levels above the normal range (Table 4.7).

Table 4.7 Hormone levels in vegetarians and non-vegetarians

HORMONES	V (22) N (%)	nV (30) N (%)	Total (52) N (%)	NRI*
Testosterone (nmol/L)				
Normal	22 (100)	23 (77)	45 (87)	8.7 – 34.7
Above normal	0 (0)	7 (23)	7 (13)	>34.7
Oestradiol (pmol/L)				
Normal	13 (59)	26 (87)	39 (75)	14.6 – 345.0
Above normal	9 (41)	4 (13)	13 (25)	>345.0
V=Vegetarian	nV=Non-Vegetarian		NRI=Normal Reference Interval	

* Cumming and Wali (1985)

4.5.3 Mean serum hormone levels of participants

There were significant differences in the levels of testosterone ($p=0.022$) and oestradiol ($p=0.037$) between vegetarians and non-vegetarians. Vegetarians had significantly lower ($p=0.022$) mean testosterone levels than non-vegetarians. Conversely, vegetarians had significantly higher mean oestradiol ($p=0.037$) levels than non-vegetarians.

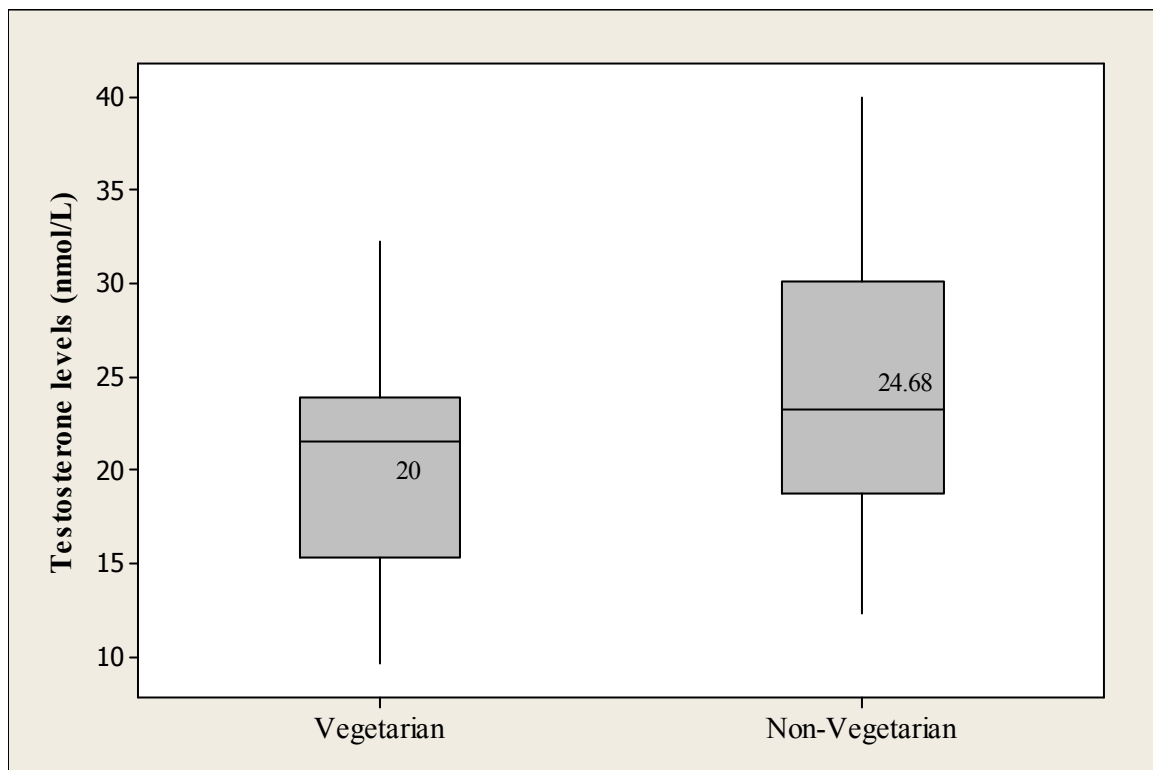


Figure 4.10 Boxplot of mean serum testosterone levels

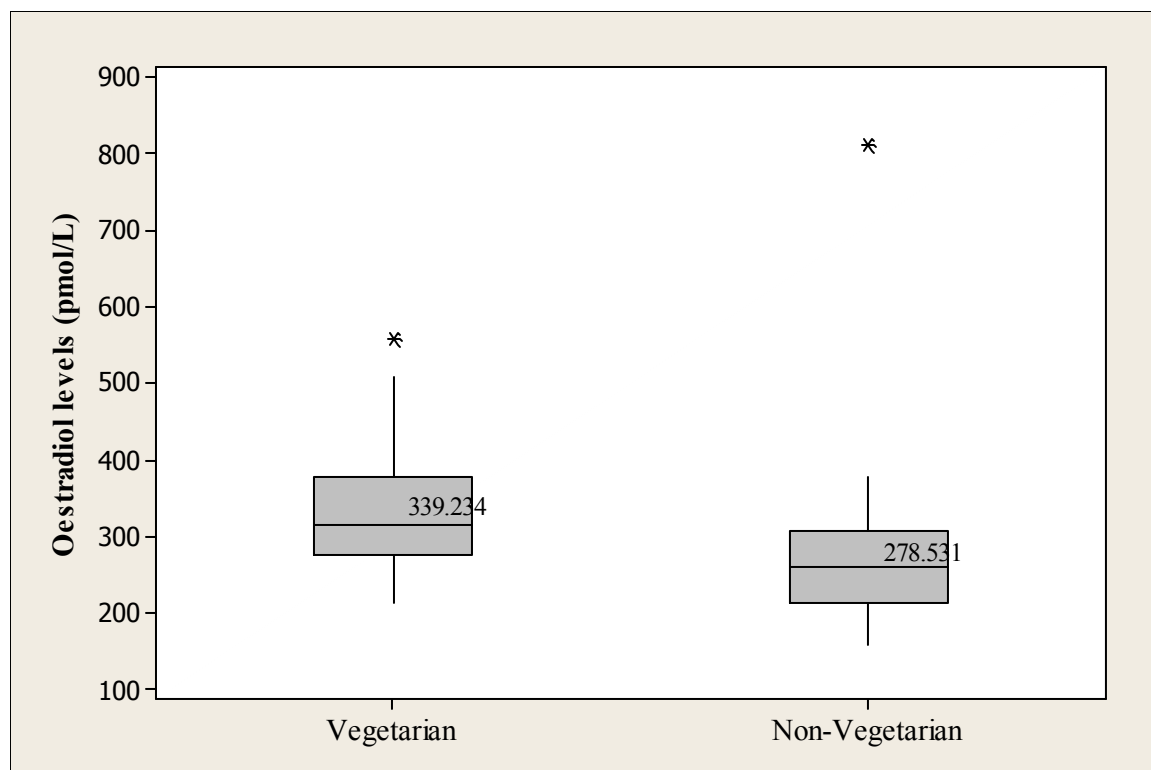


Figure 4.11 Boxplot of mean serum oestradiol levels

There were significant differences between the ages of participants and endogenous levels of testosterone and oestradiol all at $p=0.001$. There were inverse associations between age and hormone levels of testosterone both in vegetarians and non-vegetarians although insignificant. Direct but weak associations ($r=0.278$) were observed between age and oestradiol levels of non-vegetarians although this was insignificant.

There were significant differences ($p=0.001$) between protein intake and levels of endogenous hormones. Inverse associations were also found in the intake of protein and hormone levels. With vegetarians, the association was insignificant at $p=0.301$ ($r=-0.231$) for testosterone and $r=-0.152$ for oestradiol at $p=0.499$. In non-vegetarians the inverse association ($r=-0.007$; $p=0.971$) was similarly insignificant between protein intake and testosterone as well as for oestradiol ($r=-0.057$; $p=0.765$).

The mean protein intake of non-vegetarians who did not consume soy compared to their mean serum concentration of oestradiol showed direct but insignificant associations ($r=0.007$; $p=0.978$). Insignificant differences ($p=0.092$) were also found when the mean serum oestradiol concentration (339 ± 88 pmol/L) of vegetarians who consumed soy was compared to mean serum oestradiol (276 ± 138 pmol/L) of non-vegetarians who did not consume soy.

4.5.4 Association of age, protein and soy intake on mean serum hormone levels

Table 4.8 Association of age, protein, soy intake and hormone levels showing correlation coefficients (r).

		Vegetarian n=22	Non-Vegetarian n=30		
Age vs. Hormone		Mean ± SD	Mean ± SD	<i>p-value</i>	<i>r (p-value)</i>
Testosterone	Age (yrs)	57.6 ± 10.9	43.3 ± 10.4	0.001*	-0.069 (0.759)¶
	T (nmol/L)	20.0 ± 6.2	24.6 ± 7.9		-0.239 (0.204)
Oestradiol	Age (yrs)	57.6 ± 10.9	43.3 ± 10.4	0.001*	-0.087 (0.706)¶
	E(pmol/L)	339 ± 88	279 ± 116		0.278 (0.136)
Protein vs. Hormone					
Testosterone	Protein (g)	37.6 ± 14.6	54.6 ± 13.3	0.001*	-0.231(0.301)¶
	T (nmol/L)	20.0 ± 6.2	24.6 ± 7.9		-0.007 (0.971)
Oestradiol	Protein (g)	37.6 ± 14.6	54.6 ± 13.3	0.001*	-0.152 (0.499)¶
	E(pmol/L)	339 ± 88	279 ± 116		-0.057(0.765)
			n (20)		
Protein vs. Oestradiol	Protein (g)		56.1 ± 12.6†	0.001*	0.007 (0.978)
	E (pmol/L)		276 ± 138†		
Oestradiol levels of soy consumers (n=22) vs. non-consumers (n=20)					
Oestradiol	E (pmol/L)	339 ± 88	276 ± 138†	0.092	
¶ Vegetarian † Consume no Soy					

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study sought to assess the levels of endogenous sex hormones in vegetarians and non-vegetarians. Most of the vegetarians in this study were between the ages of 50-69 years. Vegetarians in this study were relatively older than non-vegetarians. This finding is contrary to results in the EPIC-Oxford cohort study of meat eaters and non-meat eaters (Davey *et al.*, 2003). In that study, the median age of meat-eaters (51 yrs) was higher than the median age (39 yrs) of vegetarians.

Vegetarians often offer different ideological bases (Lindeman and Sirelius, 2001) for choosing plant versus meat diet. All vegetarians in this present study were religious, following the practice of vegetarianism as a benefit to uphold beliefs and practices. This affirms Fraser's (2003) observation that, the abstinence of meat and animal products may be an element of religious practice for some vegetarians. Almost half of the practicing vegetarians rooted their dietary lifestyle in the religious adhering to religious beliefs, the abstinence of food from animal sources being one of such practices. Other studies (Beardsworth and Keil, 1992; Fox and Ward, 2008; Hussar and Harris, 2010) which looked at peoples' motivation for choosing plant-based diets over meat-based diet identified one of two choices; health or ethical reasons, as choice for a dietary lifestyle which avoids animal products.

Some quantitative and qualitative researches have also confirmed that health and ethical reasons were most often cited as motivations for choosing to follow a vegan diet (Ruby, 2012; Dyett *et al.*, 2013). Only a few vegetarians in this study considered both health and ethical reasons for choosing a vegetarian lifestyle.

All vegetarians had abstained from meat and animal products, some for more than two years and for others close to forty years. It can be said that although for some people, the desire and determination to maintain the vegetarian lifestyle may not be static (Beardsworth and Keil, 1992), vegetarians in this study reportedly had been vegetarians for two years and more.

In a group poll of vegetarians, most respondents claimed to be lacto-ovo vegetarians (Stahler, 2006). In this study, half of the vegetarians mentioned they were lacto-ovo vegetarians, thus they included egg and dairy products in their diets (Melina and Davies, 2003). It reflects a conscious effort by vegetarians to ensure adequacy and balance in some nutrients (calcium, iron and zinc) thought to be essential nutrients in vegetarian meals that needs attention (Marsh *et al.*, 2012). Furthermore, some nutrients (calcium) may be obtained from fortified soymilk, yoghurt and other plant foods rich in the nutrient.

As was presumed among the vegetarians there was no reported intake of alcohol and smoking. Vegetarians usually abstain from smoking and alcohol consumption when compared with non-vegetarians (Davey *et al.*, 2003; Hansen, 2005). This study reports an occasional consumption of alcoholic beverages like ‘bitters’, ‘beers’ and ‘spirits’ by less than half of non-vegetarians. A few non-vegetarians reported consumption of alcohol

between two and four servings of ‘spirits’ and ‘bitters’ once or three times within the week. No significant associations were found between alcohol consumption and levels of endogenous sex hormones although animal (Badr and Barke, 1974) and human (Gordon *et al.*, 1976) studies have shown reduction in plasma concentrations of testosterone, with the latter finding reporting a decrease in the mean plasma concentration of testosterone in chronic alcohol consumers.

5.2 *Anthropometric and Blood Pressure Measurements*

No significant findings were found between the body mass indices (BMI) of vegetarians and non-vegetarians. A BMI of 22.5 to 25.0 kg/m² is considered ideal (Mahan and Escott-Stump, 1999). However by the World Health Organizations’ (2010) classification, a BMI of 18.5-24.9 kg/m² is considered normal. It had been observed (Alewaeters *et al.*, 2005) that individuals following a vegetarian diet typically have lower BMI and tend to be leaner than their meat-eating counterparts. In an online study of vegetarian lifestyle choices, Radnitz *et al.* (2015) found majority of participants to be of normal weight (60.43%), 6.3%, 24.2% and 8.9% were underweight, overweight and obese respectively.

Most of the vegetarians in this present study had a normal BMI, similar to findings by Tonstad *et al.* (2009) who compared lifestyle choices of vegetarians and non-vegetarians. In their study, average BMI of vegetarians was 23.6 kg/m², similar to the mean BMI of 23.6 kg/m² in this study. On the other hand, BMI of non-vegetarians (28.8 kg/m²) was relatively higher than the BMI of 24.8 kg/m² in the Ghanaian non-vegetarians. Thus, the vegetarian diet is considered useful as a weight reducing diet if planned well. The findings of a few vegetarians being overweight or obese may need further investigation

also bearing in mind that other factors may be considered in determining overweight and/or obesity.

Some studies have shown that individuals following a vegetarian diet have lower blood pressure. Appleby *et al.* (2002) in a study of blood pressure and hypertension found that vegetarians had lower blood pressure, similar to findings from this present study. No significant differences were found in the systolic blood pressure of vegetarians compared to non-vegetarians. Using classification from the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of high blood pressure (Chobanian *et al.*, 2003), 13.6% of vegetarians (n=3) and 16.7% non-vegetarians (n=5) had systolic BP between 140-150 mmHg. Seventy percent (70%) of the study population had normal diastolic BP with only one (1) individual from each group having diastolic BP ≥ 100 mmHg.

Participants in the study with Stages I and II (Chobanian *et al.*, 2003) systolic and diastolic BP were advised to seek regular checks to assist in control and management of high pressures. Reported values were not conclusive of blood pressure status since a definitive diagnosis would require more than a single days reading.

5.3 Food Consumption Pattern

There were no significant differences in the frequency of consumption of starches, tubers and plantain. Vegetarians consumed more starches than non-vegetarians. In Ghana, starches represent the most abundant and primary source of carbohydrate rich foods in most meals. Vegetarians also consumed more grains and cereals than non-vegetarians. Consumption of maize products was significantly higher in vegetarians who consumed

the grain about four times weekly than non-vegetarians. A few non-vegetarians however consumed the grain daily than was observed among vegetarians.

In the EPIC-Oxford study, Davey *et al.* (2003) reported that vegetarian diets provide relatively large amounts of cereals and grain products.

The significant difference in the consumption of fish, meat and poultry products was expected because of the population under study. Vegetarians exclude all or some animal products from the diet. Therefore it was obvious that comparison with non-vegetarians will bring out clear differences in consumption of meat, fish and poultry. Sixty percent (60%) of non-vegetarians consumed fish daily.

No significant differences were observed in the frequency of consumption of legumes like cowpea and bambara beans except soy bean ($p=0.001$) which was consumed more frequently by vegetarians than non-vegetarians. Vegetarians and especially vegans often consume a substantial amount of soy bean or foods made from soy (Peeters *et al.*, 2003). Vegetarians in this study consumed soy bean in the form of drinks, steamed cuts affectionately referred to as ‘vegetarian chunks’ made into khebab.

Fruits were occasionally consumed by both groups. Some of the fruits mentioned in the study were seasonal fruits and the observation that most of the participants reportedly consumed some of the fruits occasionally is understood. Seasonal variation in the time most fruits are ready for market consumption may make some fruits available and some fruits scarce at certain times within the year. More vegetarians in this study consumed pawpaw once or twice weekly than non-vegetarians ($p=0.003$). Vegans consumed more fruits and vegetables (≈ 650 g/day) than non-vegetarians in the Adventist Health Study II

of more than 50,000 participants (Tonstad *et al.*, 2009). In a more recent online study of vegetarian lifestyle choices, the authors (Radnitz *et al.*, 2015) found vegetarians who were more concerned about their health to consume more fruits than ethical vegetarians. The health benefits of protective compounds (phytochemicals and antioxidants) found in fruits and vegetables have been linked to the prevention of cancer initiation as well as retardation of cancerous growth (Dewell *et al.*, 2008). Consumption of green leafy vegetables was significantly higher in vegetarians with $\approx 55\%$ of them consuming them daily compared to non-vegetarians, thus affirming that vegetarian diets include a healthy portfolio of fruits, vegetables, whole grains, nuts and seeds (Sabaté, 2003).

5.4 Nutrient Intake of participants

Total energy intake was higher in non-vegetarian diets than in vegetarian diets. Carbohydrates usually provided the bulk of energy for most vegetarian diets (Davey *et al.*, 2003), providing a little more than sixty (60) percent of energy, higher than what was observed in non-vegetarians in this study. Although this study did not try to distinguish nutrient consumption of various vegetarian types, vegans have been known to obtain higher energy from carbohydrates (Davey *et al.*, 2003). Also, even though the mean energy intakes were low in both diet groups, the percentage contribution of carbohydrate to energy intake met the recommended daily intake in both groups, higher in vegetarians than in non-vegetarians.

The vegetarian diet is known to provide less protein to meet recommended intakes. However, an adequate energy intake, including a portfolio of plants, legumes, whole

grains and soy products may provide adequate protein in vegetarian diets (Young and Pellet, 1994; ADA, 2003).

The mean protein intake by the vegetarian diet was only $\approx 12\%$ (37.4 g), significantly lower than what was observed in non-vegetarian diets (54.6 g, $\approx 59\%$). Similarly, the percentage protein contribution to energy intake was higher, exceeding recommended daily intake in non-vegetarians compared to vegetarians who only consumed ($\approx 12\%$) a little above the lower limit (10-15 %) for the recommended intake (WHO, 2003). Major sources of protein for vegetarians included soy bean, mushroom, cowpea and peanuts. Non-vegetarians consumed more fish, meat, poultry and cowpea.

Total fat intake was higher in non-vegetarians. Davey *et al.* (2003) found significant differences in fat intake in a cohort study of vegetarian and non-vegetarian men. This present study found differences although insignificant in fat intake between the two groups. The mean total dietary fat intakes of both groups did not meet the recommended daily intakes, however the percentage fat contribution to total energy intake was consistent with what is desired for good health (WHO, 2003) and helps prevent unhealthy weight gain in an adult population (Hooper *et al.*, 2012; WHO, 2003; FAO, 2010).

In the EPIC-Oxford study, the authors (Davey *et al.*, 2003) found a 41% higher intake of dietary fibre in vegan men than meat-eaters. Mean dietary fibre intake was similar between vegetarians and non-vegetarians in this current study. Mean dietary cholesterol however was significantly higher in non-vegetarians. Major foods contributing to dietary cholesterol in vegetarians included vegetable soups and eggs consumed by vegetarian while for non-vegetarians major contributors were fish and poultry. This difference was

observed and may be attributed to the reason that only a few vegetarians consumed eggs and the food item provided a considerable amount of dietary cholesterol. In a cross-sectional analysis of some meat-eaters, fish-eaters and vegetarians from the EPIC-Oxford cohort, the authors (Bradbury *et al.*, 2013) found that vegans had lower cholesterol than meat-eaters and fish-eaters.

Calcium intakes of lacto-ovo vegetarians are usually similar to, or higher than those in non-vegetarians (Messina *et al.*, 2004). Calcium intake in vegans may be lower than in lacto-ovo vegetarians and non-vegetarians, falling below recommended daily intakes because of the avoidance of dairy products (Messina *et al.*, 2004). However, calcium needs may be met by the inclusion of calcium fortified foods and dietary supplements in the diet (Weaver *et al.*, 1999). For the vegan, fortified foods such as fruit juices, soy milk and some breakfast cereals may contribute to calcium intake (Messina *et al.*, 2003). Comparison between groups showed the mean intake of vegetarians in this present study was significantly higher. In this study, foods contributing to calcium consumed by vegetarians included soy bean, cowpea, mushroom, whole wheat meal and some soups like palm and 'kontomire' soup. Fish, meat, eggs, peanuts, bread as well as okro, palm and 'kontomire' soups were food sources of calcium in non-vegetarian meals.

Iron content of vegetarian diets has been mentioned to be similar to that of non-vegetarian diets. The bioavailability of iron raises concern because plant foods contain non-haeme iron (Davey *et al.*, 2003), which is sensitive to inhibitors (phytates and calcium) and enhancers of iron absorption (Coudray *et al.*, 1997).

Iron absorption may be enhanced to reduce inhibitory effects of phytate to improve iron status by including a healthy combination of vitamin C rich and organic acid fruits and vegetables (Hallberg and Hulthén, 2000; Fleming *et al.*, 1998). Vegetarians in this study had significantly lower mean intake of iron than non-vegetarians, although not common in men (Key *et al.*, 2006). Major sources of iron containing foods consumed by vegetarians in this study included cowpea, 'koose', plantain, and 'kontomire' while fish, meat, poultry and cowpea were the major sources of iron consumed by non-vegetarians.

The bioavailability of zinc from vegetarian diets is lower due to higher phytic acid content of vegetarian diets (Hunt, 2003). Some research has shown zinc intakes of vegetarians to be near recommended allowances (Davey *et al.* 2003). Mean dietary zinc intakes of vegetarians in this study were significantly lower, similar to previous findings (Ball and Bartlett, 1999; Janelle and Barr, 1995). There has been difficulty in determining the effects of low zinc absorption in vegetarian diet (Hunt, 2003) due to difficulty in evaluating marginal zinc status. Major sources of zinc sources in vegetarian diets include soy products, legumes and grains and absorption may be enhanced by including organic acids found in citrus containing foods (Lönnerdal, 2000).

5.5 *Hormone levels in vegetarians*

Studies on levels of sex hormones in men have often associated insulin and glucagon levels (McCarty, 1999, Kuo *et al.*, 2000) with the metabolic syndrome (Saad, 2009) and cardiovascular health (Thorogood *et al.*, 1994; Muller *et al.*, 2003; Szeto *et al.*, 2004) among others. Vegetarians in this study had significantly low mean serum testosterone levels compared to non-vegetarians.

In a study to investigate the effects of changing from a high-meat diet to a lacto-ovo vegetarian diet in a cross-over design (Raben *et al.*, 1992), mean testosterone concentration after six weeks on the vegetarian diet decreased significantly from baseline.

Whereas their study (Raben *et al.*, 1992) was conducted in vegetarians for a relatively short duration, inference may be made that, the consumption of the vegetarian diet (low fat, high fibre) as opposed to the high-meat or high protein diet resulted in a decrease in the mean serum testosterone concentration of participants who consumed the vegetarian diet. Findings in this study are consistent with Howie and Schultz (1985) who found significant differences in the mean testosterone concentration (18.7 nmol/L) of Seventh-Day Adventist (SDA) vegetarians to be lower than non-Seventh-Day Adventist meat-eaters (6.66 ng/ml). Other lifestyle factors (alcohol, smoking) not accounted for in this study may be attributed to mean differences in hormone levels. Mean oestradiol levels were significantly higher in non-vegetarians contrary to what Howie and Schultz (1985) found in a study of SDA and non-SDA vegetarians and meat-eaters.

Age related decline in sex hormones has been reported in men (Gray *et al.*, 1991; Vermeulen, 1996; Leifke *et al.*, 2000) and even hypothesized to promote age-related health problems including sexual function (Burris *et al.*, 1992), bone density (Rudman *et al.*, 1994) and atherogenic lipids (Simon *et al.*, 1996). Leifke *et al.* (2000) showed significant association in the decline of both testosterone and oestradiol beginning from the third decade of life. Significant differences were observed in the age and hormone levels of both vegetarians and non-vegetarians ($p=0.001$).

Pearson's correlation of age and testosterone levels in vegetarians showed inverse but insignificant association ($r=-0.069$; $p=0.759$) in vegetarians consistent with findings from Key *et al.* (1990) and also an inverse association ($r=-0.239$; $p=0.204$) for non-vegetarians in this study.

In a previous study (Key *et al.*, 1990), no correlation was found in oestradiol and age, similar to findings in this study even though there was a direct but insignificant correlation in age and oestradiol levels in non-vegetarians. Age was indirectly correlated to oestradiol levels in vegetarians.

In a study among forty male meat-eaters and vegetarians, no nutrient correlated with testosterone concentrations even after adjusting for age, BMI and type of diet (Key *et al.*, 1990) consumed by participants. The influence of protein intake on hormone levels showed significant differences ($p=0.001$) between both groups; however there were inverse but insignificant association between intake and hormone levels.

When the mean protein intake of non-vegetarians were compared to mean serum oestradiol levels, direct but insignificant association were found ($r=0.007$; $p=0.978$). There were insignificant differences ($p=0.092$) between the mean serum oestradiol levels of vegetarians who consumed soy and non-vegetarians who did not consume soy. It has already been reported (Lu *et al.*, 1998) that soya bean consumption in the form of 355 ml soya milk supplements for four weeks had no effect on serum testosterone concentration.

5.6 Conclusion

There were significant differences in weight, diastolic BP, and visceral fat but no significant differences in BMI, systolic BP, and body fat between vegetarians and non-

vegetarians. Significant differences were observed in the frequency of consumption of maize products, fish, meat and poultry as well as soya bean between vegetarians and non-vegetarians. With nutrient intake, significant differences were found in the intake of protein, cholesterol, calcium, iron and zinc between both groups.

In comparison of sex hormone levels, mean serum testosterone was significantly lower in vegetarians and mean serum oestradiol concentration was significantly higher in vegetarians than non-vegetarians. Additionally, inverse association were found between age and testosterone levels in both groups but the association (inverse) was not replicated in the oestradiol levels in both groups except in vegetarians, a direct association was found in age and oestradiol levels in non-vegetarians. Inverse associations were also found between protein intake and levels of sex hormone in both groups. It cannot be conclusively said that the consumption of vegetable protein as opposed to animal proteins resulted in the significant differences observed in the mean oestradiol and testosterone concentrations of the study group since other factors may influence hormone levels other than diet or dietary lifestyle.

5.7 Limitations of the study

Firstly, there was difficulty in obtaining consent from groups due to the sensitivity of the research. This is the first study examining levels of endogenous sex hormones in male vegetarians and non-vegetarians in Ghana. As a result only few vegetarians and non-vegetarians were recruited in the present study and associations may be limited to similar groups of vegetarians and not the entire vegetarian population of the country.

Secondly, the likelihood of bias in under-reporting and over-reporting estimates of dietary intake may have introduced some difficulty in evaluating actual intakes of participants. In the same vein, estimation of soup, stews and other soy products alternatively consumed by vegetarians may have been inconsistent with actual intake.

It is recommended that;

1. A more diverse group of vegetarians and non-vegetarians be included in subsequent studies on hormone levels.
2. The significant differences in protein consumption between vegetarians and non-vegetarians are compared to clearly establish factors for hormone differences.
3. There should be the development and validation of estimates for some more local foods.
4. Alternatives and non-invasive methods of sample collection should be considered in comparing hormone levels in similar sample populations.

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APPENDIX I

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

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**My Ref. No. SAHS/ 10231437
Your Ref. No.**



P. O .Box KB 143
Korle Bu
Accra
Ghana

2nd June, 2014.

Mr. Yauniuck Yao Dogbe,
Dept. of Dietetics,
SAHS,
Korle-Bu,

Dear Mr. Dogbe,

ETHICS CLEARANCE

Ethics Identification Number: SAHS – ET. /10231437/AA/3A/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: “Levels of Endogenous Sex Hormones in Vegetarian Males”

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.

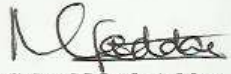
You are required to report all serious adverse events related to this research to the Committee within seven (7) days verbally and fourteen (14) days in writing.

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
 Senior Assistant Registrar

APPENDIX II

PARTICIPANT'S INFORMATION SHEET

This is a study on vegetarianism and health being conducted by Yauniuck Yao Dogbe, from the Department of Nutrition and Dietetics of the School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana. This research is under the supervision of Dr. Matilda Asante and Dr. George Asare.

The main focus of this proposed study is to examine the levels of endogenous sex hormones in males. If you decide to take part in this study, you will be asked to answer questions on your food intake, some lifestyle behaviours and also provide some information about yourself. You will be asked to provide about 3 mls (about 1 teaspoonful) of venous blood to be drawn by trained laboratory scientists for tests on levels of endogenous sex hormones. The laboratory scientists will be extra careful when drawing the blood sample to minimize bruises and/ or discomfort. In case you experience bruises or discomfort at the site of blood drawn, first aid will be provided. Your body weight and height will be measured. The information obtained from you will not be harmful in any way. If the information is published in any scientific journal, you will not be identified by name. There is no risk involved in the study except the little discomfort and bruises you might experience at the site of the blood drawn. Participating in this study is without any cost. Results of tests will be provided to participant on request. This study may contribute to the existing knowledge on vegetarianism and health. The researcher will be available and willing to answer any further questions about the research, now or during the course of the project.

CONSENT**Researcher's copy**

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 this form to take part in this research project and I understand I will receive a signed copy of this consent form for my records.

Participant's name: Participant's ID

Mobile number: Date:

Signature/Thumbprint:

Name of Researcher: Yauniuck Yao Dogbe

Date:

Mobile number: 026 7038177

Signature:

.....

CONSENT**Participant's copy**

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 this form to take part in this research project and I understand I will receive a signed copy of this consent form for my records.

Participant's name: Participant's ID

Mobile number: Date:

Signature/Thumbprint:

Name of Researcher: Yauniuck Yao Dogbe

Date:

Mobile number: 026 7038177

Signature:

APPENDIX III**QUESTIONNAIRE**

Participant's ID..... Date.....

Section A – Social demography

1. Age(years) 18-29 [] 30-39 [] 40-49 [] 50 -59 [] 60 - 69 [] 70-79 []
2. Marital Status i. single [] ii. Married [] iii. Divorced []
iv. Widowed [] v. separated []
3. Religion i. Christian [] ii. Muslim [] iii. Others (please indicate).....
4. Educational Background i. No formal education [] ii. Basic education (middle/JHS) [] iii. SHS/O –Level [] iv. HND/Diploma Certificate []
v. Bachelor Degree [] vi. Post Degree []
5. Employment Status: i Employed [] ii Unemployed [] iii Retired []
iv. Student []

Section B – Lifestyle behaviour and choices

6. Are you a vegetarian? YES [] NO []
- 7a. If yes, for how long?, if no skip to question (9).....
- 7b. Please indicate the type: i.Vegan [] ii. Lacto-vegetarian [] iii. Lacto-ovo vegetarian [] iv. Ovo-vegetarian [] v. Non vegetarian []vi. Other (please indicate).....
8. What is your main reason for becoming a vegetarian?
i. Personal health concerns []
ii. Adherence to Religious belief and practices []

iii. Moral concern and respect for animal health and welfare []

iv. No known reason []

v. Other (please indicate).....

9a. Do you have any medical condition? YES [] NO []

9b. If yes please state the condition

10a. Are you on any medication? (steroid related) YES [] NO []

10b.If yes, mention.....

11a. Do you smoke? YES [] NO []

11b. if yes how often? Daily [] Weekly [] monthly [] occasionally []

12a. Do you drink alcohol? YES [] NO []

12b. If yes how often Daily [] Weekly [] Monthly [] occasionally []

One servings of alcohol: 148ml of wine (½ medium glass of dry wine)

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

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

60 ml of (brandy, vermouth, aperitif)

12c. On average, how many servings of alcohol do you take?

Section C - Anthropometric and Physical Measurements

13. Weightkg 14.Height.....meters 15.BMI.....Kg/m²

16. Body Fat.....% 17. Visceral Fat.....% 18. Blood Pressure..... mmHg

D. Food Frequency Questionnaire (Adapted from Aggrey-Aboagyeh, 2011)

Please tick how often within the week 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						
Cowpea						
Groundnuts						
Bambara						
Soybean						
Melon seeds[Agushie]						
Fruits						
Orange						
Mango						
Pineapple						
Watermelon						
Banana						
Avocado Pear						
Pawpaw						
Vegetables						
Tomatoes						
Garden eggs						
Green leaves						
Okro						
Fats and oils						
Palm oil						
Refined oil eg coconut oil						
Margarine						

E. 24-hr Recall (Current Food Intake)

Time		Meal	Quantity
Breakfast	Weekend 1	1.	1.
		2.	2.
		3.	3.
	Weekday 1	1.	1.
		2.	2.
		3.	3.
Weekday 2	1.	1.	
	2.	2.	
	3.	3.	
Mid-morning Snack			
Lunch	Weekend 1	1.	1.
		2.	2.
		3.	3.
	Weekday 1	1.	1.
		2.	2.
		3.	3.
Weekday 2	1.	1.	
	2.	2.	
	3.	3.	
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			