UNIVERSITY OF GHANA
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FOLATE AND VITAMIN B_{12} STATUS OF A RURAL POPULATION IN THE UPPER MANYA KROBO DISTRICT OF GHANA

BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Ms Martha Kafui Akwetea towards the award of the Master of Science Degree in Dietetics in the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana.

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ABSTRACT

Background: Folate is a B vitamin found in green vegetables and fortified breakfast cereals. It plays a crucial role in the prevention of human diseases including heart disease, cancer, anaemia (megaloblastic), dementia in adults as well as neonatal defects. Despite the numerous benefits of folate**,** only sparse information exist on the folate status of Ghanaians. There is therefore the need to assess the folate status of different populations in different regions of the country, in order to determine possible health risk.

Aim: The study sought to assess the folate status of a rural adult population in Asesewa, Upper Manya Krobo Distruict of Ghana.

Methods: The study employed a cross-sectional design and a total number of 100 participants in Asesewa were recruited. In collecting the data a structured questionnaire was administered to obtain information on socio-demographic parameters. A validated food frequency questionnaire (FFQ) and a 3-day 24-hour recall were used to collect dietary data. Anthropometric and blood pressure measurements were taken. Fasting blood samples (5 ml) were collected for serum folate and vitamin B_{12} and analysed using ELISA kits.

Results: Out of the 100 who were recruited and completed all other proceedings, 63 participants were available for the blood samples collection. The results showed a high prevalence (71%) of folate deficiency in the study population with no deficiencies in vitamin B_{12}. Significant differences were found in energy, carbohydrate, protein, dietary fibre, folate and vitamin C ($p \leq 0.05$) among gender. No associations were found between dietary folate and serum folate or serum vitamin B_{12}. Age was the predictor of serum folate ($p=0.05$) in the population.

Conclusion: Folate deficiency was high in this population. The main predictor of folate in this population was age. Dietary folate was not associated with serum folate. There is a need for nutrition intervention to help improve folate status in this population.

DEDICATION

I dedicate this work to my lovely family whose support and guidance has earned me this success. All I have to say is that the God who sees in secret will reward you openly for all eyes to behold.

ACKNOWLEDGEMENTS

The plans I have for you are of good and not of evil, plans to prosper you and bring you to an expected end (Jeremiah 29:11).

My first thanks goes to God the Father, God the Son and God the Holy Spirit for how far He has brought me. Without the help of God I would not have come this far. Secondly, I thank my supervisors, Major (Rtd) Dr. George Asare and Dr. Matilda Asante who were so supportive throughout the thesis writing. I acknowledge their countless sacrifices and ideas which guided me through successfully. I also show my appreciation to Dr. Charles Brown, Mrs. Freda Intiful and all my lecturers and staff of the School of Biomedical and Allied Health Sciences who stood by my side all through and were always ready to offer me their help. I am also grateful to my family who supported me financially, emotionally, spiritually and physically. Finally I want to thank all my friends and loved one whose prayers, encouragement and assistance have earned me this success. May God reward all of you richly.

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LIST OF ABBREVIATIONS

FFQ	Food Frequency Questionnaire
CVD	Cardiovascular Diseases
THF	Tetrahydrofolate
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
MTHF	Methyltetrahydofolate
FTHF	Formyltetrahydrofolate
OH	Hydroxyl group
CN	Cyanide group
CVA	Cerebrovascular Accident
HoloTC	Holotranscobalamin
FBC	Full Blood Count
MMA	Methylmelonic Acid

CHAPTER ONE

1.0 BACKGROUND

1.1 INTRODUCTION

The B vitamins are water soluble vitamins that are needed in minute quantities to meet the body's daily requirement. Due to their ability to be soluble in water, they are absorbed into the small intestines by simple diffusion and are scattered in the aqueous parts of the cells (Mahan and Escott-Stump, 2008). Folate and vitamin B_{12} are B vitamins which depend on each other to perform numerous functions. They work together to enhance cell division as well as DNA synthesis (Schernhammer *et al.*, 2007). Due to their importance in DNA synthesis, it is very crucial at the early stage of development. Foetal growth requires rapid cell division and folate is needed for this activity, hence, there is an increase in demand during pregnancy (Scholl and Johnson, 2000; Lamers, 2011). Low intake of folate at this stage could result in neonatal birth defects such as cleft lip and palate and spina bifida. These deformities could retard growth leading to long term health issues. Low levels may also lead to other pathological states (O'Leary and Samman, 2010). Adequate intake during pregnancy is therefore necessary (Institute of Medicine, 2001).

Adequate consumption of folate rich foods mainly found in dark green leafy vegetables, fresh fruits, yeast, liver, nut, milk, eggs, beans, lentils and some fortified breakfast cereals may be helpful in improving folate status (U.S. Department of Agriculture, 2012). To help prevent deficiencies commonly consumed foods in countries and communities could be fortified with folic acid. In Ghana similar to Ethiopia, among all the cereals fermented maize is frequently consumed (Gibson *et al.*, 2008; Abdelrahim *et al.*, 2009), hence fortification of this food item will be helpful in improving folate status of individuals. On the other hand, vitamin B_{12} is only found in animal and animal products but some breakfast cereals could be fortified with the vitamin (U.S. Department of Agriculture, 2011).

Both vitamins function in erythrocyte and leucocyte formation which helps to prevent nutritional anaemias. Furthermore, adequate intake of folate and vitamin B_{12} is necessary to prevent pernicious, megaloblastic anaemias and certain cancers such as breast and cervical cancers (Castillo *et al*., 2012; Johnson, 2007, Ericson *et al*., 2007; Hernandez *et al*., 2003). Tinnitus, depression, multiple sclerosis, low sperm counts, diabetic neuropathy and asthma may result if vitamin B_{12} level in the body is below normal levels (Schneede and Ueland, 2005; Carmel, 2008). Folate deficiency also leads to raised concentrations of homocysteine which is a risk factor for CVDs (Clarke *et al*., 2010).

Folate deficiency was found to be common among developing countries compared to the developed countries. Prevalence of folate and vitamin B_{12} deficiencies was observed in some countries in the Sub-sharan African where intake of folate and other micronutrients were found to be low (Torheim *et al*., 2010). In Africa, studies have revealed that countries such as Nigeria, Zimbabwe, Sudan, Ethiopia and South Africa had some deficiencies in folate but there has been an improvement over the years (McLean *et al*., 2008, Haider *et al*., 2010). Currently, it is uncommon to observe folate deficiencies in developed countries such as USA and Canada (MacFarlane *et al*., 2011; Bailey *et al*., 2010). These countries showed low or no folate deficiency across all age groups in the population. Vitamin B_{12} deficiency is often not common in population but it is a major problem among older adults 50 years and above with atrophic gastritis

(Johnson, 2007; Andrés *et al*., 2005; Institute of Medicine, 1998; Huritz, 1997). Other researchers have shown that lifestyle practices such as smoking and alcoholic consumption can lead to folate deficiencies (Bentley *et al*., 2006; Ortega *et al*., 1994; Piyathilake *et al*., 1994).

Researches done among individuals in developed and developing countries showed that consumption of folic acid fortified foods reduced the prevalence of folate deficiency (Modjadji and Alberts, 2007; Hoey *et al*., 2007). Mandatory fortification of cereal and grain products was implemented in 1998 in the United States (Rader, 2002). In the UK, several foods particularly breakfast cereals are voluntarily fortified with folic acid by manufacturers. Folic acid fortification of foods is rare in developing countries including those in Africa although some communities have been shown to have high incidence of folate deficiency. In South Africa, it is reported that pregnancy and lactation related folate deficiency remained a common cause of megaloblastic anaemia (Ingram *et al*., 1999). In Ghana, fortification of foods with folic acid is not widely spread and there are few studies on folate status of populations. This makes it difficult to determine the prevalence of megaloblastic anaemia in the Ghanaian population. A cross-sectional study conducted by Owusu *et al*, (2010) among Ghanaians in UK and an urban population in Accra, found an improved folate status in the UK population and this was associated with consumption of fortified food and the use of folic acid supplements. This study was done in two urban populations and the adequacy of folate intakes in rural populations is unknown. There is therefore the need to assess folate intakes in specific groups due to its numerous health benefits. Assessing the folate status of the Asesewa community in Ghana will provide baseline data for future studies in rural areas. There is therefore the need to assess folate intakes in specific groups due to its numerous health benefits.

1.2 PROBLEM STATEMENT

Nutritional anaemia is caused by many factors including folate, vitamin B_{12} and iron deficiencies (Bolaman *et al*., 2003; Kuzminski *et al*., 1998). In Ghana, most published data on nutritional anaemia have focused on iron deficiency anaemia (Mockenhaupt *et al*., 2000) with limited data on megaloblastic anaemia. Iron deficiency anaemia has been established as a major nutritional problem in Asesewa. It is recorded among the main causes of morbidity in the town (Upper Manya District Health Directorate, 2012). It is unknown whether other types of anaemia associated with folate deficiency are also a problem in this community. An understanding of the relationship between diet; folate and vitamin B_{12} status in this population is needed. Knowing the numerous ill effects of folate and vitamin B_{12} deficiencies there is the need to assess the adequacy of these vitamins in this population.

1.3 SIGNIFICANCE OF STUDY

Findings from this research will provide information that can be used by dietitians and other nutrition-related health professionals to plan evidence-based dietary guidelines for the population in Asesewa. This research will also add to data on nutritional anaemias in the Upper Manya Krobo district. It will also provide baseline data for further studies on folate and vitamin B_{12} status in this population.

1.4 HYPOTHESIS

Dietary intakes of the adult population in Asesewa will have no significant effect on their folate and vitamin B_{12} status.

1.5 AIM AND OBJECTIVES

1.5.1 Aim

The aim was to assess folate and vitamin B_{12} status of the adult population in Asesewa, a rural community in the Upper Manya Krobo District of Ghana.

1.5.2 Objectives

The objectives were:

i. To determine the prevalence of folate and vitamin B_{12} deficiencies in the population.
ii. To compare folate and vitamin B_{12} intakes among gender.
iii. To determine the relationship between dietary folate and serum folate level as well as dietary vitamin B_{12} and serum vitamin B_{12}.
iv. To determine the predictors of serum folate and vitamin B_{12} in this population.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FOLATE

2.1.1 Background

Most often folate is used interchangeably with folic acid (folacin). Folate and folic acid are used to describe natural and synthetic sources respectively. The synthetic form of folate is the form used in food fortification and supplementation. Pteroylmonoglutamic acid and pteroylmonoglutamate are also used to refer to folic acid and folate respectively. According to Gregory (2001), folate is less bioavailable compared to folic acid which is mostly found as small tablets or used in minute forms in foods. In addition, folate is destroyed by prolonged boiling and may be lost in the process (Eitenmiller and Landen, 1999; Mckillop *et al*., 2002) hence such cooking methods are to be avoided or limited.

Folate is mostly found in nature as folyl polyglutamates, containing most of the active forms, which is often hydrolysed into monoglutamate during digestion (Institute of Medicine, 1998). Some sources of this vitamin include meat, liver, beans, lentils, mushrooms; dark green leafy vegetables such as spinach, *kontomire, aleefu, boma, bokoboko, shuwaka, ademe,* dandelion; and fortified or enriched foods such as bread, flour, cornmeal, pastas, rice and other grain products (Carmel, 2005; U.S. Department of Agriculture, 2012).

2.1.2 Functions

Folate functions in the synthesis, repair and methylation of DNA (Weinstein *et al*, 2003) other vitamins such as cobalamin, pyridoxine and riboflavin also play a role in remethylation of homocysteine (Mckinley *et al*., 2001). This vitamin is very important in the methylation of cystosine base at the G-C site in DNA, which is the main site for turning on or off genes (Shane, 1995). Folate is needed in transferring a methyl group to deoxyuridylate for the conversion to thymidylate an important substance for cell proliferation (Carmel, 2005). Thymidyalate is also important in the conversion of histidine to glutamic acid which when absent may lead to the accumulation of waste products such as formimonoglutamic acid (Mahan and Escott-Stump, 2008). Folate is important for the conversion of homocysteine to an amino acid called methionine. Homocysteine is a blood toxin which when accumulated in the blood may increase the risk of CVD (Homocysteine Studies Collaboration, 2002) but adequate amount of folate in the body may reduce the risk.

2.1.3 Digestion, Absorption and Storage

During digestion folate is hydrolyzed and changed into monoglutamate. This form is then reduced to tetrahydrofolate (THF) prior entering the blood. Tetrahydrofolate (THF) is further converted into 5-methyl-tetrahydrofolate (5-MTHF) and 10-formyl-tetrahydrofolate (10-FTHF) (Fig 2.1). The 5-MTHF is the form of folate found in plasma (Bailey and Gregory, 2006).

The bioavailability of dietary folate is variable due to the nature of its different forms, the presence and absence of conjugase inhibitors (Pentieva *et al*., 2004), folate binders and the nutritional status of the host. Many foods (dairy products), herbs (green and black tea), other substances like alcohol, tobacco and some drugs antidepressants, anticonvulsants; non-steroidal anti-inflammatory drugs – NSAIDs – such as ibuprofen and asprin) may inhibit the absorption of folate (Alemdaroglu *et al*., 2008; Nygren-

Babol and Jagerstad, 2012; Pfeiffer *et al*., 2013; Apeland *et al*., 2001). About 85% of folic acid is estimated to be bioavailable when consumed while 50% of natural folate is bioavailable (Carmel, 2005; Institute of Medicine, 2000). Another study by Winkels *et al*., (2007) revealed that, the bioavailability of folate from vegetables and fruits as well as liver is about 80% compared to folic acid. Research has shown that release of dietary folate during digestion may be dependent on the uptake from plant cells (Castenmiller *et al*., 2000; Pfeiffer *et al*., 1997).

Folate is transported actively through the intestines and plays a key role in regulating body folate homeostasis. Absorption of folate in the intestines requires a special acidic pH-dependent carrier-mediated process that is involved in the reduced folate carrier (RFC) (Fig. 2.1) and proton-coupled folate transporter, heme carrier protein 1 (PCFT/HCP1). Folate in the body adds up to 10 – 30 mcg, out of this about half of it is stored in the liver (Bailey and Gregory, 2006; Carmel, 2005) and the remaining in other tissues.

2.1.4 Deficiencies

Many diseases can arise when folate intake is inadequate. Intake below the recommended intakes of 400 mcg may lead to folate deficiency resulting in mutation and epigenetic methylation (Kim *et al*., 1997). During pregnancy, folate, plays a role in DNA synthesis. Deficiencies could lead to neural tube defect (NTD) (Lamers, 2011),

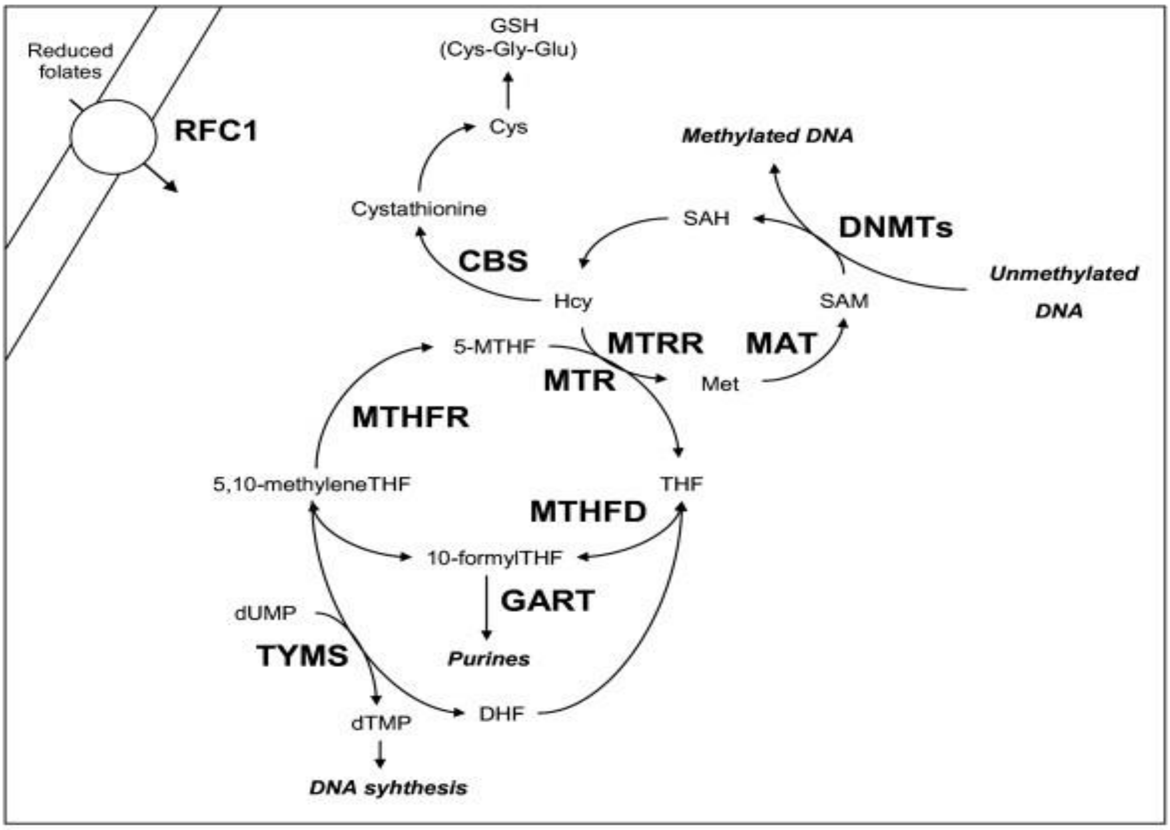

Figure 2.1: Folate metabolic pathway – Reduce folate carrier (RFC1) transports folate. Methylenetetrafolate reductase (MTHFR) reduces 5,10-methylenenetetrahydrofolate (5,10-MTHF) to 5-MTHF. A methyl group is transferred by methionine synthase (MTR) from 5-MTHF to homocysteine (Hcy) to form methionine (Met) and tetrahydrofolate (THF). Met is then changed to S-adenosylmethionine (SAM) by methionine adenosyltransferase (MAT). SAM is then changed into S-adenosylhomocysteine (SAH) by DNA methyltransferases (DNMTs) methylating DNA. Cobalamine a cofactor of MTR is maintained by methionine synthase reductase (MTRR). Hcy can also be condensed with serine to form cystathionine with the enzyme cystathionine β-synthase (CBS) which needs vitamin B_6 as a cofactor. Glutathionine (GSH) is formed with cystathionine. THF derivatives are used by thymidylate synthase (TYMS) and methylenetetrahydrofolate dehydrogenase (MTHFD) for nucleic acid precursor synthesis de novo. THF derivatives are converted by MTHFD for the synthesis of purine (with the help of phosphoribosylglycinamide transformylase (GART), Met, thymidylate. TYMS uses 5,10-MTHF and deoxyuridine monophosphate (dUMP) for deoxythymine monophosphate (dTMP) and dihydrofolate (DHF) production for the synthesis of pyrimidines (Coppedè *et al*., 2010).

low birth weight and foetal growth retardation (Scholl and Johnson, 2000). Folate is important for the conversion of homocysteine to methionine, an essential amino acid therefore, deficiencies of this vitamin leads to increased levels of homocysteine (Bailey and Gregory, 2006; Carmel, 2005). Homocysteine is a toxin which may impede the normal function of a lot of crucial compounds hence high levels in the blood may lead to oxidative stress and inflammation eventually resulting in atherosclerosis (Malinow, 1999). Depression may also occur during deficiency of the vitamin since it is heavily involved in the metabolism of many neurotransmitters, such as dopamine and serotonin, which regulate mood (Coppen and Bolander-Gouaille, 2005; Morris *et al*., 2003). Megaloblastic anaemia is a manifestation of folate deficiency (Bailey and Gregory, 2006) but was initially recognized medically as macrocytic anaemia and was distinguished from pernicious anaemia lately (Lanska, 2009). Dementia (loss of memory) a condition mostly found among the elderly could be a result of low folate levels in the blood (Clarke, 2008).

2.2 FACTORS THAT AFFECT FOLATE STATUS

2.2.1 Dietary Intake

Folate status depends on the intake from food and supplements as well as on the bioavailability of the various forms of this vitamin. Deficiency of vitamin C and minerals like iron may impair folate utility (Stokes *et al*., 1975). Dairy products and tea especially green tea have been found to inhibit dietary folate absorption (Alemdaroglu *et al*., 2008; Nygren-babol and Jagerstad, 2012). Increased consumption of coffee can also inhibit the absorption of folate (Verhoef *et al*., 2002). Adequate intake of folate for both males and females aged 19 years and above, is 400 mcg. Pregnant and lactating mothers need higher intakes of 600 and 500 mcg respectively (Institute of Medicine, 2001). Adequate folate intake is very importance at all stages in the life cycle especially foetal development, therefore pregnant women are given folic acid to meet their daily requirement (Scholl and Johnson, 2000).

2.2.2 Smoking

Tobacco contains carcinogenic and volatile chemicals which can interfere with folate uptake or may alter DNA synthesis thereby affecting health (Hernandez *et al*., 2003; Pfeiffer *et al*., 2013). Depletion of folate is therefore common among smokers leading to poor DNA formation (DeMarini, 2004). Prolonged use may lead to poor health and impaired cell division making individuals at a high risk of cancer (Shrubsole *et al*., 2004; Beilby *et al*., 2004; Hussien *et al*., 2005). A research conducted in New Zealand pointed out that, smoking reduces the serum folate by 10% which could result in rapid depletion with prolong used (Bradbury *et al*., 2014).

2.2.3 Alcohol

Alcohol intake has a direct effect on the liver which plays a role in folate storage and can also lead to cancer (Llewellyn, 2004; Rodriguez *et al*., 2004; Shiu and Chen 2004; Petridou *et al*., 2002). Folate deficiency can speed up alcohol-induced effect in hepatic methionine metabolism also resulting in enhanced oxidative liver injury and the histopathology of alcoholic liver disease (ALD) (Halsted *et al*., 2002; Björck *et al*., 2006, Prohit *et al*., 2007).

2.2.4 Drugs

Some drugs, especially those that are used to prevent seizures, such as phenobarbital, phenytoin, and methotrexate can inhibit folate absorption (Linnebank *et al*., 2011; Ortiz et al., 2000). Other drugs which account for low serum folate concentrations include anti-inflammatory drugs which include non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and oral contraceptives (Apeland *et al*., 2001).

2.2.5 Methylenetetrahydrofolate reductase (MTHFR) polymorphism

Methylenetetrahydrofolate reductase (MTHFR) polymorphism functions in the distribution of one-carbon units for nucleotide synthesis (Fig. 2.1). A common mutation in the MTHFR polymorphism (677C$\rightarrow$T) has been associated with high concentrations of plasma homocysteine and vascular disease. The mutation impairs the remethylation of homocysteine to methionine which may lead to hyperhomocysteinemia which is a risk factor for hypertension and cancer (Dong *et al*., 2008). The prevalence of the genotype varies among different ethnic groups. It is rare in African populations, and ranges between 5% and 15% in Europe and North America respectively (Adjalla *et al*., 2003; Botto and Yang, 2000) therefore folate deficiency in these countries is likely to be the major cause of hyperhomocysteinemia.

2.3 VITAMIN B_{12}

2.3.1 Background

Vitamin B_{12}, also known as cobalamin, (Fig. 2.2) is a structure made up of a corrin ring (four pyrroles) with mineral element called cobalt (metal) in the central position (Weir and Scott, 1999).

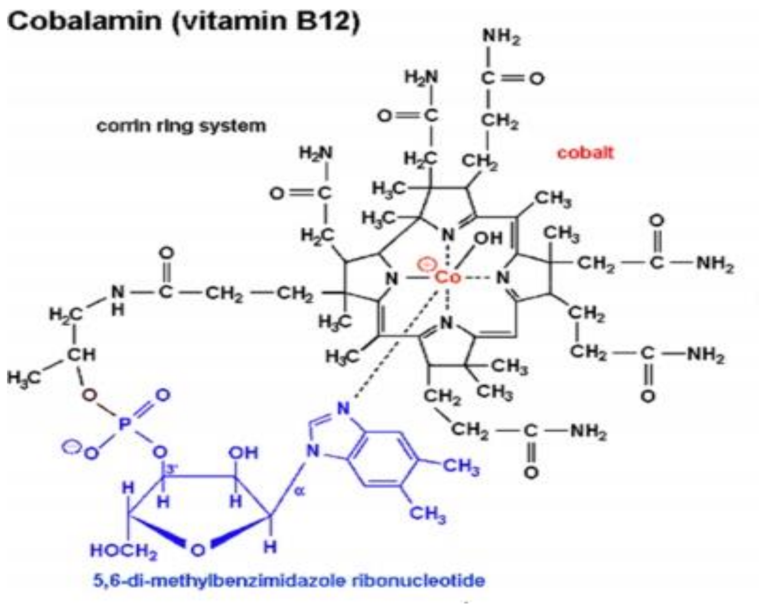

Figure 2.2: Structure of cobalamin

There are many examples of cobalamin however, 2-methylcobalamin and 5-deoxyadenosylcobalamin, are active in human metabolism (Institute of Medicine, 1998). Other forms found in nature are hydroxycobalamin (-OH group attached to the cobalt) and aquacobalamin (-H_2O group attached to the cobalt). Cynocobalamin (-CN attached to the cobalt) is the semi-synthetic form of coblamin which is mostly used in food supplements as wells as fortified foods (Institute of Medicine, 1998). Hydroxycobalamin, aquacobalamin and cynocobalamin are finally converted to active forms (methylcobalamin or deoxyadenosylcobalamin) through enzymatic acitivity.

Vitamin B_{12} is a very essential nutrient which is synthesized in bacteria and algae, but not plants. Bacteria in the human gut are also known to synthesize this B vitamin. Research has shown that the cells of mammals have two of these enzymes which are methionine synthase (utilizes methylcoblamin) and L-methylmalonyl Coenzyme A mutase (utilizes 5-deoxyadenosylcobalamin) (Scott and Weir, 1994). Animal products are the only source of Vitamin B_{12} however, some foods such as flour and breakfast cereals could be fortified with vitamin B_{12}. Strict vegetarians who may lack this vitamin in their diet may make it up with fortified foods such as marmite (Subar *et al*., 1998; Institute of Medicine, 1998).

2.3.2 Functions

2.3.2.1 Neurological function

Cobalamin in the form of methylcobalamin helps nourish the neurons and nerve tissues keeps them very healthy (Mahan and Escott-Stump, 2008). Vitamin B_{12} is needed in the formation of myelin sheaths, an axon enwrapping sheath composed of protein and fatty materials which acts as an electric insulator to carry electric impulses along the neurons (Scalabrino, 2009). These myelin sheaths protect the neurons in the brain, spinal cord and the peripherals) from neurotransmitter glutamate capable of exciting nerves. The hyper-selectivity of the blood brain barrier (BBB) prevents substances, able to excite cells, from entering nerve tissue. Vitamin B_{12} finds it difficult to cross the barrier even when levels are normal (Hinde, 2005).

2.3.2.2 Caloric level enhancer

Vitamin B_{12} has the ability to boost energy wiping out fatigue and weakness, which are symptoms of anaemia. Due to its property, it is used to enhance performance among sportsmen. Individuals with normal levels of this B vitamin will have their energy enhanced unlike those who are nutritionally deficient (Lukaski, 2004).

2.3.2.3 Haematopoeisis and ontogenesis

Constant and rapid cell dividing cells such as erythrocytes and leucocytes are dependent on vitamin B_{12} for their production and maturation. Vitamin B_{12} performs this function together with folate. During blood production iron is able to function well in the presence of these B vitamins (Planinz, 2015).

2.3.3.4 Cofactor

Vitamin B_{12} acts as a cofactor in two metabolic pathways which are very essential to human life. These pathways are: Homocysteine conversion to methionine and Methylmelonic acid (MMA) conversion to succinyl-Coenzyme A.

A. Homocyteine conversion to methionine

The active form of the vitamin methylcobalamin is needed for the normal activity of methionine synthase. This enzyme is needed for the production of homocysteine, methionine and amino acids. Methionine is then used in the synthesis of S-adenosyl methionine (SAM) which makes methyl groups available to DNA, RNA (genetic materials) and proteins (Fig. 2.1) (Coppedé *et al*., 2010). The methylation may turn a gene on or off increasing or decreasing the onset of cancer respectively (Choi and Mason, 2000).

B. Methylmelonic acid (MMA) conversion to succinyl-Coenzyme A

In this pathway, 5-deoxyadenosylcobalamin is needed by L-methylmalonyl-Coenzyme A mutase to change L-methylmalonyl-Coenzyme A to succinyl-Coenzyme A. Afterwards succinyl-Coenzyme A is used in the breakdown of lipids and protein for energy during the period of fasting or starvation (Holt and Riddle, 2003).

2.3.3 Digestion, Absorption and Storage

It is well established that B_{12} is only produced in bacteria, including those in the gut. As such the production of the synthetic form of the vitamin is only made possible by bacteria fermentation (Antony, 2000; Ansbacher *et al*, 1949). Vitamin B_{12} is bounded to protein forming a protein-B_{12} complex. This protein is then dissociated from the vitamin in the presence of hydrochloric acid (HCl) and pepsin (active form of pepsinogen) secreted by the parietal and gastric chief cells respectively (Institute of Medicine, 1998). The unbound B_{12} is quickly attached to R-binder (B_{12} binding protein), also called transcobalamin I forming holotranscobalamin I (holoTC I) (Scalabrino, 2001).

R-binder is a collection of glycoprotein which is distributed in many human body fluids such as serum. During digestion, the glands in the stomach and mouth (salivary glands) secrete these R-binders which inhibit the alteration of B_{12}, in its unbound state (Scalabrino, 2001). Gastric parietal cells also secrete intrinsic factors, glycoprotein, which moves to the small intestines to enhance the complex absorption of the vitamin. Again along the gastrointestinal tract, the pancreatic proteases detach B_{12} from the R-binder in the duodenum. The neutral pH in the duodenum makes it very conducive for the B_{12} to bind to intrinsic factor (IF). The B_{12}-IF complex is then absorbed in the distal part of the ileum through the ileal receptors, on the enterocytes, by endocytosis (Klee, 2000). In the enterocytes B_{12} is again dissociated from IF and is then bound to transcobalamin II forming holotranscobalamin II (holoTC II). Finally, the complex moves through the blood and cerebrospinal fluid to all body tissues such as the bone marrow. The transcobalamin II is cleaved off making the vitamin available to the body for its functions. In the cells, B_{12} is given off as hydroxocoblamin which is changed to its active forms and is used for their functions (Groff and Gropper, 2000).

The synthetic form of cobalamin (cynocobalamin) is readily available that is, it is not bound to a protein. During manufacturing certain substances are added to this form of cobalamin to increase its absorption. Cynocobalamin is absorbed into the blood stream at a faster rate compared to the form in animal products. When intake is poor, absorption of B_{12} is elevated which may prolong deficiency to the period of 20 – 30 years (Herbert, 1994). It is easier to determine the period of deficiency onset among strict vegetarians who are not on supplements.

Individuals who consume animal and animal products are able to store about 2000 – 3000 μg of B_{12} and may eliminate as little as 3 μg/day (Institute of Medicine, 2000). More than half (60%) of the absorbed vitamin B_{12} is stored in the liver, bound to transcobalamin III, while only about 30% is stored in the muscle making liver a rich source of the vitamin (Messina and Messina, 1996). Research has shown that about 0.1 – 0.2% of B_{12} is eliminated daily and the highest limit may occur in pernicious anaemia (Institute of Medicine, 2000).

2.3.4 Deficiencies

Adequate intake of cobalamin could be met through frequent intakes of animal and animal products. Foods fortified with vitamin B_{12} as well as nutritional supplements help to meet the daily requirement. Older adults, who are mostly prone to poor absorption, are encouraged to depend on fortified foods and food supplement to prevent deficiency diseases and improve upon their status (Institute of Medicine, 1998).

Vitamin B_{12} deficiency is known as hypocobalaminemia indicating low levels of cobalamin in the blood. This deficiency is mainly associated with poor absorption not necessarily low intake as individuals who take in more of B_{12} are also prone to deficiency diseases (McBride, 2000). Since the liver stores most of the B_{12} in the body hepatic disorders may lead to poor storage resulting in deficiency. Individuals with deficiency may also present with megaloblastic anaemia, neuropathy and poor metabolic activity (Babior, 1995). It has been observed that neurological defects appear before B_{12} deficiency anaemia but sometimes it can be without anaemia (Clarke, 2008). Since B_{12} is needed in cell division, when there is deficiency state erythrocytes are unable to divide leaving large undivided cells known as megaloblasts (Koury and Ponka, 2004). These types of cells in the blood are dysfunctional leading to megaloblastic anaemia. Deoxyribonucleic acid (DNA) synthesis also depend on this vitamin hence during deficiency this process is impaired and blood production could be affected leading to megaloblastic anaemia. This condition may appear with symptoms like weakness, fatigue, light-headedness (especially when standing), palpitation, pale skin, glossitis, gingivitis, diarrheoa, weight loss or constipation (Antony, 2012).

Neuronal demyelination and axon degeneration may result from B_{12} deficiency leading to complete distracton of the neurons (Dharmarajan *et al*., 2003). These may lead to symptoms and disease conditions such as dementia, depression, cerebrovascular accident (CVA), paraesthesia, psychosis, among others (Schulz, 2007). Dementia is mostly common among the aged and may lead to a lifelong damage if deficiency is not treated. Depression is also strongly related to B_{12} deficiency (Penninx *et al*., 2000; Tiemeier *et al*., 2002). Infants suffering from B_{12} deficiency may present with disease conditions such as anaemia, failure to thrive (FTT), poor motor development and impeded growth (Monsen and Ueland, 2003).

Some studies have shown that there are no effects of high intake of vitamin B_{12} on the body, no related diseases have been identified (Lonn, 2006; Bønaa *et al*., 2006). Currently, there is no upper limit for dietary B_{12}, and also consuming more than 1000µg in supplements and fortified foods does no harm (Institute of Medicine, 1998).

2.4 FACTORS AFFECTING VITAMIN B_{12} STATUS

2.4.1 Stomach Malfunction and Age

Absorption of B_{12} is aided by essential substances produced by the gastric cells. Diseases affecting the gastric mucosa may impair the production of gastric juices secreted by gastric glandular cells. Hypochlorhydria mostly affects people with atrophic gastritis and may lead to the proliferation of normal intestinal bacteria which may as well utilise this nutrient (Suter *et al*., 1991). This condition is marked by chronic inflammation leading to the distraction of parietal cells in the stomach lining changing them to a less functional form (Vannella *et al*., 2012) as a result, hydrochloric acid, pepsin and intrinsic factor production are hindered. Atrophic gastritis (AG) could be autoimmune or caused by *Helicobacter pylori* (Weck *et al*., 2009) and it is also found to be the end result of chronic gastritis. This condition is found to be common among older adults (Wolters *et al*., 2004), nutritional supplements and fortified foods will be helpful. The synthetic cobalamin which is in its free state is easily absorbed irrespective of the disease condition.

2.4.2 Intestinal Diseases

Vitamin B_{12} is mostly absorbed in the intestine hence diseases affecting this organ, specifically the ileum, may affect absorption. Inflammatory disease conditions such as Crohn's and coeliac disease which afflicts the ileum may be very crucial in inhibiting B_{12} absorption. Low or poor pancreatic juice secretion may lead to the inability of the R-protein to dissociate from B_{12} (Andrés *et al*, 2007). People with other intestinal disorders such as environmental enteropathy, small intestine bacterial overgrowth are also at risk of deficiency diseases (Semrad, 2011). With small intestine bacterial overgrowth, the bacteria attach themselves to the vitamin to utilize it for its own functions making B_{12} unavailable to its host. Acquired Immuno-deficiency Syndrome (AIDS) patients are prone to inflammatory conditions which may eventually result in poor B_{12} uptake by the body.

2.4.3 Gastrointestinal Surgeries

The parietal cells in the gastric glands produce hydrochloric acid and intrinsic factor, removal of all or part of the stomach may lead to loss of these cells resulting in malabsorption of protein bound B_{12} (Sumner *et al.*, 1996; Brolin *et al.*, 1998). The alkalinic environment and pancreatic juice secretion in the duodenum provides an excellent medium for B_{12} binding to intrinsic factor. Surgical removal of the duodenum will disturb normal function. The distal ileum is the major site for absorption of the cobalamin hence its removal may result in low levels in the body. Before and after surgery patients should be assessed for any deficiencies (Commonwealth of Massachusetts, 2007).

2.4.4 Pernicious Anaemia

Pernicious anaemia is an autoimmune disease condition whereby immunological activities leads to the distraction of the parietal cells necessary for hydrochloric acid and intrinsic factor production (Antony, 2012) and may be caused by atrophic gastritis (Bizzaro and Antico, 2014). The absorption of vitamin B_{12} into the blood stream as well as from bile is impaired. Again 1-2% of adults, 50 years and above are at an increased risk hence may be treated with an increased oral dose of cynocobalamin for more passive absorption (Johnson, 2007). Intramuscular B_{12} injections about 1000µg/month are also useful treatments for pernicious anaemia (Andrés *et al*., 2007).

2.4.5 Vegetarianism

The vegetarian diet is high in plant source foods and low in animal source foods. Animal products such as milk, eggs and cheese are completely avoided in the diet of vegans unlike lacto-ovo-vegetarians and lacto-vegetarians who include eggs and milk; and milk in their diet respectively (Donaldson, 2000). Unfortunately, animal and animal products are the only sources of protein bound B_{12} making strict vegetarians as well as vegans prone to deficiency diseases. Infants born to mothers who are strict vegetarian or vegans may end up with low stores of the vitamin which may result in lifetime neurological defects (von Schenck *et al*., 1997). Fortified food and nutritional supplements should be consumed by pregnant and lactating women as well as infants born to such mothers to help meet their daily requirement (Institute of Medicine, 1998).

2.4.6 Drugs

Many drugs have the ability to alter B_{12} absorption especially those for gastrointestinal disorders. Drugs needed for the suppression of gastric acid production such as proton pump inhibitors (PPI) and hydrogen receptor antagonists (H_2-RA) are examples (Howden, 2000; Kasper, 1999). Protein pump inhibitor is used to treat gastroesophageal reflux diseases (GERD) and peptic ulcer disease whereas H_2-RA is used to treat peptic ulcer disease. Nutritional supplements are not able to improve serum B_{12} levels among individuals who have used these drugs for a lengthy period of time (Dharmarajan *et al.*, 2003). Unfortunately, there is not much proof to their association to the malabsorption of B_{12} even for extremely long use (Termanini *et al.*, 1998). Prolonged use of Metformin® a glycaemic lowering drug has the ability to reduce B_{12} levels (Andrés *et al.*, 2002). Deficiency of B_{12} leading to neurological diseases, may mimic that of diabetic neuropathy which makes it a great concern in the usage of this drug. Less than half (10-30%) of individuals on the medication experience these effects (Buvat, 2004; Bauman, 2000).

Table 2.1: Dietary Reference Intakes (DRI) of Vitamin B_{12}

AGE (YEARS)	MALES AND FEMALES (mcg/day)
0 – 0.5	0.4 (AI)
0.7 – 1	0.5 (AI)
1 – 3	0.9 (RDA)
4 – 8	1.2 (RDA)
9 – 13	1.8 (RDA)
14 +	2.4 (RDA)

Source: Institute of Medicine, 1998

2.5 THE RELATIONSHIP BETWEEN FOLATE AND VITAMIN B_{12}

Folate and cobalamin perform many functions in the body such as prevention of anaemia and some congenital diseases (Scholl and Johnson, 2000; de Benoist, 2008). In folate metabolism, vitamin B_{12} and folate play major roles in converting homocyteine into methionine, an important amino acid which is needed for methylation. Vitamin B_{12} is important for the conversion of 5-methyltetrahydofolate into THF, activated folate, in the folate metabolic pathway (Fig 2.1) (Institute of Medicine, 1998).

Folate and cobalamin are needed in blood and DNA synthesis hence deficiencies in both results in megaloblastic anaemia (Clarke, 2008; Stabler, 2000). In DNA synthesis, vitamin B_{12} has the ability to form a methyl-folate trap which helps to assist the conversion of uracil to thymidine, a necessary building block of DNA (Mahan and Escott-Stump, 2008). Since both help prevent anaemia it is quite difficult to detect cobalamin deficiency in the presence of large folic acid doses as folate has a tendency in masking the B_{12} deficiency (Johnson, 2007). Some recent studies have shown that the function of folate and vitamin B_{12} in controlling homocysteine levels helps to improve upon bone health therefore preventing osteoporosis among older adults (Dhonukshe-Rutten *et al*., 2005; van Meurs *et al*., 2004; Tucker *et al*., 2005; Morris *et al*., 2005).

2.6 PREVALENCE OF FOLATE AND VITAMIN B_{12} DEFICIENCY

Currently, there is no known global prevalence of both folate and vitamin B_{12} deficiencies there have been several studies on folate and vitamin B_{12} deficiencies in different countries, among rural and urban populations. Most of the studies were carried out in females of different ages and physiological conditions (Gibson *et al*., 2008).

A study conducted by Korkalo *et al*., 2015 among Mozambique young females, found out that among all the micronutrients selected for the study there was 4.1 % of the population had low serum folate levels. Among Ethiopian women it was revealed that among all the selected regions in the rural areas the prevalence of folate deficiency was 87% (Haider *et al*., 2010). Even though WHO views the different prevalence rate as unreliable due to the small sample size normally used, all the studies have proved that deficiencies of these B vitamin are of a public health concern (Conclusion of a WHO Technical Consultation, 2008). South Africa recorded a low prevalence of folate deficiency after an intervention with folic acid fortification (Modjadji and Alberts, 2007). Similarly the countries such as Nigeria, Sudan and Zimbabwe have shown an improvement in the prevalence of folate deficiency and other developed countries shows no prevalence (McLean *et al*., 2008).

Most countries do not often carry out with vitamin B_{12} fortification as the prevalence among young adults is found to be low (Vidal-Alaball *et al*., 2005; Butler *et al*., 2006; Subar *et al*., 1998) but the prevalence is rather high among the elderly (Carmel, 2011). Variations may occur due to the differences in the immunoassays used for the various testing.

2.7 PREDICTORS OF FOLATE AND VITAMIN B_{12} STATUS

Deficiencies of these B vitamins may occur in both male and females across all age ranges. Studies have shown that males are found to be more folate deficient compared to females (Clarke *et al*., 2003; Carmel *et al*., 1999). Several studies have shown age as a predictor of folate status (Girelli *et al*., 1998; Friso *et al*., 2002; de Bree *et al*., 2003;

Barnabé *et al*., 2015). However, ages above 50 years may help predict vitamin B_{12} deficiency.

Literature has shown that obese (Body mass index, BMI ≥ 30 kg/m^2) individuals, especially women, are mostly at risk of folate deficiency (Mojtabai, 2004). There is therefore a percentage reduction in serum folate concentration with a unit increase in BMI (Bradbury, 2014). Also chronic alcohol intake and smoking may inhibit the absorption of folate leading to a deficiency. Methylmalonic acid (MMA) is found to be a predictor of vitamin B_{12} status as well as homocysteine which also predicts folate status (Conclusion of a WHO Technical Consultation, 2008). Helicobacter pylorus was also a good predictor of vitamin B_{12} concentration in the blood (Carmel *et al*., 2001).

2.8 ASESEWA, A FARMING COMMUNITY

Asesewa is a rural community in the Upper Manya Krobo district of the Eastern Region of Ghana. The district shares boundary with the Volta Lake in the north, Fanteakwa District in the west, Asuogyaman District in east, Yilo Krobo District in the south-west and Lower Manya Krobo in the south-east. Farming is the main income yielding occupation among the people in the district. Majority of the people (80%) are subsistence farmers with a smaller number being commercial farmers. The crops cultivated by these farmers include cassava, maize, plantain, vegetables as well as oil palm and mangoes. Others engage in animal farming and the animals they rear include cattle, sheep, goats, pigs, poultry and grasscutters. Some of the community members go fishing as an occupation in the district they engage in deep, shallow and domestic fishing. The district has 3 main markets, one in Asesewa and other communities such as Akateng and Sekesua. Most buyers from different parts of the country often make their purchasing at Asesewa (HALF YEAR REPORT, 2012).

Figure 2.3: Map showing Asesewa

CHAPTER THREE

3.0 METHODS

3.1 STUDY DESIGN

A cross-sectional design was employed in this study.

3.2 STUDY SITE

The study was conducted at the University of Ghana Nutrition Research Centre in Asesewa, the capital of the Upper Manya Krobo district in the Eastern region. The Research Center is adjacent to the Asesewa District Hospital.

3.3 PARTICIPANTS

The study population included adults between the ages of 25 to 65 years residing in Asesewa.

3.3.1 Inclusion Criteria

Adults who were between ages 25-65 years and had lived in Asesewa for more than a year were recruited.

3.3.2 Exclusion Criteria

Individuals who were on any dietary regimen, pregnant and lactating women were excluded from the study.

3.4 SAMPLE SIZE CALCULATION

Cochrane's formula was used to calculate the sample size:

$$N = \frac{Z^2 \times P\,[1-P]}{(E)^2}$$

N (sample size)

Z (z-score associated with the confidence level of 95%) = 1.96

P (prevalence rate within a population, unknown hence 50%) = 0.5

E (margin of error (the required precision) = 0.10

$= (1.96)^2 (0.5)\,(0.5) / 0.10^2 \qquad = 96.04$

However the sample size was rounded up to 100 to cater for dropouts during the blood sample collection.

3.5 PROCEDURE FOR DATA COLLECTION

Convenience sampling was used to select 100 participants for the study. After consultation with the opinion leaders in the town, an announcement with the town's Public Address (PA) System was used to circulate the information about the study. Participants who reported at the nutrition centre were screened for eligibility. Written consent was obtained from those who met the inclusion criteria before they were recruited into the study. After a thorough explanation of procedures involved in the study to consenting participants, anthropometric measurements were taken. Structured questionnaires were administered to participants to obtain information on social, medical histories and other lifestyle activities. Afterwards fasting blood samples were taken from the participants.

3.4.3 Pre-test of questionnaire

The questionnaire was tested using 10 participants (5 males and 5 females) before the main study was carried out. After the pre-test the medical and family histories were simplified into a table form to help reduce the bulk of the papers. Information on the family history of smoking as well as physical activity levels were excluded since they were not directly relevant in the study.

3.5.1 Dietary Assessment

A validated FFQ **(Verkleij-Hagoort *et al.*, 2007)** was modified and used to assess usual dietary intakes. The FFQ included food items of specific food groups that were consumed over a period of time. Participants' food consumption patterns were assessed by multiple responses in which respondents were asked to estimate how often a particular food or beverage was consumed. Categories ranging from 'daily', '1-2 times/week', '3-4 times/week', '5-6 times/week', 'once a month', 'twice a month' 'seldom', or 'never', were included in the questionnaire and participants chose one of these options. A three day 24-hour recall (two weekdays and one weekend) was used to obtain information on current food intake with the aid of food models. The Ghanaian handy measures which included sardine tin sizes; soup and stew ladles; match box among others were then converted into grams before the data was analysed using the Microdiet nutritional analysis software version 3.1 (Derbys, UK).

3.5.2 Anthropometric Assessment

Body weight, body mass index (BMI), visceral fat (VF) and percentage body fat (BF) of the participants were measured with an Omron Bio impedance analyzer (Shahdara, India). Body weight was measured to the nearest 0.1 kg with participants standing in upright positions without any foot wear and any heavy object Measurement were compared with standard reference ranges. Body Mass Index (BMI) was classified as <18.5 kg/m^2, 18.5-24.9 kg/m^2, 25.0-29.9 kg/m^2 and ≥ 30.0 kg/m^2 which indicated underweight, normal, overweight and obese respectively (WHO, 2012). Height was measured to the nearest 0.5 cm with a Seca Stadiometer (Hamburg, Germany), with participants standing erect in a Frankfurt plane, heels together against the stadiometer and hands on the sides.

Blood pressure (BP) was measured using an Omron Blood Pressure Monitor (Kyoto, Japan) after 5 minutes rest. In an upright position, the cuff of the blood pressure monitor was placed around the upper left arm 1-2 cm above the elbow. Two BP measurements were taken (spaced 3 minutes apart) for each participant and the mean of these two measurements was calculated as the final reading.

3.4.4 Blood sample collection and handling

Five milliliters (5ml) of fasting blood samples were taken by a trained phlebotomist. The blood was put into gel separator tubes (plain tube) and centrifuged at 3000 rpm for 15 minutes. The serum was aliquotted into eppendorf tubes and placed in a cool box. All samples were transported to the Chemical Pathology Laboratory of the School of Biomedical and Allied Health Sciences and frozen at -20°C until the analysis.

3.5 DATA ANALYSIS

3.5.1 Dietary analysis

Dietary intakes collected from the 24 hour recall were converted to energy and nutrients and analysed using the Microdiet nutrition analysis software version 3.1 (Derbys, UK). Data from questionnaires and the food frequency checklist were coded and inputted into Statistical Package for the Social Sciences (SPSS) version 20 and analysed.

3.5.2 Laboratory analysis

Folate and vitamin B_{12} were analysed using enzyme-linked immunosorbent assay (ELISA) kits (Bensheim, Germany). The analysis was performed following the manufacturer's instructions but a brief summary of the procedure is outlined below.

3.5.2.1 Preparation for folate and vitamin B_{12} test

Observing a strict protocol, the assay for the test was prepared after it had been brought to room temperature. A freshly prepared medium containing 1 ml of treatment buffer and 10 ml of water was securely closed and shook very well. The bottle was then heated in a water bath at a temperature between 90-100°C and immediately cooled at 30°C. In a sterile environment 10 ml medium was filtered with a 0.2 millipore filter into a 15 ml centrifuged test tube. Standards were prepared and a standard curve was drawn using predetermined standard concentrations, Blank-0, Standard 1 – 0.04 µg/l, Standard 2 – 0.08 µg/l, Standard 3 – 0.16 µg/l, Standard 4 – 0.24 µg/l and Standard 5 – 0.32 µg/l, to obtain an equation for individual folate serum concentration. A 0.125 ml of distilled water in the test box was added to the control bottles. Controls were prepared in duplicated, (2) CTRL 1 and (2) CTRL 2.

Similar procedure was followed for the test preparation of vitamin B_{12} but in the preparation of controls, 0.3 ml of water was added to the control bottles without duplicating the controls. The pre-determined standard concentrations which included; Blank – 0 ng/l, Standard 1 – 6 ng/l, Standard 2 – 18 ng/l, Standard 3 – 27 ng/l, Standard 4 – 36 ng/l, Standard 5 – 54 ng/l were used to draw a curve to obtain an equation for individual serum concentrations.

3.5.2.2 Testing procedure and measurement

One hundred and fifty microlitre (150 µl) of folic acid assay medium were put in the cavities. Another 150 µl of standard, controls and samples were pipetted ino the cavities. With precaution the cavities were sealed in a microtiter plate coated with *Lactobacillus rhamnosus* and incubated at 37°C for 48 hours. After the incubation period a HumaReader (Wiesbaden, Germany) was used to determine the absorbance of each sample.

The same procedure was followed for the vitamin B12 test but the microtiter plate used was coated with *Lactobacillus delbrueckii subsp. Lacis*.

3.5.2.3 Obtaining results

The manufacturer recommended 4-Parameter-alogarithm for the result calculation for both test but, 2-Parameter-alogarithm was found to be more appropriate for the calculation of folate concentration. Also the reference ranges by the manufacturer were not used; instead reference ranges by Conclusions of a WHO Technical

Consultation (2008) as well as that of Barnabé et al., (2015) were used to determine folate and vitamin B_{12} deficiency respectively. This was so because there were no specific reference ranges stated for vitamin B_{12} by WHO. Also, reference ranges by Barnabé et al., (2015) were from current literature. For serum folate <4 μ/l and ≥ 4 μ/l indicated folate deficiency and normal serum folate levels respectively. The reference ranges for vitamin B_{12} were <200 ng/l-deficient, 200-300 ng/l-normal, >300 ng/l- above normal.

3.5.3 Statistical analysis

Categorical data were summarised as frequency and percentages. Means and standard deviation of continuous variables were determined using the Independent T-Test after checking for normalcy. Pearson-*r* correlation was used to determine the relationship between the dietary intake and status. Multiple linear regressions were used to determine the predictors of sera folate and B_{12} status of the study population. Results were considered significant at $p \leq 0.05$ with a 95% confidence level. All analyses were done using SPSS (version 20).

3.6 ETHICS

Approval for the study was obtained from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences (SBAHS). All ethics protocols were observed to ensure complete anonymity of all participants before, during and after the research period. Participants had the right to withdraw from the study at any time. The purpose, risk and benefits of the study were explained to participants in the language of their understanding before they were recruited into the study. An informed written consent form was signed (or thumbprinted) by participants before their participation in the study. Written permission to carry out the study in Asesewa was obtained from the District Health Administration of the Upper Manya Krobo District before the commencement of the study. Participating subject's confidentiality was ensured throughout the study.

CHAPTER FOUR

4.0 RESULTS

4.1 BACKGROUND DATA

A total number of 100 participants were recruited for the study. All participants (100) completed the questionnaires and the 3 day 24-hour recall. However, for the blood sample collection only 63 of the respondents reported on the day of collection. Out of the 63 respondents some of the values obtained after the test analysis showed a few outliers hence results of 59 (93.7%) and 58 (92%) of the respondents were used to determine mean serum folate and B_{12} levels respectively.

4.2 DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

4.2.1 Socio-demographic characteristics of respondents

The mean ages were similar for both males and females. More than half of participants were married. Almost half (42%) of the participants had completed Junior High School (JHS) and a few had tertiary education. All these are summarised in Table 4.1.

Table 4.1: Socio-demographic characteristics of respondents

Variables	Male (n= 50)	Female (n= 50)	All (n=100
Age (years)			
Mean ± SD	45.10 ± 10.44	45.11 ± 16.22	45.11 ± 13.33
Marital Status (%)			
Single	10	5	15
Married	34	36	70
Separated/divorced	6	7	13
Widowed	0	2	2
Educational Background (%)			
University/Graduate	4	1	5
HND	3	1	4
SHS	8	3	11
JHS	22	20	42
Primary/Less	13	24	37
No school	0	1	1

4.2.2 Socio-economic characteristics of respondents

Table 4.2 shows the socio-economic characteristics of respondents. Majority of the participants were employed Thirty six (36%) were unskilled workers, artisans among others. Twenty-seven percent (27%) were engaged in trading. Twenty two percent (22%) of the study participants were farmers. Forty eight percent (48%) of participants had lived in Asesewa for more than 21 years and 19% of the participants had lived in the community between 0-5 years. Less than half of the participants had a monthly income between 0-100 GHC (30%) and 101-500 GHC (31%).

Table 4.2: Socio-economic status of respondents

Variables	**Males (n=50)**	**Females (n=50)**	**All (n=100)**
Employment Status (%)			
Employed	46	46	92
Unemployed	4	4	8
Occupation (%)			
Farmer	12	10	22
Teacher	2	2	4
Trader	3	24	27
Civil Servant	2	0	2
Mechanic	2	0	2
Other occupation	26	10	36
Not working	3	4	7
Years lived in Asesewa (%)			
0-5	7	12	19
6-10	4	3	7
11-15	7	8	15
16-20	4	5	9
21 and above	28	20	48
Don’t know	0	2	2
Approximate Household Income in GH (%)			
0-100	11	19	30
101-500	20	11	31
501-1000	4	3	7
1001-1500	0	1	1
1501-2000	1	1	2
Don’t know	14	15	29

4.3 ANTHROPOMETRIC AND BLOOD PRESSURE MEASUREMENT

With the anthropometric measurement, all except systolic blood pressure (SBP) showed a significant difference (p≤0.05) among gender (Table 4.3 shows results).

Table 4.3: Anthropometric and blood pressure measurements of participants

Variable	Males (n =50) Mean ± SD	Females (n =50) Mean ± SD	All (n= 100) Mean ± SD	p-value
Visceral Fat (%)	4.96 ± 2.97	7.12 ± 2.72	6.04 ± 3.03	0.00*
BMI (kg/m^2)	21.97 ± 2.82	26.34 ± 5.23	24.16 ± 4.73	0.00*
Body fat (%)	15.17 ± 6.43	37.47 ± 8.55	26.32 ± 13.50	0.00*
SBP (mmHg)	125.86 ± 17.36	129.86 ± 19.38	127.86 ± 18.42	0.28
DBP (mmHg)	76.96 ± 14.49	82.48 ± 13.07	79.72 ± 14.01	0.05*

*Significant p≤0.05

4.4 HEALTH STATUS, MEDICATION AND LIFESTYLE

In table 4.4, the medical history showed that 20% of the total population had heart-related diseases which included hypertension, stroke among others. Forty-eight percent (48%) of the relatives of the participants had heart related diseases. The use of herbal medications and nutritional supplements although not common was significantly higher in males than females. A few participants (5%) who were mostly males were past smokers.

Table 4.4: Health status, medication and lifestyle

Variable	Male (n=50)	Female (n=50)	All (n=100)
Medical History (%)			
Diabetes	2	4	6
Heart-related diseases	10	10	20
High cholesterol	1	1	2
Arthritis	1	1	2
Family History (%)			
Diabetes	6	6	12
Heart-related diseases	21	27	48
Cancer	0	1	1
Arthritis	2	0	2
Medication			
Herbal medication	5	13	18
Nutritional supplement	0	5	5
Smoking			
Past smoker	4	1	5
Never used	46	49	95

4.5 FOOD FREQUENCY CONSUMPTION OF RESPONDENTS

4.5.1 Consumption of cereals and grains, starchy roots and plantain

Table 4.5 presents frequency of consumption of cereals and grains; starchy roots and plantain. Frequency of consumption of bread, wheat, spaghetti and millet was low. Almost three-quarter of the respondents (71%) consumed maize and 77% had never consumed wheat. Among the starchy roots and plantain group, cassava was commonly consumed followed by plantain and yam.

Table4. 5: Cereals and grains, starchy roots and plantain

Food Group	Varieties	Daily	5-6x/ week	3-4x/ week	1-2x/ week	Twice a month	Once a month	Seldom	Never
Cereals and Grains	P. rice	8	2	19	38	3	6	17	7
	B. rice	3	0	4	7	1	2	17	66
	Wheat	1	0	0	2	0	3	17	77
	Maize	71	0	17	11	0	0	1	0
	B. bread	9	1	12	23	1	3	25	26
	T. bread	12	2	13	32	0	2	20	19
	Br. bread	4	0	4	7	0	1	23	61
	Spaghetti	8	2	6	5	0	3	26	50
	Millet	7	1	3	8	1	1	22	57
	Gari	11	1	10	20	0	4	32	22
Starchy Roots And plantain	Yam	23	5	20	24	2	2	16	8
	Cocoyam	17	5	6	16	2	3	27	24
	Cassava	43	6	22	21	2	0	5	2
	Plantain	33	8	21	23	3	1	6	5
	Potato	10	2	3	19	1	3	35	27

P. rice-Polished rice, B. rice-Brown rice, B. bread-Butter bread, T. bread-Tea bread, Br. bread-Brown bread

4.5.2 Hot and cold beverages

Among the hot beverages milo (chocolate drink) was frequently consumed followed by tea. Nineteen percent (19 %) of the study population consumed milo daily and 24% had intakes of 1-2 times each week. Carbonated drinks and fresh juice were consumed by 15% and 9% of the respondents respectively on a daily basis. It was noted that a relatively high number of the participants (20%) consumed carbonated drinks 1 to 2 times each week. Reported intake of alcoholic drinks was low (Table 4.6).

Table 4.6: Beverages, alcohol and processed foods

Food Group	Varieties	Daily	5-6x/ week	3-4x/ week	1-2x/ Week	Twice a month	Once a month	Seldom	Never
Beverages/ Alcohol	C. drink	15	3	5	20	3	5	33	16
	Coffee	11	0	3	3	4	2	13	64
	Tea	12	1	4	14	2	3	27	37
	Milo	19	3	7	24	2	3	24	18
	F. juice	9	0	5	6	1	1	13	65
	Beer	2	1	0	3	1	4	17	72
	Wine	0	0	0	1	0	5	13	81
	Guinness	1	0	0	3	0	3	15	78
	Local gin	2	1	0	6	1	1	7	82
Processed food	Pastries	9	5	5	10	0	4	23	44
	C. foods	11	1	3	12	3	4	30	36
	D. fruits	11	0	0	3	0	3	11	72

C. drink-Carbonated drink, F. Juice-Fruit juice, C. foods-Canned foods, D. fruits-Dried fruits

4.5.3 Consumption of fruits and vegetables

Mangoes were among the fruits consumed frequently on a daily basis (32%) during its season. Also both banana and pawpaw were consumed by 23% of the study population. Citrus fruits comparatively were consumed less (14%) on daily basis. Nearly all the respondents consumed onions and tomatoes daily and more than half (64%) of the participants consumed garden eggs daily. Apart from *kontomire* which was consumed daily by 34% of participants, other local green leafy vegetables were less consumed. Table 4.7 presents the frequency of fruits and vegetables intake.

Table 4.7: Fruits and vegetables

Food Group	Varieties	Daily	5-6x/ week	3-4x/ week	1-2x/ Week	Twice a month	Once a month	Seldom	Never
Fruits	Citrus	14	0	12	14	0	1	35	24
	Mangoes	32	1	1	2	0	3	33	28
	Banana	23	3	6	17	0	5	25	21
	W. melon	20	2	1	9	0	5	37	26
	Pawpaw	23	2	7	12	2	4	28	22
	Pineapple	19	2	2	7	4	4	35	27
	Coconut	15	3	4	18	4	4	33	19
	Pear	18	1	8	13	4	4	32	23
Vegetables	Okro	34	10	14	17	1	1	10	13
	Onion	100	0	0	0	0	0	0	0
	G. eggs	64	6	14	9	0	0	7	0
	T. berry	48	6	14	12	1	1	13	5
	Tomatoes	99	1	0	0	0	0	0	0
	Kontomire	37	6	24	15	4	4	5	3
	Bokoboko	1	1	1	0	0	0	5	92
	B. leaf	0	1	0	0	0	0	3	94
	Ayoyo	2	1	2	0	1	1	7	87
	C. leaf	0	1	1	0	0	0	4	94
	Cabbage	11	3	12	7	1	1	29	36
	Lettuce	5	3	3	4	0	0	25	59
	Carrot	5	4	3	5	0	0	28	53
	G. pepper	13	3	4	5	0	0	27	47
	Dandelion	0	0	3	2	0	0	14	79

W. melon-Water melon, G. Eggs-Garden eggs, T. berry-Turkey berry, B. leaf- Bitter leaf, C. leaf-Cassava leaf, G. pepper-Green pepper

4.5.4 Consumption of animal and animal products

Majority of the respondents (82%) consumed fish on a daily basis rather than other meat products. Among the crustaceans and molluscs mentioned, crabs (10%) were mostly consumed as compared to oyster (1%), snail (2%) and shrimps (2%). Consumption of milk and milk products were shown to be relatively low among the respondents. Palm nut, in the form of soup, was consumed by half of the study population (43%) on a daily basis. Daily consumption of legumes such as beans (18%) and groundnuts (22%) was observed to be low.

Table 4.8: Animal and animal product, legumes, oil seeds and nuts

Food Group	Varieties	Daily	5-6x/ week	3-4x/ week	1-2x/ Week	Twice a month	Once a month	Seldom	Never
Animal and animal product	W. milk	5	1	4	13	0	2	34	41
	Low fat	5	2	6	7	1	3	23	53
	Cheese	2	0	4	7	0	0	10	77
	Yogurt	4	1	3	15	1	6	28	42
	Burkina	0	1	0	1	0	0	10	88
	Mutton	5	0	1	9	0	5	33	47
	Chicken	9	6	8	24	2	12	26	13
	Fish	82	3	4	4	0	0	6	1
	Pork	3	1	1	3	0	2	12	78
	G. meat	11	3	1	9	5	8	35	28
	Game	8	0	0	2	5	5	39	40
	Crab	10	0	2	6	2	3	41	36
	Lobster	6	0	1	2	0	1	30	60
	Oyster	1	1	0	5	0	3	33	57
	Snail	2	0	0	1	0	2	11	84
	Shrimp	2	1	0	8	0	3	33	53
Legumes/ oil seeds/ Nuts	Beans	18	1	13	24	0	3	26	15
	G'nut	22	5	8	22	3	2	25	13
	Agushie	8	1	6	8	1	3	37	36
	Palm nut	43	4	16	28	1	3	4	1

W. milk-Whole milk, G. Meat-Goat meat, G'nut-Groundnut

4.5.5 Consumption of fats, oils and fried foods

Fifty-one percent of the respondents used palm oil on a daily basis mostly in the preparation of stew or other savouries. Only 16% of the respondents consumed fried yam (Table 4.9).

Table 4.9: Fats, oils and deep fried foods

Food Groups	Varieties	Daily	5-6x/ week	3-4x/ Week	1-2x/ week	Twice a month	Once a month	Seldom	Never
Fats/oils	Butter	7	1	3	9	1	4	26	49
	S. oil	2	2	5	6	0	1	5	79
	Palm oil	51	10	17	15	1	1	2	3
	C. oil	15	1	2	6	0	4	17	55
	Soya oil	8	2	6	2	0	1	6	75
	Frytol®	26	4	14	18	0	4	8	23
	P. k. oil	17	0	2	9	0	2	17	49
Deep Fried Foods	*Kelewele*	2	0	1	4	0	3	19	71
	F. yam	16	1	3	15	1	4	28	32
	F. c.yam	15	1	2	16	0	2	30	34
	Koose	12	3	3	12	1	0	28	41
	Maasa	1	0	0	2	0	1	16	80

S. oil-Sunflower oil, C. oil-Coconut oil, P. k. oil-Palm kernel oil, F. yam-Fried yam, F. c.yam-Fried cocoyam

4.6 NUTRIENT INTAKES OF PARTICIPANTS

4.6.1 Energy and macronutrient intake

Mean energy intake for both males and females were below recommended intakes and significantly different between groups ($p \leq 0.05$). There were significant differences in the intakes of all the macronutrients except fat and cholesterol. The mean macronutrient intake of the female respondents was obviously low compared to the male respondents. The mean percentage energy intake was shown to be within the normal range among the genders. Mean folate and vitamin C was significantly higher in males than females. Vitamin B6, B12 and iron were not significantly different (Table 4.10).

Table 4.10: Mean macro and micro nutrients intake of respondents

Variable	Male (n 50) Mean ± SD	Female (n 50) Mean ± SD	All (n 100) Mean ± SD	p-value
Macronutrient				
Energy (kcal)	2307 ± 1014	1720 ± 676	2014 ± 907	0.00*
Carbohydrate (g)	412 ± 187	289 ± 119	350 ± 168	0.00*
% Energy intake	66.66 ± 7.71	62.92 ± 10.51	64.79 ± 9.36	0.05*
Fat (g)	61.59 ± 24.48	57.59 ± 26.73	59.59 ± 25.58	0.23
% Energy intake	21.00 ± 7.04	25.28 ± 9.75	23.14 ± 8.73	0.49
Protein (g)	62.31 ± 30.06	48.19 ± 24.38	55.25 ± 28.14	0.01*
% Energy intake	11.72 ± 4.65	11.16 ± 3.22	11.44 ± 3.99	0.01*
Cholesterol (mg)	51.78 ± 41.71	48.19 ± 44.96	49.99 ± 43.18	0.68
Dietary fiber (g)	6.84 ± 11.38	2.90 ± 4.56	4.87 ± 8.84	0.03*
Micronutrient				
Vitamin B_6 (mg)	1.05 ± 0.73	2.40 ± 11.06	1.73 ± 7.83	0.39
Folate (mcg)	175 ± 118	123 ± 61.04	149 ± 97.23	0.01*
Vitamin B_{12} (mcg)	2.40 ± 3.24	2.22 ± 2.21	2.31 ± 2.78	0.75
Vitamin C (mg)	92.54 ± 101	53.50 ± 30.82	73.02 ± 76.8.	0.01*
Iron (mg)	20.78 ± 17.13	16.27 ± 8.81	18.53 ± 13.74	0.10

*p<0.05 is considered significant. Recommended daily intake (RDI) - Energy (males-2600 and females-2100 kcal), Protein (males-49 and females 41g). RDI for the micronutrient- folate (400 mcg), vitamin B_{12} (2.4 mcg), vitamin B_6 (100mg), iron (45mg), vitamin C (2000mg) (Institute of Medicine of the National Academy of Sciences, 2010); WHO/FAO (2003) states healthy % energy intake as carbohydrate (55-75%), fat (15-30%) and protein (10-15%)

4.7 MAIN FOODS CONTRIBUTING TO FOLATE AMONG RESPONDENTS

According to Table 4.11, the main foods mainly contributing to folate intake among the respondents included *waakye* (58%), *kontomire* and okro dishes (49%) and fermented corn foods (41%). Groundnut soups contributed the least.

Table 4.11: Main foods contributing to folate intake among respondents

Main foods contributing to folate	**Percentage contribution (n=100)**
***Waakye* (beans and rice)**	58
***Kontomire**, okro (stew, soup)**	49
Fermented corn foods (*banku*, porridge)	41
Beans (boiled)	26
Eggs (boiled)	24
Oranges	23
Plantain (*fufu*, boiled)	21
Tomato stew or gravy	15
Peanut (roasted, soup)	13

*Spinach was used as an equivalent of *kontomire*

4.8 PREVALENCE OF SERUM FOLATE DEFICIENCY

The study showed that, the prevalence of folate deficiency among respondents was 71% with males being more folate deficient compared to females (Fig 4.1).

4.9 BIOCHEMICAL ANALYSES

Table 4.12 shows the mean sera folate and vitamin B_{12}. After the analysis the results of 4 and 5 participants were excluded as outliers for serum folate and serum B_{12} Using the reference ranges, serum folate levels <4 µ/l indicates deficiency and ≥ 4 µ/l indicates normal levels, the mean serum folate levels of both male and female indicated folate deficiency. However, there were no vitamin B_{12} deficiencies (reference values, <200 ng/l, 200-300 ng/l and >300 ng/l indicated deficiency, normal and above normal respectively).

Table 4.12: Serum folate and vitamin B_{12} concentrations of respondents

Variable		Mean ± SD	p-value
Serum Folate (μ/l)*	Male (n=31)	3.5 ± 9.2	
	Female (n=28)	3.0 ± 4.9	
	All (n=59)°	3.3 ± 7.3	0.75
Serum B_{12}(ng/l)**	Male (n=32)	336 ± 312	
	Female (n=26)	308 ± 341	
	All (n=58)°	322 ± 325	0.66

°Sample size exclude outliers, 63 blood samples were actually used; *ng/ml=μ/l; **pg/ml=ng/l

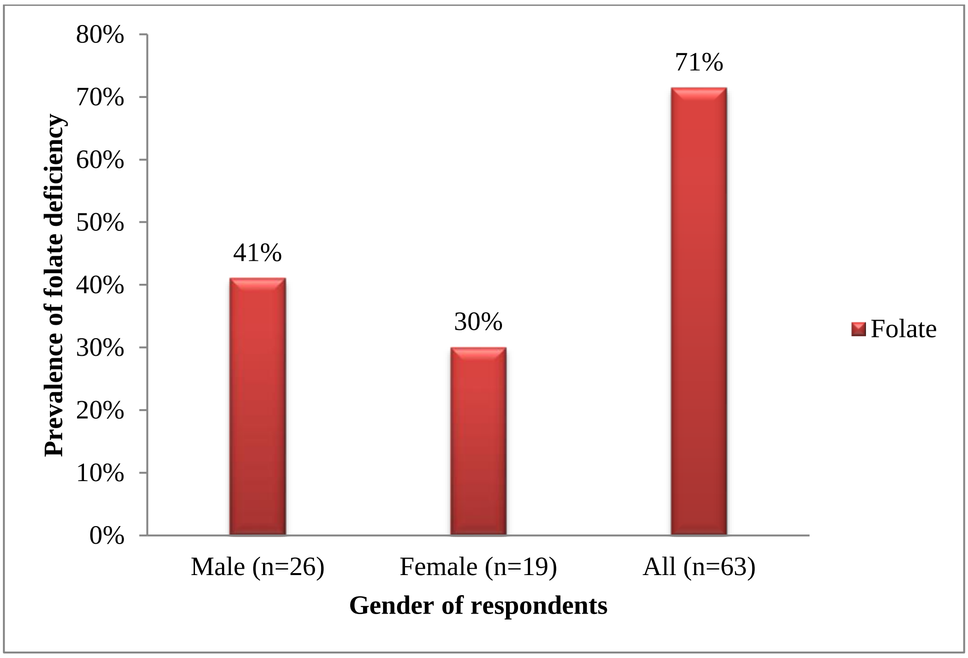

Figure 4.1: Prevalence of serum folate deficiency

4.10 CORERLATION BETWEEN SERUM FOLATE, VITAMIN B12 AND DIETARY FOLATE AND VITAMIN B12

There was no significant correlation between dietary vitamin B_{12} and serum vitamin B_{12} ($r = -.06$, $p = 0.58 > 0.05$) and dietary folate and serum folate ($r = .08$, $p = 0.42 > 0.05$) (Table 4.13).

Table 4.13: Pearson-*r* correlation between Dietary Folate, B_{12} & Sera Folate, B_{12} respectively

Variable	Serum B12	p-value	Serum Folate	p-value
Dietary B12	-.06	0.58	-	-
Dietary Folate	-	-	.08	0.42

*Significant at p-value≤ 0.05, (n =63)

4.11 PREDICTORS OF SERUM FOLATE AND SERUM B_{12} LEVELS

4.11.1 Predictors of serum folate

Multiple regression analysis was used to test for the predictors of serum folate level. From Table 4.14, the results of the regression indicated that the 7 predictors explained 9% of the variability [R^2 = .09, $F_{(7, 92)}$ = 1.26, p > 0.05] in serum folate levels. However, age was found to predict serum folate level in this population (p=0.05).

Table 4.14: Multiple regression showing predicators of serum folate level

	Serum Folate Level			
Predicators	**R^2**	**Beta**	**B**	**p-value**
(Constant)			78.61	0.10
Age		-.22	-5.58	0.05*
Gender		.01	0.38	0.96
Smoking	0.09	-.19	-13.16	0.08
Alcohol		-.07	-0.53	0.51
BMI		.06	0.41	0.58
SBP		.05	-0.08	0.81
DBP		.01	-0.02	0.96

*Significant at p ≤ 0.05

4.11.2 Predictors of serum B_{12}

Table 4.15 also indicated that the 2 predictors accounted for 2% of the variability [R^2 = .02, $F_{(2,97)} = 0.80$, $p > 0.05$] in serum vitamin B_{12} levels. None of the tested predictors significantly predicted serum vitamin B_{12} independently.

Table 4.15: Multiple regression showing predicators of serum vitamin B_{12}

		Serum Vitamin B_{12}		
Predicators	**R^2**	**Beta**	**B**	**p-value**
Stage 1				
(Constant)			521.12	0.00
Age	.02	-.06	-39.40	0.58
Gender		-.12	-34.65	0.24

*Significant at p ≤0.05

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Folate and vitamin B_{12} are very important nutrients in preventing disease conditions such as cancer, neuropathies, depression, megaloblastic anaemia, diabetes, dementia, low birth weight, some congenital deformities such as cleft lip and palate and neural tube defect. The aim of this study was to assess the folate status of a rural adult population in Asesewa, Upper Manya Krobo District of Ghana.

Results of the study showed a high level of folate deficiency (71%) among the study population which puts them at an increased risk of folate deficiency diseases afore mentioned. Similarly, a study among Ethiopian women in rural communities showed an equally high prevalence of folate deficiency (87.4%) (Haider *et al*., 2010). In South Africa, a study conducted among non-pregnant rural women showed a 27.6% of folate deficiency before fortification. However, after mandatory fortification of maize and wheat, folate deficiency was eradicated (Modjadji and Alberts, 2007) thus their status was improved. A study by Blencowe *et al*., 2010, showed that public health activities on folic acid fortification were not usually carried out in low income earning countries. Another research with urban adult population and an adult Ghanaian population in the UK showed an improved folate status in the UK population. (Owusu *et al*., 2010). This was attributed to the consumption of folic acid fortified foods consumed in the form of breakfast cereals and margarines and nutritional supplements. Breakfast cereals contributed 5% and folic acid supplements contributed 25% of total folate intake in the UK population. This highlights the need for intensive nutrition education and the introduction of folic acid fortified foods in the Asesewa community. Among this study population it was observed that fermented maize products was frequently consumed by participants on a daily basis hence fortifying this food item in rural communities could be helpful in improving the folate status.

The major contributing foods to folate in this present study were *waakye* (rice and beans) *kontomire* and okro (okra) dishes and fermented corn foods. In their study on folate intakes Owusu *et al*., (2010) identified local Ghanaian soups such as light soup, palm nut and peanut soup as the major food contributors to folate intake (22%) in the Accra population. This was followed by Ghanaian vegetable stews (*kontomire*, okro and garden egg (aubergine) stews) which contributed 20% and fermented maize dishes (11%). In this present study rice and beans was the major food contributing to folate intake (58%) followed by vegetable stews (*kontomire* and okro) and fermented corn foods (41%). It was observed from the dietary recalls that *waakye* was a common breakfast meal for many participants so it was not surprising that this food appeared as a major contributor to folate. This bean dish is commonly sold on the streets in Ghana and is preferred by many possible because it is relatively cheap, can be enjoyed even when no fish or meat is added and also because of the high fibre it makes one full for a period of time.

Plant sources of folate are not bioavailable compared to the synthetic form, folic acid (Pentieva *et al*., 2002). Although the study site was a farming community, their intake of green leafy vegetables was relatively low. It could clearly mean that food items produced in this community is rather sold or less frequently consumed. Most of the study participants mentioned that they had never consumed *bokoboko, bitterleaf*, *ayoyo* and *cassava leaf*. These green leafy vegetables were available in the community but it was clear that the low consumption might be because most of them did not consider these vegetables as food. A study by Abbey *et al.*, (2006) also supported that most Ghanaians less consumed readily available vegetables due to poor knowledge of these food items. Also the result showed that fruits were consumed occasionally aside an increase availability of these food items in the Asesewa markets (Bendech *et al*., 2014). Mangoes were mostly consumed which was not surprising as many homes in the community had the trees. However, the participants mentioned that their consumption of fruits is dependent on whether they were in season. Therefore, it will be more appropriate to assess their food intake seasonally to help determine their actual food intake within a period of time. A study conducted among Mozambique women showed that adequate intake of micronutrients reduced upon high mango consumption (Arimond *et al*., 2010). During the mango season, it has been observed that there is an increased number of houseflies in mango growing communities, these insects are known to be causative agents of diarrhoea. Also poor food hygiene may result in this disease condition. Frequent bowel movement may affect absorption of nutrients resulting in the loss of useful micronutrients such as water soluble vitamins. The study revealed that both males and females did not meet their recommended daily intake (RDI) for vitamin B_6, B_{12}, folate, and vitamin C; and iron. These micronutrients are helpful in blood production hence deficiencies may result in anaemia which is a common condition found in this study population (Upper Manya Krobo District Health Directorate, 2012). Throughout the world especially the developing countries, deficiencies of certain micronutrient may lead to anaemia of which iron deficiency is leading, seconded by folate deficiency then vitamin B_{12} deficiency (Yip and Ramakrishna, 2002).

No associations were found between mean folate intake and serum folate or mean vitamin B_{12} intakes and serum B_{12}. Similarly, Owusu *et al.*, (2010) did not find any associations in their study. The authors attributed it to underestimation of folic acid from fortified foods and misreporting of food intake by participants. Misreporting of food intake may be the main reason in our present study since consumption of fortified foods was present in this study. Bread which could be a source of folate because of fortification of wheat flour was not frequently consumed in this population. A few of the participants who reported intakes of nutritional supplements did not use folic acid supplements and also took these supplements occasionally.

Folate deficiency may be seen among individuals of all ages but vitamin B_{12} is more prominent among the elderly due to the poor function of the gastrointestinal tract (GIT) – related to increasing age (Lindenbaum and Allen, 1995; MacFarlane *et al*., 2011). This finding is in close relation with the present study since the mean age was found to be below 50 years (45.11 ± 13.33 years). However, age was a predictor of serum folate in this present study. Other studies have also found age as a predictor of serum folate status (Girelli *et al*., 1998; Friso *et al*., 2002; de Bree *et al*., 2003; Barnabé *et al*., 2015). Other factors which may affect the folate status of individuals include lifestyle practices such as smoking, increased alcohol intake among others, but it was found out that, among the study group there was low consumption of alcoholic beverages and smoking. Therefore, it is obvious that these would not be predictors of serum folate. But the result in this study shows clearly that as age (β=-.22), smoking (β=-.19) and alcohol intake (β=-.07) increases serum folate decreases.

The major source of animal protein in Asesewa was fish which was consumed on a daily basis. This result was not surprising since the community was flanged with rivers and lakes and may lead to an increased availability of less costly fish. Low consumption of milk and its product could be as a result of its cost which is found to be relatively expensive. Also one tin of milk could be shared by the entire household which could mean that nutrients obtained from this product may not meet the requirement of household members. Among the *Krobo's*, consumption of snails is taboo hence it is not surprising that the result showed low consumption. However, all these factors did not affect their protein intake as their percentage energy intake was within the normal range (10-15%) which will help prevent risk of diet related chronic diseases (WHO/FAO, 2003).

Apart from diastolic blood pressure (DBP), the anthropometric measurement of the respondents showed a significant difference among genders (p=0.05). For mean body fat, the result obtained showed that females had a high body fat and body mass index (BMI). This is not surprising since women tend to have more body fat compared to men. According to WHO (2008), 35% of all adults above 20 years were overweight, with 35% of that population being women. Bradbury (2014) reported that increased BMI may lead to a reduction of serum folate. Therefore, most of the females are more likely to be at risk of folate deficiency. The mean BMI (24.16kg/m^2) of participants in this present study however was lower than the BMI in Accra participants in the study by Owusu *et al.*, (2010) which follows the trend observed in studies. Furthermore, there was no significant difference in the systolic blood pressure (SBP) among gender, however, females showed relatively high SBP (130 ± 19.8 mmHg) compared to males. These values show that females in the rural communities also have a high risk of noncommunicable diseases (NCD) aside the increased prevalence of folate deficiency. Due to urbanization there has been a nutrition transition which could be attributed to the increased risk of NCDs in the rural communities. The result showed that 20% of the study participants consumed carbonated drinks 1 to 2 times each week. This was found to be unusual but alterations in dietary habits could be a factor. Heart-related diseases among other NCD, was found to be common in this study population and may be related to the low consumption of fruits and vegetables, as well as dietary fibre (4.87 ± 8.84 g) in this rural community (WHO, 2003).

5.2 CONCLUSION

The study showed that folate deficiency was relatively high in this population with no vitamin B_{12} deficiency. This puts the study population at a high risk of acquiring CVDs, stroke, anaemia, dementia, depression, among others. Also, congenital deformities such as neural tube defects (NTDs), cleft lip and palates are likely to be seen in the infants born to folate deficient mothers. This research finding showed that intake of dietary folate and vitamin B_{12} was not associated with serum folate and vitamin B_{12} concentrations. The recommended dietary intake (RDI) of most of the macro and micronutrients were not met by the respondents. Folate intake was seen to be four times less than the RDI for both males and females. Therefore, fortification of foods mainly contributing to folate in this community will help curb any rise in deficiency diseases.

5.2.1 Limitations of the Study

1. The software for the nutrient analysis did not have some of the local foods as well as values for dietary folate hence similar foods were used instead.
2. There was a likelihood of over or under reporting for the 3 days 24-Hour recall as participant might try to impress
3. An increased number of respondents would have been a good representation of the community in Asesewa but due to financial limitations more respondents were not recruited.

5.2.2 Recommendations

Based on this study I therefore recommend that:

1. Public health and nutrition institutions in Asesewa should help educate the rural population on the consumption of food grown in their communities.
2. Consumption of foods fortified with folic acid should be encouraged in this community.
3. Women of child-bearing age should be encouraged to take in adequate amount of folate-rich foods as well as folic acid supplement daily.
4. Since homocysteine level is a good marker of both folate and vitamin B_{12} status further studies should be carried out to find out the levels in the participants blood.
5. Erythrocyte folate could also be measured to help determine the status of the individuals over a long period of time.
6. A larger population size may be used to help represent the entire population.

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APPENDIXES

Appendix I

RESEARCH PARTICIPANT INFORMATION SHEET

Dear Sir/Madam,

I am a graduate student of the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, University of Ghana. I am undertaking a research on the "Folate and Vitamin B_{12} Status of the adult Population in Asesewa''. In simple terms, folate is a substance which is found in green leafy vegetables like kontomire and it helps in preventing birth defects, anaemia and other chronic diseases like cancer. Vitamin B_{12} is also a substance found only in animal foods and fortified breakfast cereals and it helps in the normal function of the nervous system. For the purpose of the study which is to improve the health of the community, I thereby seek your involvement, cooperation and commitment, as a participant.

Explanation of Procedure

If you agree to participate, you will be required to provide information about yourself and your usual food intake. We will also take measurement on your weight, height, blood pressure and body fat.

Risks and Discomforts

By agreeing to participate in this research, you are likely to experience some minor inconveniences such as delay during the interview and blood sample collection. The blood sample collection will involve pain with a needle prick. However, this will disappear later.

Benefits

You will receive some dietary advice on healthy eating and importance of folate and vitamin B_{12} after the interaction. The findings of this research will also be used in planning dietary intervention programmes for the Asesewa community that will ultimately improve health.

Confidentiality

Your real name or any other data that can be used to trace you will only be used to communicate your final laboratory results but will not be used in the written report. Your identity as a participant will not be disclosed to unauthorized people.

Withdrawal from project

Your participation in this research is voluntary. You have the right to withdraw from this research at any point in time, for any reason and without prejudice.

Costs for participation

You will incur no costs for participating in this research. You will also not be paid for participation in this research.

Rights and Complaints

To clarify any questions concerning the research project, participants can call Ms Martha Kafui Akwetea on Mobile No.: 0207730947. Questions regarding any rights as a person in this research project and in the case of injury due to the project should be directed to the chairman of the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Tel: 0302687975.

Appendix II

CONSENT FORM

Consent to Participate in Research

I____, confirm that, Ms Martha Kafui Akwetea, has briefed me on the nature of the research and my role as a participant. I understand that the researcher is a student of the Department of Nutrition and Dietetics, of the School of Biomedical and Allied Health Sciences, University of Ghana and this research is the student's dissertation.

Hence, I ____, freely and voluntarily give my consent for the use of my data in this research, with the knowledge that I have the right to withdraw from this research at any point in time without explanations and prejudice. I permit the Department of Nutrition and Dietetics, University of Ghana to use the findings from this research as they deem fit on the condition that my name will be disassociated from the results.

____________________	____________________	____________________
Name of Participant	Date	Signature/ Thumbprint

____________________	____________________	____________________
Name of Witness	Date	Signature /Thumbprint

Appendix III

CODE: 0000/ /2015

INDIVIDUAL INTERVIEW

Thank you for agreeing to participate in this study. Please be assured that all answers you provide here will be kept strictly confidential. We ask that you be as honest as possible with your responses.

DEMOGRAPHICS/ BACKGROUND INFORMATION

1. Gender: (1) Female (2) Male

2. What is your age? _____________ years.

3. What is your marital status? (1) single (2) married (3) separated/divorced

(4) Widowed (7) other, specify: __________________________

4. What is your religion? (1) Christian (2) Moslem (3) Traditionalist

(4) Other, specify: ________________________

5. What is the highest level of education you have completed?

(1) University/ Graduate (2) HND (3) SHS (4) JHS (5) Primary school/Less (6) No school

6. Are you currently employed? (1) Employed (2) unemployed (3) retired

7. What is/was your main occupation? _________________________

8. How many years/months have you lived in Asesewa? _________________ years.

9. What is your approximate household (gross) income/month (GH₵)? (1) 0-100 (2) 101-500 (3) 501-1000 (4) 1001-1500 (5) 1501-2000 (6) Don’t know

PERSONAL MEDICAL HISTORY

10. Have you been told by a doctor or other health care worker that you have (had) any of the following:

Condition	Yes	No	Age	Conditions	Yes	No	Age
Heart-related diseases				Diabetes			
Arthritis				High cholesterol			
Cancer (type)				Lung problems			

FAMILY MEDICAL HISTORY

11. Has any member in your family been told by a doctor or other health care worker that they have (had) any of the following:

Conditions	Yes	No	Age	Conditions	Yes	No	Age
Heart-related diseases				Diabetes			
Arthritis				High cholesterol			
Cancer (type)				Lung problems			

SMOKING

12. Have you smoked cigarette before? (1) Past smoker (2) Never used, if stopped at what age? _______________ years.

DIETARY QUESTIONNAIRE

18. How many times do you eat in a day? ______ times.

19. Do you take any special foods for your health? (1) Yes (2) No

If Yes: a) Garlic ___________________________________

b) Moringa ___________________________________

c) Dandelion ____________________________________

d) Other: ____

20. Do you take any nutritional supplements? (1) Yes (2) No, If yes which type _____________

21. Do you take herbal medicines? (1) Yes (2) No, If yes type __________quantity________

22. How many times in a week do you usually eat away from home? _______

23. a. Are you currently a vegetarian (no meat, poultry or fish)? (1) Yes (0) No

b. If yes, since what age? (0) Birth or _____ years of age

Appendix IV

FOOD FREQUENCY QUESTIONNAIRE

Food Groups	**Daily**	**5- 6x/ week**	**3-4x/ week**	**1-2x/ week**	**Twice a month**	**Once a month**	**Seldom**	**Never**
Cereals and Grains								
Polished Rice								
Local Rice								
Wheat								
Maize								
Brown Bread								
Spaghetti/Macroni								
Millet								
Gari								
Others								
Starchy Root And Plantain								
Yam								
Cocoyam								
Cassava								
Plantain								
Potato								
Legumes/oilseeds/ nuts								
Beans								
Groundnut								
Agushie								

Palm nut								
Fruits								
Citrus								
Mangoes								
Banana								
Water melon								
Pawpaw								
Pineapple								
Coconut								
Pear								
Others								
Vegetables								
Okro								
Onion								
Garden eggs								
Turkey berries(bedru)								
Tomato								
Kontomire								
Bokoboko								
Bitter leaf								
Ayoyo								
Cassava leaves								
Cabbage								
Lettuce								
Carrot								
Green pepper								

Dandelion								
Others								
Animal and animal Products								
Milk – whole								
Milk - low fat								
Cheese/Waagashie								
Yogurt								
Burkina								
Mutton								
Chicken								
Fish								
Pork								
Goat meat								
Game								
Crab								
Lobster								
Oyster								
Snail								
Shrimp								
Beverages								
Carbonated cold drinks (cola, fanta)								
Coffee								
Tea								
Milo								

Fresh juice								
Guinness								
Wine								
Beer								
Hard liquor								
Processed food items								
Pastries								
Canned Foods								
Dried fruits								
Fats /Oil								
Butter/Margarine								
Sunflower oil								
Palm oil								
Frytol®								
Palm kernel oil								
Other vegetable oils								
Deep Fried Foods And Pasteries								
Kalawele								
Fried yam								
Fried cocoyam								
Koose								
Maasa								
Others								

Appendix V

Code: 0000/ /2014

Name: ………………………………………………………… Age: ……………………

Occupation: ……………………………………………… Day: ……………………

24-HOUR RECALL

Meal time	Meal	Quantity
Breakfast		
Mid-morning Snack		
Lunch		
Mid-afternoon Snack		
Supper		
Evening Snack		

Appendix VI

PARTICIPANTS LABORATORY RESULTS

B VITAMINS	SERUM LEVEL
VITAMIN B12	
FOLATE	

Appendix V

PICTURES SHOWING SOME OF THE ACTIVITIES PERFORMED