ASSESSMENT BY DIETARY INTAKE AND HAEMOGRAM OF IRON LEVELS OF VEGETARIANS IN A SELECTED COMMUNITY IN ACCRA, GHANA

BY

JOANA OFORI-KORANTENG

(10363020)

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DECLARATION

This is to certify that this thesis is a result of an independent research work undertaken by Joana Ofori-Koranteng, under the supervision of Dr. George Asare and Dr. Matilda Asante towards the Master of Science degree in Dietetics at the Department of Dietetics, School of Allied Health Sciences, University of Ghana. References to the work of other authors cited have been dully acknowledged.

Signature…………………………….

Date……………………………………..

Joana Ofori-Koranteng

(candidate)

Signature…………………………….

Date……………………………………..

Dr. George Asare

(Supervisor)

Signature…………………………….

Date……………………………………..

Dr. Matilda Asante

(Supervisor)

DEDICATION

To the glory of the most high God

To my husband Emmanuel, and my wonderful children Nhyira, Freddy and Daniella

for their support and Love at each stage of this academic ladder.

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I express my profound and deepest gratitude to God Almighty and my Lord and Savior Jesus Christ. Your grace and mercy has enabled me to make this work a success.

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ABSTRACT

Background: Anaemia is a condition where the number of red blood cell (RBC) is reduced below the recommended value. It is diagnosed when a blood test shows haemoglobin concentrations below established levels. Iron deficiency anaemia is the most common type of anaemia. According to the World Health Organization (WHO), as many as 30% of the people in the world have anaemia due to prolonged iron deficiency. A good source of iron (haem iron) is meat and non-haem iron from plant sources. However, the haem iron is readily absorbed compared to the non-haem iron. Vegans and vegetarians who do not replace meat with other iron rich foods are at high risk of suffering from iron deficiency anaemia.

Objective: The objectives of this study was to assess the dietary intake and full blood count levels of vegetarians and non-vegetarians.

Methods: The study groups included 50 vegetarians, and 50 non-vegetarians between the ages of 25 years and 70 years. Both groups were made up of 19 females and 31 males. The study design used was a case control study design. A purposive sampling technique was used in selecting the participants. Venous blood was taken from participants to perform full blood count (FBC) test to determine those who were anaemic (low Hbs < 13.0g/dl for males and < 11.5 g/dl for females). A thin film comment was performed on cases with hemoglobin levels < 13.0g/dl and < 11.5 g/dl for males and females respectively, to determine the type of anaemia. Diet history and anthropometric measurements were also taken to determine the effect of vegetarian diet on body mass index (BMI) and visceral fat.

Results: The mean BMI of the vegetarians (25.19 kg/m^2) was significantly lower (p=0.013) than the mean BMI of the non-vegetarians (27.73 kg/m^2).

Vegetarians had significantly lower energy (p=0.030), protein (p= 0.001), total fat (p=0.030), cholesterol (p=0.001) and vitamin B_{12} (p=0.001) intakes than non-vegetarians. The Hb levels of participants showed that 24% of vegetarians and 26% of non-vegetarians were anaemic. The mean MCV, MCH, MCHC and RDW were all within normal limits for both vegetarians and non-vegetarians. The blood thin film of anaemic cases did not show marked microcytic and hypochromic RBCs. No significant association was found between dietary iron and Hb levels (p=0.071).

Conclusion: The prevalence of iron deficiency anaemia among vegetarians in Accra is low. Vegetarians had low intakes of protein, total fat, cholesterol, folate and vitamin B_{12}. Further research is needed to determine the relationship between diet and iron deficiency in a larger study.

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LIST OF ABBREVIATIONS

BMI	-	Body Mass Index
GRA%	-	Granulocyte percentage
GRA#	-	Granulocyte absolute value
Hb	-	Haemoglobin
HCT	-	Haematocrit
HGB	-	Haemoglobin
IDA	-	Iron Deficiency Anaemia
LYM%	-	Lymphocyte percentage
LYM#	-	Lymphocyte absolute value
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
MON%	-	Monocyte percentage
MON#	-	Monocyte absolute value
MPV	-	Mean Platelet Volume
PLT	-	Platelets
RBC	-	Red Blood Cells
RDW	-	Red cell Distribution Width
WBC	-	White Blood Cells
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

The World Health Organization (WHO) describes iron deficiency as the top nutritional disorder in the world. Research suggests that about 80 percent of people in the world do not have enough iron in their bodies (WHO, 2008). Anaemia which is defined as haemoglobin concentrations below established levels is a significant public health problem with major consequences for human health and socioeconomic development (WHO/UNICEF/UNU, 2001).

Anaemia is an indicator of both poor nutrition and poor health (Kotey, 2012). It is highly associated with poverty, with people in the lower socioeconomic group having a double risk compared to those in the middle or upper class. In Ghana, anemia was ranked as the fourth leading cause for hospital admissions and the second factor contributing to death after a review of the disease profile and pathology reports of selected hospitals (Kotey, 2012). Anaemia can cause headaches, weakness and many other symptoms and can lead to long-term health problems if not treated (Cafasso, 2012).

There are many causes of anemia, but iron deficiency anemia is the most common type of anemia. Iron deficiency is usually due to blood loss but may occasionally be due to poor absorption of iron (Cafasso, 2012). Insufficient iron for hemoglobin production leads to iron deficiency anaemia (Cheesbrough, 2006). Iron deficiency is a worldwide problem that affects mostly women and children. It is the most prevalent nutritional deficiency

(Clark, 2008). According to the World Health Organization (WHO), as many as 30% of people in the world have anaemia, due to prolonged iron deficiency (WHO, 2011). In Ghana, the prevalence of iron deficiency anaemia among vegetarian children and non-vegetarian children is 25% (Osei-Boadi *et al,* 2012).

Dietary iron is found in two forms, the haem and the non- haem. The haem iron is mostly found in hemoglobin containing animal foods such as meat, liver and spleen. It has a constant absorption rate and is not affected by the ingestion of other foods. The non-haem iron is obtained mostly from plants such as vegetables, cereals and beans. The absorption rate of non-haem iron varies greatly and is influenced strongly by a number of factors such as the iron status of the individual, the integrity of the gut mucosa, the solubility of the iron and the presence of absorption inhibitors or facilitators (Milman, 2011).

Vegetarian diets are mostly plant-based and contain the non-haem iron. There are different types of vegetarian diets. The most common types of vegetarian diets adapted from Robinson and Hackett (1995) are; Demi or Semi vegetarian, Piscatarian, Lacto-vegetarian, Lacto-ovo-vegetarian, ovo-vegetarian, vegan, Frutarian and macrobiotic.

Demi or Semi vegetarian occasionally eats meat, poultry and fish. Piscatarian eats fish and possibly other seafood but excludes red meat and poultry. Lacto-vegetarians exclude all meat, shell fish, egg and ingredient derived from them, example gelatine and rennet. They eat dairy product. Lacto-ovo-vegetarian excludes all meat, shell fish, egg and ingredient derived from them, but eat dairy product and eggs. Ovo-vegetarian includes eggs but avoids dairy meat, poultry, fish and other seafood (Thomas and Bishop, 2007).

Vegan excludes all animal flesh a d products derived ingredients and additives. They avoid animal products not only in their diet but also in every aspect of their life. Frutarian is a type of vegan diet which consit mainly of raw fruit, vegetables, nuts seeds, sprouted pulses and grains. Usually consists only of foods which do not kill the plant of origin. Macrobiotic is based on the Chinese philosophy of Yin and Yang. Aims to balance foods which contain qualities of these through opposing but complementary forces of nature. It has 10 levels which becomes increasingly restrictive. Lower levels are most varied and may contain meat or fish. The highest level consist of whole grains and limited fluids (Thomas and Bishop, 2007).

1.2 Problem Statement

Animal sources of food have rich stores of haem-iron and vitamin B_{12} thus vegetarian diets are more likely to be marginal in nutrients such as haem-iron and vitamin $B_{12.}$ Vegetarians therefore have a higher risk of iron deficiency anaemia compared to non-vegetarians whose diets contain reasonable amounts of meat (Sanders and Reddy, 1994). Although the iron content of vegetarian diets is similar to that of non-vegetarian diets, it is mainly in the form of non-haem iron and its bioavailability is lowered by the presence of phytate and polyphenol compounds in the plant foods. A high dietary iron intake does not assure optimum bioavailability, thus it is necessary to promote dietary practices that enhance absorption of iron from plant foods (Osei-Boadi *et al*, 2012). Vegetarianism is increasing in Ghana but there is paucity of data on the adequacy of their dietary intakes and their effects on health.

1.3 Significance of Study

Plant-based diets have high phytate content. Diets that have a high content of phytate and other modifiers of mineral absorption are associated with an increased prevalence of iron-deficiency anemia (Sanders and Reddy, 1994; Osei-Boadi *et al*, 2012). There is little data on the iron status of vegetarians in Ghana. Among the few studies done on vegetarians, the subjects were children. This study will therefore provide data on adult vegetarians that could be used for further studies. Information derived from this study will be useful to dietitians, nutritionist and other health related professionals who provide dietary advice to individual vegetarians. Appropriate dietary recommendations will be made to vegetarians based on the findings of this study.

1.4 Hypothesis

Iron deficiency anemia is not significantly higher between vegetarians than non-vegetarians.

1.5 General objective

To assess dietary intake and determine the prevalence of iron deficiency anaemia in vegetarians in a selected community in Accra, Ghana.

1.5.1 Specific Objectives

1. To measure and compare weight, height, BMI and visceral fat of vegetarians and non- vegetarians
2. To assess dietary patterns and nutrient intake of vegetarians and non-vegetarians.

3. To perform full blood count (FBC) and thin film comment on vegetarians and non-vegetarians to determine the type of anaemia if any.
4. To determine the impact of dietary intake of vegetarians and non-vegetarians on iron and heamoglobin (Hb) levels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Anaemia

2.1.1 Definitions

Anaemia is defined as a reduction in the red blood cell (RBC) count below the lower level of the normal range (< 13.0g/dl for men and < 12.0g/dl for women) (Greer *et al.*, 2008). Anaemia is also a condition in which the number of RBC or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, gender, altitude smoking, and pregnancy status. In clinical practice, anaemia is defined by a haemoglobin concentration which is below the recommended lower threshold established by epidemiological population surveys or by the local laboratory (Kotey, 2012)

The definition of anaemia in the context of the WHO criteria is haemoglobin concentration less than 13.0 g/dl in men and less than 12.0 g/dl in women (Cheesbrough, 2006). Using the WHO recommended haemoglobin thresholds make it convenient to perform useful comparison between different populations and countries on the burden of the disease (De Benoist *et a*l., 2008; World Health Organization, 2007).

2.1.2 Public Health Concern

"The world is plagued by anaemia, a common and intractable nutritional health problem affecting billions of people in Africa" (Kotey, 2012). The WHO estimates that two billion people of the world's population are anemic. This is over 30% of the world's population (World Health Organization, 2007).

Globally, the public health problem of anaemia affects both developing and developed countries. Nutritional anaemia due mainly to iron deficiency, is widely prevalent in many parts of the world. Anaemia affects health and reduces productivity and a high prevalence of anemia has profound socioeconomic consequences. About 50% of the cases of anaemia are attributed to iron deficiency (WHO, 2001). Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Iron deficiency exacts its heaviest overall toll in terms of ill-health, premature death and lost earnings (Kotey, 2012).

2.1.3 Aetiology of Anaemia

The main causes of anaemia include: dietary iron deficiency; infectious diseases such as malaria, hookworm infections and schistosomiasis; micro nutrient deficiencies including folate, vitamin B_{12} and vitamin A; or inherited conditions that affect RBC such as thalassaemia and sickle cell disease (WHO, 2007). The most significant contributor to the onset of anaemia is iron deficiency. Iron deficiency anaemia (IDA) has often been used as synonymous to anaemia, and the prevalence of anaemia used as proxy for IDA (De Benoist *et al*., 2008).

Low dietary intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and the period of life when iron requirement are especially high (as in growth and pregnancy) are the main risk factors of IDA (De Benoist *et al*., 2008).

Blood loss during menstruation, parasitic infestation such as hookworm, ascariasis and schistomiasis contribute to the lowering of haemoglobin concentration resulting in anaemia. Malaria, cancer, tuberculosis and HIV can also contribute to the burden of anaemia.

2.1.4 Pathophysiology

There are many possible causes of iron-deficiency anaemia. The condition can arise from:

1. Inadequate dietary intake secondary to a poor diet without supplementation (Mahan *et al*., 2012).
2. Inadequate absorption resulting from diarrhoea, achlorhydria, intestinal disease such as coeliac disease, atrophic gastritis, partial or total gastrectomy, or drug interference (Mahan *et al*., 2012).
3. Inadequate utilization secondary to chronic gastrointestinal disturbances.
4. Increased iron requirement for growth, which occurs during infancy, adolescence, pregnancy, and lactation (Mahan *et al*., 2012).
5. Increased excretion because of excessive menstrual blood (in females); haemorrhage from injury; or chronic blood loss from a bleeding ulcer, bleeding haemorrhoids, oesophageal varices, regional enteritis, ulcerative colitis, parasitic or malignant disease (Mahan *et al.*, 2012).
6. Defective release of iron from iron stores into the plasma and defective iron use caused by a chronic inflammation or other chronic disorders with few exceptions (Mahan *et al*., 2012).

2.1.5 Mechanism of Anaemia

Anaemia occurs by three mechanisms: blood loss, increased RBC destruction and decreased blood production. One of these mechanisms may be dominant in any anaemia presented, however more than a single cause may occur (Conrad, 1990). Increased blood loss will produce anaemia and this may be due to an acute or chronic condition. Trauma and gastrointestinal bleeding leads to this blood loss (Rudolph *et al.*, 2002).

The second other mechanism which involves increased destruction of RBC occurs in haemolytic anaemia. Extrinsic and intrinsic factors may be responsible for the increased cell destruction (Coyer *et al*., 2008). Hereditary diseases make up the intrinsic factors. Spherocytosis and elliptocytosis are conditions that cause anaemia because of a disorder in the red cell membrane. Enzyme disorders in the red cell such as glucose-6-phosphate dehydrogenase and pyruvate synthesis disease contribute to the cause of anaemia. Sickle cell disease and thalassaemia also cause anaemia because of structural abnormalities in the red blood cells (Kumar *et al*., 2003). The extrinsic factors include blood transfusion reactions, hemolytic anaemia, thrombocytopaenia purpura and disseminating intravascular coagulation (Coyer *et al*., 2008).

Impaired erythropoiesis occurs when there is disturbance of proliferation and distribution of stem cells. This is the third mechanisms by which anaemia occurs. Conditions which cause impaired cell production include reduced erythropoietin, aplastic anaemia, bone marrow dysfunction, renal cell aplasia, renal failure and endocrine disorders. Impaired cell production is the outcome of defective DNA synthesis. Defective haemoglobin synthesis is the pathological process responsible for iron deficiency anaemia, thalasseamia and the anaemia of chronic infections (Brill *et al*., 2000; Kumar *et al*., 2003).

2.1.6 Signs and Symptoms

A patient with anaemia may present with fatigue, weakness, dizziness and drowsiness. Mild anaemia may produce little clinical signs or symptoms; in severe cases of anaemia with hemoglobin concentration less than 5.0 g/dl, high output heart failure may develop (Metivier *et al*., 2000). Anaemia results in increased cardiac output in order to compensate for tissue hypoxia, and an associated systolic ejection murmur may occur as a result of increased aortic flow (Karnath, 2004). Pica, defined as the compulsive eating of non-food substances is an unusual symptom of IDA (Rose *et al*., 2000). Plummer-Vision syndrome characterized by dysphagia and oesophageal webs formed of thin mucosal membranes may also be seen among patients with iron deficiency (Hoffman *et al*., 1995). Other signs of anaemia include; pallor of the conjunctivae (Karnath, 2004), face nail beds and palmer creases, although the absence of pallor does not rule out anemia (Nardone *et al*., 1990).

2.1.7 Diagnosis of Iron Deficiency Anaemia

Iron-deficiency anaemia is diagnosed by blood tests (Cafasso, 2012). Some of the tests include a complete blood count and serum ferritin iron. Ferritin is a protein that helps store iron in your body, and a low level of ferritin usually indicates a low level of stored iron, Total iron-binding capacity, and transferrin are also measured (Lewis *et al*., 2006).

In an individual who is anaemic from iron deficiency, these tests usually show the following results:

1. Low haemoglobin and hematocrit
2. Low mean cellular volume
3. Low ferritin
4. Low serum iron
5. High transferrin or total iron-binding capacity
6. Low iron saturation (Hoffbrand *et al*., 2006; Lewis *et al*., 2006; Moore *et al*., 2010).

The peripheral smear of blood that is iron deficient will show microcytic hypochromic red blood cells.

2.1.8 Red Blood Cell Indices

Red blood cell indices are measurements that describe the size and haemoglobin content of red blood cells. The indices are used to help in the differential diagnosis of anaemia. Anaemia includes a variety of conditions with the same outcome that the blood cannot carry as much oxygen as it should. A healthy person has an adequate number of correctly sized RBC that contains enough hemoglobin to carry sufficient oxygen to all the body's tissues. An anaemic person's RBCs are either too small or too few in number. As a result, the heart and lungs must work harder to make up for the lack of oxygen delivered to the tissues by the blood (Lewis *et al*., 2006; http://medicaldictionary.thefreedictionary.com)

Anaemia is caused by many different diseases or disorders. The first step in finding the cause is to determine what type of anemia the person has. Red blood cell indices help to classify the anaemias. Anaemia of any type affects the results of one or more of the common blood tests. These tests are the haematocrit, haemoglobin, and RBC count. The hematocrit measures RBC mass, or the space occupied by RBCs. The haemoglobin test measures how much haemoglobin protein is in the blood. The RBC measures the number of red blood cells present in the blood. RBC indices are additional measurements of RBCs based on the relationship of these three test results. The relationships between the hematocrit, the haemoglobin level, and the RBC are converted to RBC indices through mathematical formulas. These formulas were worked out and first applied to the classification of anaemia by Maxwell Wintrobe in 1934. The indices include these measurements: Mean Corpuscular Volume (MCV); Mean Corpuscular Haemoglobin (MCH); Mean Corpuscular Haemoglobin Concentration (MCHC); and Red cell Distribution Width (RDW). They are usually calculated by an automated instrument as part of a complete blood count (Hoffbrand *et al*., 2006; Moore *et al*., 2010; http://medicaldictionary.thefreedictionary.com).

2.1.9 Mean Corpuscular Volume (MCV)

MCV is the index most often used. It measures the average volume of a red blood cell by dividing the hematocrit by the RBC. The MCV categorizes RBCs by sizes. Cells of normal size are called normocytic, smaller cells are microcytic, and larger cells are macrocytic. These size categories are used to classify anaemias. Normocytic anaemia has normal-sized cells and a normal MCV; microcytic anaemia has small cells and a decreased MCV; and macrocytic anaemia has large cells and an increased MCV (Lewis *et al*., 2006; http://medicaldictionary.thefreedictionary.com).

2.1.10 Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC measures the average concentration of haemoglobin in a red blood cell. This index is calculated by dividing the haemoglobin by the hematocrit. The MCHC categorizes RBCs according to their concentration of haemoglobin. Cells with a normal concentration of haemoglobin are called normochromic; cells with a lower than normal concentration are called hypochromic. Because there is a physical limit to the amount of haemoglobin that can fit in a cell, there is no hyperchromic category. Just as MCV relates to the size of the cells, MCHC relates to the color of the cells. Haemoglobin contains iron, which gives blood its characteristic red color. When examined under a microscope, normal RBCs that contain a normal amount of haemoglobin stain pinkish red with a paler area in the center. These normochromic cells have a normal MCHC. Cells with too little hemoglobin are lighter in color with a larger pale area in the center. These hypochromic cells have a low MCHC. Anaemias are categorized as hypochromic or normochromic according to the MCHC index (Lewis *et al*., 2006; Moore *et al*., 2010; http://medicaldictionary.thefreedictionary.com).

2.1.11 Mean Corpuscular Haemoglobin (MCH)

The average weight of haemoglobin in a red blood cell is measured by the MCH. The formula for this index is the sum of the haemoglobin multiplied by 10 and divided by the RBC. MCH values usually rise or fall as the MCV is increased or decreased.

2.1.12 Red Cell Distribution Width (RDW)

The RDW measures the variation in size of the red blood cells. Usually RBCs are a standard size. Certain disorders, however, cause a significant variation in cell size.

The category into which a person's anaemia is placed based on the indices provides a significant clue as to the cause of the anaemia, but further testing is needed to confirm a specific diagnosis.

The most common causes of macrocytic anaemia (high MCV) are vitamin B_{12} and folic acid deficiencies. Lack of iron in the diet thalassemia (a type of hereditary anaemia), and chronic illness are the most common causes of microcytic anaemia (low MCV). Normocytic anaemia (normal MCV) can be caused by kidney and liver disease, bone marrow disorders, or excessive bleeding or haemolysis of the RBCs. Lack of iron in the diet and thalassemia are the most common causes of hypochromic anaemia (low MCHC). Normocytic anaemias are usually also normochromic and share the same causes (normal MCHC). The red cell distribution width (RDW) measures the variation in size of red blood cells and is increased in anaemias caused by iron, vitamin $B_{12,}$ or folic acid deficiencies. Abnormal haemoglobins, as seen in sickle cell anemia, can change the shape of red blood cells and cause them to haemolyze. Haemolysis increases the RDW. Conditions that cause more immature cells to be released into the bloodstream, such as severe blood loss, will increase the RDW. The larger size of immature cells creates a distinct size variation (Moore *et al*., 2010; Lewis *et al*., 2006; Hoffbrand *et al*., 2006; http://medicaldictionary.thefreedictionary.com).

2.1.13 Health Consequences of Anaemia

Anaemia indicates poor nutrition and poor health (De Benoist *et al.*, 2008). This condition can lead to poor pregnancy outcome and also increase the risk of maternal and child mortality (Bothwell and Charlton, 1981; Macgregor, 1963; Scholl *et al.*, 1994).

Iron deficiency with or without anaemia has an important consequence on human health and child development: anaemic women and their infants are at a greater risk of dying during the prenatal period (WHO, 2007). IDA can impair physical and cognitive development of children and the physical performance of adults, particularly their work productivity (WHO, 2001).

2.1.14 Haemoglobin Synthesis

The formation of normal red blood cells involves a series of processes including the biosynthesis of nucleic acid, porphyrin, haem and proteins. Limitations placed on any of these processes could lead to anaemia (Richert *et al.*, 1959). Haemoglobin is composed of four polypeptide chains, which in adults consist of two alpha globin chains and two beta globin chains. Each polypeptide has a haem prosthetic group attached, where each haem can bind one oxygen molecule. There are four haem groups per haemoglobin molecule that together bind four oxygen molecules. The oxygen binding property of the haemoglobin is determined by the precise sequence of amino acid of the alpha and beta chains. Oxygen binds reversibly to the ferrous iron atom in each haem group.

2.1.15 Iron and Erythropoiesis

Iron remains an essential mineral for man and an important component of metalloproteins involved in oxygen transport and metabolism. It is approximated that in a well-nourished individual, the body contains 3-4g of iron. Nearly two thirds of all the iron present in the body is contained in the haemoglobin (Milman, 2011). Iron is an essential nutrient for haemoglobin and myoglobin formation and is vital for health and peak performance. Much of our iron requirement is met through recycling of the iron in RBCs (Sammons, 2007).

Deficiency of iron supply to the bone marrow leads to impairment of hemoglobin synthesis and a decline in the circulating red blood cells. This subsequently leads to IDA with a low haemoglobin concentration. The major factors which control iron absorption in a healthy individual are physiological need of iron, dietary iron intake, iron bioavailability in dietary intake and adaptation (Cook, 1990). Iron deficiency is mainly as a result of insufficient dietary iron intake.

Dietary iron is found in two forms, the haem and the non-haem. The haem iron is mostly found in animal foods and has a good bioavailability. The non-haem iron is obtained mostly from plants and it has a low bioavailability. Iron is prevalent in a wide variety of plant foods, especially beans and grains.

2.1.16 Iron absorption

Iron intake of vegans is higher than non-vegetarians. In meat, 65% of iron is bound to the haem molecule (from haemoglobin and myoglobin), which is relatively easily absorbed. The rest of the iron in meat and all iron in plants is non-haem iron (Groff and Gropper, 2000). Phytates, found in legumes and grains, and polyphenols can inhibit the absorption of plant iron. However, vitamin C is a strong enhancer of plant iron and can overcome the inhibitors in plant foods.

In a study conducted by Seshadri *et al*. (1985), vegetarian children with IDA and low vitamin C intakes in India were given 100 mg of vitamin C at both lunch and dinner for 60 days. They saw a drastic improvement in their anaemia, with most making a full recovery (Seshadri *et al*, 1985). Vitamin C is found in citrus fruits, strawberries, green leafy vegetables (Kontomire, aleefu, bokoboko and spinach), tomatoes and pepper.

Cooking foods in cast iron pans can increase iron consumption. A study in Brazil showed that cooking tomato sauce in an iron skillet increases the amount of iron in the sauce and also increased iron status among teen-aged and young adult lacto-ovo vegetarians (Quintaes *et al*, 2007). The amino acid, L-lysine, plays a part in the absorption of iron and zinc. L-lysine is only found in high amounts in legumes such as peanuts, beans, lentils, peas.

2.2 Vegetarians

Vegetarianism involves not just an eating pattern but a philosophy that affects the whole lifestyle (Thomas and Bishop, 2007). A vegetarian diet is a meal that is mostly plant based and contains vegetables, fruits, whole grains, legumes, seeds, and nuts, with little or no animal products.

There are a variation of vegetarian dietary practices. The most common types of vegetarian diets adapted from Robinson and Hackett (1995) are; Demi or Semi vegetarian, Piscatarian, Lacto-vegetarian, Lacto-ovo-vegetarian, ovo-vegetarian, vegan, Frutarian and macrobiotic.

Demi or Semi vegetarian occasionally eats meat, poultry and fish. Piscatarian eats fish and possibly other seafood but excludes red meat and poultry. Lacto-vegetarians exclude all meat, shell fish, egg and ingredient derived from them, example gelatine and rennet. They eat dairy product. Lacto-ovo-vegetarian excludes all meat, shell fish, egg and ingredient derived from them, but eat dairy product and eggs. Ovo-vegetarian includes eggs but avoids dairy meat, poultry, fish and other seafood. Vegan excludes all animal flesh a d products derived ingredients and additives. They avoid animal products not only in their diet but also in every aspect of their life. Frutarian is a type of vegan diet which consist mainly of raw fruit, vegetables, nuts seeds, sprouted pulses and grains. Usually consists only of foods which do not kill the plant of origin. Macrobiotic is based on the Chinese philosophy of Yin and Yang. Aims to balance foods which contain qualities of these through opposing but complementary forces of nature. It has 10 levels which becomes increasingly restrictive. Lower levels are most varied and may contain meat or fish. The highest level consist of whole grains and limited fluids (Thomas and Bishop, 2007).

Vegetarian diets most often lead to healthier outcomes such as lower levels of obesity, reduced risk for heart disease and lower blood pressure. Compared to non-vegetarians, vegetarians usually eat fewer calories from saturated fat, fewer overall calorie and more fiber, potassium, and vitamin C. A well-planned, vegetarian diet can deliver good nutrition. Dietary recommendations vary with the type of vegetarian diet. For children and adolescents these diets need to be carefully planned, because it may be difficult to get all the nutrients needed for growth and development. Vegetarian diets are high in fiber. High-fiber diets may lack some of the calories children need for growth, and can cause some growth problems.

The practice of vegetarianism involving the exclusion of all meat and animal products from the diet may confer some health advantages to adults due to high levels of fibre and low levels of saturated fat (Osei-Boadi *et al*., 2012). When the diet becomes more restrictive, as in the case of vegans, it is necessary to carefully select, diversify and plan meals (Sanders and Reddy, 1994; Jacobs and Dwyer, 1988). Vegetarian diets are more likely to be marginal in nutrients such as iron and vitamin B_{12} (Sanders and Reddy, 1994; Dagnelie *et al*., 1989) compared to other non-vegetarian diets that contain reasonable amounts of meat, eggs and milk products (Sanders and Reddy, 1994; Jacobs and Dwyer, 1982). Although the iron content of vegetarian diets is typically similar to that of non-vegetarian diets, the bioavailability of the iron is lower because of the absence of haem-iron and the reduced availability due to the high phytic acid content in plant foods (Key *et al*., 2006). Iron deficiency is a prevalent nutrient deficiency in the world and affects up to two-thirds of children in most developing countries (WHO, 2000), who typically receive homemade complementary foods that are poor sources of bio-available iron (Gibson *et al*., 1988). This is because a high dietary iron intake does not assure optimum bioavailability, it is necessary to promote dietary practices that enhance absorption of iron from plant foods.

2.2.1 Iron Deficiency Anaemia and Vegetarians

Marx (1997) reviewed the prevalence of iron deficiency in developed countries, the influence of lifestyle factors that may contribute to its occurrence, and dangers of population directed prevention. Marx stated that "*although iron deficiency anemia is not a major health problem in developed countries, specific groups of the population remain endangered. These groups are young children, adolescents, pregnant women, the elderly, blood donors, vegetarians, endurance athletes and migrants"*. He suggested that measures to prevent iron deficiency should be specifically aimed at population groups at risk.

Generally, vegetarians who consume variety of foods have better iron stores and are not at risk of IDA. IDA in a vegetarian could be as a result of eating a monotonous diet containing high amount of phytate and polyphenols that inhibit the absorption of iron in the food. Craig, (1994) said that the incidence of iron deficiency anaemia among vegetarians is not significantly different from that of omnivores (non-vegetarians) and that western vegetarians who consume a variety of foods have better iron stores than those in the developing countries who consume limited diets based on unleavened, unrefined cereals.

A study by Osei-Boadi *et al*. (2012) compared the diets and iron status of 26 vegetarian children between the ages of 9 months and 11 years with matched controls of 26 non-vegetarian children of similar ages and same sex living within the same communities in Accra and Cape Coast. From that study the mean intakes of energy and several other nutrients were similar among vegetarian and non-vegetarian children except for vitamin

B_{12} which was very low in the diet of vegetarian children (Osei-Boadi *et al*., 2012). The dietary diversity pattern of vegetarian and non-vegetarian children based on the 24-hour dietary recall showed that more than 90% of children in both groups consumed foods from cereals, other vegetables (non-green leafy vegetables) and fats and oil food groups. Consumption of foods from the green leafy vegetables and fruit groups was significantly higher in vegetarian children than in non-vegetarian controls. Haemoglobin concentration was not significantly different between the two groups and the prevalence of IDA was 25% in both groups. It was concluded that the typical diets of Ghanaian children lack variety and both vegetarian and non-vegetarian diets are insufficient to support adequate iron status**.** There is no local published data on adult vegetarians. Therefore little is known about their status.

A review of the diets and growth of children raised on vegetarian diets indicated that excessive bulk combined with low energy density can be a problem for children aged 5 years and below and can lead to impaired growth and that diets that have a high content of phytate and other modifiers of mineral absorption are associated with an increased prevalence of rickets and iron-deficiency anemia (Sanders and Reddy, 1994).

Another study examined the pattern of anaemia and its relation to nutritional status and dietary habits among 3633 pre-school children of 108 selected centers in rural areas of Kerala State during the period 1996 to 1998. That study reported anaemia among both vegetarians and non-vegetarians and a low consumption of dietary iron below recommended levels in both groups (George *et al*., 2000).

Nutritional education on how to increase the absorption of non-haem iron in the body is very important. An appropriately well planned vegetarian diet is compatible with an adequate iron status. Whereas phytate, polyphenols and other plant constituents found in a vegetarian diet inhibit non-haem iron absorption, Vitamin C, citric acid and other organic acids facilitate the absorption of non-haem iron (Craig, 1994). Thus knowledge of foods that will enhance the absorption of plant-based diets as seen in vegetarian diet is important.

A study conducted by Verma *et al.*, (1998), used a cross-sectional study design to assess the prevalence of anaemia among urban school children of Punjab. The results showed that more menarcheal girls were anaemic compared to non-menarcheal children. This could be the result of losing blood through menstruation by the menarcheal girls. The mean Hb levels of vegetarians were also lower compared to non-vegetarians. The commonest blood picture seen was microcytic hypochromic red blood cells. The researchers concluded that vegetarians and girls especially after menarche, were more at risk to develop anaemia.

2.2.2 Dietary Intakes of vegetarians

The nutritional status of Chinese lacto-ovo-vegetarian children aged 4-14 years were investigated by Leung *et al*. (2001). The results show that the mean daily energy intake and protein intake met the United States recommended dietary allowance. When the results were compared to that of the non-vegetarian diet, the vegetarian diet was closer to the recommended healthy diet with lower fat, more fibre and better polyunsaturated to saturated fatty acid ratio. Serum folate and vitamin B_{12} were within the normal range. The results showed that 25% of boys and 15% of girls were obese. Three boys had hyperlipidaemia. The researchers concluded that the Hong Kong Chinese vegetarian diet appears healthy, providing adequate iron and vitamin B_{12} nutrition, but the prevalence of obesity was high (Leung *et al*., 2001).

Woo *et al*. (1998) also studied the nutritional status of elderly Chinese vegetarians. The total energy, fat, protein, thiamine, riboflavin and niacin intakes were lower in vegetarians than non-vegetarians, while carbohydrate, calcium, potassium, retinol equivalent and ascorbic acid intakes were higher in that study. Furthermore the mean haemoglobin level was lower in vegetarians and the prevalence of anaemia was 30%, with deficiencies in B_{12} and/or iron accounting for 64% of the anaemia, compared with only 30% in non-vegetarians. Serum B_{12} concentration below the reference range occurred in 54% of the vegetarian subjects. It was concluded that while the Chinese vegetarian diet may result in a favourable risk-factor profile for ischaemic heart disease, it was deficient in many B vitamins and gave rise to a high frequency of nutritional anaemias.

A study by Waldmann *et al*. (2004), evaluated the iron status of German female vegans. The results show that the mean daily iron intake was higher than recommended intakes by the German Nutrition Society. In addition 42% of the female vegans < 50 years had a daily iron intake of < 18 mg/day, which was the recommended allowance by the US Food and Nutrition Board. The main dietary sources of iron were vegetables, fruits, cereals and cereal products. Although the mean iron intake was above the recommended level, 40%

of the females < 50 years were considered iron-deficient. It was suggested that young women on a vegan diet should have their iron status monitored and should consider taking iron supplements in case of a marginal status.

In a study to evaluate the dietary intakes and lifestyle factors of German vegans, it was concluded that in order to reach favourable vitamin and mineral intakes, vegans should consider taking supplements containing riboflavin, cobalamin, calcium, and iodine, and the intake of total energy and protein should also be improved (Waldmann *et al*., 2003). This will ensure that vegetarians are meeting all their micronutrient needs.

2.2.3 Body Mass Index (BMI) and Lifestyle Factors of Vegetarian

Appleby *et al*. (1998) examined the associations of diet and other lifestyle factors with body mass index (BMI) using data from the Oxford Vegetarian Study. The results of the study showed lower mean BMI in non-meat eaters than in meat eaters in all age groups for both men and women. Overall age-adjusted mean BMIs in kg/m^2 were 23.18 and 22.05 for male meat eaters and non-meat eaters respectively ($p < 0.0001$) and 22.32 and 21.32 for female meat eaters and non-meat eaters respectively ($p < 0.0001$). In addition to meat consumption, dietary fibre intake, animal fat intake, social class and past smoking were all independently associated with BMI in both men and women; alcohol consumption was independently associated with BMI in men. Generally, non-meat eaters were thinner than meat eaters and this may be partly attributed to a higher intake of dietary fibre, a lower intake of animal fat, and a lower alcohol intake in men.

Haddad *et al*. (1999) assessed diet and the nutritional status of vegans in the United States and found that BMI of the vegans were significantly lower compared to non-vegetarians.

Another study by Kennedy *et al*. (2001) examined the association between a range of health and nutrition indicators and popular diets groups. The results showed that the mean BMI was higher in the meat-eaters (24.41 kg/m^2 in men, 23.52 kg/m^2 in women) and lower in the vegans (22.49 kg/m^2 in men, 21.98 kg/m^2 in women). Fish-eaters and vegetarians had similar, intermediate mean BMI. Differences in lifestyle factors including smoking, physical activity and educational levels accounted for less than 5% of the difference in mean BMI between meat-eaters and vegans. The study concluded that fish-eaters, vegetarians and especially vegans had lower BMI than meat-eaters. Differences in macronutrient intakes accounted for about half the difference in mean BMI between vegans and meat-eaters and that high protein and low fibre intakes were the factors most strongly associated with increasing BMI.

Waldmann *et al*. (2003) evaluated the dietary intakes and lifestyle factors of German vegans and found that all the subjects had a comparatively low BMI with an extremely low mean consumption of alcohol and tobacco (96.8% were nonsmokers).

In another study by Newby *et al*., (2005) the prevalence of overweight or obesity (BMI $\geq$ 25) was 40% among omnivores, 29% among both semi-vegetarians and vegans, and 25% among lacto-vegetarians. It was concluded that semi-vegetarian, lacto-vegetarian, and vegan women have lower risk of overweight and obesity than omnivorous women. The consumption of more plant foods and less animal products which help control weight was recommended.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

A case control study design was used.

3.2 Study Site

This study was carried out among members of the Ghana Vegetarians Association at Weija and among non-vegetarians at Martyrs of Uganda Church, Mamprobi.

3.3 Sample Size Determination

A sample size of 50 vegetarians was calculated based on Epi Info Statistical software, using an absolute precision of 5% and a confidence interval of 95%.

$$n = \frac{[z]^2 [p] [1-p]}{E^2}$$

$$n = \frac{[1.96]^2 [0.05] [1-0.05]}{[0.06]^2}$$

$$n = 50$$

Where n is the estimated sample size of vegetarians

E is the desired margin of error

Z is the critical z-score on the desired level of confidence 95%

P is the prevalence of vegetarians in Ghana.

3.4 Participants

A total of 100 participants, 50 vegetarians and 50 non-vegetarians were recruited in the study. The non-vegetarians formed the control group and were matched by age and gender to the vegetarians.

3.5 Inclusion and Exclusion Criteria

The participants were adults between 25 years and 70 years and females were not to be pregnant.

3.6 Sampling Technique

A purposive sampling technique was used in selecting the participants.

3.7 Ethical Consideration

The study was undertaken after approval had been obtained from the Ethics and Protocol Review Committee of the School of Allied Health Sciences, College of Health Sciences (SAHS-ET./10363020/AA/7A/2012-2013). Permission to conduct the study was obtained from Martyrs of Uganda church and the Ghana vegetarians association.

Voluntary written informed consent and permission was sought from participants before recruitment into the study. The risks and benefits of the study were explained to the participants before the study began.

3.8 Data Collection

3.8.1 Procedure for Data Collection

Questionnaires were administered to participants to fill and information on their demographics was obtained. The weight and height of participants were measured and their body mass index (BMI) calculated using the formula; weight in kilograms divided by height squared in meters (Kg/m^2). The scale used to measure the weight of the participants recorded the visceral fat as well and participants were later grouped into those with visceral fat 1-9 (normal) and those with visceral fat greater than 9 (obese).

The diet history of participants was taken using the food frequency questionnaire and 24-hour recall of dietary intake of all meals and snacks. The 24-hour recall involved the participants recalling all the meals and snacks taken within 24 hours. Questions were asked about the type of meal the participants took (breakfast, lunch or supper), the time the meals or snacks were taken, and the food taken (example rice and vegetable stew). Handy measures were used to estimate the quantities of the meals and snacks (Appendix I). The food frequency questionnaire used in this study contained some selected foods and the participants were to tick how frequent they ate such foods; whether, daily, weekly, monthly, occasionally or never (Appendix I).

The biochemical data was obtained by drawing 5 ml of blood samples from participants into tubes containing ethylenediaminetetraacetic acid (EDTA). The blood samples were taken by a qualified Phlebotomist. Laboratory tests such as Full Blood Counts (FBC) and thin film comment were performed on the blood samples.

3.8.2 Technique of making and staining thin blood film (Cheesbrough, 2006)

1. A drop of blood was placed on the end of a clean dry slide

2. The blood was drawn back using a clean smooth edge spreader, and allowed to extend along the edge of the spreader.

3. The blood was then spread at an angle of about 30 degrees to make a film of about two thirds of the length of the slide.

4. The end of the spreader was wiped and the film immediately air dried by waving the slide back and forth.

5. The dried film was then fixed in absolute methanol for a minute.

6. The fixed film was covered with undiluted leishman stain.

7. Twice the volume of buffered water (pH 6.8) was added to the stain and mixed well with a plastic bulb pipette.

8. The stain was washed off with tap water and the back of the slide was wiped and dried in a draining rack.

3.9 Full Blood Count Analyser- ABX MICROS ES60

The ABX Micros ES60 (user manual) is a fully automated hematology analyser used for in-*vitro* diagnostics testing of whole blood specimens and whole blood component concentrates. The ABX Micros ES60 is available in 16 and 18 parameters. The system has an internal dilution system, and a graphic printer for recording all test results. The

ABX Micros ES60 performs automated blood counts and requires no manual operations for aspirating blood, dilutions, measuring, calculations, print-outs and computer transfer of data. The parameters are presented in table 3.0

Table 3.0 Parameters and their interpretation

PARAMETER	INTERPRETATION
WBC	White Blood Cells
LYM%	Lymphocyte percentage
LYM#	Lymphocyte absolute value
MON%	Monocyte percentage
MON#	Monocyte absolute value
GRA%	Granulocyte percentage
GRA#	Granulocyte absolute value
RBC	Red Blood Cells
HGB	Haemoglobin
HCT	Haematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red cell Distribution Width
PLT	Platelets
MPV	Mean Platelet Volume

3.9.1 Reagents

Reagents used were ABX Lyse reagent and ABX diluents. The ABX Lyse reagent contained potassium ferricyanide [Fe(Cn)]K, and potassium cyanide [KCN]. Blood specimens used were venous blood as recommended. Whole blood was mixed well before the sample was run.

3.9.2 Assay Procedure with Whole Blood Sample Using Analyser

1. Blood collection tube cap was removed.

2. The tube was placed beneath the sampling needle.

3. The tube was moved upwards and the sampling needle entered into the blood

4. The manual sample bar was pressed and 10µl of blood was aspirated.

5. Needle carriage assembly moved to the left, over the WBC/HGB chamber.

6. The external sampling needle was rinsed.

7. Blood sample was delivered into the WBC chamber for the first dilution.

8. Aspiration of 28.3µl of diluted blood from the WBC chamber.

9. Needle carriage assembly moved to the right, over the RBC/PLT chamber.

10. The external sampling needle was rinsed.

11. Diluted blood sample was delivered into the RBC chamber for the RBC/PLT measurement.

3.11 Data management plan

The raw data from questionnaires, laboratory results, and anthropometric indices were entered and saved in database files, pass-word protected and locked. The papers were then kept in a metal safe and locked to prevent results from circulating thereby breaching confidentiality.

3.12 Data Analysis

Statistical Package for Social Sciences (SPSS Version 20) was used to analyze the data. Data were summarized as means, standard deviations, frequencies, percentages and presented as tables and graphs. Nutrients analysis was performed using the ESHA F-PRO. The *p*-value was used to determine whether significant differences existed between the nutrient intake of the vegetarians and non-vegetarians. Dietary iron contents of the participants were grouped according to the age and gender of both the vegetarians and non-vegetarians. Analysis of variance was used to determine whether significant differences existed in the mean levels of iron intake among vegetarian and non-vegetarian males and females grouped according to their ages.

The association between haemoglobin levels and dietary iron of participants were determined using Spearman's correlation. The haemoglobin concentrations of vegetarians and non-vegetarians were also compared using t-test. Independent t-test was used to compare the mean levels of anthropometric measurements among vegetarians and non-vegetarians.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic and Socioeconomic Status of Vegetarians and Non-Vegetarians

A total of one hundred (100) participants (50 vegetarians and 50 non-vegetarians) were involved in this study. For both vegetarians and non-vegetarians, 38% were females and 62% were males. The participants were between the ages of 20 to 75 years. The vegetarians had higher level of education than the non-vegetarians. Most of the non-vegetarians were employed compared to the vegetarians. Smoking was not common in both groups, however, 72% of the non-vegetarians drunk alcohol.

Table 4.0 Demographic and socioeconomic status of vegetarian and non-vegetarians

	Vegetarians (n=50)		**Non Vegetarians (n=50)**	
Gender	**frequency**	**%**	**frequency**	**%**
Female	19	38	19	38
Male	31	62	31	62
Level of Education				
No formal education	1	2	0	0
Basic education	11	22	33	66
Secondary	4	8	0	0
Tertiary	34	68	17	34
Employment Status				
Employed	19	38	38	76
Self employed	8	16	0	0
Unemployed	3	6	7	14
Student	3	6	1	2
Retired	17	34	4	8
Lifestyle Behaviours				
Alcohol Intake	0	0	36	72%

4.2 Anthropometric measurements

The study did not show significant differences between the mean height of the vegetarians 1.67m compared to 1.65m of the non-vegetarians (*p*=0.154). The mean BMI of the vegetarians (25.19 Kg/m2) was significantly lower (*p* =0.013) than that of the non-vegetarians (27.73 Kg/m^2).

Classification into BMI categories showed that none of the non-vegetarians was underweight compared to two (2) vegetarians who had BMI below 18.5 Kg/m^2. Fifty-two percent (52%) of the vegetarians and 38% of non-vegetarians had normal weight. Overweight was found in 34% and 38% of vegetarians and non-vegetarians respectively. Ten percent (10%) of the vegetarians and 22% of non-vegetarians were obese.

Table 4.1 Anthropometric measurements of participants

Variables	Group	Mean	S.D.	*p*-value	95% C.I.	
Weight (kg)	Vegetarian	70.02	12.71	0.074	-11.007	0.511
	Non Vegetarian	75.27	16.11			
Height (m)	Vegetarian	1.67	0.07	0.154	-0.009	0.053
	Non Vegetarian	1.65	0.08			
Body Mass Index (Kg/m^2)	Vegetarian	25.19	4.29	0.013*	-4.547	-0.537
	Non Vegetarian	27.73	5.71			
Visceral FAT	Vegetarian	8.52	4.12	0.325	-2.225	0.745
	Non Vegetarian	9.26	3.33			

***significant n=50**

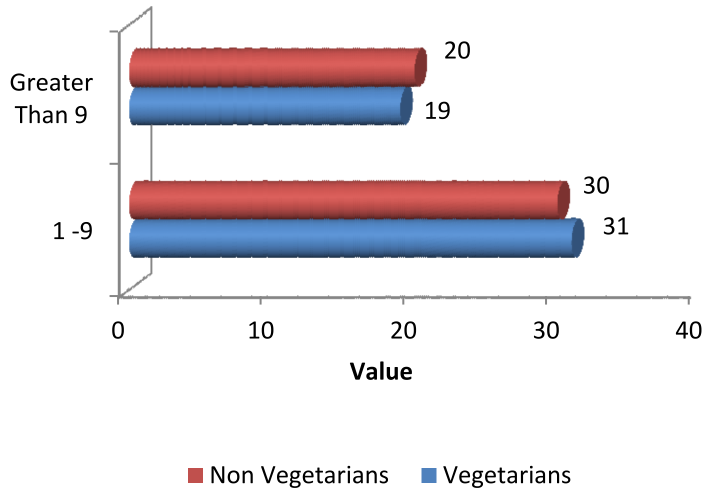

Figure 4.0 A graph showing the Visceral fat of vegetarians and non-vegetarians between 1-9 and greater than 9. 31 vegetarians and 30 non-vegetarians had normal visceral fat (between 1-9).

4.3 Types and Years of Vegetarianism

Vegetarians who participated in the study were vegans, lacto-vegetarians and ovo-lacto vegetarians. They had been practicing vegetarianism between 3 and 50 years. Reasons for practicing vegetarianism were stated as promoting reverence for life, supporting personal health and healing, protecting the environment and upholding religious and philosophical principles. The type of vegetarians with the highest percentage (56%) was the lacto-vegetarians, followed by vegans with 40% and then ovo-lacto vegetarians with 4%.

4.4 Dietary and Meal Pattern

About half of the participants from both groups (50% of the vegetarians and 52% of the non-vegetarians) ate two meals per day whiles 48% of the vegetarians and 44% of non-vegetarians ate three meals per day. The results are illustrated in Figure 4.3

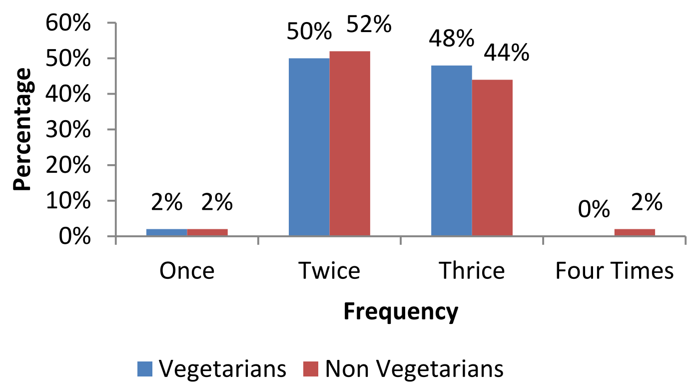

Figure 4.1 A graph showing the percentage of vegetarians and non-vegetarians and the number of meals they ate per day.

Thirty-nine vegetarians ate fruits daily as compared to 21 non-vegetarians. On a whole the consumption of fruits and vegetables was much higher in vegetarians than in non-vegetarians.

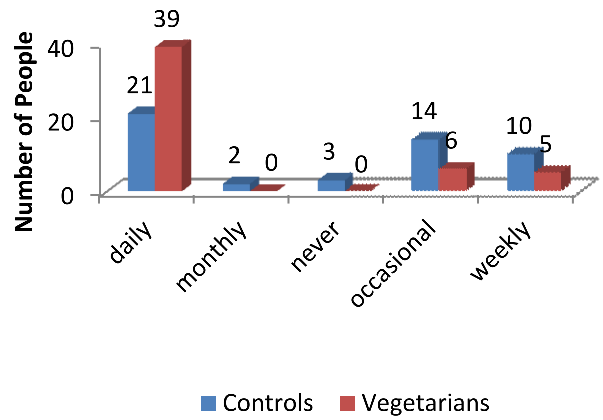

Figure 4.2 Frequency of fruits and vegetable intake of vegetarians and non-vegetarians (control).

4.5 Diet History

4.5.1 Comparison of nutrient intake between vegetarians and non-vegetarians

The mean energy, protein, fat, cholesterol and vitamin B_{12} intake were significantly lower (all p <0.05) in vegetarians than the non-vegetarian. There were no significant differences in carbohydrate, fibre, folate, vitamin C and iron intakes between the two groups.

Table 4.2 Comparison of nutrient intake between vegetarians and non-vegetarians

	Group	N	Mean	S.D.	*p*-value	95% C.I.	
Energy	Vegetarian	50	1432	680	0.030*	-698.71	-29.73
	Non Vegetarian	50	1796	979			
Protein (g)	Vegetarian	50	39.47	22.09	0.001*	-55.72	-11.96
	Non Vegetarian	50	73.31	74.77			
Carbohydrate(g)	Vegetarian	50	270	128.82	0.250	-95.15	24.75
	Non Vegetarian	50	306	170.40			
Fat (g)	Vegetarian	50	26.68	21.30	0.030*	-21.58	-1.40
	Non Vegetarian	50	38.18	28.97			
Cholesterol(mg)	Vegetarian	50	8.83	11.91	0.001*	-122.22	-61.78
	Non Vegetarian	50	100.83	107.01			
Dietary_fibre (g)	Vegetarian	50	26.29	14.66	0.360	-10.42	3.79
	Non Vegetarian	50	29.60	20.63			
Vit.B12(μg)	Vegetarian	50	0.25	0.35	0.001*	-2.71	-1.61
	Non Vegetarian	50	2.41	1.93			
Folate (μg)	Vegetarian	50	148.53	81.44	0.650	-53.19	33.19
	Non Vegetarian	50	158.53	130.59			
Vitamin_C(mg)	Vegetarian	50	100.43	105.08	0.480	-27.60	58.24
	Non Vegetarian	50	85.11	111.12			
Iron (mg)	Vegetarian	50	15.03	7.30	0.340	-5.07	1.77
	Non Vegetarian	50	16.67	9.76			

***significant**

4.6 Dietary Iron

The results of the mean iron values for the age range and gender are seen in table 4.2

Analysis of variance did not show any significant difference in the mean levels of iron intake among vegetarian males and females using the age groups (p>0.050)

Table 4.3 Mean iron intake of vegetarians and non-vegetarians

Vegetarians					
Males	**N**	**Mean**	**S.D.**	**F-value**	**P-value**
20-30	3	14.1	11.15	0.238	0.79
31-50	5	17.78	2.03		
51-80	23	15.96	7.68		
Total	31	16.07	7.28		
Females					
20-30	1	10.6	.	3.16	0.07
31-50	5	7.4	3.24		
51-80	13	15.81	7.21		
Total	19	13.32	7.19		

Non Vegetarians

Males	**n**	**Mean**	**S.D.**	**F-value**	***p*-value**
20-30	2	7.71	4.511	1.041	0.366
31-50	16	17.782	10.264		
51-80	13	18.558	9.951		
Total	31	17.458	9.994		
Females					
20-30	1	4.01	.	2.389	0.124
31-50	5	21.86	10.04		
51-80	13	13.777	8.39		
Total	19	15.39	9.489		

4.7 Vegetarians and non-vegetarians with low Hb Levels

In all a total of twelve 12 (24%) vegetarians and 13 (26%) non-vegetarians were anaemic as shown in table 4.3.

Table 4.4 Participants with low Hb Levels

Participants	Vegetarians	Non-vegetarians
Males (Hb < 13.0)	7	7
Females (Hb<11.5)	5	6
Total	12	13
Percentage	24%	26%

Reference range for males 13.0g/dl and females 11.5g/dl (according to haematology analyser)

4.8 Mean Full blood count values for participants

The mean lymphocyte (LYM), granulocyte percentage and RDW were significantly lower ($p<0.05$) in the vegetarians than non-vegetarians. The MCV in the vegetarians on the other hand was significantly higher ($p<0.05$) in the vegetarians than non-vegetarians. There were no significant differences in HGB (Hb), HCT, MCHC, MCH, RBC and WBC between the groups.

Table 4.5 Mean Full Blood Count of participants

	Group	n	Mean	S.D.	*p*-value	95% C.I.	
WBC	Vegetarian	50	3.10	1.19	0.099	-4.303	0.375
	Non Vegetarian	50	5.07	8.25			
LYM_Percentage	Vegetarian	50	52.47	10.08	0.842	-4.436	3.624
	Non Vegetarian	50	52.88	10.23			
MON_Percentage	Vegetarian	50	18.44	3.65	0.001*	3.599	6.205
	Non Vegetarian	50	13.53	2.87			
GRA_Percentage	Vegetarian	50	29.09	8.75	0.022*	-8.334	-0.658
	Non Vegetarian	50	33.59	10.51			
LYM#	Vegetarian	50	1.54	0.59	0.028*	-1.449	-0.083
	Non Vegetarian	50	2.31	2.36			
MON#	Vegetarian	50	0.53	0.27	0.747	-0.257	0.185
	Non Vegetarian	50	0.57	0.74			
GRA#	Vegetarian	50	1.03	0.54	0.121	-2.635	0.311
	Non Vegetarian	50	2.19	5.22			
RBC	Vegetarian	50	4.74	0.55	0.828	-0.210	0.262
	Non Vegetarian	50	4.71	0.64			
HGB	Vegetarian	50	13.30	1.47	0.666	-0.560	0.872
	Non Vegetarian	50	13.14	2.08			
HCT	Vegetarian	50	41.76	4.50	0.177	-0.707	3.783
	Non Vegetarian	50	40.22	6.61			
MCV	Vegetarian	50	88.12	5.33	0.036*	0.180	5.140
	Non Vegetarian	50	85.46	7.05			
MCH	Vegetarian	50	28.13	2.48	0.591	-0.783	1.367
	Non Vegetarian	50	27.84	2.92			
MCHC	Vegetarian	50	31.87	1.65	0.052	-1.244	0.007
	Non Vegetarian	50	32.49	1.50			
RDW	Vegetarian	50	13.06	1.04	0.030*	-0.922	-0.048
	Non Vegetarian	50	13.54	1.16			

***significant**

4.8.1 Mean Hb levels for vegetarians and non-vegetarians

The mean Hb for the vegetarian females, anaemic vegetarian females and anaemic vegetarian males were significantly lower (p<0.05) than in non-vegetarian females, anaemic non-vegetarian females and anaemic non-vegetarian males. There were no significant differences between male vegetarians and male non-vegetarians.

Table 4.6 Mean Hb levels for vegetarians and non-vegetarians

Hb	Group	Mean	S.D.	P-value
Males	Vegetarian	14.00	2.20	0.588
	Non Vegetarian	13.80	1.40	
Females	Vegetarian	11.80	0.80	0.001*
	Non Vegetarian	12.50	1.20	
Anaemic Females	Vegetarian	10.10	0.40	0.009*
	Non Vegetarian	11.10	0.40	
Anaemic Males	Vegetarian	11.60	0.70	0.001*
	Non Vegetarian	12.40	0.80	

***significant**

4.9 Relationship between Haemoglobin (Hb) levels and dietary Iron

There was no significant correlation between haemoglobin levels and dietary iron of vegetarians and non-vegetarians (r=0.564; p=0.071).

4.10 Blood film

Blood thin film comment showed that for 80% of the vegetarians the sizes of the red cell had microcytosis (+) and macrocytoses (+). Again there was an indication of a dimorphic picture in terms of haemoglobin that is hypochromasia (+) and hyperchromasia (+). The blood picture of the rest (20%) of the vegetarians showed a normal blood film.

The blood film of the non-vegetarians showed 60% with few poikilocytosis (difference in the shape of RBC) (+) and hypochromasia (+). Also, 40% of the non-vegetarians showed a dimorphic picture in terms of the red cell sizes. The red cell also showed microcytosis (+) and macrocytosis (+). The haemoglobin content of the RBC were hypochromasia (+) and hyperchromasia(+).

CHAPTER FIVE

5.0 DISCUSSION

The demography of the participants revealed that there was not much difference in age and gender of both vegetarians and non-vegetarian group. This was expected since subjects were matched by age and gender. Comparing the educational status of the two groups show that, a higher proportion of the vegetarians (64%) had higher educational level than the non-vegetarians (34%). This however did not agree with the study conducted by Key *et al.* (1999) which stated that the variations in level of education between the vegetarians and non-vegetarians were small and inconsistent.

The higher educational level of the vegetarians could have increased the nutritional knowledge of the vegetarians since there were indications from the dietary history that many of them ate a balanced diet. This however did not reflect in the data since most of them were eating twice daily and the quantities of meals they ate were comparatively smaller than the non-vegetarians.

The employment status of participants gave an idea of the economic status of the individual. It was observed that more non-vegetarians were employed compared to the vegetarians. Although more than half of both the vegetarians and non-vegetarians were employed, it did not affect the number of meals consumed per day. About half of both the vegetarians and non-vegetarians ate 2 meals per day. Some employed participants stated their busy work schedule did not give them enough time to eat. Studies conducted revealed that most workers skipped meals because of time constraints and financial reasons (Park, 2009).

Anthropometric data is used to evaluate health and dietary status, disease risk, and body composition changes that occur over the adult lifespan (NHANES, 2007). The mean weight, height and visceral fat of the non-vegetarians were not significantly different. The mean BMI of the vegetarians was significantly lower (p =0.013) compared to the non-vegetarians. Animal products contain much more fat than plant-based foods. Vegetarian diets are naturally low in fat. Thus vegetarians may put on less weight than meat eaters. This was consistent with findings of similar studies by Appleby *et al* (1998) and Spencer *et al* (2003). Classification into BMI categories also showed that more vegetarians (52%) than non-vegetarians (38%) had their BMI within the normal range.

The protein sources of the two groups were quite different as the vegetarians ate more of plant based protein foods while non-vegetarians ate more of animal source of protein. Proteins are composed of amino acids, and a common concern with protein acquired from vegetable sources is having adequate intakes of the essential amino acids, which cannot be synthesised by the human body (Young and Pellett, 1994). The essential amino acids can also be obtained by eating a variety of complementary plant sources that, in combination, provide all eight essential amino acids. In the lacto-ovo vegetarians, dairy and egg products can provide all the essential amino-acid needed in the body. According to Emery and Sanders (2002) protein intake in vegetarian diets is only slightly lower than in meat diets and can meet daily requirements for any person. From this study however though the vegetarians had an average protein of 39.5 g which was significantly lower than the mean protein intake of the non-vegetarians 73.3 g (p=0.001) it did not meet the recommended daily allowance for protein which is 0.85 g/kg body weight (Brown *et al*., 2008). Studies at Harvard University as well as other studies conducted in the United States, United Kingdom, Canada, Australia, New Zealand and various European countries, have reported that vegetarian diets do provide sufficient protein intake as long as a variety of plant sources are available and consumed (Davis and Melina, 2003). Thus it is possible that the vegetarians in this study were not eating a variety of plant sources.

The significantly lower mean total fat and mean cholesterol intake of the vegetarians could be attributed to the fact that the vegetarian diet is mainly plant based and has lower levels of animal sources of fat.

Dietary fibre intake of vegetarians though lower than the non-vegetarians was not statistically significant. However, both the vegetarians and non-vegetarians met their RDA of dietary fibre within the range of 26 to 38 g/day (Brown *et al*., 2008).

The total energy, total fat and cholesterol was lower in vegetarians than non-vegetarian and agrees with the study done by Woo et al. (1998).

The vegetarians had significantly lower dietary intake of vitamin B_{12} than the non-vegetarians (p=0.001), which was also below the RDA of vitamin B_{12} (2.4 μg) (Brown *et al*., 2008). According to Davey *et al.* (2003) vegetarians are frequently deficient in vitamin B_{12}, because plant foods are not sources of vitamin B_{12}. According to the United States National Institutes of Health, natural food sources of vitamin B_{12} are limited to foods that come from animals. Since vegetarian's diet is mainly plant based, there is the likelihood of vegetarian being deficient in vitamin B_{12} levels. This may explain why most of the vegetarians had low vitamin B_{12} levels. Though lacto vegetarians and lacto-ovo vegetarians do take in some animal products, the diet history of these vegetarians showed that most of them took dairy product and/or eggs once a week.

According to Sanders and Reddy (1994) vitamin B_{12} deficiency is a real hazard in un-supplemented or unfortified vegan and vegetarian diets.

The mean folate of the vegetarians was lower than the non-vegetarians, however there was no significant difference between the values (p=0.650). The RDA for folate is 400μg (Brown *et al*., 2008). Both vegetarians and non-vegetarians did not meet the RDA for folate. The primary cause for folate deficiency is low intake of food sources rich in vitamins such as legumes and green leafy vegetables (Allen, 2008).

Vitamin C intake in the vegetarians was not significantly higher than in non-vegetarians (p=0.480). Although consumption of foods from the green leafy vegetables and fruit groups were higher in vegetarians than in non-vegetarian and majority of the vegetarians (78%) ate fruit daily compared to 42% of the non-vegetarians, the differences in the means were not statistically significant. The RDA for vitamin C is 90 mg for males and 75 mg for females (Brown, 2008). The vegetarians met the RDA for vitamin C

The dietary iron contents of the participants were grouped according to age and gender for both non-vegetarians and the vegetarians. The mean dietary iron intake among male participants was not significantly different between the vegetarians of different age groups.

All the males, from both groups met their RDI of 8 mg (Mahan et al, 2012). However, with the females, only those in the age range of 51-70 years met their daily iron intake. This may be due to the fact that women in this age-group had reached menopause and had lower iron needs of 8 mg.

All the women in the age group of 20-30 years from both groups did not meet their RDI of 18 mg. Most of the women in this age group were likely to have monthly menstruation period which leads to loss of blood and iron, hence the body's needs for iron is high among this group. This is agreeable to the study conducted by Verma *et al.*, (1998), which showed that more menarcheal girls were anaemic as compared to non-menarcheal girls. Due to the high iron need in this age group, it is important to replace the iron that could be lost through menstruation. It was observed that vegetarians in the age group of 31-50 years did not meet their daily iron intake.

The proportion of anaemic participants in both the vegetarians and non-vegetarians were not very different. There were no significant differences in most of the red cell Indices except for MCV and RDW. The results of the red cell indices show that both the vegetarians and non-vegetarians did not have iron deficiency anaemia.

The blood picture of the anaemic cases did not show marked microcytic hypochromic red blood cell in both vegetarians and non-vegetarians which is a feature of iron deficiency anaemia, indicating that the anaemia was not as a result of iron deficiency.

5.1 Limitations of the study

The use of self-reported dietary intakes may result in under-reporting or over-reporting of actual dietary intakes.

Samples may not be representative of populations due to potential subjectivity of researcher

The Esha F Pro software used for nutrient analysis did not have nutritional information on some of local foods and as such they were substituted with similar foods. This could also introduce bias in the dietary intake data.

5.2 Conclusion

The prevalence of iron deficiency anaemia in vegetarians in the study is low. Comparisons among vegetarians and non-vegetarians showed significantly low levels of protein, total fat, cholesterol and vitamin B_{12} and BMI among vegetarians. The mean protein level for vegetarians was 39.47 g and 73.31g for non-vegetarians. The mean fat for vegetarians and non-vegetarians were 26.68 g and 38.18 g respectively. The mean cholesterol level for vegetarians was 8.83 mg and that for the non-vegetarians was 100.83 mg. the mean vitamin B12 level for the vegetarians and non-vegetarians were 0.25 and 2.41 respectively. The mean BMI for vegetarians was 25.19 Kg/m2 and 27.73 Kg/m2 for non-vegetarians.

5.3 Recommendations

Dieticians, Nutritionist and Public Health professionals should educate the general public on the need to eat balanced and nutritionally adequate meals every day.

The Ghana Vegetarians Association should invite Dieticians or Nutritionists to educate their members on the need to fortify their foods with vitamin B_{12} and how to combine various plant foods to meet their daily requirements for protein.

Also, the Ghana Vegetarians Association should screen its members for folate and vitamin B_{12} deficiency since some were anaemic and had significantly low dietary intake of vitamin B_{12}. The serum iron levels of the vegetarians should also be checked to determine whether their iron levels are within normal levels.

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APPENDIX I

UNIVERSITY OF GHANA

SCHOOL OF ALLIED HEALTH SCIENCES

INFORMED CONSENT FORM

I, **Joana Ofori-Koranteng**, wish to conduct a research on the dietary intake and prevalence of iron deficiency anaemia among vegetarians and non-vegetarians in Accra. I am a Dietetic student of the School of Allied Health Sciences, College of Health Sciences, University of Ghana, Korle Bu.

The Aim of this study is to examine the dietary intake and the prevalence of iron deficiency anaemia among vegetarians and non-vegetarians.

About 5 ml of venous blood will be drawn from you to perform a full blood count and a thin film test. Body weight and height will also be measured to determine your nutritional status.

The information provided by the participants will be kept confidential. The participant is assured of confidentiality by the researcher, your data will only be known to the researcher. If the information is published in any scientific journal, you will not be identified by name. This study may contribute to the existing knowledge of vegetarian diet and their iron status.

There is no risk involved in the study except a little discomfort and bruises you might experience at the site where blood is drawn. Participating in this study is voluntary, without any cost. You are free to withdraw from the study at any point in time.

The results of blood analysis will be given to participants to enable them to know their Haemoglobin levels and other Red Blood Cell parameters.

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

CONSENT

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

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

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

TELEPHONE NUMBER: ……………………………..

NAME OF PARTICIPANT: ……………………………………………………….

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

MOBILE NUMBER: …………………………………

APPENDIX II

QUESTIONNAIRE FOR DIETARY INTAKE AND PREVALENCE OF ANAEMIA

Participant's ID ……………………….

Date …………………………….

Section A: Socio - Demographic Status (Please Tick ✓ where applicable)

1. Age of participant (years) …………………..
2. Gender Male ☐ Female ☐
3. Marital Status Single ☐ Married ☐ Divorced ☐ Widowed ☐ Separated ☐
4. Religion Christian ☐ Muslim ☐ Buddhists ☐ Hindu ☐ Others, please specify……………………….
5. Educational Background
   - No formal Education ☐
   - Basic Education (middle/JHS) ☐
   - Basic Education (middle/JHS) ☐
   - HND/Diploma Certificate Bachelor ☐
   - Degree Post Degree ☐
6. Employment Status Employed ☐ Unemployed ☐ Retired ☐ Student ☐

Section B: Vegetarianism (Please Tick ✓where applicable)

7. Are you a vegetarian? YES ☐ NO ☐

If No, please skip to section C.

8. If yes, how long have you been a vegetarian? …………………………………
9. Please indicate the type Vegans ☐ Lacto-vegetarians ☐ Ovo-lacto vegetarian ☐ Non vegetarian ☐

10. What is/are your main reason(s) for becoming a vegetarian?
    - To support personal health and healing ☐
    - To promote reverence for life ☐
    - To protect the environment ☐
    - To uphold religious and philosophical principles ☐

Section C: Dietary and Meal Pattern (Please Tick ✓ where applicable)

11. How many times do you eat in a day?
    Once ☐ Twice ☐ Thrice ☐
12. Do you normally skip breakfast?
    Yes ☐ No ☐
13. Do you take snacks in between your meals?
    Yes ☐ No ☐
14. If yes, what snacks do you take regularly?
    - Fruits and Vegetables ☐
    - Soft Drinks ☐
    - Pastries ☐
15. How often do you take fruits and vegetables?
    Daily ☐ Weekly ☐ Monthly ☐
    Occasionally ☐ Never ☐
16. What animal product do you consume regularly?
    Fish ☐ Red Meat ☐ Chicken ☐

Section D: Health Status (Please Tick ✓ where applicable)

17. Do you have any medical condition? YES ☐ NO ☐
18. If yes, please state the condition …………………………………………
19. Are you on cholesterol lowering medication? YES ☐ NO ☐
20. Do you take other medication or herbal treatment? YES ☐ NO ☐

21. If yes, please state ……………………………………………………..

22. Do you take food supplements? YES ☐ NO ☐

Section D: Life Style Behaviours (Please Tick ✓ where applicable)

23. Do you smoke? YES ☐ NO ☐

24. If yes, how often? Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐

25. Do you drink alcohol? YES ☐ No ☐

26. What type of alcohol do you drink regularly?

Wine ☐ Beer ☐ Spirits ☐ Brandy ☐

27. If yes, how often? Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐

28. On average, how much alcohol do you take daily?

$^1/_2$ Medium glass of wine ☐

1 Mini Guinness or Beer ☐

1 Tot of Spirit or Whisky ☐

Others please state. ………………………………..

FOR OFFICIAL USE

Anthropometric and Physical measurements

Weight ………………………….kg

Height………………….meters

BMI……………….Kg/m2

Visceral fat ………...%

24 HOUR RECALL

Type of Meal & Time eaten	Food Eaten	Handy Measure	Quantity (g)
Breakfast			
Snack			
Lunch			
Snack			
Supper			
Snack			

FOOD FREQUENCY QUESTIONNAIRE (Adapted from Asare.J, 2011**)**

FOOD ITEMS	Daily	Weekly	2-3/Wk	Monthly	Occasionally	Never
Animal protein (Fish, meat, snail, poultry, egg, liver, etc.)						
Milk						
Soft drinks sweets & ice-cream (Fanta, coke, sprite, yoghurt, fanice						
Vegetable protein (Cowpea, bean, soya bean, bambara, agushie, groundnuts, cashew nuts etc.)						
Vegetables (Carrots, cabbage, nkontomire, aleefu, okro, tomatoes, onions, garden eggs etc.)						
Cereals and Grains (Corn, millet, rice, sorghum, oats, wheat etc.)						
Tubers (Sweet potatoes, yam, plantain, cocoyam etc.)						
Fruit (Orange, pawpaw, pineapple, banana, watermelon, apple avocado etc.)						
Fruit juice (Ceres, Don Simon etc.)						
Others (specify)						

APPENDIX III

COMPLETE BLOOD COUNT MEASUREMENT PRINCIPLES

Red Blood Cells/Platelets

The RBCs and PLTs were measured by an electronic impedance variation principle, meaning an electronic field was generated around the micro-aperture through which the blood cells passed. The cells created a resistance in the electronic field as they passed through the calibrated micro-aperture. This in turn caused an electronic pulse to be generated which was amplified, measured and mathematically calculated to create a numerical value.

Haemoglobin (Hb) determination

The hemoglobin measurement was based on a startup cycle. The cycle included hemoglobin blank test sequence of two haemoglobin blank measurements. Each analysis cycle run after start-up, also had an Hb blank measurement which was compared to the initial start-up Hb blank. Each analysis cycle run thereafter compared the Hb blank read to the previous cycle Hb Blank reading. During the WBC analysis cycle, 0.5ml of ABX Lyse reagent was added to 2.05 ml of diluted blood in the WBC chamber. The Lyse reagent contained potassium ferricyanide [Fe(Cn)K] and potassium cyanide (KCN). The lysing reagent broke down the RBC cell membrane and released the haemoglobin within the RBC. The hemoglobin then combined with the KCN to form a chromogenous cyanmethaemoglobin compound. This chemical compound was measured by spectrophotometry, through the optical pathway in the WBC chamber at light wavelength measurement of 550nm.

Results: Hb results were were calculated as follows:

Hb = Log (Blank value/Sample value) x Calibration Coefficient.

Haematocrit (HCT) determination

The haematocrit is a combination measurement of electronic pulses and mathematical calculations. All the RBC pulses were grouped into various sizes. Each group pulse height was then averaged. All the pulse height averages were then averaged one final time for a mean average of all the RBC pulse heights [a function of the numeric integration of the mean cell volume (MCV)]. Results were given as a percentage of that integration.

Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

MCV was calculated directly by the autoanalyser from the entire RBC histogram. MCH was calculated from the hemoglobin value and the RBC count.

Calculations were as follows:

MCH (pg) = Hb/RBC x 10(pg) Picograms

MCHC was calculated according to the hemoglobin and hematocrit values. Calculation was as follows:

MCHC (g/dl) = HGB/HCT x 100

Red Cell Distribution Width (RDW)

The RDW is used to determine erythrocyte abnormalities linked to anisocytosis. The RDW enabled one to follow the evolution of the width of the RBC histogram in relation to the number of cells and their average volume. RDW was calculated as follows:

RDW (%) = K x SD/MCV

K: Calibration coefficient for RDW.

SD: Standard Deviation according to statistical studies on cell distribution.

MCV: (Mean Cell Volume) of the erythrocytes.

Mean Platelet Volume (MPV)

The MPV was directly calculated from the platelet histogram distribution curve. The calculation was almost the same as the MCV.

Plateletcrit (PCT)

Thrombocrit was calculated according to the formula:

PCT% = PLT (103/mm3) x MPV (μm3) / 10 000