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VITAMIN D DEFICIENCY AND ANAEMIA AMONG ALLIED HEALTH STUDENTS IN THE UNIVERSITY OF GHANA

BY

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DECLARATION

I, Rosemond Noel Duncan hereby declare that this dissertation is the result of my own research work carried out in the Department of Dietetics, School of Biomedical and Allied Health Sciences, University of Ghana, under the supervision of Dr. Freda Dzifa Intiful, and neither the whole nor any of part of it has been or is being submitted for another degree at this or any other university. All references cited have been fully acknowledged.

…… 16/ 10/ 2020 ……

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(Supervisor)

DEDICATION

To two of my favourite people in the world: Babs and Zoe-Grace, thanks for bearing with me especially when I was not around to be wife and mummy to you respectively; I hope our home will reap the benefits of the precious time invested in this programme. Finally, to the person for whose sake I decided to embark on this journey; my mum -Suzanne Beshara Millen - of blessed memory. This is for you mummy, I hope I made you proud!

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ABSTRACT

Background: Young adults transitioning from late adolescence to early adulthood (18-24 yrs) experience a growth spurt that imposes an increased need for both macronutrients and micronutrients. Adequate vitamin D levels mainly from sun exposure and diet improves bone function, especially attaining maximum bone strength and density in young adults. Also, the extra-skeletal functions of vitamin D which includes muscle strengthening and improved cognitive performance are all essential for growth. High prevalence of VDD have been reported in countries with abundant sunlight supply year round and in these areas VDD has been associated with poor diet, skin pigmentation, clothing and lack of sun exposure. Anaemia is another major public health problem known to be high especially among females of child-bearing age. Recent studies have reported an association between Vitamin D and anaemia indicating potential roles for vitamin D in iron homeostasis and erythropoiesis. Unfortunately, there is very little data on the vitamin D status of Ghanaians and moreso among healthy young adults.

Aim: To determine the relationship between vitamin D deficiency and anaemia among undergraduate Allied Health students in University of Ghana, Legon.

Methods: A cross-sectional study design was used in this study. One Hundred and Twenty (120) study participants were randomly selected amongst undergraduates aged 18- 24 years from different departments in SBAHS, Univeristy of Ghana. A self-reported questionnaire was administered to obtain information on the demographic and socio-economic characteristics and anthropometric measurements of students. Also a FFQ was used to assess the dietary intakes of foods rich in vitamin D and iron. A Sunlight Exposure Questionnaire (SEQ) was also used to estimate sunlight exposure of students. Serum Vitamin D levels and Hb levels were determined from the blood samples collected from each student; using an Elisa Kit and the flow cytometry method respectively. Statistical Package for Social Sciences

(SPSS) Version 23.0 was used to analyse the data obtained. Chi-square test, Binary Logistic Regression and Correlation tests were used to determine the association between the variables. An independent t-test was used to find the differences between mean vitamin D and haemoglobin status of the students' gender. Statistical significance was set as $p<0.05$.

Results: The prevalence of vitamin D deficiency was 77.5%. More males (80.3%) than females (74.6%) were vitamin D deficient. Thirty-eight percent (38%) of students were anaemic, majority being females (74.6%). Males had a much lower likelihood of being anaemic levels [AOR = 229.971, (95%CI = 30.141- 1754.641), $p< 0.001$]. Intake of some vitamin D- rich foods (Offal and processed meat), exposure to sunlight on weekends and normal muscle mass were significantly associated with vitamin D status. There was no significant association between vitamin D status and haemoglobin levels of students.

Conclusion: Vitamin D deficiency was very high amongst the students although most of them spent at least 30 minutes in the sun, especially on weekdays. Also, student intake of foods rich in vitamin D and iron was poor and almost all students rarely or never took any form of supplementation. There was no significant association between vitamin D and anaemia. However, the high prevalence of anaemia especially among female students goes to confirm global health reports on anaemia.

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LIST OF ABBREVIATIONS

1, 25$(OH)_2$D	—	1,25-dihydroxyvitamin D
7DHC	—	7-dehydrocholecalciferol
25(OH)D	—	25-hydroxyvitamin D
DBP	—	Vitamin D Binding Protein
Hb	—	Haemoglobin
LC-MS/MS	—	Liquid chromatography – tandem mass spectrometry
IDA	—	Iron Deficiency Anaemia
IOM	—	Institute of Medicine
NHANES	—	National Health and Nutrition Examination Survey
PTH	—	Parathyroid hormone
RDAs	—	Recommended Daily Allowances
SBAHS	—	School of Biomedical and Allied Health Sciences
UV	—	Ultraviolet
UVB	—	Ultra-violet B
VBP	—	Vitamin Binding Protein
VDD	—	Vitamin D Deficiency
VDR	—	Vitamin D receptor
WHO	—	World Health Organization

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Young adulthood refers to an age period during which young people transition from late adolescence (16 – 19 years) to early adulthood (20 – 30 years) (Bonnie, Stroud, & Breiner, 2015; WHO, 2017). During this period, young adults still experience a growth spurt albeit less striking than what occurs during childhood and early adolescence; their bones reach maximum strength and density, known as peak bone mass; their strength and physical performance is at its highest and so is their cognitive development (Bonnie et al., 2015; Levine, 2012). Therefore, young adults require adequate nourishment during this integral phase to protect their health and lay a good foundation for healthy development over the life course.

Vitamin D is an essential fat-soluble vitamin which is essential for calcium and phosphate absorption as well as bone growth and accretion (Wakayo et al., 2015).Vitamin D also functions as a prohormone nutrient and its function extends beyond skeletal functions including muscle strengthening, cellular proliferation and differentiation, immune system modulation, inhibition of renin synthesis and insulin production (Sim et al., 2010; Liu et al., 2015).

Recent studies indicate that insufficient amounts of vitamin D not only leads to rickets in children and osteomalacia in adults, but vitamin D deficiency (VDD) increases the risk of several diseases as hypertension, diabetes, metabolic syndrome, cancer, cardiovascular diseases, autoimmune and infectious diseases, among others, (Atkinson et al., 2014; Nair & Maseeh, 2012; Palacios & Gonzalez, 2014; Saggese, Vierucci, Boot, Czech-Kowalska, Weber, Camargo, Mallet, Fanos, Shaw, & Holick, 2015) .

The term vitamin D refers to both ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). According to Holick (2010), vitamin D3 is the predominant natural source of Vitamin D which is mainly synthesized from exposure to Ultra-Violet rays B (UVB) of sunlight on the human skin. To a lesser extent, both vitamin D3 and D2 can be obtained naturally from animal and plant foods respectively (Pludowski et al., 2018).

The animal sources of vitamin D3 include oily fishes as salmon, mackerel, herrings; fish oil such as cod liver oil; egg yolk and milk; only small amounts of vitamin D2 is obtained from plants, specifically in some UV-irradiated mushrooms (Pludowski et al., 2018). Other sources of dietary vitamin D can be gotten from fortified foods such as breakfast cereals, orange juices, breads and milk and supplements (mostly produced in temperate regions due to seasonal variations) and vitamin D can also be gotten from dietary supplements, usually in the form of vitamin D3 (Bhatt et al., 2019).

The vitamin D status of an individual can be assessed by measuring serum 25-hydroxyvitamin D [25(OH)D], which is the main circulating form of vitamin D in the body (van Schoor & Lips, 2017). There have been several deliberations on the cut off points for VDD using serum 25(OH)D based on different theories by scientific institutions and scholars; however, according to the Endocrine Society Clinical Guidelines, VDD is defined when serum concentration of 25(OH)D is less than 20ng/ml (50 nmol/L) (Holick et al., 2011). The Endocrine Society and IOM both recommend an RDA of 600 IU/day as the minimum requirement for adults 18 years and over in order to maximize bone health and muscle function (Holick et al., 2011; Ross et al., 2011).

Another micronutrient which is of global health concern due to its high deficiency prevalence especially among females of child bearing age is iron (Salam et al., 2016). The World Health Organization (WHO) estimates that about 2 billion people in the world suffer from anaemia of

which approximately 50% are diagnosed as iron deficiency anaemia (IDA) (Liu et al., 2015). One is said to be anaemic if their level of haemoglobin falls below 12.0g/dL in females and below 13.0 g/dL in males according the World Health Organization (Cappellini & Motta, 2015).

Anaemia may be caused by both nutritional and non-nutritional factors however in most young adults, common causes of anaemia are as a result of poor nutrition, chronic blood loss and infection; although cases of chronic or genetic diseases and malabsorption can also lead to anaemia (Balarajan et al., 2011; Liu et al., 2015). In Ghana, studies by Egbi et al. (2014), Intiful et al. (2016) and Mawuko Hamid et al. (2010) have indicated that parasitic infestations by malaria parasites which destroy red blood cells, and hookworm infestations are major contributing factors of anaemia especially among children and women of child bearing age, including pregnant women.

According to WHO (2017), nutritional anaemias result when the intake of certain nutrients is insufficient to meet the demands for synthesis of haemoglobin and erythrocytes. Iron deficiency anaemia (IDA) is the most common nutritional anaemia and the most prevalent nutritional disorder worldwide accounting for about 75%-80% of the total burden of anaemia ; although some nutritional anaemias are less frequently caused by folate and vitamin B12 deficiencies (WHO, 2017). Haem-iron found in animal food sources like meat, fish, poultry and their products, contain the highest amounts of iron and is more bioavailable to the body than non-haem sources of iron, examples of which include dark green leafy vegetables, legumes, nuts and seeds (Hurrell & Egli, 2010).

Due to the ongoing growth spurt in young adults, low haemoglobin levels leading to anaemia results in fatigue, reduced physical activity, low cognitive performance , reduction in immune

competence and increased morbidity from all infections (Bagni, Luiz, & Da Veiga, 2013; Egbi et al., 2014).

In view of the extra skeletal functions of vitamin D, some of which are still being discovered, several studies over the past decade have confirmed an association between VDD and anaemia across the life cycle on both healthy and diseased individuals. (Atkinson et al., 2014; Chowdhury et al., 2019; Kartal & Kartal, 2015; Sim et al., 2010; Smith & Tangpricha, 2015). According to Liu et al. (2015), vitamin D is said to influence the pathogenesis of anaemia through its role in erythropoiesis and iron homeostasis, hence its association with anaemia. Therefore, it has been suggested that sufficient vitamin D status (≥ 30ng/ml) may be important in preventing anaemia, particularly in diseases characterized by inflammation (Smith & Tangpricha, 2015).

1.2 Problem statement

Despite the availability of sunlight all year round, low vitamin D statuses have been reported to be highest in the Middle East and Africa particularly in girls and women (Palacios & Gonzalez, 2014). A recent systematic review by Mogire et al. (2020) on the vitamin D status in Africa reported that approximately 1 in 5 people living in Africa have inadequate 25(OH)D concentrations (with a threshold of <12 ng/ml) and the prevalence was higher in newborn babies and people living in urban populations.

In Ghana, only few studies on the prevalence of VDD have been conducted, however those studies showed a wide prevalence range of VDD from as low as 4% in healthy adults to as high as 92% in diabetic patients (Durazo-Arvizu et al., 2014; Fondjo et al., 2017; Gernand et al., 2019). This presupposes that there is a high prevalence of VDD in Ghana among people with different age groups and health conditions.

Anaemia, is still a nutritional deficiency of global health concern and its prevalence has been reported to be highest in developing countries especially among children and females of child bearing age (Salam et al., 2016; Yasutake, et al., 2013). The Ghana Demographic Health Survey (2014) reported that the prevalence of anaemia among young women (15-49 years) is 42% and anaemia was found to be more prevalent in younger adult females (15 -19 years) than older females. Although the prevalence of anaemia is reported to have decreased from 59% in 2008 to 42% in 2014 according to the survey, its occurrence is still classified as a severe public health problem by the World Health Organization (GSS, GHS, & ICF, 2015; Yasutake et al., 2013).

According to the 2010 Population and Housing Census (GSS, 2011), about one third of Ghana's population is made up of the youth from the ages of 15 – 35 years. This implies that a significant number of Ghanaians are transitioning from adolescence into adulthood and therefore require adequate nutrition in order to meet their physiologic and metabolic needs.

Inadequate amounts of Vitamin D during young adulthood may lead to osteoporosis later on in life as a result of not reaching their maximal bone mass during this developmental phase (Soliman et al., 2014). Vitamin D deficiency in young adults will also result in diminished muscle strength, negative cardiovascular outcomes, insulin resistance, obesity, and neurological disorders (Pérez-López et al., 2010).

Furthermore, the iron requirement is also very high in young adults because of the intense growth and muscle development during this phase which results in an increase in blood volume, sexual maturation and menstrual losses (Salam et al., 2016). Also, iron is significant in psychomotor development and cognitive performance, improving attention span and intelligence which is important to young adults who may be schooling or working (de Andrade Cairo, et al., 2014).

Unfortunately, due to urbanization and advances in technology many people especially the youth tend to spend most of their time indoors engaged in television watching or using technological gadgets or even working behind computers instead of participating in outdoor activities and as such do not receive enough sunlight exposure to maintain adequate levels of vitamin D (Wakayo et al., 2015). Furthermore, due to the fast paced city life, many people have adapted poor eating habits and have resorted to "western style" convenience foods such as fast foods and processed snacks which are relatively cheaper but contain low amounts of vitamin D and iron (Vorster et al., 2011).

Moreover, unknowing to many, studies have reported that people with more melanin (dark skin pigmentation) have natural sun protection hence require at least 3-5 times longer sunlight exposure to make the same amount of vitamin D as a person with a white skin tone (Holick et al., 2011). The vast majority of Ghanaians have dark skin pigmentation and may be susceptible to VDD if they do not spend adequate time being exposed to sunlight. Furthermore, due to the notion that there is ample supply from sunlight in tropical countries as Ghana, most locally produced foods are not fortified with vitamin D (Nimitphong & Holick, 2013).

Regardless of the increased risk factors of VDD and anaemia that young adults are predisposed to as a result of epidemiological changes, there is scanty data on the prevalence of VDD and its associated factors among healthy young adults in Ghana. In addition, studies to investigate the relationship between VDD and anaemia among young adults in Ghana are lacking. Therefore this study would be beneficial in providing empirical data on the prevalence of VDD and anaemia as well as identify specific predictors of VDD and anaemia peculiar to this age group; among undergraduate students in the School of Biomedical and Allied Health Sciences, University of Ghana, Legon.

1.3 Significance of Study

This study provides the prevalence and identify possible risk factors of VDD and anaemia among young adults. It also provides relevant information on their dietary intake of vitamin D and iron- rich foods; their nutritional status; sunlight exposure; vitamin D and haemoglobin status. Policy makers and health providers will be more informed in developing intervention programs and guidelines for vitamin D and iron fortification and supplementation through the findings of this study. The data collected serves as a guide for dietitians and other public health providers in dietary education or counselling with the intention to address VDD and Anaemia.

1.4 Hypothesis

H_0: There is a high prevalence of deficiency of serum vitamin D levels less than 20 ng/ml among young adults in Ghana.

H_0: There is a high prevalence of anaemia (Hb < 12g/dl) among young adults in Ghana

H_0: There is no association between vitamin D deficiency and anaemia among young adults in Ghana

1.5 Aim and Objectives

1.5.1 Aim

To determine the relationship between vitamin D deficiency and anaemia among Allied Health students of the School of Biomedical and Allied Health Sciences in University of Ghana, Legon.

1.5.2 Objectives

The specific objectives are:

1. To determine the frequency of consumption of vitamin D rich foods and iron rich foods among undergraduate Allied Health students.
2. To determine the length of sunlight exposure during the day among undergraduate Allied Health students.
3. To determine the serum concentrations of vitamin D among undergraduate Allied Health students.
4. To determine the haemoglobin concentrations among undergraduate Allied Health students.
5. To determine the association between vitamin D status and anthropometric status; sunlight exposure and Hb levels among undergraduate Allied Health students.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Sources of Vitamin D

Vitamin D is commonly known as a fat-soluble vitamin important for its role in bone and mineral metabolism (Pludowski et al., 2018). However based on the pleiotropic functions of vitamin D which are still being discovered, vitamin D is also defined as a classic steroid hormone required in regulating several biological processes in the human body by regulating gene expression (Weydert, 2014). By means of activation of plant and animal sterol fractions - ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) respectively- through the Ultra Violet B (UVB) rays of sunlight, Vitamin D is produced (Weydert, 2014).

The major source of vitamin D is from sunlight exposure (Holick & Chen, 2008). However, according to Hossein-nezhad & Holick (2013) due to the oblique nature of the sun's rays, only a small amount of UVB rays reaches the earth's surface in the early morning or late afternoon hours of the day. Thus, the most efficient way of obtaining vitamin D through UVB rays is when the sun is most perpendicular to the earth's surface between 10 am and 3 pm (Hossein-nezhad & Holick, 2013). Therefore, exposure of at least 30% of the body surface area to sunlight for about 15 to 30 minutes a day when UVB rays are most efficient, can produce averagely 10,000 to 20,000 IU of vitamin D (Pilz et al., 2018).

In contrast, the natural food sources of vitamin D are limited and may contribute only100 to 200 IU of vitamin D per day although this may vary considerably depending on factors such as season/sun exposure habits, latitude, nutrition/supplement intake or ethnicity (Hossein-nezhad & Holick, 2013). Most of the dietary sources of vitamin D comes from animal sources in the form of vitamin D3 and include oily fish such as salmon, sardines, tuna, oils from fish such as cod-liver oil, egg yolks (Pludowski et al., 2018). Vitamin D2 however is gotten from

plant sources and fungal sources such as mushrooms and yeast exposed to sunlight or UV radiation (Pilz et al., 2018). Nevertheless, adequate amounts of Vitamin D in the form of vitamin D3 (cholecalciferol) can also be gotten from dietary supplements and fortified foods (Pilz et al., 2018b). Some foods fortified containing vitamin D include cereals, fruit juices, dairy products and some margarines while supplement sources of vitamin D usually have a daily recommendation of 600-800 IU (Hossein-nezhad & Holick, 2013). Due to the fact the vitamin D is fat soluble, both vitamin D and its metabolites may also be stored and released from the body's adipose tissue when needed (Pilz et al., 2018).

2.2 Vitamin D Metabolism

In humans and animals, 7-dehydrocholesterol (7-DHC), the vitamin D precursor found primarily in the epidermal layer of the skin is activated when exposed to ultraviolet B (UVB) rays of sunlight at a wavelength of 290–315 nm (Weydert, 2014). Upon absorbing the UVB rays of the sun, 7-DHC is converted to pre-vitamin D3 and through thermal isomerization further converted to vitamin D3 which then binds to vitamin D binding protein (VBP) (Pludowski et al., 2018). Bound to the vitamin D binding protein, Vitamin D3 is transported to the liver where it is hydroxylated by vitamin D-25-hydroxylase to form 25-hydroxyvitamin D [25(OH)D] (Weydert, 2014). 25(OH)D is the major circulating form of vitamin D and is also referred to as a pro-hormone although it has no innate hormone activity in this state (Weydert, 2014). For dietary vitamin D, it is absorbed predominantly in the small intestine via chylomicrons then enters the lymphatic system that drains into the superior vena cava before reaching the liver (Pludowski et al., 2018). Dietary sources of Vitamin D3 and D2 share, in general, the same metabolism.

From the liver, 25(OH)D is transported to the kidneys and through further hydroxylation by the enzyme 25-hydroxyvitamin D-1-α-hydroxylase (CYP27B1) is converted into the biologically active form, 1,25 di-hydroxyvitamin D [1,25$(OH)_2$D], also called calcitriol (Weydert, 2014). The conversion of the pro-hormone 25 (OH)D to its active form, 1,25$(OH)_2$D is tightly regulated by parathyroid hormone (PTH), calcium, and phosphorus levels(Pludowski et al., 2018).According to Weydert (2014), calcitriol regulates more than 200 different genes, directly or indirectly, by binding to vitamin D nuclear hormone receptors (VDR), these receptors are known to drive a wide variety of biological processes. In this activated state, vitamin D has classic endocrine effects and regulates serum calcium and bone metabolism (Weydert, 2014).

According to Pilz et al., (2018) whereas serum 1,25$(OH)_2$D levels mainly derive from the kidneys and therefore exert classic endocrine functions, there is also a wide expression of extra-renal 1- α -hydroxylase that converts 25(OH)D to 1,25$(OH)_2$D on a tissue level thereby contributing to autocrine and paracrine functions of 1,25$(OH)_2$D. Pilz et al., (2018) also indicated that given the fact that VDR are found ubiquitously in the nucleus of all the cells of the immune system and can respond to the activated 1,25$(OH)_2$D for gene expression; it provides a sound scientific basis to postulate that vitamin D is important for overall human health.

Vitamin D metabolism is self-regulated through negative feedback mechanisms, serum phosphate and calcium, fibroblast growth factors (FGF-23), and parathyroid hormone (PTH) (Wintermeyer et al., 2016). According to Ritter & Brown (2011), the overall reduction of 25(OH)D causes an increase in PTH levels by a negative regulation mechanism: by binding to the parathyroid vitamin D receptor, active vitamin D inhibits PTH gene transcription .This then may increase bone turnover and consequently bone loss by increasing bone FGF-23gene

expression ; FGF-23, subsequently, might enhance vitamin D inactivation both positively and negatively by 1a-hydroxylase and 24-hydroxylation of 25(OH)D (Wintermeyer et al., 2016).

2.3 Functions of Vitamin D in Young Adults

2.3.1 Musculo-Skeletal Functions

According to Pludowski et al. (2013) the most widely known vitamin D functions are related to intestinal calcium absorption, serum calcium and phosphate homeostasis, and bone formation or resorption processes in order to guarantee the mineralization of the skeleton and support muscle function. Inadequate amounts of vitamin D induces defects of bone mineralization leading to clinical manifestations such as rickets in children and osteomalacia in adults (Holick et al., 2011).

According to Carpenter et al. (2017), rickets is a bone disease that is associated with low serum calcium and low serum phosphate, and is characterized by widening and delay of mineralization of growth plates in bones. Rickets is usually found in infants and children. Osteomalacia on the other hand constitutes defective mineralisation of existing (old) bone during the remodelling process and therefore always goes along with rickets in growing children (open growth plates) and occurs ubiquitously in bones of adults or adolescents (closed growth plates) (Uday & Högler, 2017). Rickets and osteomalacia can lead to bone deformation, as well as isolated and global bone pain and muscle weakness (myopathy) (Pilz et al., 2018). Furthermore, it can also lead to osteoporosis, a disorder characterized by low bone mineral density (BMD) and increased risk of fracture resulting in the majority of cases from impaired muscular function leading to falls (Pludowski et al., 2013).

In the intestine, calcitriol, the active form of vitamin D, enhances calcium and phosphorus absorption from approximately 10 - 15% up to 30 - 40% for calcium and intestinal phosphorus

absorption from approximately 60% to 80% (Hossein-Nezhad & Holick, 2013). 1,25(OH)2D also increases the expression of a calcium binding protein (CaBP) and other proteins that helps the transport of calcium through the intestinal absorptive cell to the serosal side depositing it into the circulation (Holick, 2011). In humans, serum calcium concentration is maintained at a very narrow range of about 2.45–2.65 mmol/L and therefore adequate levels of vitamin D is required for effective absorption and maintenance of normal blood levels of calcium and phosphate, which in turn are required for normal bone mineralization (Holick, 2011) .

In the kidneys, calcitriol stimulates PTH-dependent tubular reabsorption of calcium (Pludowski et al., 2018). Consequently, when the blood ionized calcium concentration decreases below the normal range, a series of anti-hypocalcemic events will occur to restore calcium levels back to the physiologic range (Pludowski et al., 2018). 1,25(OH)2D interacts with its VDR in the parathyroid glands and negatively feedback regulates parathyroid hormone (PTH) production (Holick, 2011).

In the skeletal tissues, calcitiol and PTH works in conjunction to control bone turnover. During bone turnover when calcitriol enters the skeleton it interacts with its VDR in the osteoblast to increase the expression of RANKL (receptor activator of NFκB ligand) (Holick, 2011). Monocytic preosteoclasts have the receptor RANK which interacts with RANKL causing signal transduction resulting in the formation of multi-nucleated osteoclasts which are capable of releasing HCl to dissolve the bone mineral and collagenases to destroy the matrix releasing calcium into the circulation (Holick, 2011).

Adolescence and early adulthood is a crucial phase for bone development because it is the time when the most rapid bone accrual occurs. According to Saggese et al.,(2015) skeletal mass doubles between the beginning of puberty and adulthood where there is maximum increase in strength and density of the bones, known as the peak bone mass. Peak bone mass and lean body

mass are attained at the end of skeletal maturation, between the age of 18–20 years in girls and 20–23 years in boys Saggese et al. (2015). Bone mass is a key determinant of fracture risk; therefore maximizing bone mineral mass during childhood and adolescence may contribute to fracture risk reduction during adolescence and possibly in the elderly (Rizzoli et al., 2010). Osteomalacia in girls has been found to cause pelvic deformities and lead to obstructed labour later in life, as reported by (Uday & Högler, 2017).

In addition to the musculoskeletal functions of vitamin D, a review by Rizzoli et al. (2010) reported that low vitamin D levels lead to muscle weakness which inadvertently increased the risk of falls and fractures in adults. According to Pludowski et al. (2013) it has been postulated that the increases in muscle mass (force) drives the increase in bone strength reflecting the functional adaptation of bone to its function. Therefore, a decrease in muscle mass (force) is expected to decrease bone mass (BMD) because a decreased rate of muscle loading is applied on bone (Pludowski et al. 2013). There is also evidence that vitamin D increases calcium influx in muscle cells and thus may have both direct and indirect calcium-related effects on muscle, specifically on muscle contraction (Girgis et al., 2013).

Vitamin D deficiency thus can cause myopathy, a disease of the muscle tissue, which tends to be more marked in proximal muscles (Ward et al., 2009). In their study to determine if vitamin D status affected muscle function among post menarchal adolescent girls, Ward et al.(2009) reported that serum vitamin D levels was positively associated with muscle power, force, velocity, and jump height. As such, adolescent girls with low-serum 25(OH)D concentration generated less power, and so jump height and velocity were lower than those with higher concentrations of 25(OH)D (Ward et al., 2009).

2.3.2 Extra Skeletal Functions

In recent years, there has been a significant interest in the extra skeletal functions of vitamin D other than its skeletomuscular functions, due to its ubiquity in the human body (Holick, 2011). According to Bikle (2016) the vitamin D receptor (VDR) is found in nearly all, if not all, cells in the body and the enzyme that produces calcitriol and ligand for VDR, namely CYP27B1, is also widely expressed in many cells of the body.

Apart from regulating calcium and phosphorus metabolism, Vitamin D has a profound effect on various biological processes, which include cell proliferation, differentiation, apoptosis, immune regulation, genome stability, and neurogenesis ; Bhatt, 2019). Several evidence based studies have reported the significant role of vitamin D in cancer prevention, improved cardiovascular function, diabetes prevention, prevention of obesity, improved muscle function, enhanced barrier function of the skin, hair follicle cycling, and prevention of immune related diseases (Bikle, 2016; Holick et al., 2011; Pludowski et al., 2013).

2.3.2.1 Vitamin D and Immunity

According to Pludowski et al. (2013) vitamin D receptors (VDR) have also been found on the cells of the immune system agents namely the lymphocytes, monocytes and dendritic cells. Vitamin D binds to VDRs on various cells participating in immune responses, thereby modulating both the activation and deactivation of the innate and adaptive responses. Additionally, both humoral and cellular adaptive responses are affected by vitamin D. Hence Vitamin D has been defined as a natural modulator and regulator of most immune-mediated processes (Agmon-Levin et al, 2013).

Vitamin D signalling is reported to affect the maturation of macrophages which are a type of white blood cells that engulf and ingests foreign materials in the body and also stimulates the

action of other immune cells (Pludowski et al., 2013). In addition, vitamin D helps in regulating the production of macrophage-specific surface antigens and the secretion of the lysosomal enzyme acid phosphatase and hydrogen peroxide; all as a defence mechanism to protect the human body (Pludowski et al., 2013). Unfortunately, these features of antimicrobial functions have been found to be impaired during vitamin D deficiency. As such vitamin D protects against bacteria infections a tuberculosis, acute respiratory infections and even dental caries. A study from Sweden revealed that vitamin D supplementation had a protective effect against respiratory tract infections, leading to a decrease in the number of antibiotic-prescriptions (Pludowski et al., 2013).

As a natural immune modulator, the role of Vitamin D is evident in adaptive immunity since nuclear VDR and vitamin D-activating enzymes are present in both T and B cells (Prietl et al, 2013). Vitamin D aids in reducing the production of auto-reactive antibodies by controlling B-cell activation and proliferation; evidence of which have been reported in epidemiological studies showing an association between vitamin D deficiency and the increased incidence of autoimmune diseases (Prietl et al,, 2013). These autoimmune diseases which include type 1 diabetes mellitus, multiple sclerosis (MS), rheumatoid arthritis (RA), and Crohn disease (CD), have been found to be predominant among people living in higher latitudes, further away from the sun (Prietl et al,, 2013). A longitudinal study in Finland showed that children who received vitamin D supplementation were less likely to develop Type 1 DM compared to those who did not receive supplementation (Weydert, 2014).

2.3.2.2 Vitamin D and Cognitive Impairment

Many vitamin D receptors have been found in different regions of the brain that affect cognition and mood, suggesting that low vitamin D levels may be associated with cognitive decline and

symptoms of depression (Bhatt et al., 2019). Vitamin D is also known to be involved in neuroprotective mechanisms such as modulating the production of nerve growth, amyloid phagocytosis and clearance and vaso-protection (Schlögl & Holick, 2014). However, low concentrations of vitamin D have been associated with impairments in cognitive functions such as memory and orientation, executive function impairments and diagnosis of dementia and Alzheimer's disease (Dean et al., 2011).

Most of the studies showing the association between vitamin D and neurological function are related to adults, According to Bhatt et al. (2019) the risk for cognitive decline increases radically with increasing age of a person and low levels of vitamin D have been associated with substantial cognitive decline in older adults. Nonetheless, a study by (Ganji et al., 2010) to determine the association between serum vitamin D and depression among US young adults populations 15-39 years (from the NHANES III) showed that the likelihood of having depression in persons with vitamin D deficiency is significantly higher compared to those with vitamin D sufficiency. Contrary to this however, a randomized controlled trial conducted by Dean et al. (2011) revealed no association between vitamin D and cognitive or emotional functioning in healthy adults; contrary to similar studies conducted on the elderly.

Furthermore, some studies have postulated that the increase in the prevalence of autism over the last decades could be due to the recommendations to avoid sunlight exposure and generally low vitamin D levels (Pérez-López et al., 2010). In addition, a recent study by Jia et al.(2015) indicated that core symptoms of autism improved in a 32-month-old boy on the Autistic spectrum after vitamin D supplementation. According to Weydert (2014), adequate vitamin D levels in developing babies in the womb and infants ensures normal receptor transcriptional activity vital for brain development and mental functioning. Also Vitamin D is said to influence cognitive function by affecting the amino acid building blocks directly involved in learning,

memory, motor control, and social behaviour and is closely associated with the executive function of the brain (Weydert, 2014).

2.3.2.3 Vitamin D and Cardiovascular Diseases

The cardiovascular system is a target tissue for vitamin D since VDRs as well as vitamin D metabolizing enzymes are expressed in arterial vessels, heart and almost all cells and tissues with relevance for the pathogenesis of cardiovascular diseases (Pludowski et al., 2013). Vitamin D plays a protective role in the cardiovascular system through downregulating the renin–angiotensin–aldosterone system, cardiac remodelling, regulating the endothelial response to injury and blood coagulation by increasing thrombus formation and tissue factor activity (Schlögl & Holick, 2014).

Systematic reviews and meta-analyses of observational studies have confirmed that low levels of 25(OH)D are associated with cardiovascular risk factors such as diabetes mellitus, dyslipidemia and arterial hypertension and predicts cardiovascular events including strokes (Pludowski et al., 2013). In a meta-analysis including 65,994 participants and 6123 cardiovascular disease cases, the risk of cardiovascular events significantly increased with decreasing 25(OH)D levels below 24 ng/mL (60 nmol/L) (Pludowski et al., 2013).

2.4 Vitamin D Testing

Vitamin D status is assessed by measuring serum 25-hydroxyvitamin D [25(OH)D], the main circulating form of vitamin D in the body (van Schoor & Lips, 2017). Although 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) or calcitriol is the biologically active form of vitamin D in the human body, 25(OH)D is used in testing for vitamin D status because it has a much higher

serum concentration and a longer half-life lasting about 3 weeks whilst calcitriol has a short half-life of about 1 day (Pilz et al., 2019).

However, the Endocrine Society Clinical Practice Guidelines suggests using serum 1,25$[OH]_2$D only in monitoring certain conditions, such as acquired and inherited disorders of vitamin D and phosphate metabolism (Holick et al., 2011).

The methods used for vitamin D metabolite measurement can be divided into two main groups namely the immunochemical methods which includes radioactive, enzymatic or chemiluminescence detection; and physical detection methods which includes High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Tandem Mass Spectrometry method (LC-MS/MS) (van den Ouweland, 2016). According to Giustina et al., (2019) and Wejse et al., (2007) the LC-MS/MS method is currently the gold standard in testing for vitamin D and its metabolites. This is because the LC-MS/MS method is more sensitive and provides more accurate results. Nonetheless, only about 10% of clinical laboratories use the LC-MS/MS method because this technique requires expensive hardware, technical expertise and can be laborious as it requires optimization of many steps (van den Ouweland, 2016).

Conversely, most clinical laboratories use the immunoassay method for vitamin D testing since the procedure is simpler, automated and not as expensive as the physical detection method (van den Ouweland, 2016). The principle of the immunochemical tests are mainly based on competitive principle: antibody (Ab) or protein binding formats; however the tests results may not be as sensitive and dynamic as LC-MS/MS (van den Ouweland, 2016).

Serum vitamin D levels can be expressed in nanograms per millilitre (ng/ml) or as nanomoles per litre (nmol/L); nmol/L can be converted to ng/ml by dividing by 2.5 (Bentley, 2015).

2.5 Vitamin D Status and Recommended Intake

There have been several controversies by scientific societies and experts about the exact serum concentration of vitamin D to be used in defining vitamin D deficiency and sufficiency (Pludowski et al., 2018). One school of thought as indicated in the Institute of Medicine (IOM) guidelines for preventing and treating vitamin D deficiency, believes the cut-off for vitamin D sufficiency should be 20 ng/ml (50 nmol/L) based only on the skeletal functions of vitamin D (Hossein and Holick, 2013). According to the IOM, there is still no conclusive evidence of the extra skeletal functions of vitamin D at a population level (Hossein-Nezhad & Holick, 2013).

The Endocrine Society on the hand recommends that serum vitamin D levels of 30 ng/ml (75 nmol/L), based on the extra skeletal functions of vitamin D, is required for vitamin D sufficiency (Holick et al., 2011). According to their guideline for evaluating, preventing and treating vitamin D deficiency, the Endocrine society stated, the risk of cancer, infectious diseases, autoimmune diseases and cardiovascular diseases were higher in study participants who had serum vitamin D concentrations below 20 ng/ml (50 nmol/L). (Holick et al, 2011).

Majority of guidelines and societies including IOM and the Endocrine society, nonetheless have come to a general consensus that vitamin D deficiency be defined as serum 25(OH)D concentration less than 20ng/ml (<50nmol/L) ; Vitamin D insufficiency as (21–29 ng/mL (51–74 nmol/L) and Vitamin D sufficiency as >30 ng/mL (75 nmol/L) (Holick et al., 2011)

The Endocrine Society's Clinical Guidelines for evaluation, prevention and treatment of vitamin D deficiency suggests that adults aged 19–50 year require at least 600 IU/d of vitamin D to maximize bone health and muscle function (Holick et al, 2011). However, in order to raise the blood level of 25(OH)D consistently above 30 ng/ml one may require at least 1500–2000 IU/d of vitamin D (Holick et al., 2011).

2.6 Determinants of Vitamin D Deficiency

2.6.1 Inadequate Sunlight Exposure

According to Holick (2017), the major cause for the vitamin D deficiency pandemic is the lack of appreciation that sun exposure has been and continues to be the major source of vitamin D deficiency for most children and adults. Sunlight is the best source of vitamin D and humans are physiologically adapted to produce vitamin D in response to sun exposure, specifically UVB radiation (Baggerly et al., 2015).

Unfortunately the advancement in technology coupled with urbanization and westernization has created an "indoor generation", where people, especially children and the youth, prefer spending time indoors (Parva et al., 2018). The younger population prefer engaging with technological gadgets such as phones, gaming devices, computers and the television rather than going outside to play, exercise or perform other outdoor activities as gardening.

According to a review by Palacios & Gonzalez (2014), low vitamin D status is a problem even in countries with sun exposure all year round such as in the Middle East and Africa. This was confirmed in a recent systematic review of vitamin D deficiency in Africa by Mogire et al., (2020) which reported that rapid urbanisation and associated lifestyle changes in Africa could explain why 25(OH)D concentrations were lower than expected. In addition, populations living in urban areas were observed to have lower 25(OH)D concentrations than rural populations (Mogire et al., 2020).

Another important reason for low sun exposure among people living near the equator can be attributed to their clothing. In Arab countries like Saudi-Arabia where sunshine is plentiful year round, many women especially in their reproductive years have been found to suffer from low vitamin D status (Alzaheb, 2018). Limited sun exposure is a major risk factor for vitamin D deficiency amongst these women because Middle Eastern women generally dress

conservatively, covering up most of their skin while outside including their limbs, head, and often their face and hands, hence receive little to no sun exposure needed for vitamin D production (Alzaheb, 2018).

However, a high mean serum vitamin D concentration of 46 ng/ml was reported among traditionally living populations in East Africa who also received continuous sunlight exposure (Luxwolda et al., 2012). According to Luxwolda et al., (2012), the two tribes, the Masai and the Hadza who were a group of pastoral farmers and hunters and gatherers, spent most parts of the day outdoors and lived in rural areas.

In the temperate regions where there is seasonal sunshine availability, the prevalence of nutritional rickets and Osteomalacia was endemic until vitamin D, sourced from the sun, was discovered in the early 1900's (Hoel et al., 2016). As a result, interventions to fortify foods and supplement with vitamin D were mandatorily implemented to treat and prevent the consequences of vitamin D deficiency as well as to meet the population's recommended requirement; this has drastically reduced the incidence of vitamin D deficiency in temperate regions (Pilz et al., 2018).

2.6.2 Inadequate Intake of Dietary Sources of Vitamin D

The natural food sources of vitamin D which includes oily fish, meat, egg yolk and dairy are limited and are generally not widely consumed in adequate amounts and usually when consumed frequently, is not enough to meet recommended dietary allowance in day (Yang, et al., 2013). In addition, most of the youthful population in developing countries have adapted western diets which is convenient yet low in vitamin D food sources hence inadequate in meeting daily requirement (Forrest & Stuhldreher, 2011).

A review by Pilz et al., (2018) reported that median vitamin D intake collated from several studies globally is below 5 µg (200 IU) per day. This implies that without food fortification and supplementation of Vitamin D, it is unfeasible for most adults to meet the IOM Estimated Average Requirement of 10 µg/d; considering that most people also have inadequate sunlight exposure. Fortunately, in most western countries with seasonal variations, frequently consumed foods such as fruit juices, UHT milk, oils , margarine, butter and most breakfast cereals have been fortified with vitamin D, therefore coupled with supplementation most of their population are able to meet their daily requirement despite limited sunshine supply (Pilz et al., 2018).

Unfortunately, this is not the case in most tropical countries located near the equator, especially in Africa, where vitamin D food fortification and supplementation may be overlooked due to the assumption that the endogenous synthesis from the sun is adequate (Nimitphong & Holick, 2013). Mandatory and voluntary food fortification with vitamin D in most western countries as in the United States and Europe has proven to be the most effective method in increasing serum concentrations of vitamin D (Black et al., 2012). In Finland, for example, evidence from an 11 year follow up study on vitamin D fortification of fluid milk and fat spreads showed that a marked improvement in the vitamin D status especially among children and the adult population (Jääskeläinen et al., 2017) .

2.6.3 Skin Pigmentation

According to Libon et al., (2013), skin pigmentation is largely determined by the concentration and type of melanin in skin. Melanin is a large opaque polymer that is responsible for pigmentation of the skin, hair and eyes; the overall melanin density correlates with the darkness of skin implying that the darker one's skin pigmentation, the more melanin they have

(Schlessinger & James, 2018). Melanin is said to hinder UV transmission hence prevents the transformation of 7-dehydrocholesterol to vitamin D3; preventing cutaneous vitamin D production (Prentice, et al., 2009).

In view of the function of melanin as a natural sun blocker, in order to receive adequate vitamin D in a day, Holick (2011) stated that people with more melanin (dark skin pigmented persons) would need 10 to 15 times more sun exposure to produce equivalent amounts of vitamin D as people with less melanin (light skin pigmented persons). Unfortunately, there have not been many studies in Africa determining the consequence of skin pigmentation and duration of sun exposure on vitamin D status. Therefore, most of Africans, majority of whom are dark skinned are unaware that it takes a longer time for vitamin D production and may probably be vitamin D deficient or insufficient as a result (Palacios & Gonzalez, 2014).

Numerous studies carried out mostly in temperate countries, comparing black and white young and old participants in different countries have reported that black people have lower 25(OH)D levels than white or light skinned people (Poopedi et al., 2011). A study by Poopedi et al., (2011) aimed at determining the vitamin D status in a cohort of healthy 10-year-old urban children in South Africa reported that white children had significantly higher 25(OH)D than their black peers. Another similar study by Rajakumar et al., (2011) amongst African-American and Caucasian school age children revealed that the African-American children had significantly lower vitamin D levels during summer and autumn than the Caucasian children.

2.6.4 Obesity

A meta-analysis by Saneei et al., (2013) confirms a significant inverse correlation between serum 25(OH)D concentrations and BMI in adult population. In view of the fact that vitamin D is a fat-soluble vitamin, adipose tissue, which is largely present in obese individuals, is a

major storage site for vitamin D, hence its bioavailability is said to be reduced in obese or overweight people (Poopedi et al., 2011; Kagotho et al., 2018).

In their research to determine the vitamin D status among healthy adults in Nairobi, Kenya, Kagotho et al.(2018) observed that females were more likely to be vitamin D deficient possibly due to their high BMI and increased subcutaneous tissue. Hannemann et al., (2015) also confirmed the inverse relationship between serum vitamin D concentrations and adiposity among an adult population in Germany and Denmark. Invariably, within the same population or group of people, persons who lost weight or had more muscle mass than fat had higher vitamin D levels.

Furthermore, in their study, Belenchia, et al. (2013) indicated that the correction of poor vitamin D status through supplementation can be effective in treating obesity and other metabolic diseases in adolescents.

2.7 Prevalence of VDD

Vitamin D deficiency, once pandemic among children in the industrialized countries, is now found to be extremely widespread, with approximately 30% and 60% of children and adults worldwide being vitamin D deficient and insufficient respectively (Holick, 2017). A high prevalence of vitamin D deficiency among children and adolescents has been reported even in countries with abundant sunshine especially in Asia, Africa and the Middle-East (Soliman et al., 2014). Children and young and middle-aged adults are at equally high risk for vitamin D deficiency and insufficiency worldwide (Holick et al, 2011).

The prevalence of vitamin D deficiency varies globally based on several factors especially geographical location, skin pigmentation and dietary intake of vitamin D (Holick, 2017).

According to Hilger et al., (2014), the global prevalence of vitamin D deficiency defined as serum 25(OH)D cutoff of less than 20ng/ml, is 37%. In the United States of America, a prevalence of 18% was reported by Herrick et al., (2019), whilst in Europe a higher prevalence of 40% was reported by Cashman et al. (2016). The highest prevalence however have been reported in China (72%) and the Middle East, specifically Iran and Jordan (as high as 90% in some places) (Arabi et al., 2010; Zhang et al., 2013) The high prevalence of vitamin D deficiency in the Middle East has mainly been attributed to the lifestyle of the inhabitants who do not spend a lot of time outdoors and also the conventional style of dressing for women (Alzaheb, 2018).

In Africa, although few prevalence studies on vitamin D deficiency have been done, a pooled prevalence of 34.22% for cut-off serum 25(OH)D concentration less than 20ng/ml was reported in a systematic review by Mogire et al. (2020); similar to the prevalence in Europe. According to Mogire et al. (2020), the mean serum 25(OH)D concentrations were lower in populations living in northern African countries or South Africa compared with sub-Saharan Africa since countries in sub-Sahara Africa are closer to the equator hence closer to the sun.

Additionally, vitamin D status was generally lower in urban areas compared with rural areas, in women compared with men, and in newborn babies compared with their mothers (Mogire et al., 2020). It was however observed that studies with the highest 25(OH)D concentrations in Africa were in populations that were still practising traditional lifestyles, including nomadic animal rearing, hunting, and gathering (Luxwolda et al., 2012; Mogire et al., 2020).

Very few studies on the vitamin D status of Ghanaians have been conducted despite the global high prevalence of vitamin D deficiency even in countries with sunlight supply year round. In their study to determine the vitamin D status of adults (25-45years) in 5 different populations among people of African ancestry located in varying latitudes , Durazo-Arvizu et al., (2014)

observed that among the Ghanaian population sampled from Kumasi, the prevalence of vitamin D deficiency was 4.6% (for serum 25(OH)D <20ng/ml).

Another study by Fondjo et al., (2017) to determine the vitamin D status of among Ghanaian type 2 diabetics (T2DM) in a Diabetic clinic in the Ashanti region showed a prevalence of vitamin D deficiency of 92.4% among T2DM cases and 60.2% among the non-diabetic controls. Furthermore, a vitamin D deficiency prevalence of 13.3% (for serum 25(OH)D <20ng/ml) was reported in a recent study by Gernand et al., (2019) among women of reproductive age in Asesewa, a sub-urban area in the Eastern region of Ghana.

2.8 Anaemia in Young Adults

2.8.1 Sources and Function of Iron in Young Adults

Dietary iron occurs in two forms as heme from animal origin and non-heme from plant origin (Abbaspour et al., 2014). The primary sources of heme iron are haemoglobin and myoglobin gotten from consumption of meat, poultry and fish whereas non-heme iron sources can be gotten from cereals, pulses, legumes, fruits and vegetables (Abbaspour et al., 2014). The bioavailability of heme iron (15%-35%) is higher than that for non-heme iron (2% - 20%) and may be strongly influenced by the presence of other food components; nonetheless, most diets are largely non-heme iron based (Hurrell & Egli, 2010).

The human body absorbs about 1-2mg of iron from dietary intake (Camaschella, 2015). Other sources of iron for iron homeostasis are obtained by recycling iron from the breakdown of red blood cells and the retention of iron in the absence of an excretion mechanism (Camaschella, 2015). Excess iron levels can be toxic to the body so in order to maintain iron homeostasis, about 1-2mg of iron is lost daily as a result of menstrual bleeding, sweating, skin desquamation and urinary excretion (Lopez et al., 2016).

Iron is an essential component of haemoglobin in red blood cells and of myoglobin in muscles which contains about 60% of total body iron (Lopez et al., 2016). Iron is also necessary for biologic functions including respiration, energy production, DNA synthesis and cell proliferation (Camaschella, 2015). During adolescence and early adulthood, iron requirement is high because of intense growth and muscle development which results in an increase in blood volume, sexual maturation and menstrual losses (Salam et al., 2016). The depletion of iron stores in women starts during adolescence with the onset of menarche hence female adolescents especially are at risk of low iron stores if it is not replenished in their diet (Salam et al., 2016).

Iron rich diets are also very important in psychomotor development and cognitive performance especially for infants, children and adolescents (De Andrade Cairo et al., 2014). Several studies have been done to confirm this correlation including a study conducted in rural India by More et al., (2013) on the effect on iron deficiency on cognitive function among adolescents. They observed that cognitive performance was decreased in iron deficient secondary school adolescent girls.

The RDA for iron for males is 8mg/d and for female is 18mg/day (Hurrell & Egli, 2010)). For vegetarian, however, the RDA for iron is 1.8 times higher than in people who eat meat. This is so because heme iron from meat is more bioavailable than non-heme iron from plant-based foods (Hurrell & Egli, 2010).

2.8.2 Haemoglobin Testing and Definitions

Anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status (WHO, 2008). Anaemia occurs when there is a decrease in the level of haemoglobin below 12.0g/dL in women and below 13.0 g/dL in men according the World

Health Organization [WHO] (Cappellini & Motta, 2015). Iron Deficiency Anaemia (IDA) is thought to be the most common cause of anaemia globally (50%), although other conditions, such as folate, vitamin B12 and vitamin A deficiencies, chronic inflammation, parasitic infections, and inherited disorders can all cause anaemia (WHO, 2008).

Nutritional anaemias are caused by deficiency in iron, Vitamin B12 and folate (Scully, 2013); with Iron Deficiency Anaemia (IDA) being the most common cause of anaemia globally (50%)(WHO, 2008). Anaemia is generally associated with fatigue, weakness, dizziness and drowsiness (WHO, 2008).

Iron is an important component of haemoglobin, the substance in red blood cells that carries oxygen from the lungs and transports it throughout the body (Camaschella, 2015). About 70% of the body's iron stores are found in haemoglobin; hence, haemoglobin (Hb) concentration is the most reliable indicator for anaemia at the population level and also a good indicator for Iron Deficiency Anaemia (IDA) (Clark, Imran, Madni, & Wolf, 2017).

The main risk factors of IDA results from insufficient dietary intake of iron, poor absorption of non-heme iron and period of life when iron requirements are especially high as occurs in adolescence and pregnancy (WHO, 2008). Iron forms a major component of myoglobin in muscles and haemoglobin in the blood; hence it is essential during the growth spurt in adolescents due to sharp increase in lean body mass, blood volume, and red cell mass (Biesalski Hans & Jana, 2018). Iron is also vital in sex maturation and menstrual losses especially in female adolescents (Salam et al., 2016).

In adolescents, especially in developing countries, IDA is caused by rapid growth spurt, hormonal changes, malnutrition and starting menstrual periods, insufficient intake of iron resulting from poverty, malnutrition and a diet which is mainly plant based (Camaschella, 2015). Other causes of anaemia which are pathologic and common in developing countries

including Ghana are intestinal worm infestation such as hookworm infections and schistosomiasis, and malaria (Camaschella, 2015). IDA is also common in overweight and obese children (Hutchinson, 2016).

Anaemia, specifically IDA in adolescents will cause reduced physical and mental capacity as well as diminished concentration in work and educational performance (Bagni, Luiz, & Veiga, 2013). It also results in higher vulnerability to infections and poses a major threat to female adolescents during motherhood (Tesfaye et al., 2015).

2.8.3 Prevalence of Anaemia

Anaemia is the most prevalent micronutrient deficiency globally Salam et al. (2016). The WHO global Database on anaemia for 1993 to 2003 states that anaemia affects about 25% of people globally of which 47.4% are preschool children and 35% non-pregnant women (WHO, 2008). The WHO Global prevalence of Anaemia in 2011 stated that South-East Asia, Eastern Mediterranean and African countries had the lowest mean blood haemoglobin concentrations and the highest prevalence of anaemia across population groups (WHO, 2015). The report also indicated the prevalence of anaemia had risen from 37.7% to 41.5% for non-pregnant women and from 38.9% to 48.7% for pregnant women in these regions (WHO, 2015).

In addition, more than half of children in the South-East Asia and African Regions (53.8% or more) were classified as having anaemia. Severe anaemia was highest in the African Region including most countries in West Africa such as Ghana, Togo, Burkina Faso, Cote D'Ivoire and Nigeria, according to the WHO Global prevalence of Anaemia in 2011 (WHO, 2015).In Ghana, the prevalence of anaemia is 42% and that of Iron-Deficiency Anaemia (IDA) is 16% among females of child bearing age according to Ghana Demographic Health Survey (2014).

2.9 Association between VDD And Anaemia

Recent advances in understanding the association between vitamin D and anaemia suggest that maintenance of sufficient vitamin D status may be important in preventing anaemia, particularly in diseases characterized by inflammation (Smith & Tangpricha, 2015). According to Yin & Agrawal (2014), one major anti-inflammatory function of Vitamin D is down-regulating pro-inflammatory cytokines and hepcidin such as the hepcidin-ferroportin axis, that controls iron homeostasis. Hepcidin is a peptide hormone that regulates systemic iron homeostasis and Vitamin D is said to limit the function of hepcidin by increasing iron availability thereby preventing anaemia (Ruchala & Nemeth, 2014).

In their review article, Smith & Tangpricha (2015) also stated that there is evidence that vitamin D may be protective against anaemia by supporting erythropoiesis. Erythropoiesis is the process by which red blood cells are produced and this process is stimulated by the hormone erythropoietin which is produced in the kidneys (Fandrey & Hallek, 2015). During inflammation, inflammatory cytokines are released that impair erythropoiesis by inhibiting the production of erythropoietin in the kidneys (Nemeth & Ganz, 2014). This leads to reduced red blood cell production and lifespan hence anaemia (Smith & Tangpricha, 2015). Vitamin D is thus said to decrease the effect of these pro-inflammatory cytokines and also has a synergistic effect with the hormone erythropoietin to enhance erythropoiesis (Smith & Tangpricha, 2015).

A recent study by Lee et al., (2015) examining the association between VDD and anaemia in a nationally representative sample of Korean children and adolescents showed a strong significance between VDD and anaemia especially IDA in healthy female children and adolescents. However, this association was attenuated after adjustment for iron deficiency suggesting vitamin D's association to IDA. Nonetheless, it is not yet known if VDD is the cause of anaemia.

In their review on the association between vitamin D and anaemia, Smith & Tangpricha (2015) also observed that vitamin D has previously been found to be associated with anaemia in both healthy and diseased populations. However, emerging studies indicate although this association may differ between race and ethnic groups, the association between vitamin D and anaemia is particularly specific to anaemia of inflammation that is, anaemia characterized by chronic diseases (Smith & Tangpricha, 2015).

CHAPTER 3

3.0 METHODS

3.1 Study Design

A cross-sectional design with analytic component was used in this study.

3.2 Study Site

The study was carried out among Allied Health students of the School of Biomedical and Allied Health Sciences (SBAHS) within the College of Health Sciences, University of Ghana. The University of Ghana is Ghana's premier with a current student population of about 37,940 representing a male to female ratio of about 1.4:1 . About 84% out of the student population are Bachelor's Degree students, approximately 13% are Post Graduate students and about 2% offering Sub-Degrees. The University also has a few international students (about 2%) from different countries around the world. The College of Health Sciences is one of the 4 colleges run by the University of Ghana that trains health professionals. The College of Health Sciences is made of 6 institutions one of which is the SBAHS. The SBAHS specializes in biomedical science education and professional training of allied health professionals. It currently offers 7 Bachelor Degree programmes in Dental Laboratory Science, Dietetics, Medical Sciences, Medical Laboratory Sciences, Occupational Therapy, Physiotherapy, Radiography and Respiratory Therapy and Post Graduate programmes in Audiology, Dietetics, Medical Laboratory Sciences and in Speech and Language Therapy, Anatomy and Physiology.

3.3 Study Population

The study population comprised of undergraduate students aged between 18 - 24 years within in the School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana.

3.3.1 Inclusion Criteria

i. Undergraduate Allied Health students aged between 18 - 24 years in the University of Ghana.
ii. Undergraduate Allied Health students who consented to their participation
iii. Apparently healthy undergraduate Allied Health students.

3.3.2 Exclusion Criteria

i. Undergraduate Allied Health students aged 18-24 years who refused to participate in the study.
ii. Undergraduate Allied Health students with existing chronic diseases.

3.4 Sampling Technique

Stratified sampling was used in selecting students from different departments within the SBAHS. All Allied Health students who had their classes at the Korle Bu campus of the University of Ghana were given consent forms to ensure each participant was given an equal chance of being selected. Students that consented to participate were enrolled in the study.

At each Level, proportional sampling was used to determine how many students to choose. Eligible study participants were then stratified according to sex and systematically sampled. A kth number representing the sampling interval was calculated for the stratified groups in each

Level. One participant from the stratified gender list was selected at random followed by the kth person until the desired sample size number was reached.

All selected study participants were given a special code that corresponded with their names on a register. This was done to maintain anonymity during the study nonetheless ensuring that after analysis of blood samples, test results are given to their respective study participants.

3.5 Sample Size Determination

The sample size used in this study was calculated using the formula below. In studies designed to measure a characteristic in terms of a proportion, the equation for sample size is

$$N = \frac{4\,(Z_{Crit}{}^2)\;p(1-p)}{D^2}$$ (Eng, 2003)

Where:

N = the sample size

Z_{crit} = the value for 95% CI is 1.960

D = the total width of the expected CI,

p = the pre-study estimate of the proportion to be measured, (60% representing prevalence of VDD among healthy control groups in the Ashanti region of Ghana, according to Fondjo et al. (2017).

Assume D to be ± 10% (of the estimated proportion)

Hence $N = \frac{4\,(1.960)^2 \times 0.60\,(1-0.60)}{(0.20)^2}$

N = 92.2 ≈ 92

Considering a drop-out rate of 10% and a non-response rate of 10%.

N = 92 + (0.2 x 92)

N = 110.4

Hence the total sample size will be rounded up to 110 participants

3.6 Data Collection

A Questionnaire was administered to obtain information on the demographic and socio-economic characteristics of the students. Anthropometric measurements involving weight (kg) and standing height (m) of the students were measured and used to calculate their Body Mass Index (BMI). A Food Frequency Questionnaire (FFQ) was used to assess the dietary intakes of foods rich in vitamin D and hematinic (Iron rich foods) (Appendix II). A Sunlight Exposure Questionnaire (SEQ) adapted from Glanz et al. (2008) was used to estimate the participants' exposure to sunlight (Appendix II). Data were collected by self-administered structured questionnaires developed after reviewing several similar studies. Students were given explanation of the different sections within the questionnaire and how they were required to answer the questions.

3.6.1 Socio-Demographic Information

The socio-demographic characteristics of student included their age, sex, residency status, programme being offered, undergraduate level, religion, ethnicity and parent's occupation

3.6.2 Anthropometric Measurements

3.6.2.1 Standing Height

Standing height was measured using a stadiometer with a fixed vertical backboard and an adjustable head piece (National Health and Nutrition Examination Survey (NHANES), 2007). All students selected for this study had their heights measured to the nearest 1 cm using the Seca stadiometer (Seca model 213, Hamburg, Germany). Before measuring, eligible students

were asked to remove any hair ornaments, jewellery, or hair buns from the top of the head. Each participant was then asked to maintain an upright position against the backboard with their body weight evenly distributed and both feet flat on the platform, ensuring that their heels were together and toes apart. It was also ensured that the back of their heads, shoulder blades, buttocks, and heels made contact with the backboard. With each participant's head aligned in the Frankfort horizontal plane, they were asked to take a deep breath to straighten their spine whilst the head piece of the stadiometer was lowered to rest firmly on the head and their heights read on the vertical board.

3.6.2.2 Weight

The body weight of study participants was measured to the nearest 0.1 kg using a well calibrated Full Body Sensor Body Composition Monitor and Scale (Omron HB-516C, USA). The students participating in the study were asked to stand in the centre of the scale platform facing the recorder, hands at sides, and looking straight ahead. It was ensured that each participant removed their shoes, socks, emptied their pockets of any item and removed their watches and any heavy jewellery they wore before weighing.

3.6.2.3 Body Mass Index (BMI)

The BMI of each study participant was calculated from the height and the weight measurements taken using the formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{(\text{Height [m]})^2}$$

The BMI results obtained was categorized into WHO (1997) cut-off points for BMI classification in kg/m^2 where Underweight: < 18.5 , Normal : 18.5 – 24.9, Overweight : 25.0

– 29.0 , Obesity Class I : 30.0 – 34.9 , Obesity Class II : 35.0 – 39.9 and Obesity Class III : > 40.

3.6.3 Dietary Intake Assessments

Dietary intakes of vitamin D rich and Iron rich foods of all study participants were assessed using a validated qualitative Food Frequency Questionnaire (Appendix II). A Food Frequency Questionnaire is a limited checklist of foods and beverages with a frequency response section for subjects to report how often each item was consumed over a specified period of time. The FFQ was used to assess the usual intake over a period of time without estimating portion size. The questionnaire comprised of 84 food items in 10 food categories and 5 supplements in the supplement category. The frequency rating scale measured how often a study participant ate a particular food item and the 7 options provided included: 'Never", "Rarely", frequency in a week and frequency in a day.

3.6.4 Sunlight Exposure Questionnaire

The sunlight exposure questionnaire adopted from Glanz et al., (2008) was used to evaluate each study participants' average time of sunlight exposure (Appendix II). The questions inquired how long they spent outdoors on a sunny day between 10am and 4pm on weekdays and weekends. In addition, participants were asked which part of their body was usually exposed under the sun. Muslim girls were also asked about their usual clothing when they were in the company of other Muslims girls only in outdoor private settings.

3.6.5 Determination of Serum Concentrations of Vitamin D

Vitamin D was assayed using the Human Vitamin D ELISA kit from Sunlong Biotech Company Limited (Hangzhou, China) using the Sandwich-ELISA method. Two millilitres

(2ml) of blood was drawn from each participant and put into a gel separator tube. Each tube was labelled with the specific identification numbers given to each participant. The blood samples in the gel separator tubes were taken to the lab and centrifuged at 1000rpm for 4 minutes. After centrifugation, the serum (now the supernatant) was pipetted from each gel separator tube and put into Eppendorf tubes also labelled according to the respective participant ID indicated on the gel separator tubes. The serum in the Eppendorf tubes were stored at -80˚ C until they were ready to be used.

During the serum vitamin D determination, the Eppendorf tubes were taken out of the freezer and thawed to room temperature. The Micro-Elisa strip plate used for the vitamin D assay had been pre-coated with antibody specific to vitamin D. Standards and samples were then added to specific wells on the strip plate for antigens to bind to the antibody on the plate. A second antibody, Horseradish Peroxidase (HRP) was conjugated to an antibody specific to the hormone added, incubated and washed to remove unwanted components.

For colour development, TMB solution was also added to each well on the strip plate. Only wells containing the hormone and the HRP conjugated hormone antibody appeared blue and later turned yellow when the stop solution was added. The Optical density of standards and samples was measured spectrophotometrically at 450 nm. A standard curve was drawn and used to extrapolate the concentrations of the samples.

3.6.6 Determination of Haemoglobin Concentration

In determining the Haemoglobin concentration, 2ml of blood samples were collected from each study participant and put into EDTA (Ethylenediaminetetraacetic acid) tubes; each labelled according to each participant's unique ID. The EDTA tubes functions by binding to calcium and prevents the blood from clotting. The blood samples in the labelled EDTA tubes were kept in a small ice-chest container and transported to the laboratory within 6hrs of sample collection.

Using the URIT-5160 fully automated Hematology analyzer, Full Blood Counts (FBC) of each sample was determined using the Flow Cytometry principle. The Hb concentration was obtained from results of the FBC for each participant.

Flow cytometry is a sophisticated instrument measuring multiple physical characteristics of a single cell such as size and granularity simultaneously as the cell flows in suspension through a measuring device. Its working depends on the light scattering features of the cells under investigation, which may be derived from dyes or monoclonal antibodies targeting either extracellular molecules located on the surface or intracellular molecules inside the cell.

3.7 Data Management

Data from this study was kept under access restriction. All digital information was stored on a laptop under password protection by the researcher. Completed questionnaires were coded, filed and kept in an enclosed container in the Department of Dietetics, University of Ghana. All the information gathered were withheld from public access during the period of the research. All quality assurance protocols were observed before, during and at the completion of this research.

3.8 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) Version 23.0 was used in analysing the data obtained. Categorical data were summarized using frequencies and percentages whilst continuous data were summarized as means and standard deviation. Pearson's chi-square test was used to determine the association between serum vitamin D concentrations and Hb concentration status, anthropometric indices, sun exposure and dietary intakes. The Student's t-test was used in comparing differences between serum vitamin D concentrations and Hb

concentration status between males and females; late adolescents and young adults. Logistic regression analysis was used to predict the odds of having low serum concentration of vitamin D. Statistical significance was set at $p<0.05$.

3.9 Ethical Approval

Ethical approval and permission was sought from the Ethics and Protocol Review Committee (EPRC) of the College of Health Science, University of Ghana. Permission was also sought from the School of Biomedical and Allied Health Sciences (SBAHS) and the Heads of Departments of Medical Laboratory Sciences, Occupational Therapy, Physiotherapy, Radiography, Respiratory Therapy and Dietetics.

All study participants gave their written consent in order to participate in the study. The recruitment ensured voluntary participation of each study participant. A trained phlebotomist was employed to collect the blood samples and 2 research assistants trained to assist in anthropometric measurement taking. All data remained confidential and anonymous by not bearing participants names instead, unique identifications were assigned to each participant once they consented to take part in the study. There was no direct benefit for participation but information on their vitamin D status and Hb concentration status were made known to each study participants in an enclosed envelope. They were advised on how to modify their diet in order to improve upon their vitamin D and Iron levels in order to prevent VDD and anaemia; coupled with increasing their sun exposure to improve vitamin D production on their skin.

CHAPTER 4

4.0 RESULTS

A total number of 120 students were enrolled in this study from the School of Biomedical and Allied Health Sciences. All study participants consented to answering the questionnaire and agreed for blood samples to be taken in order to determine their vitamin D status and haemoglobin levels. All variables were characterised by the gender (Male and Female) of the students.

4.1 Socio-Demographic Information

Table 4.1 shows the demographic characteristics of the participants who partook in this study. The mean age of both male and female study participants was 21.53 ± 1.58 years, with a greater percentage falling under the early adult category. Most of the students (90%) who enrolled in this study resided on campus and almost all study participants were Christians. There was a significant association between gender of students and their fathers' occupations (p = 0.019).

Table 4.1: Socio-demographic characteristics of participants. (N = 120)

Characteristics	**Gender**			**X^2**	***p*-value**
	Males ***n* = 61** **n (%)**	**Females** ***n* = 59** **n (%)**	**Total** ***N* = 120**		
Age Category					
Late adolescents[a]	3 (4.9)	5 (8.5)	8 (6.7)		
Early adults[b]	58 (95.1)	54 (91.5)	112 (93.3)	0.610	0.487
Level					
200	12 (19.7)	7 (11.9)	19 (15.8)		
300	20 (32.8)	26 (44.1)	46 (38.3)		
400	29 (47.5)	26 (44.1)	55 (45.8)	2.229	0.328

Table 4.1 (continued).

Characteristics	Gender			X^2	*p*-value
	Males *n* = 61 n (%)	Females *n* = 59 n (%)	Total *N* = 120 N (%)		
Residence status					
Resident on campus	55 (90.2)	53 (89.8)	108 (90.0)		
Non-Resident on campus	6 (9.8)	6 (10.2)	12 (10.0)	0.004	0.951
Programme					
Medical Laboratory	36 (59.0)	32 (54.2)	68 (56.7)		
Dietetics	5 (8.2)	10 (15.9)	15 (12.5)		
Physiotherapy	9 (14.8)	11 (18.6)	20 (16.7)		
Radiotherapy	9 (14.8)	4 (6.8)	13 (10.8)		
Occupational Therapy	2 (3.3)	1 (1.7)	3 (2.5)		
Respiratory Therapy	0 (0)	1 (1.7)	1 (0.8)	5.246	0.379
Religion					
Christian	59 (96.7)	58 (98.3)	117 (97.5)		
Muslim	1 (1.6)	1 (1.7)	2 (1.7)		
Other	1 (1.6)	0 (0)	1 (0.8)	1.152	1.000
Ethnicity[c]					
Akan	31 (50.8)	39 (66.1)	70 (58.3)		
Ga-Dangbe	15 (24.6)	7 (11.9)	22 (18.3)		
Ewe	10 (16.4)	8 (13.6)	18 (15.0)		
Frafra	1 (1.6)	2 (3.4)	3 (2.5)		
Guan	1 (1.6)	1 (1.7)	2 (1.7)	4.894	0.435
Father's Occupation[d]					
Professional/Technical/ Managerial	33 (54.1)	35 (59.3)	68 (56.7)		
Clergy	2 (3.3)	8 (13.6)	10 (8.3)		
Sales and Services	7 (11.5)	7 (11.9)	14 (11.7)		
Skilled Manual	8 (13.1)	1 (1.7)	9 (7.5)		
Other	9 (14.8)	3 (5.1)	12 (10)	13.123	**0.019***
Mother's Occupation[c]					
Professional/Technical/ Managerial	15 (24.6)	22 (37.3)	37 (30.8)		
Clergy	3 (4.9)	0 (0)	3 (2.5)		
Sales and Services	32 (52.5)	30 (50.8)	62 (51.7)		
Skilled Manual	5 (8.2)	5 (8.5)	10 (8.3)		
Other	3 (4.9)	0 (0)	3 (2.5)	6.878	0.207

**Significance set at p ≤ 0.05 : Pearson Chi-Square and Fisher's Exact Test [a]Late adolescents: (18–19 years); [b]Early adults: (20-24 years); [c]Males: 58(45.9), Females: 57(45.8), Total: 115(95.8); [d]Males: 59(47.5), Females: 54(40.7), Total: 113(94.2).*

4.2 Body Mass Index classification of Students by Gender

Figure 4.1 shows a bar chart of the BMI classification of students based on gender for all participants who enrolled in the study. According to WHO (1999) classification of BMI, majority of the respondents were normal, with more normal males than females. The predominant gender in the obesity classification were females (10.2% and 1.7% respectively for Obesity Class I and Class III). The mean BMI for the students was 22.6 ± 4.3 kg/m^2; 21.8 ± 3.2 kg/m^2 for males and 23.5 ± 5.0 kg/m^2 for females. There was a significant association between BMI and gender of students (p = 0.023).

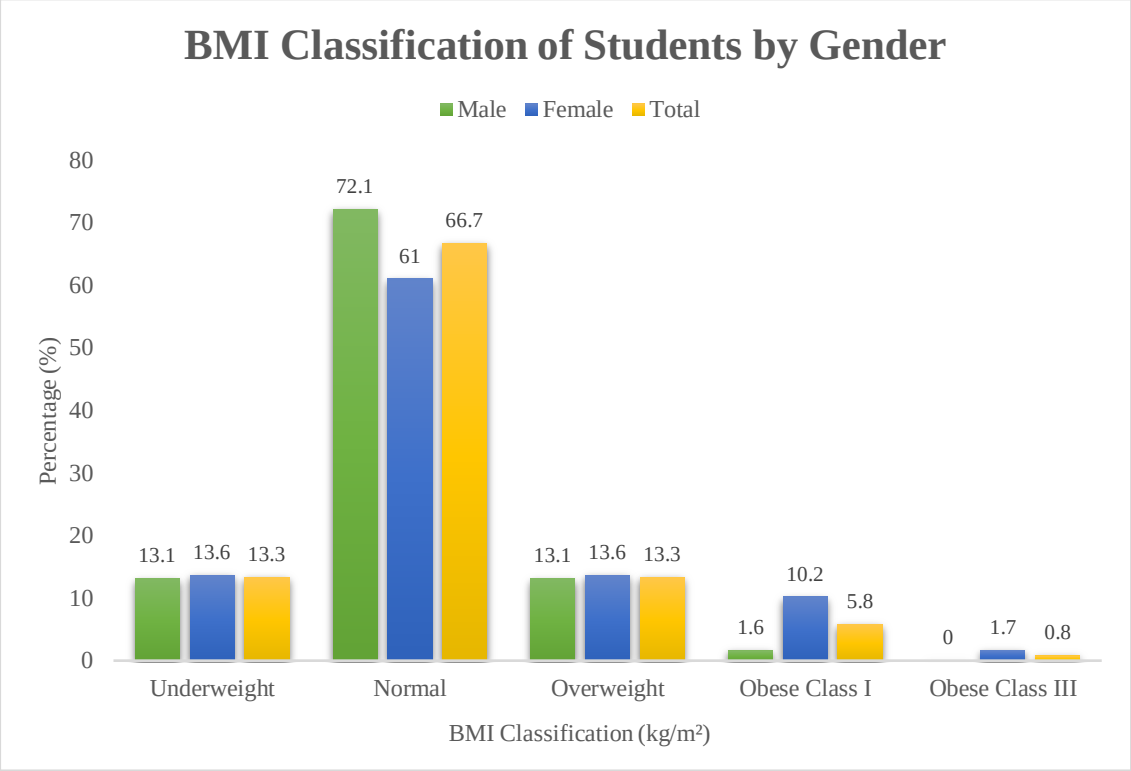

Figure 4.1: Body Mass Index Classification of Students by Gender

WHO (1999) BMI Classifications (kg/m^2): Underweight < 18; Normal 18- 24.9; Overweight 25- 29.9; Obesity Class I 30 – 34.9; Obesity Class II 35 – 39.9; Obesity Class III >40

4.2.1 Body Fat, Visceral Fat and Muscle Mass classification by Gender of students

The body fat, visceral fat and muscle mass classification is indicated on Table 4.2. There were significant associations between gender and body fat (p = 0.034), visceral fat (p = 0.006) and muscle mass (p <0.001). More females had both high and very high body fat than males. A significant number of male participants (p< 0.001) had muscle mass percentages above the normal range compared to females who were within the normal range.

Table 4.2: Body Fat, Visceral Fat and Muscle Mass classification by Gender of students. (N=120)

Characteristics	Gender			X^2	*p*-value
	Males *n* = 61 n (%)	Females *n* = 59 n (%)	Total *N*=120 N (%)		
Body Fat[a]					
Low	8 (13.1)	3 (5.1)	11 (9.2)	9.643	**0.034***
Normal	37 (60.7)	28 (47.5)	65 (54.2)		
High	7 (11.5)	15 (25.4)	22 (18.3)		
Very High	7 (11.5)	13 (22.0)	20 (16.7)		
Visceral Fat[b]					
Normal	53 (86.9)	59 (100)	112 (93.3)	7.766	**0.006***
High	5 (8.2)	0 (0)	5 (4.2)		
Muscle Mass[a]					
Low	4 (6.6)	9 (15.3)	13 (10.8)	54.965	**< 0.001***
Normal	8 (13.1)	41 (69.5)	49 (40.8)		
High	29 (47.5)	7 (11.9)	36 (30)		
Very High	18 (29.5)	2 (3.4)	20 (16.7)		

**Significance set at p≤0.05: Fisher's Exact test. [a] Males: 59 (96.8), Females:59 (100), Total: 118 (98.4). [b]Males:58(95.1), Females: 59 (100), Total: 117 (97.5) . Classification according to Omron Healthcare figures : Body Fat for Males: Low <8%; Normal 8 – 19.9%; High 20 – 24.9%; Very High ≥ 25% ; for Female Low <21%; Normal 21-32.9%; High 33-38.9%; Very High ≥ 39%; Visceral Fat Normal 1-9; High 10-14; Very High 15-30; Muscle Mass for Males: Low <33%; Normal 33.3 – 39.3%; High 39.4 – 44%; Very High ≥ 44.1% for Females: Low <24.3%; Normal 24.3 – 30.3%; High 30.4 – 35.3%; Very High ≥ 35.4%*

4.3 Dietary intake of Vitamin D rich foods and Iron rich foods (haem and non-haem) by Students

Figures 4.3 – 4.11 show the frequency of consumption of vitamin D rich foods and Iron rich foods among the students. The frequencies of food item consumption were divided into 7 categories which included: "Never", "Rarely", "1x Weekly", "2-4x Weekly", "5-6x Weekly", "1x Daily" and ">1x Daily". Most of the bar charts in the different food groups followed a similar pattern, peaking at the frequencies "Rarely" and "1x a week" followed by a drop in percentages of consumption to less than 10 in the "1x Daily" and ">1x Daily" frequencies.

4.3.1 Vitamin D rich Foods and Haem-Iron rich Foods (Figures 4.3 – 4.7):

Figure 4.3 shows the frequency of Red Meat and Red Meat products consumption of the students. Over 60% of students rarely ate liver however once weekly greater than 50% percent of them ate game, kidney and offal. Less than 10% of the students ate red meat and its products once and greater than once daily. For poultry and poultry products (Figure 4.4), students consumed mostly chicken/turkey/guinea fowl, chicken eggs and gizzard at least once weekly and chicken eggs the most frequently consumed poultry product.

Shellfish including crabs and shrimps was rarely eaten by students and within the week, fresh or smoked fish was the preferred choice of fish compared to canned fish (Figure 4.5). Figure 4.6 shows that most students consumed pasteurized yogurt and ice-cream more than whole/skimmed milk and cheese was rarely eaten. Generally students never or rarely took any supplements; up to 70% of students had ever taken either a vitamin D or Iron supplement before (Figure 4.7).

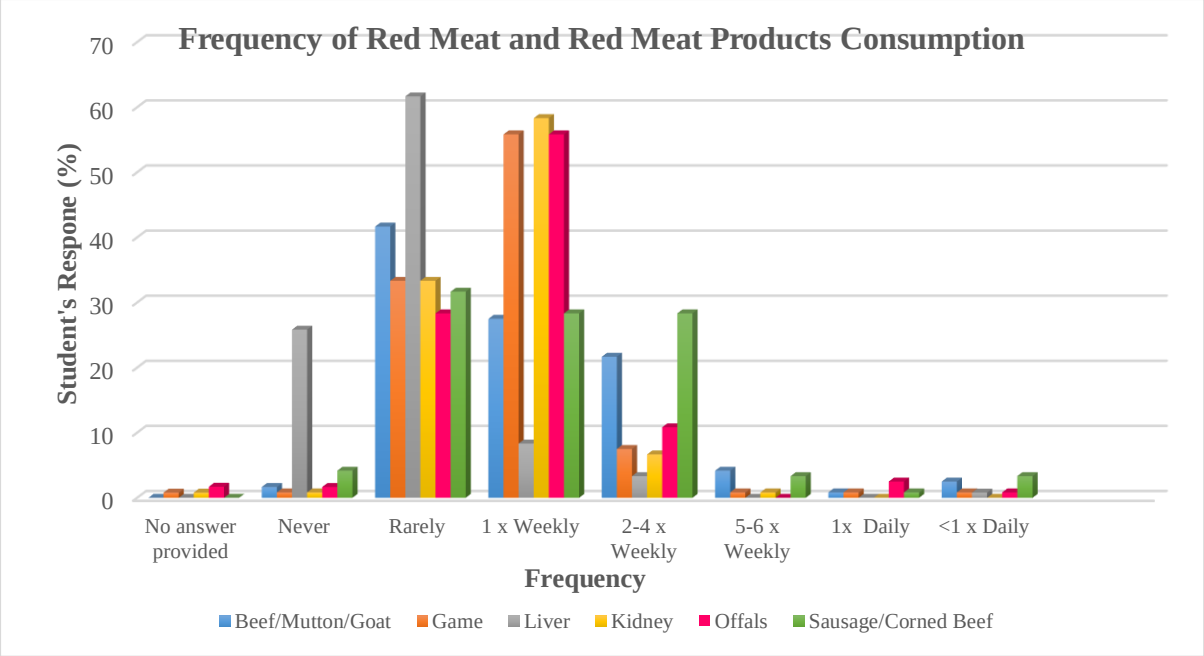

Figure 4.2: Frequency of Red Meat and Red Meat products consumption

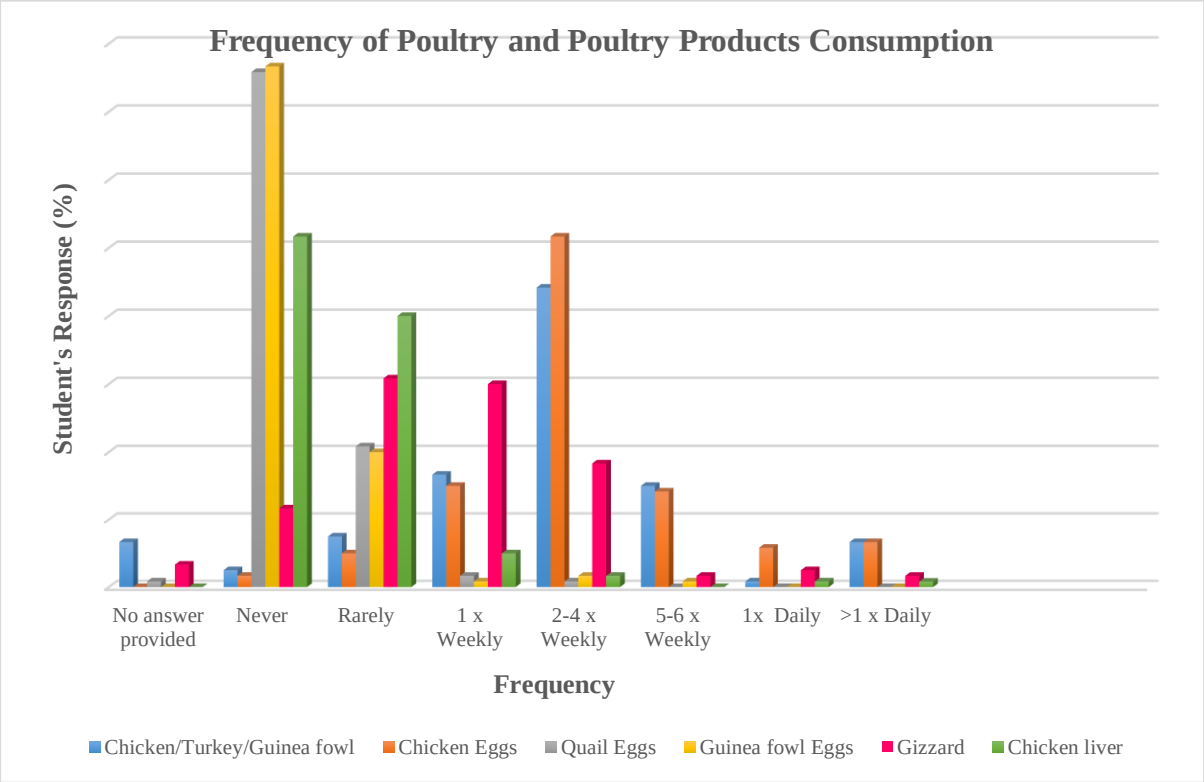

Figure 4.3: Frequency of Poultry and Poultry products consumption

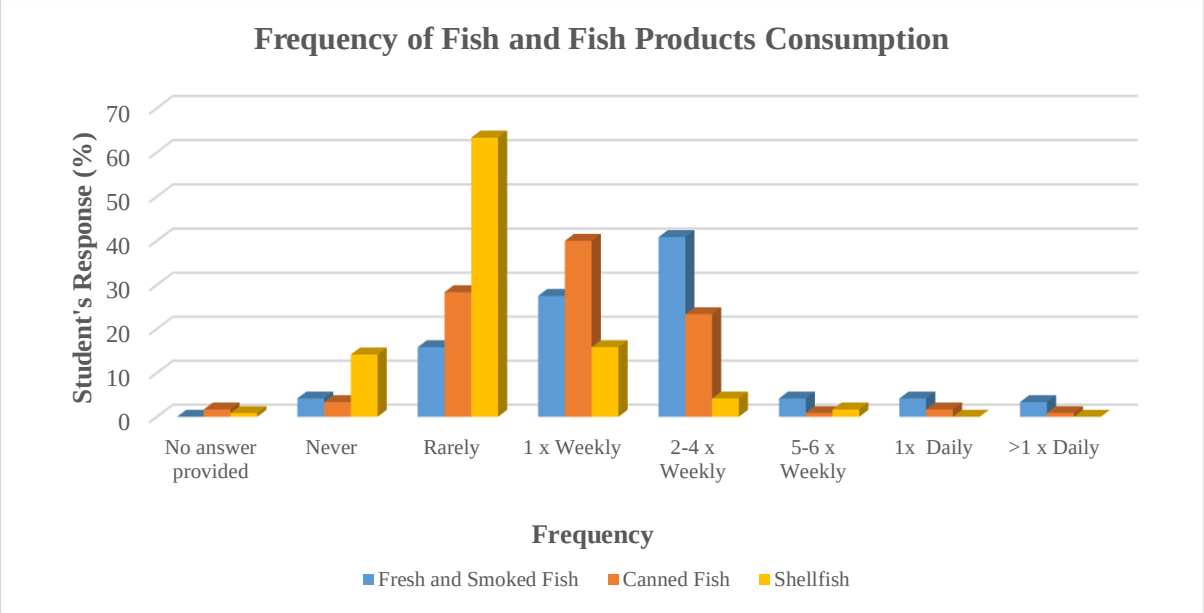

Figure 4.4: Frequency of Fish and Fish products consumption

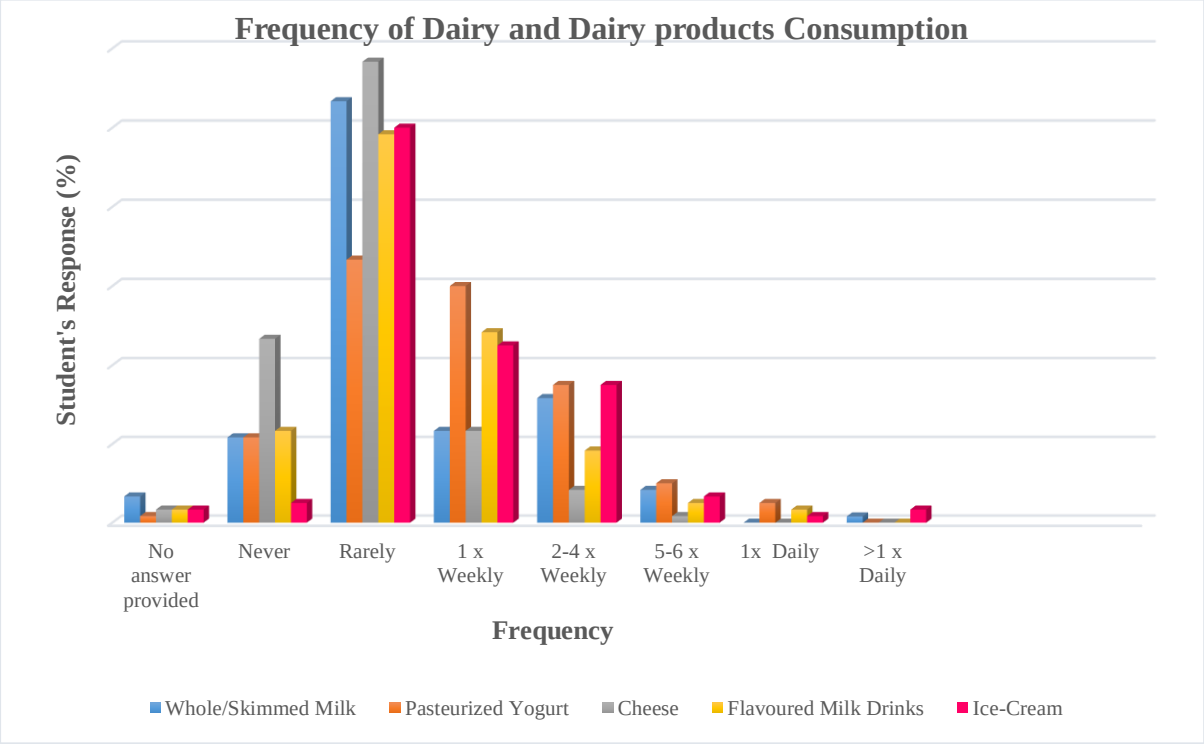

Figure 4.5: Frequency of Dairy and Dairy products consumption

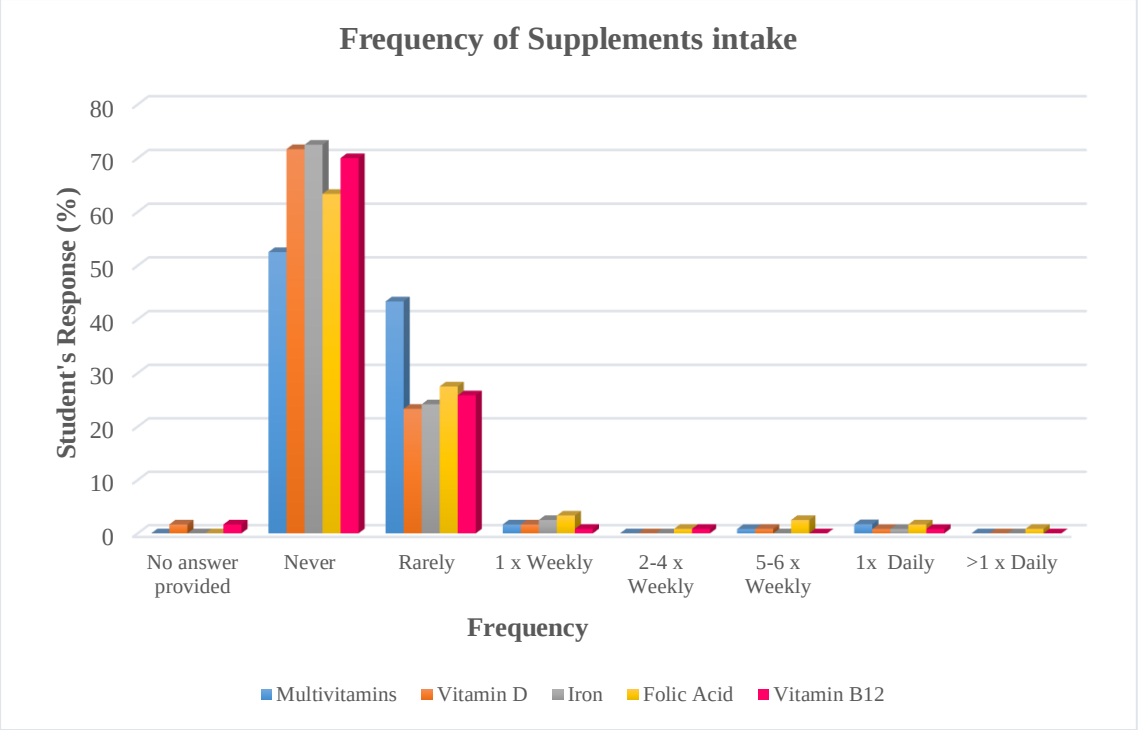

Figure 4.6: Frequency of Supplement intake

4.3.2 Non-haeme Iron rich Foods (Figures 4.8 – 4.11):

In Figure 4.8, the bar chart showing frequency of vegetables consumption, more than 60% of students ate dark green leafy vegetables at least once weekly. However fewer students (about 20%) ate iron rich turkey berries at least once weekly. For legumes/nuts/seeds consumption, students rarely consumed soybeans and seeds such as "agushi" or sesame, however they consumed more groundnuts and beans during the week (Figure 4.9).

In Figure 4.10, iron rich whole grains and cereals were poorly consumed by students; greater than 40% had never eaten weanimix (a cereal-legume blend) and brown rice before. The frequently consumed wholegrains and cereals were oats, whole wheat bread and fortified cereals, in order of decreasing frequencies. Among the fruits consumed by the students, citrus fruits were the most frequently consumed followed by pineapple (Figure 4.11). The bar graph also indicated that students consumed packaged fruit juices more frequently than freshly squeezed fruit juices.

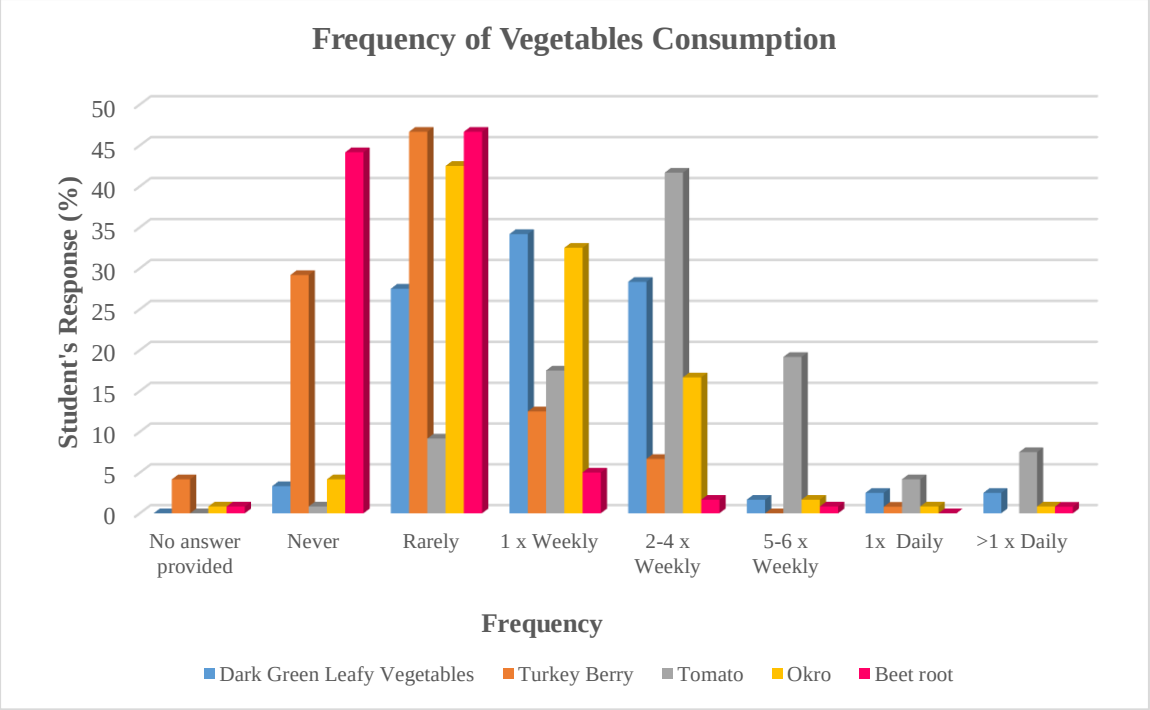

Figure 4.7: Frequency of Vegetables Consumption

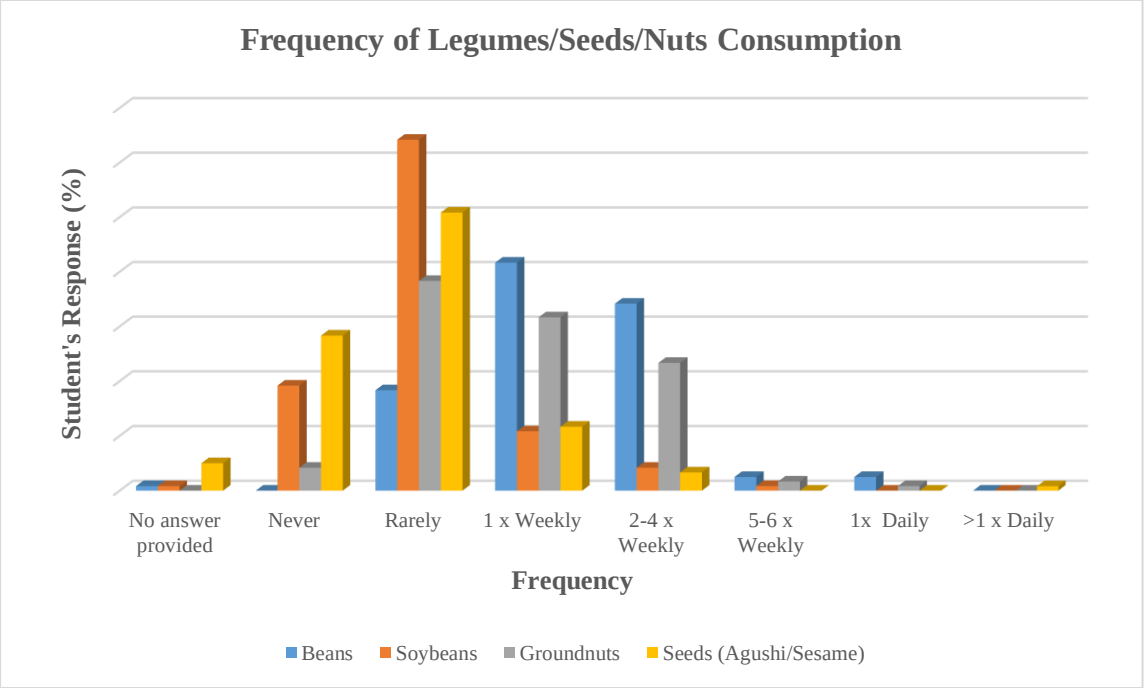

Figure 4.8: Frequency of Legumes, Nuts and Seeds Consumption

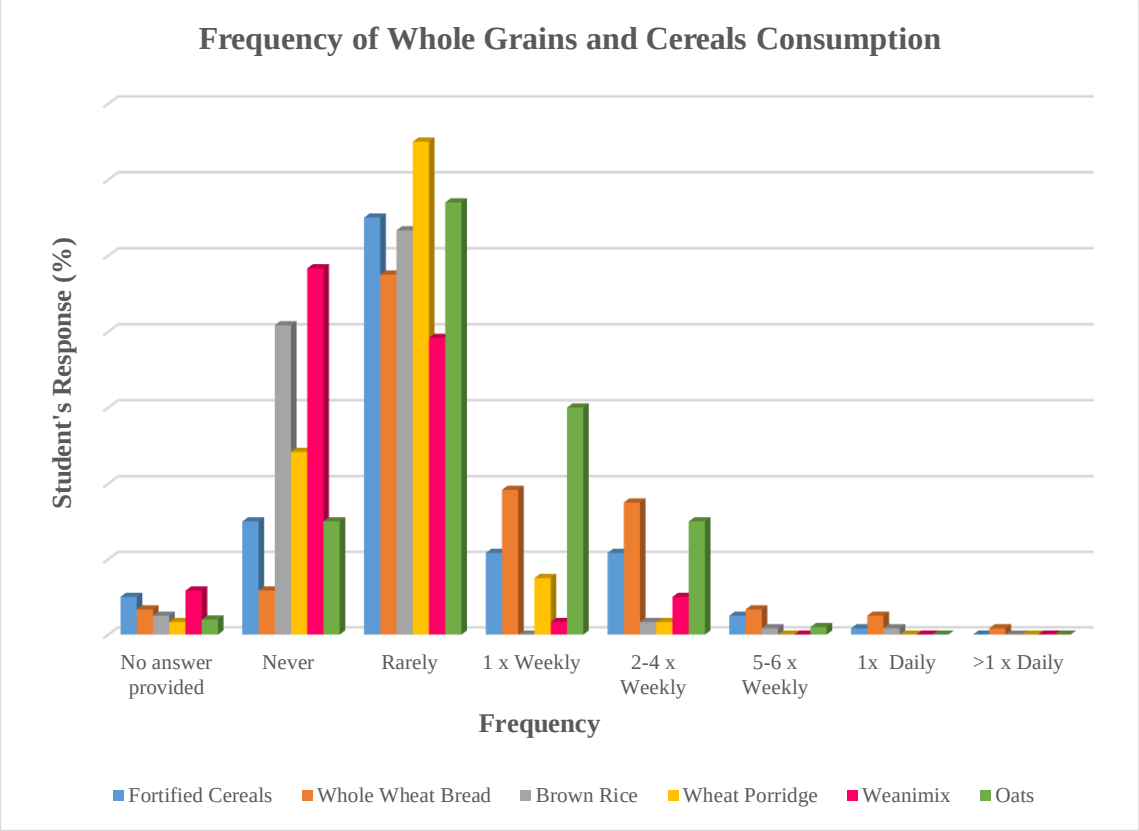

Figure 4.9: Frequency of Whole Grains and Cereals Consumption

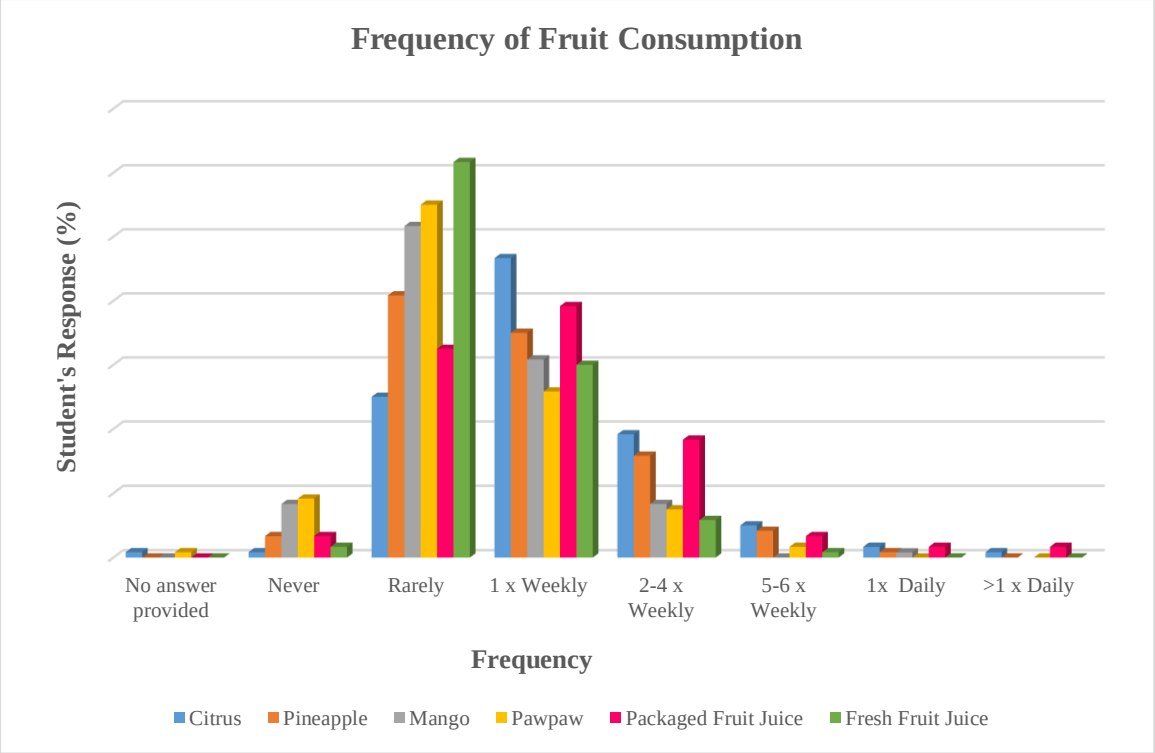

Figure 4.10: Frequency of Fruits Consumption

4.4 Length of Sunlight Exposure based on Gender of students.

The length of exposure of sunlight based on gender is presented in Table 4.3. A significant majority (*p*= 0.024) of both male and female students spent 30 minutes or more in the sun on weekends however this was similar on weekdays although there were more females who spent time in the sun (beyond 30 minutes) than males on weekends. Male students frequently spent between 10am -1pm outdoors whilst for female students, they were frequently outdoor in the sun between 7am-10am. Receiving sun exposure for at least 5 or more days in a week was common among more than half of the study participants.

Table 4.3: Length of Sunlight Exposure based on Gender of students. (N=120)

Characteristics	Gender			X^2	*p*-value
	Males *n*=56 n (%)	Females *n*=56 n (%)	Total *N*=112 N (%)		
Duration of Sun Exposure from 10am - 4pm (Weekdays)					
<30 mins/day	4 (6.6)	9 (15.3)	13 (10.8)	2.743	0.433
30 mins-1 hr/day	14 (23.0)	14 (23.7)	28 (23.3)		
>1 hr/day	38 (62.3)	33 (55.9)	71 (59.2)		
Duration of Sun Exposure from 10am – 4pm (Weekends)					
<30 mins/day	6 (9.8)	19 (32.2)	25 (20.8)	9.424	**0.024***
30 mins -1 hr/day	23 (37.7)	15 (25.4)	38 (31.7)		
>1 hour/day	27 (44.3)	22 (37.3)	49 (40.8)		
Time mostly spent outdoors under the sun during the day					
7am – 10 am	12 (19.7)	21 (35.6)	33 (27.5)	8.578	0.073
10am – 1 pm	19 (31.1)	17 (28.8)	36 (30.0)		
1pm – 4pm	13 (21.3)	15 (25.4)	28 (23.3)		
4pm – 7pm	12 (19.7)	3 (5.1)	15 (12.5)		
Average number of days exposed to sunlight in a week[a]					
1 Day	3 (4.9)	1 (1.7)	4 (3.3)	8.111	0.150
2 Days	8 (13.1)	2 (3.4)	10 (8.3)		
3-4 Days	13 (21.3)	16 (27.1)	29 (24.2)		
5 Days or more	30 (49.2)	37 (62.7)	67 (55.8)		

**Significance set at p≤0.05: Pearson's Chi-Square test. [a]Males: 54(44.9), Females 56(46.7), Total 110(91.6)*

4.4.1 Exposure of body parts to sunlight based on Gender of students.

Table 4.4 shows the percentage of body parts of students that is usually exposed to sunlight. A significant number of students (p <0.001) did not expose their arms to sunlight, although significantly more females (35.6%) than males (4.9%) exposed their full arms. Similarly, a significant number of females exposed their Lower arms, Lower legs, Back and Shoulders than male students (p = 0.005, p <0.001 and p = 0.053).

Table 4.4 Exposure of body parts to sunlight based on Gender of students. (N=120)

Characteristics	**Gender**			**X^2**	***p*-value**
	Males ***n*=58** **n (%)**	**Females** ***n*=56** **n (%)**	**Total** ***N*=114** **N (%)**		
Exposure of Face, Head and Neck region					
Yes	53 (86.9)	53 (89.8)	106 (88.3)	0.467	0.909
No	5 (8.2)	3 (5.0)	8 (6.7)		
Exposure of Full arms					
Yes	3 (4.9)	21 (35.6)	24 (20.0)	17.916	**≤0.001***
No	55 (90.2)	35 (59.3)	90 (75.0)		
Exposure of Lower arms (below the elbows)					
Yes	28 (45.9)	43 (72.9)	71 (59.2)	9.859	**0.005***
No	30 (49.2)	13 (22.0)	43 (35.8)		
Exposure of Thighs and Lower legs (below the knees)					
Yes	2 (3.3)	4 (6.8)	6 (5.0)	0.782	0.740
No	47 (77.0)	23 (39.0)	70 (58.3)		
Exposure of Lower legs (below the knees)					
Yes	11 (18.0)	33 (55.9)	44 (36.7)	19.201	**≤0.001***
No	47 (77.0)	23 (39.0)	70 (58.3)		
Exposure of Back and Shoulders					
Yes	1 (1.6)	8 (13.6)	9 (7.5)	6.184	**0.053***
No	57 (93.4)	48 (81.4)	105 (87.5)		

**Significance set at p≤0.05: Pearson's Chi-Square test*

4.5 Anaemia classification using Haemoglobin (Hb) levels based on Gender of students.

According to WHO (2011), the haemoglobin cut-offs for nutritional anaemia are defined as sever, moderate, mild and non-anaemic in order of increasing haemoglobin levels (Table 4.5). About 38% of the students were anaemic (34.2% mildly anaemic and 4.2% moderately anaemic). None of the student's Hb levels were within the severely anaemic category (<7 g/dL for Females and <8 for Males). Almost all of the anaemic students were females (96%). In addition, majority of the females were significantly mildly anaemic whilst most of the males had normal Hb values above 13g/dL.

Table 4.5: Anaemia classification using Hb levels based on Gender. (N=120)

Characteristics	**Gender**			X^2	***p*-value**
	Males	**Females**	**Total**		
	***n*=61**	***n*=59**	***N*=120**		
	n (%)	**n (%)**	**N(%)**		
Haemoglobin levels (g/dL)					
Moderately Anaemic	0 (0)	5 (8.5)	5(4.2)		
Mildly Anaemic	2 (3.3)	39 (66.1)	41 (34.2)		
Non-Anaemic	59 (96.7)	15 (25.4)	74 (61.7)	71.639	**<0.001***
Mean Haemoglobin levels (g/dL)					
Mean ± SD	14.63 ± 1.01	11.34 ± 0.99	13.01 ± 1.93		
Ranges of Haemoglobin levels (g/dL)					
Ranges	12.7 – 17.8	8.4 – 13.1	8.4 – 17.8		

**Significance set at p≤0.05 : Fisher's Exact test Haemoglobin levels cut-offs for Anaemia: for Non-pregnant females >15yrs – Severe (<7g/dL), Moderate (7 – 9.9g/dL), Mild (10 – 10.9g/dL) and Non-Anaemic (≥12g/dL); for males >15yrs – Severe(<8g/dL), Moderate(8 – 10.9g/dL), Mild(11 – 12.9g/dL) and Non-Anaemic (≥13g/dL).*

4.6 Serum Vitamin D Status based on Gender of students

There was no significant association between Vitamin D status and gender (Table 4.6). The Institute of Medicine (IOM) categorises vitamin D levels based on serum vitamin D as Deficient, Insufficient and Sufficient. About 77% of all study participants were vitamin D deficient. A higher percentage of males (80.3%) were vitamin D sufficient than females (74.6%). The standard deviation for the mean vitamin status D (29.63 ± 78.43) ng/ml was greater than the mean hence the median was used.

Table 4.6: Serum Vitamin D Status based on Gender of students. (N=120)

Characteristics	**Gender**			**X^2**	***p*-value**
	Males ***n*=61** **n(%)**	**Females** ***n*=59** **n(%)**	**Total** ***N*=120** **N(%)**		
Vitamin D Status (ng/ml)					
Deficiency	49 (80.3)	44 (74.6)	93 (77.5)		
Insufficiency	5 (8.2)	3 (5.1)	8 (6.7)		
Sufficiency	7 (11.5)	12 (20.3)	19 (15.8)	2.032	0.405
Median Vitamin D status					
Median	2.15	4.29	3.75		
Ranges of Vitamin D status					
Range	0.27 – 460.18	0.56 – 443.22	0.27 – 460.18		

Significance set at p≤0.05: Fisher's Exact Test. Serum Vitamin D cut-offs according to IOM: Deficiency (≤20ng/ml), Insufficient (21 - 29ng/ml) and Sufficiency (≥30ng/ml).

4.7 Association between Vitamin D status and BMI, Body Fat, Visceral Fat and Muscle Mass of students

There were no significant associations between vitamin D status of students and their anthropometric indicators as shown in Table 4.7. Greater than 60% of students who were vitamin D deficient/insufficient had normal BMI as was the case for students who were vitamin D sufficient. Also, 7 out of 8 of the obese students were vitamin D deficient/insufficient. Similar to the BMI results, most students with normal body fat, visceral fat and muscle mass percent were also vitamin D deficient/insufficient.

Table 4.7: Association between Vitamin D status and BMI, Body Fat, Visceral Fat and Muscle Mass of students. (N=120)

Characteristics	Vitamin D status (ng/ml)			X^2	*p*-value
	Deficiency/ Insufficiency	Sufficiency	Total		
	n = 101	*n* = 19	*N*= 120		
Body Mass Index					
Underweight	14 (13.9)	2 (10.5)	16 (13.3)		
Normal	68 (67.3)	12 (63.2)	80 (66.7)		
Overweight	12 (11.9)	4 (21.1)	16 (13.3)		
Obesity Class I	6 (5.9)	1 (5.3)	7 (5.8)		
Obesity Class III	1 (1.0)	0 (0)	1 (0.8)	1.922	0.784
Body Fat[a]					
Low	10 (9.9)	1 (5.3)	11 (9.2)		
Normal	54 (53.5)	11 (57.9)	55 (54.2)		
High	19 (18.8)	3 (15.8)	22 (18.3)		
Very High	16 (13.3)	4 (21.1)	20 (16.7)	0.895	0.944
Visceral Fat[b]					
Normal	94 (93.1)	18 (94.7)	112 (93.3)		
High	4 (4.0)	1 (5.3)	5 (4.2)	0.545	1.000
Muscle Mass[a]					
Low	10 (9.9)	3 (15.8)	13 (10.8)		
Normal	43 (42.6)	6 (31.6)	49 (40.8)		
High	28 (27.7)	8 (42.1)	36 (30.0)		
Very High	18 (17.8)	2 (10.5)	20 (16.7)	2.841	0.614

**Significance set at p≤0.05: Fisher's Exact test. [a] Deficiency/Insufficiency: n= 99, Sufficiency: n = 19; [b]Deficiency/ Insufficiency: n= 98, Sufficiency: n = 19*

4.8 Association between vitamin D status and vitamin D rich foods

The association between vitamin D status among students and some selected vitamin D rich foods is described in Table 4.8. Within the Red Meat and Red Meat Products food group, there were significant associations between the vitamin D status of students and their consumption of : Red Meat (Beef/Goat/Mutton), Offal meat and processed red meat. A significant number (47.5%) of students who never/rarely consumed Beef/Goat/Mutton were also vitamin D deficient/insufficient. In addition, 12 out of 19 students with vitamin D sufficiency statuses ate processed meats twice or more weekly.

For Poultry and Poultry products, there was no significant association with the vitamin D status of students. Over 70% of students who were vitamin D sufficient consumed poultry twice or more weekly. Chicken eggs were the most frequently consumed poultry among majority of students both vitamin D deficient/insufficient and sufficient.

There were no significant associations between vitamin D status and Fish and fish products; Dairy and Dairy products; Spreads and Supplements. Nearly 50% of students who were vitamin D deficient/insufficient consumed Fish and Evaporated milk at least twice weekly however most student never/rarely took multivitamins or vitamin D supplements.

Table 4.8: Association between vitamin D status and selected vitamin D rich foods among male and female students. (N=120)

Frequency of Vitamin D rich Foods Consumption	Vitamin D status (ng/ml) Deficiency/ Insufficiency *n* = 101 n(%)	Sufficiency *n* = 19 n(%)	Total *N*= 120 N(%)	X^2	*p*-value
Red Meat and Red Meat Products:					
Beef/Goat/Mutton					
Never/Rarely	48 (47.5)	4 (21.1)	52 (43.3)		
Once weekly	24 (23.8)	9 (47.4)	33 (27.5)		
Twice or more weekly	29 (28.7)	6 (31.6)	35 (29.2)	5.822	0.050
Liver					
Never/Rarely	88 (87.1)	17 (89.5)	105 (87.5)		
Once weekly	8 (7.9)	2 (10.5)	10 (8.3)		
Twice or more weekly	5 (5.0)	0 (0)	5 (4.2)	0.713	0.722
Other Offal Meat (Tripe, intestines, trotters)					
Never/Rarely	90 (89.1)	13 (68.4)	103 (85.8)		
Once weekly	7 (6.9)	6 (31.6)	13 (10.8)		
Twice or more weekly	4 (4.0)	0 (0)	4 (3.3)	8.078	**0.013***
Processed Meat (Corned beef/ Sausages)					
Never/Rarely	39 (38.6)	4 (21.1)	43 (35.8)		
Once weekly	31 (30.7)	3 (15.8)	34 (28.3)		
Twice or more weekly	31 (30.7)	12 (63.2)	43 (35.8)	6.582	**0.045***

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.8 (Continued).

Frequency of Vitamin D rich Foods Consumption	Vitamin D status (ng/ml) Deficiency/ Insufficiency *n* = 101 n(%)	Sufficiency *n* = 19 n(%)	Total *N*= 120 N(%)	X^2	*p*-value
Poultry and Poultry Products:					
Chicken/Turkey/Guinea fowl/Duck					
Never/Rarely	20 (19.8)	0 (0)	20 (16.7)		
Once weekly	15 (14.9)	5 (26.3)	20 (16.7)		
Twice or more weekly	63 (65.3)	14 (73.7)	80 (66.7)	5.808	0.055
Chicken Eggs					
Never/Rarely	7 (6.9)	1 (5.3)	8 (6.7)		
Once weekly	16 (15.8)	2 (10.5)	18 (15.0)		
Twice or more weekly	78 (77.2)	16 (84.2)	94 (78.3)	0.310	0.896
Chicken Gizzard					
Never/Rarely	46 (45.5)	9 (47.4)	55 (45.8)		
Once weekly	30 (29.7)	6 (31.6)	36 (30)		
Twice or more weekly	25 (24.8)	4 (21.1)	29 (24.2)	0.159	1.000
Chicken Liver					
Never/Rarely	94 (93.1)	16 (84.2)	110 (91.7)		
Once weekly	5 (5.0)	1 (5.3)	6 (5.0)		
Twice or more weekly	2 (2.0)	2 (10.5)	4 (3.3)	3.554	0.163

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.8 (Continued).

Frequency of Vitamin D rich Foods Consumption	Vitamin D status (ng/ml) Deficiency/ Insufficiency *n* = 101 n(%)	Sufficiency *n* = 19 n(%)	Total *N*= 120 N(%)	X^2	*p*-value
Fish and Fish Products:					
Fresh and Smoked Fish (Red fish, Cassava, Mackerel, Tuna, Tilapia, Herrings)					
Never/Rarely	23 (22.8)	1 (5.3)	24 (20.0)		
Once weekly	24 (23.8)	9 (47.4)	33 (27.5)		
Twice or more weekly	54 (53.5)	9 (47.4)	63 (52.5)	5.494	0.062
Canned Fish (Tuna, Sardine, Mackerel)					
Never/Rarely	31 (30.7)	9 (47.4)	40 (33.3)		
Once weekly	41 (40.6)	7 (36.8)	48 (40)		
Twice or more weekly	29 (28.7)	3 (15.8)	32 (26.7)	2.252	0.318
Shellfish (Crabs, Oysters, shrimps)					
Never/Rarely	79 (78.2)	15 (78.9)	94 (78.3)		
Once weekly	16 (15.8)	3 (15.8)	19 (15.8)		
Twice or more weekly	6 (5.9)	1 (5.3)	7 (5.8)	0.159	1.000

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.8 (Continued).

Frequency of Vitamin D rich Foods Consumption	Vitamin D status (ng/ml) Deficiency/ Insufficiency *n* = 101 n(%)	Sufficiency *n* = 19 n(%)	Total *N*= 120 N(%)	X^2	*p*-value
Dairy and Dairy Products:					
Evaporated Milk					
Never/Rarely	26 (25.7)	4 (21.1)	30 (25.0)		
Once weekly	25 (24.8)	6 (31.6)	31 (25.8)		
Twice or more weekly	50 (49.5)	9 (47.4)	59 (49.2)	0.502	0.849
Pasteurized yogurt					
Never/Rarely	45 (44.6)	9 (47.4)	54 (45.0)		
Once weekly	30 (29.7)	6 (31.6)	36 (30.0)		
Twice or more weekly	26 (25.7)	4 (21.1)	30 (25.0)	0.215	0.902
Dairy cheese					
Never/Rarely	86 (85.1)	14 (73.7)	100 (83.3)		
Once weekly	10 (9.9)	4 (21.1)	14 (11.7)		
Twice or more weekly	5 (5.0)	1 (5.3)	6 (5.0)	2.287	0.314
Dairy Ice-cream					
Never/Rarely	54 (53.5)	11 (57.9)	65 (54.2)		
Once weekly	22 (21.8)	5 (26.3)	27 (22.5)		
Twice or more weekly	25 (24.8)	3 (15.8)	28 (23.3)	0.749	0.712

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.8 (Continued)

Frequency of Vitamin D rich Foods Consumption	Vitamin D status (ng/ml) Deficiency/ Insufficiency *n* = 101 n(%)	Sufficiency *n* = 19 n(%)	Total *N*= 120 N(%)	X^2	*p*-value
Spreads:					
Butter					
Never/Rarely	81 (80.2)	12 (63.2)	93 (77.5)		
Once weekly	14 (13.9)	4 (21.1)	18 (15.0)		
Twice or more weekly	6 (5.9)	3 (15.8)	9 (7.5)	3.518	0.131
Margarine					
Never/Rarely	59 (58.4)	10 (52.6)	69 (57.5)		
Once weekly	27 (26.7)	5 (26.3)	32 (26.7)		
Twice or more weekly	15 (14.9)	4 (21.1)	19 (15.8)	0.679	0.735
Supplements:					
Multivitamin					
Never/Rarely	97 (96.0)	18 (94.7)	115 (95.8)		
Once weekly	2 (2.0)	0 (0)	2 (1.7)		
Twice or more weekly	2 (2.0)	1 (5.3)	3 (2.5)	1.425	0.584
Vitamin D Only					
Never/Rarely	98 (97.5)	18 (94.7)	116 (96.7)		
Once weekly	2 (2.0)	0 (0)	2 (1.7)		
Twice or more weekly	1 (1.0)	1 (5.3)	2 (1.7)	2.296	0.503

**Significance set at p≤0.05: Fisher's Exact test.*

4.9 Association between haemoglobin levels and Iron rich foods by students.

Tables 4.9 and 4.10 show the association between haemoglobin levels of students and selected Iron rich foods (Haem and Non-haem respectively). There was no significant association between haemoglobin level and iron rich foods (Haem and Non-haem).

From the Haem-iron rich food categories, Red meat and its products were least consumed compared to Poultry, Fish and Dairy foods (Table 4.9). At least twice weekly, the most frequently consumed red meat was processed meat (35.8% of students); chicken eggs (78.3% of students) for Poultry products; Fresh/Smoked fish (53.5% of students) for Fish and Evaporated milk (49.2% of students) for Dairy consumption. It was observed that among the non-anaemic students, a greater percentage of them (61.7%) ate haem-iron rich foods at least twice weekly.

Whole grains and cereals were the least consumed non-haem-iron rich foods among the students, compared to Vegetables; Legumes, nuts and seeds (Table 4.10). Tomatoes/Tomato paste was the most frequently consumed vegetable even amongst students with anaemia with over 72% of students consuming at least twice weekly. However nearly all students (both anaemic and not anaemic) never/rarely consumed Turkey Berries (*Solanum torvum*). Beans was also the most frequently consumed legume especially amongst students who were not anaemic (41.9%). Over 70% of students consumed at least one Citrus fruit each week and it was also observed that students consumed more pre-packaged fruit juices than fresh juices. Whole wheat bread was the most consumed whole grain and cereal product although only few students majority being non-anaemic students consumed it frequently.

There was also no significant association between haemoglobin status of students and supplements intake. Over 90% of students never/rarely took any supplements.

Table 4.9: Association between haemoglobin levels and selected Iron rich foods (Haem foods) among male and female students. (N=120)

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n (%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Haem Iron rich Foods					
Red Meat and Red Meat Products:					
Beef/Goat/Mutton					
Never/Rarely	16 (34.8)	36 (48.6)	52 (43.3)		
Once weekly	18 (39.1)	15 (20.3)	33 (27.5)		
Twice or more weekly	12 (26.1)	23 (31.1)	35 (29.2)	5.024	0.091
Liver					
Never/Rarely	42 (91.3)	63 (85.1)	105 (87.5)		
Once weekly	4 (8.7)	6 (8.1)	10 (8.3)		
Twice or more weekly	0 (0)	5 (6.8)	5 (4.2)	3.047	0.246
Other Offal Meat (Tripe, intestines, trotters)					
Never/Rarely	41 (89.1)	62 (83.8)	103 (85.8)		
Once weekly	5 (10.9)	8 (10.8)	13 (10.8)		
Twice or more weekly	0 (0)	4 (3.3)	4 (3.3)	2.236	0.386
Processed Meat (Corned beef/ Sausages)					
Never/Rarely	15 (32.6)	28 (37.8)	43 (35.8)		
Once weekly	11 (23.9)	23 (31.1)	34 (28.3)		
Twice or more weekly	20 (43.5)	23 (31.1)	43 (35.8)	1.908	0.421

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.9 (Continued)

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Poultry and Poultry Products:					
Chicken/Turkey/Guinea fowl/Duck					
Never/Rarely	8 (17.4)	12 (16.2)	20 (16.7)		
Once weekly	8 (17.4)	12 (16.2)	20 (16.7)		
Twice or more weekly	30 (65.2)	50 (67.6)	80 (66.7)	0.147	1.000
Chicken Eggs					
Never/Rarely	3 (6.5)	5 (6.8)	8 (6.7)		
Once weekly	8 (17.4)	10 (13.5)	18 (15.0)		
Twice or more weekly	35 (76.1)	59 (79.7)	94 (78.3)	0.451	0.855
Chicken Gizzard					
Never/Rarely	24 (52.2)	31 (41.9)	55 (45.8)		
Once weekly	11 (23.9)	25 (33.8)	36 (30)		
Twice or more weekly	11 (23.9)	18 (24.3)	29 (24.2)	1.565	0.469
Chicken Liver					
Never/Rarely	44 (95.7)	66 (89.2)	110 (91.7)		
Once weekly	1 (2.2)	5 (6.8)	6 (5.0)		
Twice or more weekly	1 (2.2)	3 (4.1)	4 (3.3)	1.389	0.581

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.9 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Fish and Fish Products:					
Fresh and Smoked Fish (Red fish, Cassava, Mackerel, Tuna, Tilapia, Herrings)					
Never/Rarely	8 (17.4)	16 (21.6)	24 (20.0)		
Once weekly	12 (26.1)	21 (28.4)	33 (27.5)		
Twice or more weekly	26 (56.5)	37 (50.0)	63 (52.5)	0.528	0.798
Canned Fish (Tuna, Sardine, Mackerel)					
Never/Rarely	14 (30.4)	26 (35.1)	40 (33.3)		
Once weekly	19 (41.3)	29 (39.2)	48 (40)		
Twice or more weekly	13 (28.3)	19 (25.7)	32 (26.7)	0.323	0.890
Shellfish (Crabs, Oysters, shrimps))					
Never/Rarely	35 (76.1)	59 (79.7)	94 (78.3)		
Once weekly	8 (17.4)	11 (14.9)	19 (15.8)		
Twice or more weekly	3 (6.5)	4 (5.4)	7 (5.8)	0.337	0.884

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.9 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Dairy and Dairy Products:					
Evaporated Milk					
Never/Rarely	11 (23.9)	19 (25.7)	30 (25.0)		
Once weekly	14 (30.4)	17 (23.0)	31 (25.8)		
Twice or more weekly	21 (45.7)	38 (51.4)	59 (49.2)	0.862	0.669
Pasteurized yogurt					
Never/Rarely	21 (45.7)	33 (44.6)	54 (45.0)		
Once weekly	13 (28.3)	23 (31.1)	36 (30.0)		
Twice or more weekly	12 (26.1)	18 (24.3)	30 (25.0)	0.153	0.942
Dairy cheese					
Never/Rarely	40 (87.0)	60 (81.1)	100 (83.3)		
Once weekly	4 (8.7)	10 (13.5)	14 (11.7)		
Twice or more weekly	2 (4.3)	4 (5.4)	6 (5.0)	0.719	0.795
Dairy Ice-cream					
Never/Rarely	21 (45.7)	44 (59.5)	65 (54.2)		
Once weekly	10 (21.7)	17 (23.0)	27 (22.5)		
Twice or more weekly	15 (32.6)	13 (17.6)	28 (23.3)	3.705	0.163

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.10: Association between haemoglobin levels and selected Iron rich foods (Non-Haem foods) among male and female students. (N=120)

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Non-Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Non-Haem Iron rich Foods					
Vegetables:					
Dark Green Leafy Vegetables					
Never/Rarely	15 (32.6)	22 (29.7)	37 (30.8)		
Once weekly	16 (34.8)	25 (33.8)	41 (34.2)		
Twice or more weekly	15 (32.6)	27 (36.5)	42 (35.0)	0.240	0.918
Turkey Berries (Abeduro)					
Never/Rarely	32 (69.6)	64 (53.3)	96 (80.0)		
Once weekly	9 (19.6)	6 (8.1)	15 (12.5)		
Twice or more weekly	5 (10.9)	4 (5.4)	9 (7.5)	5.085	0.077
Tomatoes/Tomato paste					
Never/Rarely	3 (6.5)	9 (12.2)	12 (10.0)		
Once weekly	8 (17.4)	13 (17.6)	21 (17.5)		
Twice or more weekly	35 (76.1)	52 (43.3)	87 (72.5)	0.961	0.713
Okro					
Never/Rarely	21 (45.7)	36 (48.6)	57 (47.5)		
Once weekly	19 (41.3)	20 (27.0)	39 (32.5)		
Twice or more weekly	6 (13.0)	18 (24.3)	24 (20.0)	3.561	0.181

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.10 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Legumes, Nuts and Seeds:					
Beans					
Never/Rarely	9 (19.6)	14 (18.9)	23 (19.2)		
Once weekly	21 (45.7)	29 (39.2)	50 (41.7)		
Twice or more weekly	16 (34.8)	31 (41.9)	47 (39.2)	0.687	0.756
Soybeans					
Never/Rarely	40 (87.0)	61 (82.4)	101 (84.2)		
Once weekly	4 (8.7)	9 (12.2)	13 (10.8)		
Twice or more weekly	2 (4.3)	4 (5.4)	6 (5.0)	0.448	0.923
Groundnuts					
Never/Rarely	21 (45.7)	30 (40.5)	51 (42.5)		
Once weekly	17 (37.0)	21 (28.4)	38 (31.7)		
Twice or more weekly	8 (17.4)	23 (31.1)	31 (25.8)	2.896	0.254
Seeds (Melon seeds "Agushi", Pumpkin, Sunflower, Chia, Sesame Seeds)					
Never/Rarely	36 (78.3)	65 (87.8)	101 (84.2)		
Once weekly	9 (19.6)	5 (6.8)	14 (11.7)		
Twice or more weekly	1 (2.2)	4 (5.4)	5 (4.2)	4.699	0.087
Tiger Nuts					
Never/Rarely	44 (95.7)	63 (85.1)	107 (89.2)		
Once weekly	1 (2.2)	7 (9.5)	8 (6.7)		
Twice or more weekly	1 (2.2)	4 (5.4)	5 (4.2)	2.986	0.269

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.10 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Non-Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Whole Grains and Cereals:					
Oats Porridge					
Never/Rarely	32 (69.6)	42 (56.8)	74 (61.7)		
Once weekly	10 (21.7)	20 (27.0)	30 (25.0)		
Twice or more weekly	4 (8.7)	12 (16.2)	16 (13.3)	2.156	0.352
Weanimix ("Tom Brown"/Blend of Cereal and Legume)					
Never/Rarely	42 (91.3)	70 (94.6)	112 (93.3)		
Once weekly	1 (2.2)	1 (1.4)	2 (1.7)		
Twice or more weekly	3 (6.5)	3 (4.1)	6 (5.0)	0.909	0.844
Fortified Breakfast Cereals					
Never/Rarely	34 (73.9)	56 (75.7)	90 (75.0)		
Once weekly	5 (10.9)	8 (10.8)	13 (10.8)		
Twice or more weekly	7 (15.2)	10 (13.5)	17 (14.2)	0.163	0.951
Brown Rice					
Never/Rarely	45 (97.8)	71 (95.9)	116 (96.7)		
Once weekly	0 (0)	0 (0)	0 (0)		
Twice or more weekly	1 (2.2)	3 (4.1)	4 (3.3)	0.311	1.000
Whole Wheat Bread					
Never/Rarely	29 (63.0)	39 (52.7)	68 (56.7)		
Once weekly	8 (17.4)	15 (20.3)	23 (19.2)		
Twice or more weekly	9 (19.6)	20 (27.0)	29 (24.2)	1.268	0.542

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.10 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Not Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Fruits:					
Citrus Fruits (Oranges, Tangerines, Lemons)					
Never/Rarely	15 (32.6)	17 (23.0)	32 (26.7)		
Once weekly	19 (41.3)	37 (50.0)	56 (46.7)		
Twice or more weekly	12 (26.1)	20 (27.0)	32 (26.7)	1.471	0.497
Apple					
Never/Rarely	33 (71.7)	42 (56.8)	75 (62.5)		
Once weekly	9 (19.6)	25 (33.8)	34 (28.3)		
Twice or more weekly	4 (8.7)	7 (9.5)	11 (9.2)	3.051	0.218
Pineapple					
Never/Rarely	20 (43.5)	33 (44.6)	53 (44.2)		
Once weekly	15 (32.6)	27 (36.5)	42 (35.0)		
Twice or more weekly	11 (23.9)	14 (18.9)	25 (20.8)	0.511	0.832
Fresh Fruit Juice					
Never/Rarely	31 (67.4)	45 (37.5)	76 (63.3)		
Once weekly	13 (28.3)	23 (31.1)	36 (30.0)		
Twice or more weekly	2 (4.3)	6 (8.1)	8 (6.7)	0.780	0.683
Packaged Fruit Juice- Fortified					
Never/Rarely	19 (41.3)	24 (32.4)	43 (35.8)		
Once weekly	14 (30.4)	33 (44.6)	47 (39.2)		
Twice or more weekly	13 (28.3)	17 (23.0)	30 (25.0)	2.425	0.308

**Significance set at p≤0.05: Fisher's Exact test.*

Table 4.10 (Continued).

Frequency of Iron rich Foods Consumption	Haemoglobin (g/dL) Anaemic *n* = 46 n(%)	Non-Anaemic *n* = 74 n(%)	Total *N* = 120 N(%)	X^2	*p*-value
Supplements:					
Multivitamins					
Never/Rarely	46 (100)	69 (93.2)	115 (95.8)		
Once weekly	0 (0)	2 (2.7)	2 (1.7)		
Twice or more weekly	0 (0)	3 (4.1)	3 (2.5)	2.401	0.276
Iron Supplement					
Never/Rarely	45 (97.8)	71 (59.2)	116 (96.7)		
Once weekly	1 (2.2)	2 (2.7)	3 (2.5)		
Twice or more weekly	0 (0)	1 (1.4)	1 (0.8)	0.746	1.000
Folic Acid Supplement					
Never/Rarely	43 (93.5)	66 (89.2)	109 (90.8)		
Once weekly	1 (2.2)	3 (4.1)	4 (3.3)		
Twice or more weekly	2 (4.3)	5 (6.8)	7 (5.8)	0.571	0.893
Vitamin B12 Supplement					
Never/Rarely	46 (100)	71 (95.9)	117 (97.5)		
Once weekly	0 (0)	1 (1.4)	1 (0.8)		
Twice or more weekly	0 (0)	2 (2.7)	2 (1.7)	1.473	0.705

**Significance set at p≤0.05: Fisher's Exact test.*

4.10 Association between vitamin D and length of sunlight exposure of students

Table 4.11 shows the association between vitamin D and how much sunlight the students were exposed to. There was no significant association between vitamin D and length of sunlight exposure. More than half of the students spent greater than an hour a day in the sun during weekdays although most of them were vitamin deficient/insufficient. The peak time for sunlight exposure during the day among the students was between 10am to 1pm. Most students were also exposed to some sunlight at least 5 times within a week.

Table 4.11: Association between vitamin D and length of sunlight exposure of students

Characteristics	Vitamin D status (ng/ml)			X^2	*p*-value
	Deficient/ Insufficient	Sufficient	Total		
	n= 94	*n*= 18	*N*=112		
	n(%)	n(%)	N(%)		
Duration of Sun Exposure from 10am - 4pm (Weekdays)					
<30 mins/day	13 (12.9)	0 (0)	13 (10.8)	4.070	0.213
30 mins-1 hr/day	25 (24.8)	3 (15.8)	28 (23.3)		
>1 hr/day	56 (55.4)	15 (78.9)	71 (59.2)		
Duration of Sun Exposure from 10am – 4pm (Weekends)					
<30 mins/day	24 (23.8)	1 (5.3)	25 (20.8)	5.383	0.125
30 mins -1 hr/day	33 (32.7)	5 (26.3)	38 (31.7)		
>1 hour/day	37 (36.6)	12 (63.2)	49 (40.8)		
Time mostly spent outdoors under the sun during the day					
7am – 10 am	27 (26.7)	6 (31.6)	33 (27.5)	2.484	0.659
10am – 1 pm	28 (27.7)	8 (42.1)	36 (30.0)		
1pm – 4pm	25 (24.8)	3 (15.8)	28 (23.3)		
4pm – 7pm	14 (13.9)	1 (5.3)	15 (12.5)		
Average number of days exposed to sunlight in a week[a]					
1 Day	3 (3.0)	1 (5.3)	4 (3.3)	3.066	0.669
2 Days	10 (9.9)	0 (0)	10 (8.3)		
3-4 Days	25 (24.8)	4 (21.1)	29 (24.2)		
5 Days or more	54 (53.5)	13 (68.4)	67 (55.8)		

**Significance set at p≤0.05: Fisher's Exact test.* *[a]Deficient/Insufficient: n = 91 , Sufficient: n = 18, Total: N = 109*

4.11 Association between Vitamin D status and Haemoglobin levels of students

There was no significant association between Vitamin D status and Haemoglobin levels of the students (Table 4.12). Out of 101 students who were vitamin D deficient/insufficient, 63% of them were not anaemic. For students who were vitamin D sufficient, greater than 50% of them were also not anaemic.

Table 4.12: Association between Vitamin D status and Haemoglobin levels of students. (N=120)

Characteristics	Vitamin D status (ng/ml)			X^2	*p*-value
	Deficient/ Insufficient	Sufficient	Total		
	n= 101	*n*= 19	*N*= 120		
	n(%)	n(%)	N(%)		
Haemoglobin levels (g/dL)					
Anaemic	37 (36.6)	9 (47.4)	46 (38.3)		
Not Anaemic	64 (63.4)	10 (52.6)	74 (61.7)	0.780	0.444

Significance set at p≤0.05: Fisher's Exact test

4.12 Correlation between Vitamin D Status, Haemoglobin levels, Age, BMI, Fat, Visceral Fat, Muscle Mass.

Table 4.13 shows the correlation between vitamin D status, haemoglobin levels, age, BMI, body fat, visceral fat and muscle mass of students. Vitamin D did not correlate with any of the anthropometric indicators (BMI, body fat, visceral fat and muscle mass), age and haemoglobin levels. However there were strong significant positive correlations between haemoglobin levels and age ($r^2 = 0.385$; $p<0.001$) and muscle mass ($r^2 = 0.518$; $p<0.001$). Also haemoglobin levels correlated significantly and negatively with body fat ($r^2 = -0.618$; $p<0.001$). BMI also correlated significantly and positively with body fat and visceral fat; with a strong significant negative correlation with muscle mass.

Table 4.13: Correlation between Vitamin D Status, Haemoglobin levels, Gender, Age, BMI, Body Fat, Visceral Fat, Muscle Mass

	Age	BMI	BF%	MM%	VF%	Hb	Vit D
Age	1						
BMI	-0.082 0.373	1					
Fat%	-0.262* 0.004	0.719* <0.001	1				
MM%	0.129 0.160	-0.237* <0.001	-0.542* <0.001	1			
VF%	0.087 0.347	0.780* <0.001	0.526* <0.001	-0.048 0.606	1		
Hb	0.385* <0.001	-0.125 0.175	-0.618* <0.001	0.518* <0.001	0.074 0.442	1	
Vit D	-0.082 0.374	0.079 0.392	0.118 0.198	-0.066 0.477	0.114 0.214	-0.036 0.694	1

BMI (Body Mass Index),BF (Body Fat), MM (Muscle Mass), VF (Visceral Fat), Hb (Haemoglobin), Vit D (Vitamin D)

**Significance set at p≤0.05: Pearson's Correlation.*

4.13 Relationship between Vitamin D, Gender, BMI, Body fat, Muscle Mass, Visceral fat, weekday and weekend sunlight exposure, length of sunlight exposure and Hb levels.

A binary logistic regression was performed to ascertain the effect of gender, BMI, body fat, muscle mass, visceral fat, weekday and weekend sunlight exposure, length of sunlight exposure and Hb levels on the likelihood that study participants have sufficient serum Vitamin D levels (Table 4.14).

After adjusting for all other covariates, gender was a significant predictor of serum vitamin D levels [AOR = 0.018, (95%CI = 0.001- 0.312), p=0.006]. Males had 98.2% reduced odds of being vitamin D sufficient compared to females.

After controlling for gender, BMI, BF, VF, weekday and weekend sun exposure, length of sunlight exposure and Hb levels, having normal muscle mass was significantly associated with serum vitamin D levels [AOR =0.032, (95% CI=0.003-0.346), p=0.005]. Students with normal muscle mass levels had 97% reduced odds of being vitamin D sufficient compared to those with high muscle mass levels.

There were no significant association between BMI, BF, VF, weekday and weekend sun exposure, length of sunlight exposure and Hb levels and serum vitamin D levels (all p-values >0.05). However, being exposed to sunlight less than 30 minutes during the weekends was significantly associated with having sufficient serum vitamin D levels (AOR= 0.024; (95% CI= 0.001-0.499), p=0.017].

Table 4.14: Relationship between serum vitamin D status and Gender, BMI, Body Fat, Visceral Fat, Muscle mass, Weekday Sunlight exposure, Weekend Sunlight exposure, Length of sunlight exposure, HB level

Variables	Odds Ratio	df	SE	95% CI		*p*-value
				Lower	Upper	
Gender		1				
Female	Ref		1.450			
Male	0.018			0.001	0.0312	**0.006***
BMI Classification		2				
Overweight/Obesity	Ref					
Normal	0.728		1.060	0.091	5.819	0.765
Underweight	0.296		1.690	0.011	8.112	0.296
Body Fat		2				0.881
High/Very High	Ref					
Normal	0.596		1.027	0.080	4.466	0.615
Low	0.598		2.259	0.007	50.120	0.820
Muscle Mass		2				**0.013***
High/Very High	Ref					
Normal	0.032		1.213	0.003	0.346	**0.005**
Low	0.205		1.518	0.010	4.027	0.297
Visceral Fat		2				
Very High	Ref					
High	0.085		1.757	0.003	2.653	0.160
Normal	0.000		40192.970	0.000		1.000
Duration of Sun Exposure (Weekdays)		3				0.929
>1 hr/day	Ref					
30 min – 1 hr/day	1.768		0.944	0.278	11.255	0.546
<30 mins/day	0.000		10218.974	0.000		0.999
Duration of Sun Exposure (Weekends)		2				0.056
>1 hr/day	Ref					
30 min – 1 hr/day	0.421		0.784	0.091	1.960	0.271
<30 mins/day	0.021		1.627	0.001	0.499	**0.017**
Time mostly spent outdoors under the Sun		3				0.195
4 – 7 pm	Ref					
1 – 4 pm	0.433		1.456	0.025	7.526	0.566
10 – 1 pm	3.628		1.329	0.268	49.037	0.332
7 – 10 am	2.894		1.345	0.207	40.428	0.430

Table 4.14 (Continued).

Variables	Odds Ratio	df	SE	95% CI		*p*-value
				Lower	Upper	
Hb Levels		1				
Not Anaemic	Ref					
Anaemic	0.484		0.930	0.078	2.994	0.435

*Multiple logistic regression test. *significance set at p < 0.05. df (degree of freedom), SE (Standard Error), CI (Confidence Interval), BMI (Body Mass Index),BF (Body Fat), MM (Muscle Mass), VF (Visceral Fat), Hb (Haemoglobin).*

4.14 Relationship between Hb levels and Gender, Red Meat (Beef/Goat/Mutton), Vegetables (Turkey berries and Okro) and Vitamin D status.

Table 4.15 shows a binary logistic regression between Hb levels of students and gender, red meat (Beef/Goat/Mutton), Vegetables (Turkey berries and Okro) and Vitamin D status.

Gender was a significant predictor of serum Hb levels [AOR = 229.971, (95%CI = 30.141-1754.641), p< 0.001]; after adjusting for all other covariates. Males were about 230 times more likely not to be anaemic compared to females.

Also, adjusting for gender, red meat and vitamin D status, eating okro once weekly was significantly associated with Hb levels [AOR =0.058, (95% CI=0.064 – 1.885), p=0.033]. Students who ate okro once weekly had 94.2% reduced odds of not being anaemic compared to those who ate it twice or more weekly.

There was no significant relationship between Hb levels of students and consumption of Beef/Goat/Mutton, Turkey Berries and Vitamin D status.

Table 4.14: Relationship between Hb levels and Gender, Red Meat (Beef/Goat/Mutton), Vegetables (Turkey berries and Okro) and Vitamin D status.

Variables	Odds Ratio	df	SE	95% CI		*p*-value
				Lower	Upper	
Gender		1				**≤0.001***
Female	Ref					
Male	229.971		1.037	30.141	1754.641	≤0.001
Beef/Goat/Mutton (Red Meat)		2				0.298
Twice or more weekly	Ref					
Once weekly	0.503		0.761	0.113	2.237	0.367
Never/Rarely	0.280		0.824	0.056	1.409	0.123
Turkey Berries (Vegetable)		2				0.565
Twice or more weekly	Ref					
Once weekly	4.042		1.483	0.221	73.905	0.346
Never/Rarely	3.400		1.201	0.323	35.768	0.308
Okro (Vegetable)		2				**0.033***
Twice or more weekly	Ref					
Once weekly	0.058		1.099	0.064	1.885	0.009
Never/Rarely	0.347		0.863	0.007	0.498	0.220
Vitamin D Status		1				
Sufficient	Ref					
Deficient/Insufficient	1.090		0.900	0.187	6.367	0.924

*Multiple logistic regression test. *significance set at p < 0.05. df (degree of freedom), SE (Standard Error), CI (Confidence Interval).*

CHAPTER 5

5.0 DISCUSSION

This study aimed at determining the relationship between vitamin D deficiency and anaemia among undergraduate Allied Health students. As young adults transitioning from puberty to adulthood there is increased nutrient requirement as a result of their growth spurt during this period (Soliman et al., 2014). The results of this study indicate that there is indeed a high prevalence of vitamin D deficiency among Allied Health students aged 18 – 24 years. Overall, 77.5% of students were vitamin D deficient (that is serum 25(OH)D less than 20ng/ml). This comprised of 80.3% of the males and 74.6% of females who participated in the study . About 38% of the students were also anaemic, majority being female students. However, there was no association between vitamin D deficiency and anaemia, as otherwise suggested in studies by Shin & Shim (2013) among Korean adult women as well as among young children in North India as reported by Chowdhury et al.(2019).

5.1 Dietary Vitamin D intakes among undergraduate students

Although sunlight is the main source of vitamin D, it can also be gotten from food, primarily from animal flesh in the form of cholecalciferol (Vitamin D3). Results from the food frequency questionnaire (FFQ) indicated that majority of students consumed poultry meat and chicken eggs more frequently than fish and red meat. This is in line with information from the 2017 Ghana Poultry Report by UDSA's Foreign Agricultural Service (2017) which stated that Ghanaians consumed more frozen poultry especially chicken more than other animal products because it was cheaper and conveniently portioned for domestic use. In addition, a national campaign in Ghana championed by the Ministry of Health to increase the consumption of eggs

due to its health benefits, aside its affordability may confirm the high consumption of chicken eggs amongst the students.

Out of the vitamin D food sources listed in the FFQ, significant associations were observed only between serum vitamin D status and consumption of Beef/Goat/Mutton; offal meat and processed meats ($p = 0.05$; $p = 0.013$; $p = 0.045$) ; all of which were in the red meat category. The results showed that majority of students who consumed Beef/Goat/Mutton offal and processed meats were also vitamin D deficient.

In contrast , a study by Yu et al. (2013) indicated that the mean serum vitamin D concentration of Korean adolescents was positively related to consumption of vitamin D food sources such as beef, oily fish and dairy. One possible explanation to this may have been that the study by Yu et al. (2013) was collected over a period of one year whereas data from this study was collected in one day, not considering seasonal variations and changes in the diet of students. Also, this study did not quantify the amount of vitamin D rich foods being consumed, hence it is likely the students may have been consuming small amounts even though the frequencies appear to be high.

Furthermore, in a review paper, Schmid & Walther (2013) confirmed from pools of studies that oily fish, egg yolk, offal and processed meat contained a considerable amount of vitamin D due to their high fat content and as such could lead to an increase in serum vitamin D levels when ingested (Schmid & Walther, 2013). This may have attributed to the significant association between Beef/Goat/Mutton; offal meat and processed meats (all of which contain a considerable amount of fat) and vitamin D.

The results also showed that less than 3% of students took multivitamins or vitamin D supplements at least once weekly. This may suggest that most of them relied on dietary intakes

for their nutritional requirement. According to Nimitphong & Holick (2013) vitamin D supplementation may be overlooked in tropical countries located near the equator due to year round sunlight and the assumption of endogenous synthesis of vitamin D consequently.

5.2 Dietary intakes of iron rich foods among undergraduate students

Poultry was the most frequently consumed haem-rich food with about 67% of students consuming it at least twice weekly; similar to the consumption of vitamin D food source. There were no significant associations between haem iron rich food consumption and haemoglobin levels of students although it was observed that most students who ate haem iron rich foods such as meat, poultry, fish, dairy and their products at least twice weekly were also not anaemic.

Some studies by Chukwu et al.(2018) and Ghose & Yaya (2018) among Nigerian adolescents and Ghanaian young women respectively, have confirmed a positive correlation between dietary iron foods sources and haemoglobin levels. In addition Leonard et al. (2014) reported that young Australian women in their child bearing age who ate flesh foods as a result of increased nutritional knowledge had higher haemoglobin levels. Haem iron in meat or animal flesh is known to be an excellent source of iron and more bioavailable than non-haem iron found in plants (Hurrell & Egli, 2010).

In comparison to this study however, results from a study by Bagni et al. (2014) also showed no association between dietary intake and anaemia among adolescents, especially in girls; they therefore concluded that other factors beyond diet could explain anaemia during puberty.

For non-haem iron rich foods, students generally consumed low amounts of fruits, vegetables and whole grains; whole grains and cereals being the least frequently consumed. A significant association between haemoglobin and consumption of okro ($p = 0.033$). Okro is a nutritious vegetable crop widely grown and used in Ghana; its green pods are used in vegetable stews and soups. The high folate content of okro as well as vitamin C and iron makes it a dietary haematinic which can help prevent anaemia when consumed frequently (Gemede, Ratta, Haki, & Woldegiorgis, 2014).

Also, although fruit intake was generally low among students, citrus fruits were preferred over the other types of fruits. Together with tomatoes, fruits provide the body with high amounts of vitamin C which increases the bioavailability of iron, especially non-haem iron, when consumed together; it also provides the body with antioxidants that protect and help mop out free radicals from biological reactions and preserve cell integrity in the body (Hurrell & Egli, 2010; Kattappagari et al., 2015).

In a cross-sectional study based on data extracted from the Ghana Demographic and Health Survey, 2008; Ghose & Yaya (2018) assessed the consumption of fruits and vegetables in relation to anaemia among non-pregnant Ghanaian women 15 - 49 years. They found out that urban women who did not maintain WHO's recommended level of fruit and vegetable consumption – which is 5 servings a day – had a significantly higher likelihood of being moderate to severely anaemic (Ghose & Yaya, 2018; "WHO | Promoting fruit and vegetable consumption around the world," 2015). Contrary to the findings of this study, the results from Ghose & Yaya (2018) alludes to the fact that the general consumption of non-haem sources of iron is significant in increasing haemoglobin levels. In addition, their study confirmed that the general consumption of fruits and vegetables among urban dweller including students- as in

this study- are generally low and do not meet the WHO recommendation (Ghose & Yaya, 2018).

Another study conducted among Nigerian adolescents 16 -19 years old also confirmed that poor dietary intake of both haem and non-haem iron rich foods was a major contributing factor to a high prevalence of anaemia (Chukwu, Dare, & Ogbonna, 2018). They also pointed out that other dietary factors such as the action of iron food inhibiters like phytates, an anti-nutrient in plant-based diets as whole grains, legumes and seeds, could possibly hinder absorption of important nutrients including iron which eventually will lead to anaemia (Astley & Finglas, 2016; Chukwu et al., 2018).

5.3 Length of sunlight exposure among undergraduate students

Sunlight is the best source of vitamin D and humans are physiologically adapted to produce vitamin D in response to sun exposure, specifically UVB radiation (Baggerly et al., 2015). However, growing evidence on the prevalence of vitamin D deficiency in sunny countries suggests that despite the abundance of sunlight, low serum vitamin D levels of people living in these regions are still being reported (Correia et al., 2014; Mendes et al., 2018).

The results from this study showed a significant association between length of sunlight exposure from 10 am – 4pm on weekends and the gender of students. Additionally, more male students spent longer periods in the sun compared to females. Similarly, a study in Bangkok which is a tropical region as Ghana, reported that male adolescent students spent more time in the sun than female students (Tempark et al., 2012). Contrary to this finding, a study in Sweden, a temperate region, observed that among a primary healthcare population of adults above 18years, females significantly spent more time in the sun than males; although it was observed

that these females also used sunscreens extensively than males did (Falk & Anderson, 2013). According to the Centre for Disease Control and Prevention, men generally tend to spend more time outside in the sun than women due to outdoor activities such as sports or gardening that they engage in (Center for Disease Control and Prevention, 2019).

In addition, this study showed that students who spent less than 30 minutes during weekends in the sun had 98% reduced odds of being vitamin D sufficient than students who spent more than an hour a day in the sun. According to Holick (2017), a research scientist who has done extensive studies on vitamin D; exposure of one's face and arms to adequate sunlight wavelength (UVB radiation of 290–370 nm) for 15–30 minutes, from 11 am–3 pm daily, should be enough to maintain adequate vitamin D status.

Similar to this finding, in Ethiopia, where there is sunlight all year round, a study to determine vitamin D deficiency among rural and urban school children 11-18 years reported that the prevalence of vitamin D deficiency was higher in urban school children who had low sun exposure compared to the rural children who spent more time in the sun on both weekdays and weekends (Wakayo et al., 2015). Furthermore, another study among a cohort of Saudi adolescents from public schools also observed a significantly low vitamin D status among students who did not engage in outdoor activities, especially among females who were required to cover their head, arms and legs (Al-Daghri et al., 2015).

However, there was no significant association between sunlight exposure and vitamin D status among a group of healthy black adults in Kenya although they were exposed to sunlight for ≥3 hours in a day (Kagotho et al.,2018).

Unfortunately the results from this study did not correlate with Holick's hypothesis, for although majority of students spent over 30 minutes in the sun more than 5 times weekly between 7 – 1pm (when the sun it at its peak); their vitamin D levels were low. Secondly,

results from the binary regression reported that spending less than 30 minutes was significantly insufficient to maintain adequate vitamin D status; contrary to what Holick (2017) reported.

One possible explanation why most students were vitamin D deficient despite receiving more than 30 minutes of sunlight daily may have been because all the students in this study had dark skin pigmentation (more melanin). According to Libon et al.(2013), people with dark skin pigmentation require a longer sun exposure period of about 2-3 times more than people with light skin pigmentation in order to meet their daily vitamin D requirement. This is because UV ray transmission is limited by melanin and reduces the capacity for endogenous vitamin D synthesis on the skin (Lisbon et al., 2013). Another possible reason why most students were vitamin D deficient may be attributed the amount of skin surface exposed to sunlight. Majority of both male and female students covered their forearms and thighs when dressed up, leaving only their lower legs and face exposed to sunlight; which has a smaller skin surface area.

5.4 Serum Vitamin D status in undergraduate students

Results from this study revealed that among the study participants, a large percentage were vitamin D deficient. These findings add to growing data on the high prevalence of vitamin D deficiency among the African population. This is attested to in a recent systematic review by Mogire et al., (2020) reported in the Lancet Global Health.

In their systematic review, Mogire et al. (2020) reported that the mean vitamin D deficiency prevalence in Ghana, pooled from 3 studies by Durazo-Arvizu et al. (2014); Fondjo et al. (2017) and Gernand et al. (2019) ranged from as low as 4% among healthy adults in Kumasi to as high as 92.4% among diabetics at Nkwaie in the Ashanti region. The vitamin D deficiency prevalence (77.5%) however in this study was higher than what was recorded in the studies pooled by Mogrie et al. (2020) from Ghana and this may have been due to several factors that

affect vitamin D levels. One of these factors could have been the fact that in the systematic review by Mogire et al. (2020), the cut-offs for vitamin D deficiency used was <12ng/ml (30nmol/L) as recommended by the IOM. However the cut-offs used in this study to define vitamin D deficiency was <20ng/ml (50nmol/L), as recommended by the Endocrine Society's Clinical Guidelines. This implied that the prevalence of VDD may have been higher in the pooled studies if they factored in all participants who had serum vitamin D concentrations below 20ng/ml.

Another factor that may have contributed to differences in the prevalence of VDD was that the study participants in each study (both this one and that of Mogire and colleges) were recruited from different regions in Ghana with different demographics and therefore was not representative of the population of Ghana. Whereas in this study, participants were recruited in Accra which is the capital of Ghana, hence mostly urbanized; in the other 3 studies by Durazo-Arvizu et al. (2014); Fondjo et al. (2017) and Gernand et al. (2019), participants were recruited from different parts of Kumasi and Asesewa, in the Upper Many Krobo District; a blend of semi-urban and rural areas. According to Norval et al. (2016), urbanisation and its associated lifestyle changes may lead to reduced personal sunlight exposure, with the potential to decrease vitamin D status. This may have been the case with participants in this study hence the high prevalence of VDD.

Additionally, the age groups of study participants varied in both this study and the 3 pooled studies in Ghana from Mogrie et al. (2019). While the age range for this study was between 18- 25 years, that for Gernand et al. (2019) was between 18 -35 years; Durazo-Arvizu et al. (2014) between 25 – 45 years and above 25 years in the study by Fondjo et al. (2017). Although a study by Gallagher (2013) reported that the endogenous metabolism of vitamin D decreases with age , it was not the case in this study as VDD was more prevalent in young adults.

A similar study also on healthy adults in a tertiary hospital in Kenya reported a VDD (<20ng/ml) prevalence of 17.6% (Kagotho et al., 2018). Furthermore, the systematic review by Mogire et al. (2020) pooled from about 129 studies indicated VDD prevalence in Africa ranged from 3- 90% with a mean prevalence of 34.2%. Both studies reported much lower VDD prevalence than this study and it can possibly be attributed to the continent's heterogeneity in latitude, geography, climate, food availability, religious and cultural practices, and skin pigmentation; all of which are determinants of vitamin D status (Mogire et al., 2020).

Similar to this study's findings, a high prevalence of VDD (up to 90%) was reported in the Middle East where there is also sunshine all year round (Bouillon, 2020). Another study on vitamin D status of healthy Iranian adults 18years and above also observed a high VDD (<20ng/ml) prevalence of 50.4% (Hovsepian et al., 2011). This prevalence was relatively closer to what was observed in this study and most likely reflective of the actual status of the adults as their data was collected over a period of one year as opposed to one day

In temperate regions like the USA and Europe that rely heavily on vitamin D supplementation and fortification due to seasonal variations, VDD prevalence of 18% and 40% respectively have been reported (Cashman et al., 2016; Herrick et al., 2019b). It was however observed in these temperate regions that people with dark skin pigmentation recorded the lowest concentration of serum vitamin D, reiterating the negative effect of melanin on the endogenous synthesis of vitamin D (Herrick et al., 2019; Kagotho et al., 2018).

From this study, a wide range of serum vitamin D concentration between 0.27 – 460.18 ng/ml was observed among the students, with a median of 3.75 ng/ml. This possibly implied that even though majority of the students were vitamin D deficient, some of those who were vitamin D sufficient had concentrations well above vitamin D toxicity levels. According Marcinowska-Suchowierska, et al. (2018), serum vitamin D concentrations above 150ng/ml is the hallmark

of vitamin D toxicity or hypervitaminosis D, a severe clinical condition characterized by hypercalcemia with symptoms such as confusion, apathy, recurrent vomiting, abdominal pain, polyuria, polydipsia, and dehydration.

It is also worth noting that because of the wide range of serum vitamin D concentration of students, the calculated mean of 29.6 ng/ml could not be used because its standard deviation was greater than the mean value, hence the median concentration of 3.75ng/ml was used.

Another observation in this study was that male students were significantly more likely to be vitamin D deficient than females. Coherent with this finding is a study by Tønnesen et al. (2016) also conducted among young healthy adults (18-25 years) in Copenhagen, Denmark where males had a significantly higher prevalence of VDD than females. According to Tønnesen et al. (2016), similar studies have reported a noticeable trend among adolescents in the same latitude as Denmark, such as in Norway, Russia and the United Kingdom. However, these studies attributed the high serum vitamin D concentrations in females to the use of oral contraception (oestrogen) and the fact that females enjoyed getting tanned during summer (Falk & Anderson, 2013; Tønnesen et al., 2016).

Contrary to this finding, some studies conducted in Africa reported otherwise. In their research to determine the vitamin D status among healthy adults in Nairobi, Kenya, Kagotho et al.(2018) observed that females were more likely to be vitamin D deficient possibly due to their high BMI and increased subcutaneous tissue. The reason for this is because vitamin D is fat soluble and adipose tissue is a major storage site for it, hence its bioavailability is reduced in obesity/overweight people (Kagotho et al., 2018). This assertion is also confirmed by Mogire et al. (2020).

5.5 Association between serum vitamin D concentration and anthropometric measures

Considering all the anthropometric measurements (BMI, body fat, muscle mass and visceral fat), having normal muscle mass was the only significant anthropometric predictor of serum vitamin D levels. This meant students with normal muscle mass levels had 97% reduced odds of being vitamin D sufficient compared to those with high muscle mass levels. There have been inconsistent studies between the relationship between muscle mass and vitamin D. According to a study by Lee (2013) taken from the KNHANES IV (Korean National Health and Nutrition Examination Survey) study, Korean men with a higher lean muscle mass were less likely to be vitamin D deficient, nonetheless no significant association between lean mass and vitamin D was found among women in this study. However, there was no significant association between vitamin D status and lean mass among black preadolescent children in South Africa (White et al., 2019)

Although male students had significantly higher muscle mass than females students which was expected since males are more muscularly built than females (Schorr et al., 2018); it was surprising to observe that more males (49) were vitamin D deficient than females (44). According to Hannemann et al. (2015) adiposity measures such as abdominal visceral or subcutaneous adipose tissue are inversely associated with serum Vitamin D concentrations in adults. Considering Hannemann et al. (2015) findings, the significantly higher visceral fat of the male students in this study may have possibly resulted in low levels of serum vitamin D concentrations amongst most of the male students.

5.6 Haemoglobin concentration in undergraduate students

In this study, anaemia was classified into three categories based on haemoglobin levels cut-offs according to WHO's recommendations for males and non-pregnant females >15yrs

respectively as follows: Severe, Moderate, Mild and Non-Anaemic (Cappellini & Motta, 2015). The prevalence of anaemia among the 120 study participants of undergraduate students was 38.2%, majority (75%) of them being female students. This is in line with the study's hypothesis that indeed there is a high prevalence of anaemia among young adults. The Ghana Demography and Health Survey (2014) also confirmed the high prevalence of anaemia of 42% among females of child bearing age, most of whom were adolescents.

A review by Yasutake et al. (2013) to assess the extent of anaemia in a critical age, adolescents and young adults ages (15 to 24) in Low and Middle Income countries observed that the prevalence of anaemia ranged between 15% to over 50%. The highest prevalence of anaemia were in Africa, with Ghana being one of the countries with moderate and severe anaemia being over 20% (Yasutake et al., 2013). A study by Intiful et al. (2016) in three hospitals in Accra, Ghana confirmed the high prevalence (76%) of anaemia among pregnant adolescents aged 15 – 19 years who were attending antenatal clinics.

Gender was found to be a significant predictor of serum Hb levels; after adjusting for all other covariates in this study. This implied that the male students were about 230 times more likely not to be anaemic compared to females. Out of the anaemic students, none of them were severely anaemic but majority were mildly anaemic; 95% of whom were females. According to Camaschella (2015), adolescents and all women of child bearing age especially have a high vulnerability to anaemia, especially iron deficiency anaemia, due to their growth spurt and the onset of menarche. Iron is a major component of haemoglobin in the blood and its role is crucial to biologic functions, including respiration, energy production, DNA synthesis, and cell proliferation; its main sources are from diet and supplementation (Hentze et al., 2010). Unfortunately due to the poor dietary intake of iron rich foods and supplementation among the

students, it was not surprising to see a high prevalence of anaemia especially amongst the females (76%).

In India, out of 200 health medical students, only 8% were anaemic; all of whom were females (Saxena et al., 2011). The high literacy rates of these students could have accounted for the relatively low prevalence in anaemia. However this was not the case in this current study as the study participants were Allied Health Students hence were literates, however a high prevalence of anaemia was reported. This high prevalence of anaemia especially among the female study participants in this study may possibly be due to inadequate intake of iron-rich foods in their diet to meet their physiological needs.

Furthermore, the prevalence of anaemia was also reported to be higher in adolescents in rural areas than urban more due to ignorance, low socio economic status and poor diet as reported in studies by Camaschella (2015) and Saxena et al. (2011).

A strong significant positive correlation was observed between haemoglobin levels and age implying that as age increased so did haemoglobin levels. In his book, Garvin (2010) stated that haemoglobin levels for male and female infants are identical then gradually rise at the same rate during childhood, but during adolescence female haemoglobin levels reach a plateau whilst in males it continues to rise throughout puberty to higher levels characteristic of adult men. However, as people grow older, haemoglobin levels become inversely associated with age, according to Zakai et al.(2013).

Another strong significant positive correlation was observed between haemoglobin levels and muscle mass ($r^2 = 0.518$; $p<0.001$) implying an increase in one's muscle mass led to higher haemoglobin levels. In line with this correlation, Abbaspour et al.(2014) stated in their review

that about 15% of iron is bound to myoglobin in the muscle whilst two-thirds of the body's iron is found in haemoglobin. Therefore, students who had high muscle mass, as evident in male students, also had a high iron stores.

Haemoglobin levels however correlated significantly and negatively with body fat (r^2 = -0.618; $p<0.001$) which meant an increase in one's body fat led to a decrease in haemoglobin levels. A review by Cepeda-Lopez et al. (2010) stated that overweight individuals are at higher risk of iron deficiency than normal-weight individuals and this is because obesity affects the absorption and sequestration of iron through the release of hepcidin. Another study by Bagni et al.(2013) confirmed that overweight was associated with low haemoglobin levels among adolescent girls in Brazil.

5.7 Association between serum vitamin D status and haemoglobin levels

In line with the hypothesis, there was no significant association between serum vitamin D status and haemoglobin levels among the students. Contrary to this finding, a cross-sectional study conducted in medical centres in California , USA by Sim et al. (2010) reported that healthy individuals >17 years who were vitamin D deficient were at a higher risk of anaemia than those who were not vitamin D deficient. Similarly, vitamin D was associated with mild anaemia among young children in the resource-poor setting of northern urban India, although the effect was independent of iron, vitamin B12, and folate deficiency (Chowdhury et al., 2019). Furthermore, Atkinson et al.(2014) confirmed an association between vitamin D deficiency and anaemia in healthy US children and adolescents from the 2001-2006 NHANES study. In addition, they noted that the vitamin D threshold levels for lower haemoglobin levels were lower in black children in comparison with white children.

In their review on the association between vitamin D and anaemia, Smith & Tangpricha (2015) observed that vitamin D has previously been found to be associated with anaemia in both healthy and diseased populations. However, emerging studies indicate although this association may differ between race and ethnic groups, the association between vitamin D and anaemia is particularly specific to anaemia of inflammation that is, anaemia characterized by chronic diseases (Smith & Tangpricha, 2015). Nonetheless, vitamin D has been reported to be a potent regulator of the hepcidin-ferroportin axis, that controls iron homeostasis, hence VDD may affect the regulation of hepcidin thereby increasing the incidence of anaemia, according to Bacchetta et al. (2014). In addition, Vitamin D is also known to play a role in erythropoiesis by influencing erythroid precursors in the bone marrow, implying when endogenous vitamin D is deficient, red blood cells synthesis may be affected and this can progress to anaemia over a period of time (Malczewska-Lenczowska et al., 2018).

5.8 Limitations

The study had some limitations:

1. Relying on self-reporting to ascertain duration of sun exposure could have introduced some biases.
2. Since it was a cross-sectional study, data was collected only during one season so did not factor in seasonal variations in diet and sunlight exposure.
3. The results do not show the heterogeneity of the population since a particular age group (18 – 24 years) was used and study participants were sampled from one location (School of Biomedical and Allied Health Sciences).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research aimed at determining if there was a high prevalence of vitamin D deficiency among young adults in a country where there is abundant sunlight all year round. It also sought to find the prevalence of anaemia among these students and whether or not an association exists between vitamin D status and anaemia.

Among the students, there was a general low consumption of both dietary vitamin D–rich foods and iron rich food. Their overall consumption of fruits and vegetables did not meet WHO's recommendation of 5 servings per day. In addition, almost all the students rarely or never took any form of supplements.

The study also showed that male students spent longer periods in the sun than female students and the frequency of sun exposure was higher on weekdays than on weekends for all students.

Vitamin D deficiency was evident among the students in this study with a concerning prevalence of 77.5% that will require public health intervention. Anaemia was also relatively high especially among female students, confirming its global prevalence, with females of child bearing age being a major risk group.

Although vitamin D status was significantly associated with intake of some vitamin D- rich foods, exposure to sunlight on weekends and normal muscle mass; there was no observable significant association with haemoglobin levels of students.

6.2 Recommendation:

Based on the findings of this study:

1. Dietitians and health practitioners need to create the awareness of the prevalence of vitamin D deficiency in Ghana despite year round sunlight exposure.

2. Health professionals must encourage students to spend at least 1 hour each in the sun between the hours of 1pm – 3pm in order to receive their daily requirement of vitamin D.

3. Although there was a significant association between vitamin D and consumption of offal and processed meat; Dieticians and Health providers should advice consuming them in limited amounts as they are also high in saturated fats and cholesterol. This is important in order to help prevent certain comorbidities as cardiovascular diseases and hypertension.

4. Further research is needed to determine the vitamin D status of different age groups, especially the vulnerable groups as infants, children, adolescents, pregnant women, the elderly and those with chronic diseases. If these studies confirm that indeed there is a high prevalence of vitamin D deficiency; then guidelines and policies must be set and implemented to fortify locally produced foods with vitamin D.

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APPENDIX I

ASSENT FORM

VITAMIN D DEFICIENCY AND ANAEMIA AMONG ALLIED HEALTH STUDENTS IN UNIVERSITY OF GHANA

My name is Rosemond Noel Duncan, a final year MSc Dietetics student of the School of Biomedical And Allied Health Sciences, University of Ghana. I am carrying out a research on "VITAMIN D DEFICIENCY AND ANAEMIA AMONG ALLIED HEALTH STUDENTS IN UNIVERISTY OF GHANA", as a partial fulfilment of my Master's degree in Dietetics.

The inclusion criteria for this study are undergraduate students aged 18 - 24 years who are students of the School of Biomedical and Allied Health Sciences.

If you agree to participate in this study, I will measure your weight, height and a qualified phlebotomist will take blood samples of 4mls from your arm for vitamin D and haemoglobin determination. You will also fill a questionnaire consisting of a food frequency table of vitamin D and iron rich foods and also how much sunlight are you are exposed to. Kindly be assured that your answers will be kept confidential and not disclosed to anyone.

At the end of the study you will be given enclosed envelopes containing information on your vitamin D and anaemia status. The information you provide will help me identify the occurrence of vitamin D deficiency and anaemia among young adults (18 to 24 years).

Participation in this study is voluntary, if you do not want to be part, you are free to opt out. You are free to ask any questions about the study at any time by reaching me on 0244063748 or via email: rosie.ampah@gmail.com.

By providing your name below, you have understood the procedures of this study, had all your questions answered and have agreed to be part of the study. Thank you for your kind cooperation.

Name of Student:

NAME	**DATE**	**SIGNATURE**	**PHONE NUMBER**

APPENDIX II

RESEARCH QUESTIONNAIRE

Vitamin D and Anaemia among Allied Health Sciences students in University of Ghana

Date: …….....

Study Participant's Code:

Instructions for filling this Questionnaire:

There are four (4) sections in this questionnaire: Socio-Demographic Information, Anthropometry, a Food Frequency Questionnaire and a Sun Exposure Assessment. Kindly fill out section **one (1)** and tick the appropriate response to sections **three (3)** and **four (4)**. Section **two (2)** will be completed by the researcher after taking each study participant's anthropometry.

SECTION ONE: SOCIO-DEMOGRAPHIC INFORMATION

1. Age: ……..
2. Gender:

   ☐ Male ☐ Female
3. Level:

   ☐ 200 ☐ 300 ☐ 400
4. Programme:

   ☐ Medical Laboratory ☐ Nutrition and Dietetics ☐ Physiotherapy

   ☐ Radiotherapy ☐ Occupational Therapy ☐ Respiratory Therapy
5. Residence status:

   ☐ Resident on Campus ☐ Non-Resident on Campus
6. Religion:

☐ Christian ☐ Muslim ☐ Traditionalist ☐ Other, specify……

7. Ethnicity: ……..
8. Father's occupation: ……..
9. Mother's occupation: …….

SECTION TWO: ANTHROPOMETRY

Parameters	Weight (kg)	Height (m)	Fat (%)	Muscle (%)	Visceral Fat
1st Reading					
2nd Reading					

SECTION THREE: DIETARY ASSESSMENT USING A FOOD FREQUENCY QUESTIONNAIRE

Kindly specify how often you consume the foods enlisted below

Food Groups	***Never***	***Rarely***	***Times per Week***			***Times per Day***	
			Once	***Two to Four***	***Five to Six***	***Once***	***More than once***
1. Whole Grains and Cereals: ***Porridges***							
Corn porridge							
Millet porridge							
Rice porridge							
Oats porridge							
Wheat porridge							
Weanimix							
"Ekuebgemi/oblayo" porridge							
Fortified Breakfast cereal :							
Corn flakes, Weetabix, Granola etc.							
Grain and Cereal products							
White Rice							
Brown Rice							
Pasta (Spaghetti/Macaroni)							

Banku/Akple/Kenkey/"Kafa"							
Bread							
Whole wheat bread							
"Butter"/ "Tea" /"Sugar" bread							
Food Groups	***Never***	***Rarely***	***Times per Week***			***Times per Day***	
			Once	***Two to Four***	***Five to Six***	***Once***	***More than once***
Pastries							
Cakes/pancakes/biscuits/Rockbuns /Doughnut/ "Bofrot"/							
Meat pie/ Spring rolls/ Samosa							
2. Beverages							
Tea (tea bag, green, herbal, fruit flavoured etc.)							
Coffee							
Cocoa (Hot or cold chocolate drinks)							
3. Meat and Meat Products							
Red Meat							
Beef/Goat/Mutton (Sheep)							
Game meat (Bush meat)							
Liver							
Kidney							
Other Offals: tripe, intestines, trotters etc.							
Processed Meats							
Corned beef/Sausages							
Bacon/ham							
Fish (Fresh and Smoked)							
Redfish (snapper), Cassava fish, Mackerel ("Saama"), Tuna, Herring, Tilapia, Salmon							
Fish Products							
Canned Tuna, Sardines, Mackerel							
Other fish products:							

fish pie, fish nuggets etc							
Shellfish: crabs, oyster, clams, shrimps							
Snails							
Food Groups	***Never***	***Rarely***	***Times per Week***			***Times per Day***	
			Once	***Two to Four***	***Five to Six***	***Once***	***More than once***
Poultry							
Chicken/Turkey/Guinea Fowl/Duck							
Poultry products							
Chicken eggs							
Quail eggs							
Guinea fowl eggs							
Gizzard (offal)							
Chicken liver (offal)							
4. Dairy and Dairy products							
Whole/Skimmed pasteurized milk							
Evaporated milk (Ideal, Peak etc)							
Powdered milk							
Pasteurized yogurt							
Cheese (soft/ hard/fermented)							
Wagashi							
Flavoured Milk drinks							
Ice cream							
5. Vegetables							
Dark green leafy vegetables (nkontomire, bokoboko, alefu , ayoyo, lettuce, etc)							
Turkey berries (“abeduru”)							
Tomatoes/ tomato paste							
Okro							
Beet root							
6. Legumes, Nuts and Seeds							
Beans							
Peas							
Soybeans							
Groundnuts							

Cashew nuts							
Almond							
Coconut							
Seeds (melon ("agushie", pumpkin, sunflower, chia, sesame,)							
Tiger nut							
Palm nut fruit (for Palm soup)							
Food Groups	***Never***	***Rarely***	***Times per Week***			***Times per Day***	
			Once	***Two to Four***	***Five to Six***	***Once***	***More than once***
Legumes, nuts and seeds products							
Soy / Almond / Coconut milk							
Soy chunks							
Seed Oil (sunflower etc)/Groundnut Oil/Olive oil							
Palm oil/ Coconut Oil							
7. Fruits and Fruit products							
Citrus (orange, tangerine, lemon)							
Avocado pear							
Apple							
Pineapple							
Watermelon							
Pawpaw							
Mango							
Grapes							
Fruit products							
Fruit juice (Fresh)							
Fruit juice (processed in a pack)							
Fruit smoothies							
8. Spreads and Sweeteners							
Butter							
Margarine							
Jam and marmalade							
Marmite							
Sugar (White/Brown)							
Honey							
9. Condiments							
Tomato ketchup							
Mayonnaise/ Salad cream							
Barbeque sauce							

10. Sweets and Other drinks							
Chocolates							
Toffees/ Candy							
Soda (fizzy drinks: coke, sprite etc.)							
Malt							
Alcoholic drinks							
Food Groups	***Never***	***Rarely***	***Times per Week***			***Times per Day***	
			Once	***Two to Four***	***Five to Six***	***Once***	***More than once***
11. Condiments							
Tomato ketchup							
Mayonnaise/ Salad cream							
Barbeque sauce							
12. Sweets and Other drinks							
Chocolates							
Toffees/ Candy							
Soda (fizzy drinks: coke, sprite etc.)							
Malt							
Alcoholic drinks							
13. Supplements							
Multivitamin							
Vitamin D *only*							
Iron only							
Folic acid *only*							
Vitamin B_{12} *only*							
Any Medication, please specify:							

SECTION FOUR: SUN EXPOSURE ASSESSMENT

Kindly tick the best response that represents the level of direct sun exposure.

1. On a sunny day, on average, how many hours are you outside per day between 10am and 4 pm on WEEKDAYS (Monday-Friday)?
    - 30 minutes or less
    - 31 minutes to 1 hour
    - 2 hours
    - 3 hours
    - 4 hours
    - 5 hours
    - 6 hours

2. On a sunny day, on average, how many hours are you outside per day between 10am and 4 pm on WEEKENDS (Saturday and Sunday)?
    - 30 minutes or less
    - 31 minutes to 1 hour
    - 2 hours
    - 3 hours
    - 4 hours
    - 5 hours
    - 6 hours

3. What time do you mostly spend outdoors under the sun?
    - 7-10 am
    - 10-1 pm
    - 1-4 pm
    - 4-7 pm

For the following questions, think about what you do when you are outside on a warm sunny day.

	Never	Rarely	Sometimes	Often	Always
4. Do you wear sunscreen on sunny days? Please specify: …….(SPF)					
5. How often do you wear long sleeve shirts to cover your arms?					
6. How often do you wear long trousers or skirts to cover your legs?					
7. How often do you wear a hat or cover your head when outdoors on a sunny day?					

8. Which **parts** of your body are usually exposed when dressed up? Choose all that applies

- Face, Head and Neck region
- Lower Arms : below the elbows
- Full Arms
- Lower Legs: below the knees
- Thighs
- Back and Shoulders
- Other, please specify ……..

9. On the average, how many days a week do you expose yourself to the sun?

- Not at all
- 1 day in a week
- 2 days in a week
- 3-4 days in a week
- or more than 5 days

10. **For Muslim Girls Only**: When you are in the company of women only in outdoor private settings, describe your usual clothing:
    - Face, head and hands exposed
    - Shorts/Skirt and top with shoulders exposed
    - Shorts/Skirt and T-shirt or similar top
    - Shorts/Skirt and long sleeves top
    - Long trousers/Skirt and T-shirt or similar top
    - Long trousers/Skirt and long sleeves top

THANK YOU

APPENDIX III

ETHICAL APPROVAL

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

EPRC/APRIL/2019 APRIL 13, 2019

Ref. No.:.....

Rosemond Noel Duncan
Dept. of Nutrition and Dietetics
SBAHS.
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.8 – 5.6/2018-2019***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its 28 March 2019 full board meeting reviewed and approved your research protocol.

Title of Protocol: "Vitamin D deficiency and anaemia among Ghanaian adolescents in selected schools in the Greater Accra Region"

Principal Investigator: **Rosemond Noel Duncan**

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

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

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

This ethical clearance is valid till APRIL 15, 2020.

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

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Dept. of Nutrition and Dietetics

• P.O. Box KB 52, Korle-Bu, Accra, Ghana Telephone: +233 (0) 302 665103/4 • Fax: +233 (0) 302 668762

APPENDIX IV

LETTER OF APPROVAL FROM ALLIED HEALTH ACADEMIC AFFAIRS

UNIVERSITY OF GHANA
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES

Ref. No.:.....

30th April, 2019

TO WHOM IT MAY CONCERN

Dear Sir/Madam,

LETTER OF INTRODUCTION - ROSEMOND NOEL DUNCAN

I write to kindly introduce to you Ms. Rosemond Noel Duncan, an MSc student from the Department of Nutrition and Dietetics, School of Biomedical and Allied Health Sciences, University of Ghana.

Ms. Duncan is conducting research for her thesis on the topic "**Vitamin D Deficiency and Anaemia among School of Biomedical and Allied Health Students of the University of Ghana**".

She has humbly requested to be allowed to interview and take blood samples of few of the students present for this purpose.

We will be grateful for any assistance you may be able to offer her.

Thank you.

Yours faithfully,

Stephen Amo-Mensah
SCHOOL ADMINISTRATOR

INTEGRI PROCEDAMUS

COLLEGE OF HEALTH SCIENCES

• P. O. Box KB 143, Korle Bu, Accra, Ghana.