UNIVERSITI PUTRA MALAYSIA

ASSOCIATION BETWEEN DIETARY CALCIUM INTAKE AND HEMOGLOBIN CONCENTRATION IN FEMALE STUDENTS IN FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

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This project entitled "Association between dietary calcium intake and hemoglobin concentration in female student in Facult of Medicine and Health Sciences, Universiti Putra Malaysia" was prepared by Nursyafiqah Aqilah binti Suhaimi and submitted to the Faculty of Medicine and Health Sciences as a partial fulfillment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

ASSOCIATION BETWEEN DIETARY CALCIUM INTAKE AND HEMOGLOBIN CONCENTRATION IN FEMALE STUDENTS IN FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

Nursyafiqah Aqilah binti Suhaimi

Anemia can be defined as a depletion in the total number of red blood cells (RBC)s, which can be measured by reduction in hemoglobin (Hb) concentration, hematocrit (Hct) or RBC count. It was estimated that 30% or approximately 2 million women of reproductive age were anemic in Malaysia. Iron deficiency anemia (IDA) was found to be the most prevalent type of nutritional deficiency worldwide. Calcium has been postulated to inhibit the absorption of iron by impeding the intake of iron by binding to the surface of intestinal absorptive cells, where non-heme iron also binds, causing a rendered iron absorption. This study was carried out to determine the association between dietary calcium intake and hemoglobin concentrations among students in Faculty of Medicine and Health Sciences, UPM. A set of questionnaire was used to determine socio demographic factors whilst anthropometric measurements were used to obtain weight and height of participants and BMI was classified using WHO cut off points. Dietary calcium intake and other dietary factors were determined using 24 hour dietary recall (24HR) and 3 day diet history (DH). Hb concentrations was used to define anemia using WHO cut off points <12 g/dl. The prevalence of anemia observed in this study was 38.5% with mean ± SD of Hb concentrations was 12.08 ± 1.30 g/dl. The mean ± SD calcium intake of participants was 479.29 ± 430.64 mg (24HR) and 460.74 ± 282.69 mg (DH). There was no significant association between dietary calcium intake measured by 24HR (r= 0.127, p= 0.188) and DH (r= -0.022, p= 0.821). There was also no significant association observed between other dietary factors (iron, vitamin C and vitamin D) and Hb concentration. Further analysis carried out reported that there was strong positive significant association between dietary iron and calcium intake (r= 0.625, p= 0.001 and r= 0.594, p= 0.001 measured by 24HR and DH, respectively). Despite the non-significant association observed between calcium and Hb concentration in this study, calcium intake was demonstrated to be associated with iron intake, which may indicate possible connection with iron absorption but not to the extent where it affects hemoglobin concentrations. With the findings, there is urgency in addressing anemia occurence and the importance of calcium intake, especially among university students as they are the posterity of the country in the future.

ABSTRAK

PERKAITAN ANTARA PENGAMBILAN KALSIUM DALAM DIET DENGAN KEPEKATAN HEMOGLOBIN DI KALANGAN PELAJAR WANITA DI FAKULTI PERUBATAN DAN SAINS KESIHATAN, UNIVERSITI PUTRA MALAYSIA

Nursyafiqah Aqilah binti Suhaimi

Anemia dapat dedifinisikan sebagai penurunan jumlah sel darah merah yang dapat diukur dengan pengurangan kepekatan hemoglobin (Hb), hematocrit atau jumlah sel darah merah. Dianggarkan 30% atau lebih kurang 2 juta wanita dalam usia reproduktif mengalami anemia di Malaysia. Anemia kekurangan zat besi didapati sebagai jenis kekurangan pemakanan yang paling banyak berlaku di seluruh dunia. Kalsium telah dianggap untuk menghalang penyerapan zat besi dengan menghalangi pengambilan zat besi di permukaan sel penyerap di usus. Kajian ini telah dilakukan untuk menentukan perkaitan antara pengambilan kalsium dalam diet dengan kepekatan hemoglobin di kalangan pelajar wanita di Fakulti Perubatan dan Sains Kesihatan, UPM. Satu set soal selidik digunakan untuk menentukan faktor sosio demografi sementara pengukuran antropometri digunakan untuk mendapatkan berat dan tinggi peserta dan BMI diklasifikasikan menggunakan titik pemotongan WHO. Pengambilan kalsium melalui diet ditentukan menggunakan pengambilan makanan 24 jam (24HR) dan sejarah diet 3 hari (DH). Kepekatan Hb digunakan untuk menentukan anemia menggunakan titik pemotongan WHO. Prevalensi anemia dalam kajian ini adalah 38.5% dengan min ± SD kepekatan hb adalah 12.08 ± 1.30 g/dl. Min ± SD pengambilan kalsium peserta adalah 479.29 ± 430.64 mg (24HR) dan 460.74 ± 282.69 mg (DH). Hasil kajian menunjukkan bahawa tidak ada hubungan yang signifikan antara pengambilan kalsium makanan yang diukur dengan 24HR ($r = 0.127$, $p = 0.188$) dan DH ($r = -0.022$, $p = 0.821$). Selain itu, hubungan yang tidak signifikan juga tidak terdapat antara faktor pemakanan lain (zat besi, vitamin C dan vitamin D) dan kepekatan Hb. Sebaliknya, analisis lebih lanjut yang dilakukan melaporkan bahawa terdapat hubungan positif positif yang kuat antara pengambilan zat besi dan kalsium makanan ($r = 0.625$, $p = 0.001$ dan $r = 0.594$, $p = 0.001$ masing-masing diukur oleh 24HR dan DH). Kajian ini mendapati bahawa kalsium hanya berkaitan dengan zat besi dan tidak menuju tahap Hb. Walaupum terdapat hubungan yang tidak signifikan antara pengambilan kalsium dan kepekatan hb dalam kajian ini, pengambilan kalsium ditunjukkan berkaitan dengan pengambilan zat besi, yang munkin menunjukkan kemungkinan hubungan penyerapaan zat besi tetapi tidak sejauh ia mempengaruhi kepekatan hb. Dengan penemuan tersebut, anemia dan masalah pengambilan kalsium perlu ditangani dengan segera terutama dalam kalangan pelajar universiti kerana mereka adalah pelapis negara pada masa akan datang.

CHAPTER 1

INTRODUCTION

1.1 Background

Anemia can be defined as a depletion in the total number of red blood cells (RBC)s, which can be measured by reduction in hemoglobin (Hb) concentration, hematocrit (Hct) or RBC count (Di Renzo et al., 2015). According to World Health Organization (WHO) (2011), anemia occurs when the number of RBC, as well as the amount of oxygen RBC can transport, is inadequate to meet the amount RBC needed by the body for normal physiological function. According to the WHO survey, it was estimated that 30% or approximately 2 million women of reproductive age were anemic in Malaysia (Milman, 2015). National Health and Morbidity Survey (2017) reported that the prevalence of anemia in Malaysia in 2015 was 24.6% and found to be higher in females (35.5%) compared to males (14.3%). A study carried out in Kuala Lumpur found that the prevalence of anemia and iron deficiency anemia (IDA) among Malaysian women lives in urban areas were 20.9% and 10.3%, respectively (Loh & Khor, 2010). In another study carried out in Sabah, it was demonstrated that there were 32% of young women aged between 12 to 19 years old were anemic and nearly

88% of those young women were reported to be anemic due to iron deficiency (ID) (Milman, 2015).

There are many types of anemia including IDA, aplastic anemia, pernicious anemia, and hemolytic anemia (National Heart, Lung and Blood Institute, 2011) and IDA is the most prevalent type of nutritional deficiency worldwide (Killip, 2017). IDA is a condition where the balance of iron intake, iron stores, and the body's loss of iron are not enough to support the production of RBC (Miller, 2013). Iron is needed to produce hemoglobin, a protein that functions to carry oxygen in the RBC (National Heart, Lung and Blood Institute, 2011). Groups that are at risk of getting IDA are infants, children, adolescents, women of childbearing age, individuals with certain diseases such as Crohn's disease, celiac disease, kidney failure, and those who have insufficient intake of iron and internal bleeding (National Heart, Lung and Blood Institute, 2011). WHO (2001) suggested that measuring hemoglobin concentration is the most valid method to determine the prevalence of anemia in a population. Daru et al. (2017) also suggested that hemoglobin concentration is an alternative gold standard to measure iron status among anemic individuals. Hence, the prevalence of anemia is often used as a proxy of IDA (World Health Organization, 2008).

In Malaysia, a higher number of ID and IDA cases among women were due to blood loss during menses, further iron depletion due to no supplementation of iron during pregnancy and iron losses due to bleeding during delivery (Milman, 2015). The main source of dietary iron for women in Malaysia was reported to be mainly from the vegetable origin (75%) which has–poor bioavailability compared to animal origins such as meat, poultry, and fish (25%) (Milman, 2015). A review by Dasa & Abera (2018) suggested that the occurrence of iron deficiency which leads to IDA can be

caused by dietary factors. Dietary constituents may affect the amount of iron absorbed by the duodenum and small intestine by acting as inhibitors or enhancers (National Coordinating Committee on Food and Nutrition, 2017).

1.2 Problem statement

Dietary factors such as phytate, polyphenols, calcium, ascorbic acid, and muscle tissues have been shown to influence iron absorption (Abbaspour et al., 2014). Ascorbic acid has been shown in various studies to be capable of effectively increasing the non-heme iron absorption (Dasa & Abera, 2018). According to the WHO (2000), adding enhancers such as organic acid citric, malic or ascorbic acid may increase the absorption of iron. On the contrary, phytic acid, polyphenols, calcium, and peptides from partially digested proteins are the major inhibitors of iron absorption (Abbaspour et al., 2014). Although the only micronutrient that might inhibit the absorption of heme and non-heme iron is calcium, the underlying mechanisms are unclear and the effect shows to be temporary (National Coordinating Committee on Food and Nutrition, 2017). Calcium has been postulated to inhibit the absorption of iron by impeding the intake of iron by binding to the surface of intestinal absorptive cells, where non-heme iron also binds, causing a rendered iron absorption (National Coordinating Committee on Food and Nutrition, 2017).

1.3 Significance of study

There were extensive studies carried out to determine the association between calcium and hemoglobin concentration or different iron biomarkers. A study carried

out in Australia to determine the factors affecting iron status in 15 to 30 years old female students showed that high calcium intake was one of the factors that can increase the possibility of an individual to become ID (Rangan et al., 1997). A cross-sectional study carried out in six European countries among adolescents girls aged 11 to 15 years old and young adult women aged 20 to 23 years old found that dietary calcium intake was inversely associated with serum ferritin levels in adolescent girls and women, respectively ($r = -0.09$ and -0.07, $p < 0.05$) (Vijver et al., 1999). It was shown that serum ferritin level decreased by -0.57 μg/L for every 100 mg/d increased of calcium intake (Vijver et al., 1999). A fortification study carried out by Hallberg et al (1991) reported that 300-600 mg of added calcium in wheat rolls and administration of 165 mg calcium as milk, cheese, or calcium chloride reduced iron absorption by 50-60%. A double stable isotope study carried out in Canada aimed to measure non-heme iron absorption with and without added calcium in 13 women with low iron status found a significant inverse association between calcium and iron absorption (-0.58, $p = 0.037$) (Benkhedda, Abbe & Kevin, 2009). The participants were provided with two types of meals consumed with 500 mg added of calcium carbonate tablet or without calcium (Benkhedda, Abbe & Kevin, 2009). A cross-sectional study in Greece, also known as The Healthy Growth Study found that girls without menses with a low amount of iron status ($p < 0.003$) had a higher intake of calcium and packed fruit juice compared to other girls without menses with normal iron status (Moschonis, 2013). A study carried out by Miranda et al (2013) reported that a single supplementation of iron and combined supplementation of iron and calcium were equally effective in reducing the prevalence of IDA among Bolivian school children aged 6 to 10 years old. The participants were divided into two groups where the first group received 700 mg calcium as calcium carbonate and 30 mg iron as ferrous sulfate

(combine supplementation iron and calcium) whilst the second group received 30 mg of iron as ferrous sulfate (single iron supplementation) for 3 months (Miranda et al., 2013). A fasting venous blood blood sample was obtained at baseline and end of the study to determine hemoglobin concentration, serum ferritin concentration and C-reactive protein (Miranda et al., 2013). It was shown that both supplementation treatments were able to significantly reduce the prevalence of IDA to 51% (iron supplementation) and 26% (combined supplementation iron and calcium) from 56% and 30% respectively ($p< 0.001$) (Miranda et al., 2013).

On the contrary, several studies found no association between calcium intake and iron status. A calcium supplementation study by Kalkwarf and Harrast (1998) reported that 6 months of 500 mg calcium supplementation in lactation mothers did not significantly affect serum ferritin concentrations ($p= 0.8$). Another calcium supplementation study carried out in Chile among 33 to 47 years old women reported that 34 days of 600 mg calcium supplementation measured by double isotopic method did not affect heme iron and non-heme iron bioavailability ($p= 0.41$ and $p= 0.65$) (Castillo et al., 2013). In a study carried out among adolescent girls aged 12 to 14 years old in Denmark reported that 1 year of 500 mg calcium supplementation did not affect iron biomarkers including hemoglobin concentration, serum ferritin concentration and transferrin receptors concentration (Mølgard, Kaestel & Michaelsen, 2005). Similarly in Denmark, a stable isotope study carried out among 21 to 34 years old women reported that 4 day periods consumption of a glass of milk with 3 main meals (breakfast, lunch, and dinner) or consumed an equivalent amount of calcium from fortified foods did not decrease non-heme iron absorption (Grinder-Pedersen et al., 2004). It was shown that there was no significant difference in non-

heme iron absorption between basic diets and basic diets supplemented with milk, calcium lactate, or milk mineral isolate (Grinder-Pedersen et al., 2004).

Although there were many studies carried out to determine the association between calcium and iron status, the studies were carried out in other countries. Besides, a large number of previous studies have used calcium supplementation and isotope-labeled methods and not dietary intake of calcium to determine the association between calcium and iron status. Different compounds of calcium have been used in previous studies including calcium carbonate, calcium citrate, and calcium citrate malate which may have caused a distinct rate of absorption in the human body. Lamy and Burckhardt (2014) suggested that calcium citrate has a better absorption compared to calcium carbonate when consumed with foods. Calcium from the diet might have different absorption rates, compared to supplement, where Napoli et al (2007) have proposed that dietary calcium may have better bioavailability compared to calcium supplements.

Besides, the evolve in foods generally warrants further investigation as it affects the dietary habits of populations which consequently create differences in dietary intake of the population from 10 years ago. According to Noraida et al (2018), rapid urbanization and good socioeconomic progress changed the lifestyle of Malaysian including dietary intake and food choices. For instance, the consumption of fast foods increased among adults and children nowadays (Noraida et al., 2018). It was reported in 2003 that both men and women in Malaysia did not meet the recommended intake of calcium (Mirnalini et al., 2008). However, it was reported that calcium-rich foods including marine fish and green leafy vegetables increased among the Malaysian population as reported in the MANS 2003 and 2014 (Noraida et al., 2018). These findings shows the shift of fluctuations in the consumption of calcium

in the Malaysian population, specifically. Other than that, previous studies mainly used other iron biomarkers including serum ferritin concentrations and isotope labelled iron and not specific to hemoglobin concentrations. There were also limited recent local studies carried out to determine the association between dietary calcium intake and hemoglobin concentration among university students. Therefore, this study was carried out to determine the association between dietary calcium intake and hemoglobin concentration.

1.4 Research Questions

The present research was carried out to address the following questions:

1) What is the hemoglobin concentration of female students?
2) How much calcium intake among female students?
3) What is the association between dietary calcium intake and hemoglobin concentration in female students?

1.5 Research Objectives

1.5.1 General Objective

To determine the association between dietary calcium intake and hemoglobin concentration in female students in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

1.5.2 Specific Objectives

a) To determine socio-demographic characteristics (age, ethnicity, status, religion, education, course of study program, year of study and monthly allowances) of female students.

b) To assess anthropometric measurements of female students.

c) To assess hemoglobin levels of female students.

d) To assess dietary calcium intake of female students.

e) To assess other dietary components (iron, vitamin D and vitamin C) that may affect hemoglobin concentration of female students.

f) To determine the association between dietary calcium intake and hemoglobin concentration of female students.

1.6 Research Hypotheses

There was an association between dietary calcium intake and hemoglobin concentration in female students in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

1.7 Research Framework

Figure 1.1 shows the research framework for this study. Measuring hemoglobin concentration is the most reliable method to determine the prevalence of anemia in a population although it does not determine the cause of anemia. (World Health Organization, 2015). Killip et al., (2007) suggested that hemoglobin and ferritin tests are the best to diagnose anemia.

Socio-demographic characteristics such as age, sex, and socioeconomic status were found to effect on anemia. IDA was found to be higher among those who were

in low socioeconomic status (World Health Organization, 2001). Other than that, the distribution of normal hemoglobin varies with age, sex, and physiological status, for example during pregnancy (World Health Organization, 2008).

Body weight status was found to affect anemia status. According to Coyer (2005), anemia is high among children among some ethnic groups but it can also affect overweight children and children that have chronic illnesses.

Calcium in foods, as well as supplementation, has been shown to inhibit iron absorption (Beck et al., 2014). Calcium was found to be associated with a lower rate of iron absorption in single meal tests which however the body compensates by up-regulates iron absorption (The North American Menopause Society, 2016).

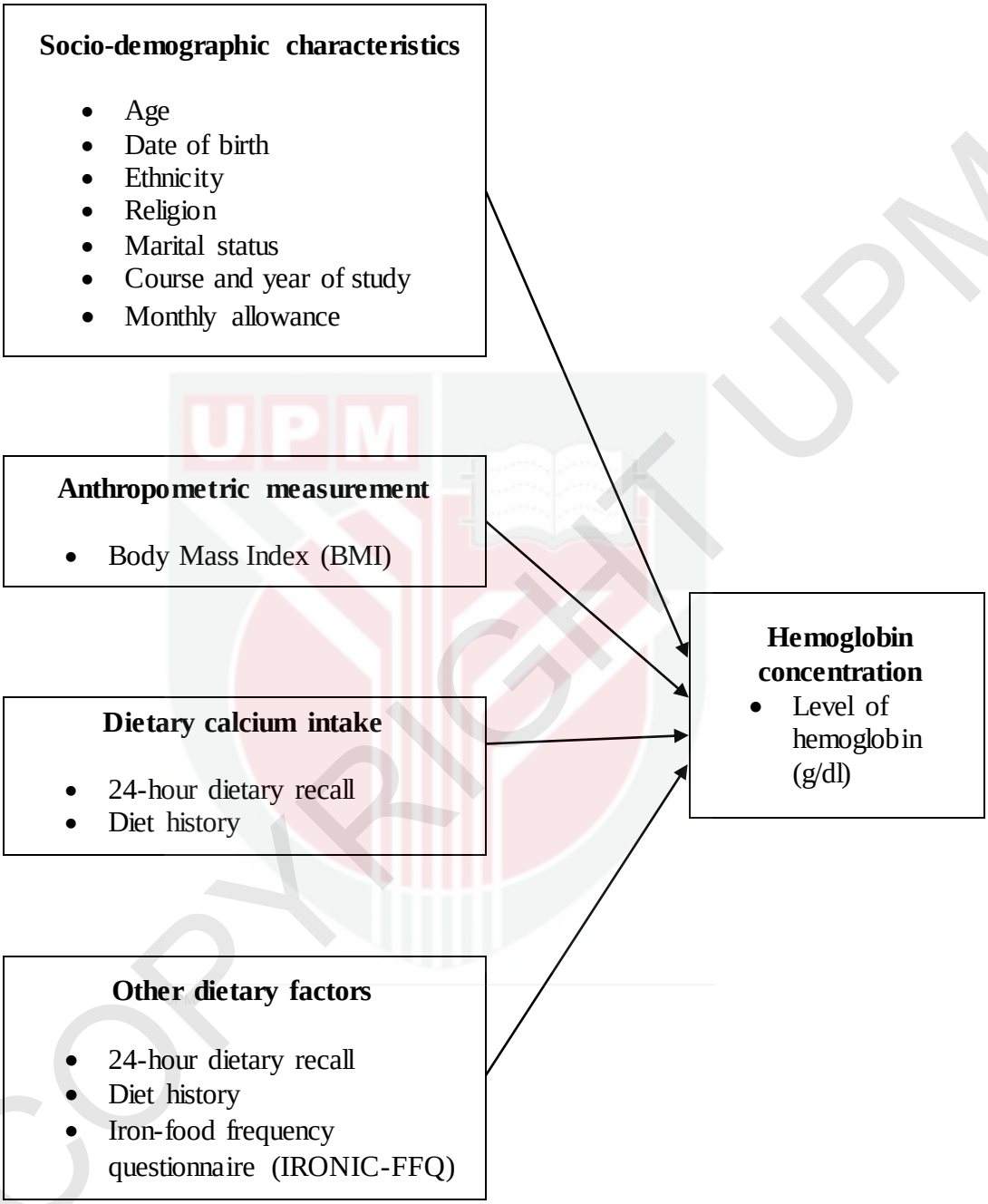

Figure 1.1. Research framework

CHAPTER 2

LITERATURE REVIEW

2.1 Iron

2.1.1 Iron metabolism

Iron is part of transition elements and categorized as one of the most available metals and basic nutrients on earth (Oliveira et al., 2014). Transition metals are elements with incomplete D sub-shells atoms (Chobot & Hadacek, 2010). It can form chelates and complexes and usually exist in two or more oxidation states (Chobot & Hadacek, 2010). This metal is important for life as iron can serve as an electron donor and acceptors renders (Oliveira et al., 2014). Iron is an important micronutrient needed by the body for growth, reproduction, energy metabolism, healing of wounds, and immune system function (Stephenson & Schiff, 2016). Iron is needed by the body for major roles in cellular processes such as synthesis of DNA, RNA, and protein; electron transport, cell respiration, cell growth, and gene expression regulation (National Coordinating Committee on Food and Nutrition, 2017). It is estimated that there are 3-5 grams of iron in an adult human body and about two-thirds of iron are integrated into the hemoglobin of developing erythroid precursor cells and mature RBC (Oliveira et al., 2014). The other remaining iron in the body is stored in ferritin, an iron storage protein, and macrophages transit pool (Oliveira et al., 2014). A small amount of iron is located in muscles within myoglobin and other components of certain proteins and

enzymes containing cellular irons (Oliveira et al., 2014). **Table 2.1** shows four main forms of biologically essential sources of iron in our body (National Coordinating Committee on Food and Nutrition, 2017).

Table 2.1: Four main forms of iron

Forms of iron	Examples	Functions
Heme protein iron	Hemoglobin Myoglobin Cytochromes	Transporting and storage of oxygen Electron transport
Iron sulfur-enzymes	Flavoproteins Heme-flavoproteins	Energy metabolism
Iron storage	Transferrin Lactoferrin Ferritin Hemosiderin	Storage and transport proteins
Enzymes containing iron	Sulfur Non-heme enzymes	Electron transfer process

Iron is important for the structure and function of hemoglobin (Miller, 2013). Hemoglobin will transport the oxygen to the whole part of the body through red blood cells, myoglobin transfers the oxygen to the cells, and cytochromes deliver energy from macronutrients through chemical reactions (Stephenson & Schiff, 2016). Ferritin is the major iron storage protein, which comprises of 24 subunits arranged to form a spherical shell with a large central cavity (Anderson & Fraser, 2017).

The central regulation of the iron in the body is controlled by the gut (Duck & Connor, 2016). Absorption of dietary iron takes place almost entirely at the duodenum and the upper portions of the jejunum (Oliveira et al., 2014). **Figure 2.1** shows the mechanism of iron absorption from the intestinal lumen of the duodenum into the bloodstream (Duck & Connor, 2016).

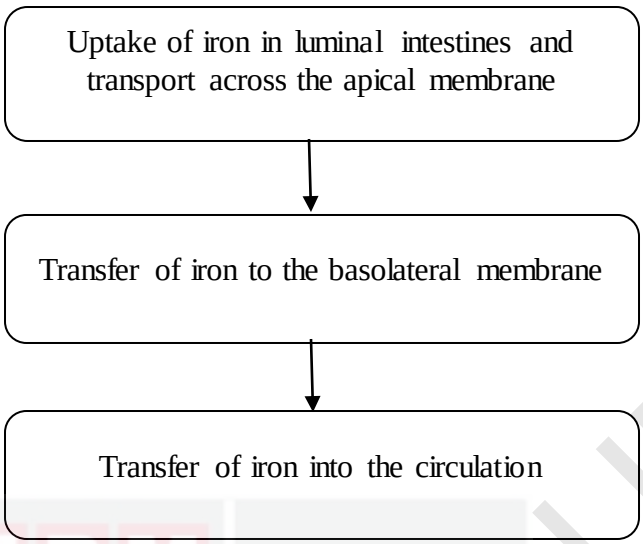

Figure 2.1. Mechanism of iron absorption from the intestinal lumen of the duodenum into the bloodstream (Duck & Connor, 2016)

In the first stage of iron absorption, iron will across enterocytes in the apical membrane through divalent metal transporter (DMT-1), a known mediator of cationic metal transport (Duck & Connor, 2016). In the cell, iron will be stored in ferritin molecule (Waldvogel-Abramowski et al., 2014). Next, iron will pass through the basolateral membrane through an iron transport protein, ferroportin (Duck & Connor, 2016). Ferroportin is incorporated with hephestin which will convert ferrous iron to ferric iron and will be transported throughout the body by transferrin (Duck & Connor, 2016). Waldvogel-Abramowski et al (2014) suggested iron loss approximately 1-2 mg daily. Hence, intestinal iron absorption occurs to balance the loss of iron (Waldvogel-Abramowski et al., 2014). Iron is excreted through three major ways of skin exfoliation, desquamation of intestinal epithelium and menstruation, urine, or feces (Duck & Connor, 2016). The regulator for body iron stores plays an important role in controlling the absorption of iron based on the needs of the body by decreasing or increasing absorption when stores are high or low, respectively (National Coordinating Committee on Food and Nutrition, 2017). Negative iron balance occurs

when the iron stores which is serum ferritin become low (National Coordinating Committee on Food and Nutrition, 2017). Iron absorption will become lower than losses which will consecutively increase dietary iron absorption regulation (National Coordinating Committee on Food and Nutrition, 2017). A continuous balance between iron uptake, transport, storage, and usage of iron is essential to maintain iron homeostasis as iron is needed for various cellular functions (Oliveira et al., 2014).

Imbalance of iron homeostasis might lead to the development of several diseases including hereditary hemochromatosis (HH), thalassemia, and IDA (Oliveira et al., 2014). Excess iron may become toxic and harmful when accumulated in different human organs which consequently leads to iron-overload disorders such as HH and thalassemia (Oliveira et al., 2014). On the contrary, low levels of iron may lead to the development of IDA (Oliveira et al., 2014). Hemoglobin concentration will be low when iron stores are depleted which will correspond to the increased iron absorption (National Coordinating Committee on Food and Nutrition, 2017). Cells could not obtain energy when the iron is not sufficient to produce hemoglobin, myoglobin, and cytochromes which explained why fatigue is the main symptom of iron deficiency (Stephenson & Schiff, 2016). Other symptoms of anemia include pale or yellowish skin, dizziness, increased thirst, sweating, rapid pulse and breathing, lower leg cramps, and heart-related symptoms (National Heart, Lung and Blood Institute, 2011).

2.1.2 Forms of iron

Dietary iron comprises of heme and non-heme iron which are found in hemoglobin and myoglobin in animal source foods and both plant and animal foods, respectively (Hurrell & Egli, 2010). There are two oxidative states of iron which

includes ferrous (Fe^{2+}) and ferric (Fe^{3+}) (Oliveira et al., 2014). In animal food sources such as meat, poultry, and seafood, iron exists as ferrous iron in hemoglobin in the heme form whilst non-heme iron exists as ferric iron in the plant-based foods (Lopez et al., 2016). The digestive tract absorbed more heme iron (15-35%) compared to non-heme iron (2-20%) (Stephenson & Schiff, 2016). The heme iron is highly absorbed because it is directly absorbed into the mucosal cells as an iron-porphyrin complex (Hallberg et al., 1979). Non-heme iron needs reduction to ferrous iron before absorption making non-heme iron incapable to use these transporters (Young et al., 2018). Also, the absorption of heme iron is less influenced by other constituents in a meal except for calcium and is also less affected by the iron status of the body (Zijp et al, 2000). A cross-sectional study carried out in Australia among young women aged 18 to 35 years old found that both heme and non-heme iron were positively associated with serum ferritin level with heme iron was found to have a stronger predictor (b=0.128, p=0.009) than non-heme iron (b= 0.037, p=0.028) (Young et al., 2018). Heme iron was found to be an independent predictor of serum ferritin levels in healthy young women which indicated that consuming heme iron foods such as meat may help in maintaining adequate serum ferritin levels (Young et al., 2018).

2.1.3 Iron bioavailability

World Health Organization (2001) reported that enhancers and inhibitors have been shown to influence the bioavailability of iron from foods. Iron absorption can vary between 2% to 35% depending on the presence of enhancers and inhibitors in meals (Brazaca & Silva, 2003). Teucher et al (2004) suggested that the most efficient enhancer of iron absorption is ascorbic acid. The stomach and proximal small intestines have low pH which keeps the iron in its soluble form to be easily accessible

for absorption (Anderson & Frazer, 2017). Most dietary iron exists in the ferric form hence, the ferric form of iron from the diet will be reduced to ferrous iron so that it can be absorbed (Anderson & Frazer, 2017). A study carried out in India to evaluate the iron status among young women age 18 to 35 years old found that when ascorbic acid added to a meal at a molar ratio of 2:1, iron absorption significantly increased by 291% in IDA group and 270% in the control group ($p<0.001$) (Thankachan et al., 2007). In addition, increased iron absorption was found for both groups when ascorbic acid was added at a molar ratio to the iron of 4:1 which was 350% and 343% respectively ($p<0.001$) (Thankachan et al., 2007). There were no significant differences in the enhancing effect of ascorbic acid between IDA the control groups which showed that the enhancing effect might not depend on the iron status of the subjects (Thankachan et al., 2007).

2.1.4 Iron status biomarkers

2.1.4.1 Hemoglobin concentration

Iron status can be determined by measuring hemoglobin concentrations, serum ferritin levels, serum iron and transferrin concentrations, and total iron-binding capacity concentrations (TIBC) (World Health Organization, 2001). Measuring hemoglobin concentration is the most valid method to determine the prevalence of anemia in a population (World Health Organization, 2011). Daru et al. (2017) also suggested that hemoglobin concentration is an alternative gold standard to measure iron status among anemic individuals. Distributions of normal hemoglobin differ with age, sex, residential elevation (altitude), smoking status, and physiological status such as pregnancy (World Health Organization, 2011). Hemoglobin concentration will be reduced below the normal levels when the RBC has insufficient iron (World Health

Organization, 2001). Hence, the prevalence of anemia is often used as a proxy of IDA (World Health Organization, 2008). **Table 2.2** shows the hemoglobin thresholds used to define anemia according to age or gender group (World Health Organization, 2011).

Table 2.2: Hemoglobin thresholds to define anemia (WHO, 2011)

Age or gender group	Hemoglobin threshold (g/dl)
Children (0.50-4.99 years)	11.0
Children (5.00-11.99 years)	11.5
Children (12.00-14.99 years)	12.0
Non-pregnant women (≥15 years)	12.0
Pregnant women	11.0
Men (≥15 years)	13.0

2.1.4.2 Other iron status biomarkers

Other than hemoglobin concentrations, serum ferritin (SF) concentration, soluble transferrin receptor (sTfR) level, zinc protoporphyrin level, reticulocyte hemoglobin concentration, serum iron concentration, hepcidin concentration, and total iron-binding capacity (TIBC) level or transferrin saturation level (TSAT) can be used to measure iron status of an individual. (Daru et al, 2017). Measurement of serum ferritin concentration is the most precise test that can be used to determine the total body iron stores but in the absence of inflammation (Lopez et al., 2016). Although serum ferritin concentrations are a universally available and standardized measurement, ferritin concentrations increased independently of iron status in acute and chronic inflammatory diseases (Lopez et al., 2016). This indicates that serum ferritin also reflects the acute-phase response in an individual body other than iron status (Suchdev et al., 2016). Ferritin is a positive acute-phase response protein that increases in infected individuals in response to infections or inflammations (Suchdev et al., 2016). Hence, cautious interpretation of serum ferritin concentration should be

in place and that extra measurement of the possibility of inflammation to make sure the validity of the assessment is achieved.

Serum iron level may be used to detect IDA because, in iron-deficient states, transferrin protein expression will increase so the iron saturation on the transferrin is lower to 15% (Miller, 2013). IDA can be detected by measuring the TSAT which is equivalent to circulating iron which serum iron divided by TIBC (Wish, 2006). Moreover, sTRF concentration can be also be used as one of the iron biomarkers because iron deficiency may cause increased release of soluble transferrin from erythroblasts (Miller, 2013).

2.2 Factors affecting iron status

2.2.1 Socio-demographic factors

Iron status depends on several factors and varies between individuals (Zijp et al., 2000). According to World Health Organization (2017), children under age 5 especially infants under 2 years of age, adolescents, women of reproductive age (15-49 years old), and pregnant women are the most at-risk groups of being anemic. One of the reasons why adolescents and women of reproductive age have a higher risk of anemia is because of regular blood loss due to menstruation (World Health Organization, 2017). A study carried out in 16 districts of India aimed to determine the prevalence of anemia by estimating the hemoglobin concentrations among pregnant women and adolescent girls found that the prevalence of anemia among women and adolescent girls was 84.9% and 90.1%, respectively (Toteja et al., 2006). The authors implied that higher prevalence of anemia in the study may be due to ineffectiveness of iron supplementation programs among the study population (Toteja et al., 2006). On the contrary, it was reported in a study carried out in Kenya that

pregnant women aged 31 years old and above were 3 times more likely to be anemic compared to women aged 18 to 24 years old (p= 0.012) (Okube et al, 2016). Hemoglobin concentration was used to diagnose anemia in this study population (Okube et al., 2016). However, the authors implied that anemia in pregnancy increases with increased parity and maternal age (Okube et al.,2016).

On the other hand, a study carried out by Hu et al. (2017) among Chinese adults aged 18 to 65 years found that women age less than 50 years old had significantly lower SF concentrations compared to women aged more than 50 years old which might be explained by the absence of menstrual cycle in those aged more than 50 years old. Fasting blood samples were used to assess various iron status including SF concentrations, hemoglobin level whilst 3 days 24 hour dietary recall was used to assess diet intake of the populations (Hu et al., 2017).

A cross-sectional survey carried out to determine the prevalence of anemia in pregnancy among Malaysian women found that there was a significant difference in hemoglobin concentration between Chinese and Malays women aged 16 to 56 years old (Haniff et al., 2007). The mean hemoglobin concentration of Chinese was observed to be significantly higher than Malay and Indian women (p= 0.033) (Haniff et al., 2007). However, there was no significant association found between the mother's age and hemoglobin concentration (Haniff et al., 2007). On the contrary, a study carried out by Loh and Khor (2010) to determine iron intake and IDA among young women in Kuala Lumpur found that 33% of the subjects have depleted iron status and among that, Malay has the lowest percentage (22.9%) followed by Chinese (25.2%) and Indians (53.9%). Iron intake of the study population were measured by diet recall whilst Hb concentrations, hematocrit, MCV and SF level were determined to assess iron status (Loh & Khor., 2010). Nevertheless, these two studies suggested

that ethnicity may affect the iron status of an individual due to variation in the dietary intake for each ethnicity.

A study carried out by Stephen et al. (2018) suggested that the main risk factors of anemia in women age ranged 15 to 46 years old in Northern Tanzania were the place of residence and education level. Education level of the participants were being categorized into none, primary and secondary or higher educational level whilst anemia was determined by measuring hemoglobin concentrations (Stephen et al., 2018). It was observed in the study that women with secondary and tertiary education have a lower possibility to be anemic (p= 0.022) (Stephen et al., 2018). It was suggested that educated pregnant women might receive better income and consumed nutritious food which lowers their risk to become anemic (Stephen et al., 2018).

Meanwhile, contradicting findings were reported in a study carried out to determine the prevalence and risk factors for anemia among rural and urban men and women of all ages in Malawi (Adamu et al., 2017). It was observed that women with higher education levels and live in urban areas were more likely to become anemic (p= 0.001) (Adamu et al., 2017). Educational levels of participants were being categorized into four categories which were no formal, standard, secondary and tertiary levels (Adamu et al., 2017). It was suggested that educated women in urban places had increased risk of nutrition-related anemia due to their consumption of food that has shifted away from traditional diets and also supplements (Adamu et al., 2017).

Besides, a significant correlation was found between anemia and family income status in a study carried out in Iran which was aimed to determine the prevalence and association of anemia in reproductive age women (Sadeghian et al., 2013). The family income status were being divided into 6 categories where lowest

range was below 100 dollar whilst highest range was more than 500 dollars (Sadeghian et al., 2013). Anemia was being determined using hemoglobin concentrations of participants (Sadeghian et al., 2013). It was reported that the lower economic group had a lower mean hemoglobin concentration compared to the upper economic group (Sadeghian et al., 2013).

A cross sectional study carried out by Qin et al. (2013) reported that Chinese women with a high and medium socio economic status (SES) had a higher prevalence of anemia than women with low SES (p=0.001). Low SES was defined as an annual income less than 1999 Yuan, medium SES as 2000-4999 Yuan and high as more than 5000 Yuan whilst anemia was reported by hemoglobin concentration (Qin et al., 2013). A cross-sectional study carried out in Kenya among pregnant mothers found that employed or self-employed pregnant women had 2.9 times and 1.9 times the risk of anemia compared to housewives, respectively (Okube et al., 2016). Hemoglobin concentrations were used to determine anemia status of the study population (Okube et al., 2016). The authors implied the observed findings may be due to insufficient resting time in employed women as a pregnant mother and more likely to skip antenatal appointment compared to housewives (Okube et al., 2016).

However, there were also several other studies suggested that socio-demographic factors may not be associated with iron status. A study carried out to determine the prevalence, risk factors and adverse perinatal outcomes of anemia by Grum et al (2018) found that age, religion, marital status, occupation, household size, and income were not significantly associated with anemia. A study carried out in Northern Tanzania among pregnant mothers also found that age, marital status, occupation, income, and alcohol intake were not associated with anemia during pregnancy (Stephen et al., 2018). It was observed that these two study determine

anemia when participants have <11 mg/dl hemoglobin concentrations which was lower than cut off point mentioned by WHO.

2.2.2 Body composition

Several studies reported that body composition may affect iron status of an individual. According to Maden (2018), body mass index (BMI) can be used to interpret the body weight status of adults and serves as a quick review of nutritional status (Maden, 2018). A cross-sectional study carried out in China to determine the associations between dietary, lifestyle and socio-demographic factors with iron status in Chinese adults age 18 to 65 years found that BMI was positively associated with SF concentration for both men and women ($p< 0.001$) (Hu et al., 2017). There were four BMI groups where the study used cut offs recommended by the Guidelines for Preventing and Controlling Overweight and Obesity in Chinese Adults (Hu et al., 2017). It was observed in the study that those with a higher BMI and younger age had higher hemoglobin concentrations (Hu et al., 2017). The author implied that this study has confirmed several dietary and non-dietary factors that associated with body iron status specifically among Chinese adult population.

In a cross-sectional study carried out in China to determine the association between anemia and body composition (BMI and waist circumference) among Chinese women aged 20 years and above found that overweight or obese women were less likely to have anemia compared to normal weight women (Qin et al., 2013). The participants were classified by BMI categories according to Chinese standards as underweight (BMI < 18.5), normal weight (BMI > 18.5 < 24), overweight (BMI ≥ 24 < 28) and obesity (BMI ≥ 28) whilst hemoglobin concentration was used to define

anemia (Qin et al., 2013). Nevertheless, the authors suggested that the main limitation of this study was due to anemia as the assessment of iron status without distinguishing whether it was caused by ID or chronic disease (Qin et al., 2013).

Another study carried out among pregnant women aged 18 to 31 years in Kenya aimed to determine the prevalence and factors associated with anemia found that pregnant women with mid-upper arm circumference (MUAC) less than 23 cm had a higher prevalence of anemia (Okube et al., 2016). MUAC was reported in two categories, more than 23 cm and low than 23 cm whilst hemoglobin concentration was used to determine anemia (Okube et al., 2016). The observed findings might be due to the fact that undernourished women were more likely to have micronutrient deficiencies (Tekeste, 2015). A study carried out in Tanzania by Msemo et al. (2018) reported that likehood of being anemic were reduced among women with increased MUAC. Determination of anemia status were reported using hemoglobin concentration with WHO cut off point whilst no category of MUAC presented (Msemo et al., 2018).

However, contradict findings were found in a cross-sectional study carried out among Iranian population to determine the association between BMI and hemoglobin concentration and iron status biomarkers (Anari et al., 2014). Fasting blood samples were collected to assess wide range of iron status biomarkers (Anari et al., 2014). It was found that BMI did not affect hemoglobin concentration, serum iron level, TIBC concentration, TSAT level, SF level and mean corpuscular volume concentration (Anari et al., 2014). The observed findings were due to the population involved in the study includes both genders and male may probability have a lower risk of getting anemia (Anari et al., 2014).

2.2.3 Diet intake

According to Zijp et al. (2000), several dietary factors may influence non-heme iron absorption by either inhibiting or enhancing the absorption. Dietary intake is also a factor for the development of ID and consequently to IDA (Beck et al., 2014). High iron bioavailability diets contain a high amounts of meat and foods that may enhance the absorption of iron with low intake of foods that inhibit the absorption of iron (Beck et al., 2014). One of the foods that promotes the absorption of non-heme iron is meat (Zijp et al., 2000).

Previous studies suggested that there was an association between dietary intake and iron status. A study has been carried out in Greece to determine the association between iron depletion and menstruation and dietary intake in pubertal girls aged 9-13 years old (Moschonis et al., 2013). A semiquantitative FFQ was used to determine the children's habitual food consumption whilst bold samples were obtained to assess wide range of iron status biomarkers but ID was determined using SF concentrations (Moshonis et al., 2013). It was reported that girls iron-depleted without menses had a lower intake of poultry ($p= 0.037$), high consumption of fast foods ($p= 0.041$) and fruit juices ($p= 0.044$) compared to girls without menses with normal iron status (Moschonis et al., 2013). These observed findings might be due to the inadequate amount of iron intake by the iron-depleted girls which can be obtained from poultry. It was suggested that diet which was low in iron-rich food sources are the major risk of ID in girls from both developing and developed countries (Moschonis et al., 2013).

In a study carried out by Hu et al. (2017), it was found that there were a positive associations between pork ($p= 0.006$) and poultry ($p< 0.001$) and SF concentrations among men. The dietary intake of participants were determined using 3 days of 24

hour dietary recall which the food consumption was grouped into 10 food groups including red meat, grains, pork, vegetables, fish, poultry, eggs, tubers, fruits, and milk (Hu et al., 2017). Meanwhile, iron status were determined using SF concentrations, hemoglobin concentrations, sTfR level and serum high-sensitivity C-reactive protein (hs-CRP) concentrations (Hu et al., 2017). Pork and poultry contain a high amount of heme iron and it was found that there was a significant association between red meat consumption and serum ferritin concentration in both men (p= 0.002) and women (p=0.03) (Hu et al., 2017).

According to Stephenson & Schiff (2016), the ferrous form of iron will be effectively absorbed compared to ferric iron. Ferric ion can convert to ferrous iron in acidic conditions, hence, medications such as antacids that reduce acidity in the stomach will lower iron absorption (Stephenson & Schiff, 2016). Foods containing oxalic acid, polyphenols, phytic acid, soy protein may also reduce iron absorption (Stephenson & Schiff, 2016). It was also shown in previous studies that, iron should not be consumed with dairy products, antacids, coffee, tea, bran, whole grains, calcium, and calcium supplements to avoid the possibility of reduced iron absorption (Coyer, 2005).

2.3 Calcium

2.3.1 Biology of calcium

Calcium is the most plentiful micronutrient in the human body which represents approximately about 1.2 to 1.4 kg and more than 99% of calcium is stored in the bones and teeth whilst less than 1% of calcium is found in extracellular serum calcium (Beto, 2015). The remainder of calcium can be found in the teeth (0.6%), soft

tissues (0.6%), the plasma (0.03%), and extracellular fluid (0.06%) (National Coordinating Committee on Food and Nutrition, 2017).

Variety of signaling mechanisms involved in controlling and coordinating cell activity in which it require the release of ionized calcium from intracellular compartments into the cytoplasm (Weaver & Heaney, 2000). External signals are being translated into internal signals in the cell by ionized calcium (Weaver & Heaney 2006). These observed function of ionized calcium are due to its small size and its affinity for protein molecules (Weaver & Heaney, 2006). Calcium ion (Ca^{2+}) is suitable for signaling ion due to the smaller size of its ionic radius compared to other prevailing ionic species including potassium ion (K^{+}) and chloride ions (Cl-) (Weaver & Heaney, 2006). Although Ca^{2+} is larger than magnesium ions (Mg^{2+}), it is small enough to fit into intracellular pores (Weaver & Heaney, 2006). It is not prone to be toxic at high concentrations or causing tissue damage under various conditions as it has only one oxidation state (Weaver & Heaney, 2006).

According to Weaver & Heaney (2006), calcium was found to be the most studied minerals related to human health. Food sources such as dairy products, vegetables, and cereals, foods fortified with inorganic or organic calcium are the main sources of dietary calcium as well as dietary calcium supplements (National Coordinating Committee on Food and Nutrition, 2017). In Malaysia, foods that are rich in calcium are fish from edible bones such as canned sardines and anchovies, beans and bean products including yellow dhal, tofu, *tempeh*, locally processed foods such as shrimp paste, *cincaluk* and *budu* as well as vegetables like spinach , watercress, mustard leaves, *cekur manis*, tapioca leaves, *kai-lan* and brocolli (National Coordinating Committee on Food and Nutrition, 2017).

2.3.2 Functions of calcium

The body needs calcium for the formation of bones and teeth, muscle contraction, blood clotting, nerve impulse transmission, and cell metabolism (Stephenson & Schiff, 2016). Other than that, research has proven that calcium is required for vascular contraction, vasodilation, muscle functions, the transmission of nerve, intracellular signaling, and secretion of hormones (Beto, 2014). Beto (2014) also suggested that the changes in serum calcium may impact one or more of these functions. The absorption of calcium occurs in the intestines and mostly in the duodenum (Stephenson & Schiff, 2016). Moreover, the absorption of calcium takes place in the gastrointestinal tract through passive absorption as a primary mechanism (Beto, 2014). It is estimated that approximately 65% of calcium is absorbed in pH 6.5-7.5 (Beto, 2014). The amount of absorbed calcium depends on the quantity of calcium present, total and segmental transit time, and amount of calcium present in each unique pH level (Beto, 2014). It was also reported that calcium supplements solubility is greatly affected by pH level (Beto, 2014). However, it has been suggested that calcium comprehends a complex dependent interaction with other nutrients in the body (Beto., 2014).

2.4 The association between calcium intake and hemoglobin concentration

Lonnerdal (2010) suggested that calcium interferes with the absorption of iron in humans. It was suggested that competitive inhibition between calcium and iron might occurs during the final stages of intracellular metabolism of iron rather than initial binding at the intestinal mucosal cells (Hallberg et al., 1992). Lynch (2000) suggested that inhibition of calcium on iron absorption may be prevented by avoiding the consumption of calcium supplement at the same time with high iron meals. It is

better to consume iron supplements at a different time with calcium or zinc supplement as these three minerals compete for absorption sites (Stephenson & Schiff, 2016).

Most of the previous studies carried out to determine the association between calcium intake and iron status comprised of the female as participants because these are one of the groups that are vulnerable to get IDA. Inconsistent findings were found in previous studies carried out to determine the association between calcium intake and iron status. Previous studies carried out have used dietary supplements whilst only limited studies used dietary calcium intake to measure the association of calcium and iron status. **Table 2.3** shows the details of previous studies aimed to determine the association between calcium intake and iron status.

Table 2.3: Previous studies to determine the association between calcium and iron status

Author	Country	Sample population & age (years)	Method		Findings	Remarks
			Calcium intake	Iron status		
Vijver et al., 1988	Denmark Finland France Italy The Netherland Poland	n= 1604 age= - 11-15 yrs - 20-23 yrs	3-day food record	Serum iron, transferrin & SF from 10 ml blood of non- fasting participants	Significant inverse correlation between dietary calcium intake and iron status (r= -0.09 for adolescent girls) (r= -0.07 for women) ($p < 0.05$ for both groups)	Did not distinguish between heme and non-heme iron
Hallberg et al., 1992	Sweden	n= 28 age= 24-65 yrs	165 mg of $CaCl_2$	*Double isotopic method	Ca significantly affect the absorption of heme iron	Male and female were included
Pendersen et al., 2004	Denmark	n= 14 age= 21-34 yrs	- Milk - Ca lactate - Milk mineral isolate	SF from venous blood	No significant difference between 3 sources of calcium and non-heme iron absorption	- Low sample population - Consumption of Ca only for 4 days
Mølgaard et al., 2005	Denmark	n= 113 age=12-14 yrs	500 mg of Ca/d for 1 year	Hb, SF & serum Tfr from blood	No significant difference between Ca supplementation and iron status	Consumption of Ca for 1 year

SF, serum ferritin; $CaCl_2$, calcium chloride; Ca, calcium; Hb, hemoglobin; Tfr, transferrin; Fe, iron; $CaCO_3$, calcium carbonate; Ca; calcium; CRP, C-reactive proteins; IDA, iron deficiency anemia; yrs, years

*A method which measured radiolabeled ^{55}Fe and ^{59}Fe by calculation of the amount of radioactivity ingested

Table 2.3 (Cont.): Previous studies to determine the association between calcium and iron status

Author	Country	Sample population & age (years)	Method		Findings	Remarks
			Calcium intake	Iron status		
Benkhedda, Abbe & Cockell., 2010	Canada	n= 13 age= 20-38 yrs	500 mg of CaCo3	SF from 15 ml venous blood	Significant inverse correlation between Ca and Fe absorption (r= -0.58, p= 0.037)	- Low sample population - Focused only on low iron status women
Castillo et al., 2013	South America	n= 26 age= 33-47 yrs	600 mg of CaCo3 for 34 days	*Double isotopic method	Ca supplementation did not affect heme and non-heme iron bioavailability	Low sample population
Miranda et al., 2013	South America	n= 195 age= 9-10 yrs	700 mg of CaCO3	SF, Hb & CRP from fasting venous blood	No significant difference between combined (Ca+Fe) supplementation and Fe supplementation to reduce IDA	Male were included

SF, serum ferritin; $CaCl_2$, calcium chloride; Ca, calcium; Hb, hemoglobin; Tfr, transferrin; Fe, iron; CaCO3, calcium carbonate; Ca; calcium; CRP, C-reactive proteins; IDA, iron deficiency anemia; yrs, years

*A method which measured radiolabeled ^{55}Fe and ^{59}Fe by calculation of the amount of radioactivity ingested

CHAPTER 3

METHODOLOGY

3.1 Study design

This was a cross-sectional study aimed to determine the association between dietary calcium intake and hemoglobin concentration in female students in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

3.1.1 Study protocol

Figure 3.1 shows the data collection procedures for this study. Data collection was carried out between December 2019 and February 2020. Prior to data collection, approval for the study protocol was obtained from JKEUPM for Research Involving Human Subjects and Dean of Faculty of Medicine and Health Sciences as shown in **Appendix B** [Reference No.: JKEUPM-2019-456]. Participant information sheet which contains brief explanation on the aims of the study and consent form were provided to the participants and the interview session was conducted for approximately 30 minutes. The participants were free to withdraw from the research at any time without giving reasons. All the information collected was kept as private and confidential including participants' identity.

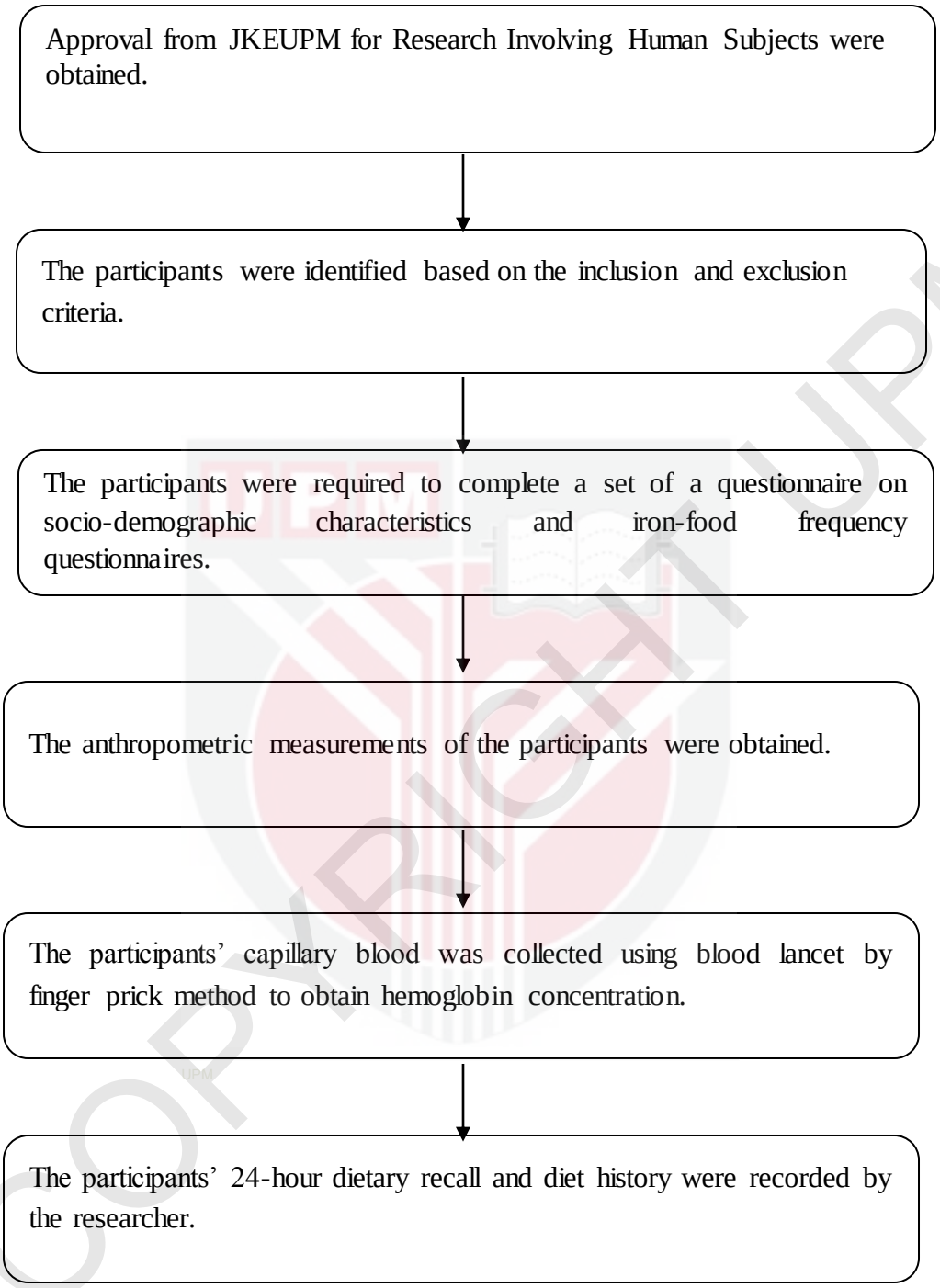

Figure 3.1. Study protocol

3.1.2 Study approval

Ethical approval was obtained from the Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia (JKEUPM) (JKEUPM-2019-456) to the data collection, which followed by obtaining permission from the Dean, Faculty of Medicine and Health Sciences.

3.2 Study location

This study was carried out in the Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM), Serdang. Data collection was carried out in the nutrition lab of the Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Serdang. The faculty was selected by convenience sampling method.

3.3 Participants

The participants of this study were recruited among students in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The participants were selected based on the following inclusion and exclusion criteria (**Table 3.1**). Women were selected because anemia has been shown to be more prevalent in childbearing age women that the prevalence was much higher in women compared to men, demonstrated in various studies carried out worldwide, including the local studies.

Table 3.1: Inclusion and exclusion criteria for the selection of participants

Inclusion criteria	Exclusion criteria
● Malaysian ● Female ● Age 19-49 years old	● Pregnant women and lactating mothers ● Menopause women ● Individuals who use contraceptives ● Donated blood for the past 6 months ● Individuals who regularly consume nutritional supplements ● Presence of gastrointestinal disorders and chronic diseases such as celiac disease, gastroesophageal reflux disease (GERD), diabetes mellitus, and metabolic disorder.

3.4 Sample size determination

A formula by Hulley et al. (2013) was used to determine the number of participants involved.

Formula:

$$N = \left[\frac{(Z_\alpha + Z_\beta)}{c}\right]^2 + 3$$

$$c = 0.5 * ln\left[\frac{(1+r)}{(1-r)}\right]$$

Where:-

The standard normal deviate for Zα = 1.96

The standard normal deviate for Zβ = 0.84

The expected correlation coefficient = r

Table 3.2: Sample size calculation

Independent variables	Correlation, r	Sample size, n
Calcium supplementation (Benkhedda, 2010).	r = -0.58	C = 0.5 * ln [(1 + (-0.58)) / (1 – (-0.58))] = -0.66 N = $[(1.96 + 0.84) / (-0.66)]^2 + 3$ = **20.9**
Calcium supplementation (Kadry et al., 2018)	r = -0.352	C = 0.5 * ln [(1+(-0.352)) / (1-(-0.352))] = -0.37 N = $[(1.96 + 0.84) / (-0.37)]^2 + 3$ = **60.3**
Calcium (Harris et al., 2003)	r = 0.34	C = 0.5 * ln [(1+0.34) / (1-0.34)] = 0.35 N = $[(1.96 + 0.84) / (0.35)]^2 + 3$ = **67**
Added calcium 0-300 mg (Hallberg et al., 1991)	r = 0.99	C = 0.5 * ln [(1+0.99) / (1-0.99)] = 2.64 N = $[(1.96 + 0.84) / (2.64)]^2 + 3$ = **4**

The adjustment was calculated as below using the data from Harris et al (2003) study:

Adjustment in computing the sample size:

i) Sample Size Effect = DEFF= 1.3

n = 67 x 1.3 = **87.1**

ii) Response rate (80%) :

$$\frac{\#\text{ of respondents you need}}{\text{expected \% response rate}} \times 100$$

n = $\frac{87.1}{80} \times 100$ = 108.8 ~ **109 participants**

A total of 109 participants were required for the study based on the findings from Harris et al (2003) study with 20% drop out rate consideration.

3.5 Self-Administrated Questionnaire

Participants were required to complete a set of questionnaires to obtain information related to socio-demographic characteristics which include age, date of birth, ethnicity, religion, marital status, level of education, course and year of study and monthly allowance (**Appendix D**).

3.6 Anthropometric measurements

Body weight (kg), height (cm) and body mass index (BMI) of the participants were determined to assess the body weight status of the participants (National Health and Nutrition Survey, 1988). Body weight was measured using the SECA 803 flat weighing scale (SECA, Hamburg, Germany) to the nearest 0.1 kg (National Health and Nutrition Survey, 1988). The participants were required to remove the shoes, socks, or any heavy items from their bodies (National Health and Nutrition Survey, 1988). The weighing scale was placed on a flat surface and zero balance was assured before taking the measurement (National Health and Nutrition Survey, 1988). A SECA 213 stadiometer (SECA, Hamburg, Germany) was used to measure the participants' height to the nearest 0.1 cm (Miranda et al., 2014). The participants were required to stand upright and barefooted on the stadiometer and the head was positioned in the Frankfort horizontal plane (Madden, 2018). After the data on body weight and height were acquired, BMI was estimated and interpreted by using the following formula and classification.

$$Body\ Mass\ Index\ (BMI) = \frac{body\ weight\ (kg)}{height\ (m^2)}$$

Table 3.3: Classification of BMI (WHO, 2000)

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal	18.5 - 24.9
Overweight	25.0 – 29.9
Obesity Class I	30.0- 34.9
Obesity Class II	35.0 -39.9
Obesity Class III	>40.00

3.7 Determination of dietary assessments

3.7.1 24 hour dietary recall and diet history

Dietary calcium intake and other dietary components (iron, vitamin C and vitamin D) of the participants was assessed by using 24-hour dietary recall (1 day) and diet history (3 days) which includes two days of weekdays and one day of the weekend. 24-hour dietary recall was used to assess food and beverages consumption high calcium foods in the last 24 hours that may potentially affect hemoglobin concentration collected during the data collection day. A 3-day diet history was used to represent the habitual diet of participants during weekdays and weekend. A 24-hour dietary recall was not sufficient to represent the typical daily intake of an individual due to the variability of food and nutrients intake day-to-day. A detailed one to one interview on the frequency of food consumed, the time taken for meals as well as the portion of foods were also reported for both diet assessment methods. Both of the diet assessment methods were obtained with the aid of household measurements such as bowls, standard measuring cups, glasses, and spoons to facilitate participants in estimating the portion size and quantities of food consumed. Total dietary calcium intake was analyzed using Nutritionist Pro Version 25 (First Data Bank Inc., 2011). The Malaysian Food Composition Table was used as a reference to analyze the dietary intake (Tee et al., 1997). The amount of dietary calcium intake of participants was compared with Recommended Nutrient Intake (RNI) for calcium for female adults

age 19 to 49 years old which is 1000 mg/day (National Coordinating Committee on Food and Nutrition, 2017).

3.7.2 IRONIC-FFQ

Dietary iron intake of the participants was assessed by using an iron-food frequency questionnaire (IRONIC-FFQ—IRON Intake Calculation-Food Frequency Questionnaire) (Głabska et al., 2017). The IRONIC-FFQ was suggested as a practical tool to assess the iron intake and analysis of dietary intervention for young women in anemia risk group (Glabska et al., 2017). The assessment of the IRONIC-FFQ was reported to have an acceptable validity level and positively validated reproducibility (Glabska et al., 2017). IRONIC-FFQ consisted of 12 groups of food products classified meat, meat products, eggs, fish, dairy products, cereal products, fruits, vegetables, potatoes, fats, nuts and seeds. The participants were required to complete open-ended questions regarding the serving sizes, types, and frequency of food products listed in the IRONIC-FFQ. The dietary iron intake of participants was estimated using the following formula where the number of servings was divided into 7 days a week to obtain the daily number of servings.

Iron intake (mg) = daily number of servings × typical iron content in one serving

The amount of dietary iron intake of participants was compared with Recommended Nutrient Intake (RNI) (National Coordinating Committee on Food and Nutrition, 2017) for iron for female adult age 19 to 50 years old which is 29 mg/day in 10% of bioavailability. This value was selected due to Malaysia diets commonly have iron

bioavailability about 4 to 12% (National Coordinating Committee on Food and Nutrition, 2017).

3.7.3 Analysis of misreporting

Zainuddin et al (2019) reported that under-reporting of energy intake (EI) was common in dietary assessment method. Misinterpretation of the individual's nutritional state and misleading association between diet and disease will occur due to this bias of reporting (Zainuddin et al., 2019). Hence, under or over reporting of EI is important in any nutritional surveys (Zainuddin et al., 2019). Goldberg equation was used to determine under reporting and acceptable reporting. The equation involves the calculation of ratio of EI and basal metabolic rate (BMR). BMR was being calculated using the following BMR predictive equations for adult Malaysians (National Coordinating Committee on Food and Nutrition, 2017).

BMR for female (18-30 years) (MJ/day) = 0.0535 (Weight) + 1.994 x 239

Underreporting and over reporting was being calculated using cut off points which were obtained by comparing cut off points with EI:BMR ratio for each participants. The cut off values was used to determine three ranges of misreporting which was <1.489 for under reporters, 1.489 - 1.613 for normal or plausible reporter and >1.613 for over reporters. (National Coordinating Committee on Food and Nutrition, 2017).

3.8 Determination on hemoglobin concentration

Data on hemoglobin concentration were collected using HemoCue© Hb 201+ Analyzer (HemoCue AB, Ängelholm, Sweden). Blood samples were obtained by a finger-prick method using blood lancet by a trained researcher with the assistance of professional lab assistants and observed by the principal investigator who is a trained phlebotomist and the other medical doctor who is also part of the research team. The process began with cleaning the middle finger with disinfectant before a prick was obtained. The first drop of blood was cleaned off and tip of the finger was pressed again to get the second drop of blood. The collected blood was placed in the micro cuvette and placed in the cuvette holder to measure hemoglobin concentration. The level of hemoglobin to determine anemia was classified using the cut off by WHO as follows (**Table 3.4**).

Table 3.4: Hemoglobin levels to diagnose anemia at sea level (g/dl)

Population	Non-anemia	Anemia		
		Mild	Moderate	Severe
Non-pregnant women (15 years of age and above)	12.0 or higher	11.0-11.9	8.0-10.9	lower than 8.0

Adapted from WHO (2000)

3.9 Statistical Analysis

Statistical analyses and normality tests were performed by using IBM SPSS Statistics 25 (IBM Corp., Armonk, NY). Normality of all the variables were checked by using skewness, histogram and plot. The findings of the continuous variables were presented as means and standard deviations whilst categorical variables were presented as frequencies and percentages. Spearman's rank correlation or Pearson

Product-Moment Correlation were used to assess the association between dietary calcium, iron, vitamin C, vitamin D intake and hemoglobin concentration. The level of significance was set at $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Socio demographic characteristics of study population

Table 4.1 shows the socio-demographic characteristics of the participants. The participants' age ranged between 20 to 25 years where the mean ± SD age of participants was 22.25 ± 1.17 years. Out of 109 participants, there were 65 (59.6%), 27 (24.8%) and 17 (15.6%) participants aged between 22-23, 20-21, and 24-25 years respectively. Regarding ethnicity, approximately 81.7% of participants were Malays 7.3% were Chinese, 6.4% were Indian, whilst the remaining 4.6% were other races. All participants were single and bachelor degree students. The mean ± SD monthly allowances of participants was RM 442.82 ± 293.856 with the lowest proportion was below RM 200 (16.5%) and highest was more than RM 1000 (2.8%). In this present study, there were no statistical analysis been carried out to determine the associations between socio demographic factors and hemoglobin concentrations. However, there were several other studies reported the association between socio demographic factors including age, ethnicity, educational levels and monthly allowances and iron status.

Table 4.1: Distributions of participants by socio demographic characteristics (n= 109)

Characteristics	Mean ± SD	n (%)
Age (years)	22.25 ± 1.17	
20-21		27 (24.8)
22-23		65 (59.6)
24-25		17 (15.6)
Ethnicity		
Malay		89 (81.7)
Chinese		8 (7.3)
Indian		7 (6.4)
Others		5 (4.6)
Status		
Single		109 (100.0)
Religion		
Islam		92 (84.4)
Buddhism		8 (7.3)
Hinduism		7 (6.4)
Christian		2 (1.8)
Education		
Bachelor		109 (100.0)
Course of study program		
B. Sc (Nutrition and Community Health)		49 (45.0)
B. Sc (Biomedical Sciences)		19 (17.4)
B. Sc (Environment and Occupational Health)		19 (17.4)
Doctor of Medicine (MD)		16 (14.7)
B. Sc (Dietetics)		3 (2.8)
B. of Nursing		3 (2.8)
Year of study		
1st year		10 (9.2)
2nd year		17 (15.6)
3rd year		40 (36.7)
4th year		31 (28.4)
5th year		11 (10.1)
Monthly allowances	442.82 ± 293.86	
≤RM 200		18 (16.5)
RM 201 – RM 400		53 (48.6)
RM 401 – RM 600		24 (22.0)
RM 601 – RM 800		4 (3.7)
RM 801 – RM 1000		7 (6.4)
≥ RM 1001		3 (2.8)

A cross sectional study carried out to determine the prevalence of IDA among university students age 17-25 years in Noakholi region, Bangladesh reported that students ranged between 20 to 22 years (43.4%) have the higher prevalence compared to 17-19 years (27.7%) and 23 to 25 years (28.9%) age groups (Shill et al., 2014). Hemoglobin concentrations were obtained to determine the status of anemia (Shill et al., 2014). It was suggested that the prevalence of anemia among university students especially female may be due to food intake which provides insufficient amount of iron and lack of awareness which majority of students are not concious about anemia (Shill et al., 2014). Other than that, it was also suggested that iron supplement and irregular breakfast intake could also be the possible reason of higher prevalence of anemia among university students (Shill et al., 2014).

Similar trend of findings were reported in a study carried out in Hodeida Province, Yemen aimed to determine the prevalence of IDA among medical university students. It was reported that students aged 20 to 22 years (59.2%) had higher prevalence compared to students aged range of 17 to 19 (25.0%) and 23 to 25 years (15.8%) (Al-alimi, Bashanfer & Morish, 2018). It was also observed that female (54.0%) had higher prevalence of anemia compared to male (46.0%) (Al-alimi, Bashanfer & Morish, 2018). The author mentioned that higher prevalence of IDA observed in the adolescents may be due to rapid growth and menarche which increased iron demand and insufficient intake of iron-rich foods (Al-alimi, Bashanfer & Morish, 2018). Other than that, frequent consumption of tea, coffee and cola with meals and improper diet intake could also be factors contributing to anemia (Al-alimi, Bashanfer & Morish, 2018).

A study was carried out in China aimed to determine the association between dietary, lifestyle and sociodemographic factors and iron status among adults aged 18 to 65 years old (Hu et al., 2019). Dietary data were collected by using 3 day 24 hour dietary recall on consecutive days whilst iron status were obtained by collecting fasting blood samples of participants (Hu et al., 2019). It was reported that women aged more than 50 years old had significantly higher SF concentrations compared to women aged less than 50 years old ($p < 0.001$) (Hu et al., 2017). It was suggested that those below 50 years still had menstrual cycle and losing blood regularly (Hu et al., 2017). Losing blood regularly by women in younger age groups may increase their risk to become anemic.

There were two local studies that found associations between ethnicity and hemoglobin concentrations. A study carried out to determine the prevalence of anemia among Malaysian pregnant women by assessing the hemoglobin concentration of participants reported that hemoglobin concentration of Chinese was significantly higher than Malay and Indian women ($p = 0.033$) (Haniff et al., 2007). On the contrary, a study carried out to determine iron intake and IDA among young women in Kuala Lumpur reported 33% of participants have depleted iron status with Malay had the lowest percentage (22.9%) followed by Chinese (25.2%) and Indians (53.9%) (Loh & Khor, 2010). Iron status measured were hemoglobin concentrations, hematocrit, MCV and SF level whilst diet recall was used to measure the participants' iron intake (Loh & Khor, 2010). These studies suggested that ethnicity may affect individual's iron status due to variations of dietary intake for each ethnicity.

A study carried out in Malawi among all ages of men and women to determine the prevalence and risk factors of anemia among rural and urban populations reported there was significant difference between educational levels and anemia (Adamu et al.,

2017). There were four categories of educational levels including no formal, standard, secondary and tertiary levels (Adamu et al., 2017). It was reported that women with higher educational levels and live in urban areas were more likely to become anemic (p= 0.001) (Adamu et al., 2017). It was suggested that the findings may be due to the diets of urban women which has shifted away from their traditional diets (Adamu et al., 2017). However, contradicted findings were reported between place of residence and educational level anemia in a study carried out among pregnant women age ranged 15 to 46 years old in Nothern Tanzania (Stephen et al., 2018). There were three educational levels classified into none, primary and secondary and higher educational level whilst anemia was determined by measuring hemoglobin concentrations (Stephen et al., 2018). It was reported that women in the secondary and tertiary educational levels had lower possibility to become anemic (p= 0.022) (Stephen et al., 2018). The author suggested that pregnant women with higher educational levels may have better income and hence, they can consume nutritious food and lowered their risk to be anemic (Stephen et al., 2018).

4.2 Physical characteristics and general dietary intake of study population

4.2.1 Anthropometric measurements

Table 4.2 shows the distributions of participants' by BMI. The mean ± SD BMI of the participants was 22.53 ± 4.03 kg/m^2 with majority of the participants had a normal BMI (67.9%), followed by overweight (19.3%) and underweight (10.1%). The remaining of participants (2.7%) were considered obese.

Table 4.2: Distributions of participants by BMI

	Mean ± SD	n (%)
Body Mass Index (kg/m^2)	22.53 ± 4.03	
Underweight		11 (10.1)
Normal		74 (67.9)
Overweight		21 (19.3)
Obesity Class I		1 (0.9)
Obesity Class II		1 (0.9)
Obesity Class III		1 (0.9)

Inconsistent findings with higher proportion of overweight and obese students were found in several local studies carried out among university students. A study carried out by Fatehah et al. (2019) reported that there were 23.0% of female students in Universiti Sultan Zainal Abidin (UniSZA) were overweight and 11.0% of the students were obese. Another local study carried out among students in five public universities found that there were 23.0% of bachelor students were overweight and 17.6% of the students were obese (Wan Mohamed Redzi et al., 2019). A study carried out by Koo et al. (2019) among Malaysian university students in private university in Klang Valley reported that there were 29.3% of female students were overweight whilst 19.2% were obese. Nevertheless, a cross sectional study carried out in 22 countries among university students found similar trend with this study where the prevalence of overweight and obese among female students were 19.3%. The findings of this study may be contradicted with other local study findings due to the BMI classification guideline used where the present study have used WHO BMI classification whilst several other studies used Asian BMI classification.

However, the findings of this study were found to be consistent with several studies carried out among medical or health sciences students. A study carried out by Oo et al. (2019) among renal care nursing students in Lincoln University College,

Selangor, reported that 18.3% of the students were overweight or obese. Another study carried out among medical students in a private university in Malaysia showed a similar trend where the prevalence of pre-obesity in female students were 13.8% and prevalence of obesity were 1.9% (Gopalakrishnan et al., 2012). A study carried out among medical students found that 1.7% of female students were obese (Boo et al., 2010). In India, a study carried out among medical and allied science students reported that 18.6% of female students were overweight and 5.0% were obese (Parajuli et al., 2019).

High consumption of fast foods may be one of the cause of high prevalence of overweight and obesity among university students. High intake of fast foods among university students are due to the price are affordable and fast foods are quickly prepared (Hakim et al., 2012). Universiti Putra Malaysia (UPM) located near central part of Malaysia and there are high availability of fast food restaurants. An intervention program on lifestyle and weight management as well as education program to encourage healthier food choices could give benefits to university students (Hakim et al., 2012).

4.2.2 Energy and macronutrient intake

Table 4.3 presents the participants' energy and nutrient intake which was analyzed by using two dietary assessment, 24-hour dietary recall (24HR) and 3-days diet history (DH). Median and interquartile range (IQR) was presented as the data was not normally distributed. The mean ± SD of energy intake of the participants was 1638 ± 689 kcal where it was lower than mean ± SD (1984 ± 76 kcal) of a study carried out by Koo et al. (2019) among university students.

Majority of participants in this study did not achieved the RNI for energy intake (71.6% recorded by 24HR and 76.1% recorded by DH). This findings were consistent with a study carried out among university students in selected universities in Selangor where 72.2% of female students did not meet RNI for energy (Hakim et al., 2012). A low energy intake in the diet may cause the absorption of other essential nutrients become poor as well (Hakim et al., 2012).

Other than that, majority of the participants (68.8% recorded by 24HR and 50.5% recorded by DH) did not meet the recommended range for carbohydrate (<50% of energy intake from carbohydrate). This may explain why the students did not meet RNI for energy as they have low intake of energy from carbohydrate. However, majority of the students were found to have higher intake of energy from fats which is >30% of energy from fats (67.9% recorded by 24HR and 73.4% recorded by DH). These results may be caused by the higher prevalence of overweight and obesity observed in this study. Hakim et al. (2012) mentioned that higher prevalence of overweight and obesity among university students were due to the availability of fast food restaurants which would lead to higher intake of fat and sugar-sweetened beverages but low intake of healthful foods.

Table 4.3: Distribution of participants' energy and nutrient intake

Nutrients	24 hour dietary recall		3 day diet history	
	Mean ± SD/ n (%)	Median (IQR)	Mean ± SD/ n (%)	Median (IQR)
Energy (kcal)	1638.32 ± 689.89	1492.21 (1890.64 – 1218.71)	1592.50 ± 702.33	1363.10 (1824.98 – 1153.53)
<RNI	78 (71.6)		83 (76.1)	
≥RNI	31 (28.4)		26 (23.9)	
Carbohydrate (g)	194.62 ± 82.82	185.56 (225.50 – 134.86)	195.33 ± 88.68	166.09 (230.82 – 134.83)
% of energy from carbohydrate				
< 50%	75 (68.8)		55 (50.5)	
50-65%	25 (22.9)		52 (47.7)	
>65%	9 (8.3)		2 (1.8)	
Protein (g)	70.18 ± 38.06	68.18 (83.19 – 46.68)	64.86 ± 32.74	55.70 (75.65 – 44.49)
<RNI	34 (31.2)		49 (45.0)	
≥RNI	75 (68.8)		60 (55.0)	
Total fat (g)	64.78 ± 37.27	55.98 (79.82 – 39.32)	64.99 ± 38.90	56.30 (74.58 – 41.52)
% of energy from fat				
< 25%	16 (14.7)		7 (6.4)	
25-30%	19 (17.4)		22 (20.2)	
>30%	74 (67.9)		80 (73.4)	

This study found that more than half of participants in this study met the RNI intake for protein (68.8% recorded by 24HR and 60.0% recorded by DH) and it was consistent with findings of a local study carried out among university students where the students were reported to have an adequate intake of protein according to RNI (Koo et al., 2019).

4.3 Hemoglobin concentrations

Table 4.4 shows the distributions of participants by hemoglobin concentration. The mean ± SD of hemoglobin concentrations was 12.08 ± 1.30 g/dl. Majority of the participants were not anemic (61.5%). In terms of severity, participants with mild anemia was 22.0%, moderate anemia was 15.6% and severe anemia was 0.9%.

Table 4.4: Distribution of participants by hemoglobin concentrations (n= 109)

	Mean ± SD	**n (%)**
Distributions of hemoglobin concentrations (g/dl)	12.08 ± 1.30	
Non-anemia		67 (61.5)
Anemia (mild)		24 (22.0)
Anemia (moderate)		17 (15.6)
Anemia (severe)		1 (0.9)

In the present study, the prevalence of anemia observed was 38.5% which was approximately aligned with the result reported by WHO that 30.1% of Malaysian adult were anemic (World Health Organization, 2008). A similar trend was also reported by WHO that stated globally, 30.2% of non-pregnant women aged 15 to 49 years old were anemic (Mclean et al., 2008). National Health and Morbidity Survey (NHMS) (2015) also reported almost similar prevalence of anemia among female which was 35.5%. A local study carried out among Malaysian adults reported that prevalence of

anemia among non-pregnant women aged 15 to 49 years was 34.8% (Awaluddin et al., 2017). The result was also consistent with a study carried out in Saudi Arabia among female undergraduate university students which reported an IDA prevalence of 35.3% (Jamea et al., 2019).

Anemia affects all population groups and appears to maintain contributing to the global burden of disease if no intervention or action is taken quickly (Awaluddin et al., 2017). WHO reported that the prevalence of anemia in Malaysia were considered as moderate public health problem (World Health Organization, 2008). National Plan of Action for Nutrition of Malaysia (NPANM) III, 2016-2025 has included intervention plan to reduce the prevalence of anemia in women reproductive age by 50% in 2025.

4.4 Calcium intake of study population

The following (**Table 4.5**) summarizes the dietary calcium intake of participants. The results are presented as median and IQR due to data was not normally distributed. Majority of the participants did not meet the recommended RNI for calcium (91.7% for 24HR and 94.5% for DR).

Table 4.5: Distribution of participants by calcium intake (n=109)

	Mean n (%)	**SD**	**Median**	**IQR**	**Mean n (%)**	**SD**	**Median**	**IQR**
Calcium (mg)	479.29	430.64	430.64	647.22-251.92	460.74	282.69	347.39	660.42-238.18
<RNI	100 (91.7)				103 (94.5)			
≥RNI	9 (8.3)				6 (5.5)			

In this study, the mean ± SD intake of calcium of participants were 479.29 ± 430.64 mg (24HR) and 460.74 ± 282.69 mg (DH) where consistent finding was found in a study carried out among university students by Hakim et al. (2012) with mean ± SD calcium intake of 420.65 ± 200.56 mg. A review study on global dietary calcium intake among adults found that low dietary calcium intake (<400 mg/day) was reported in South, East and Southeast Asia countries (Balk et al., 2017). A study carried out among female adults in Malaysia also found similar trend where more than half of participants did not meet RNI of calcium (Chai et al., 2019). A study among private university students in Klang also found that the student had an inadequate intake of calcium (Koo et al., 2019). Low dietary calcium intake of this present study population may be due to low consumption of calcium-rich foods including milk among university students which can be observed by their dietary intake. It was reported that majority of students acknowledged that milk is one of main source of calcium however, only 10% of students knew other sources of calcium (Sham et al., 2013). Sham et al. (2013) also mentioned that most university students reported that they have lack of knowledge regarding sources and recommended amount of calcium for an adult.

4.5 Other dietary components that may affect hemoglobin concentrations

Table 4.6 shows the distribution of participants by iron, vitamin C and vitamin D intake which was analyzed by using two dietary assessment, 24HR and DH. Nearly all participants did not meet the RNI for iron (89.0% for 24HR and 94.5% for DH). More than half of participants did not meet RNI for vitamin C (66.1% for 24HR and 65.1% for DH). All of participants did not meet RNI for vitamin D. **Table 4.7** shows

the distributions of participants' iron, vitamin C and vitamin D intake by weekday and weekend.

Table 4.6: Distribution of participants by iron, vitamin C and vitamin D intake (n=109)

Nutrients	24 hour dietary recall		3 day diet history	
	Mean ± SD/ n (%)	Median (IQR)	Mean ± SD/ n (%)	Median (IQR)
Iron (mg)	14.56 ± 10.17	11.84 (17.27 – 8.63)	14.47 ± 7.85	12.93 (17.81 – 8.95)
<RNI	97 (89.0)		103 (94.5)	
≥RNI	12 (11.0)		6 (5.5)	
Vitamin C (mg)	111.78 ± 248.45	36.26 (104.04 – 9.74)	109.63 ± 164.03	42.37 (113.78 – 19.82)
<RNI	72 (66.1)		71 (65.1)	
≥RNI	37 (33.9)		38 (34.9)	
Vitamin D (ug)	1.09 ± 1.91	0.31 (1.29 – 0)	0.71 ± 1.10	0.35 (0.95 – 0)
<RNI	(100.0)		(100.0)	

Table 4.7: Distribution of participants' iron, vitamin C and vitamin D intake by category of days (n=109)

Nutrients	Weekday (Day 1 and Day 2)		Weekend (Day 3)	
	Mean ± SD	n (%)	Mean ± SD	n (%)
Iron (mg)	14.11 ± 8.35		15.52 ± 11.72	
<RNI		103 (94.5)		95 (87.2)
≥RNI		6 (5.5)		14 (12.8)
Vitamin C (mg)	109.19 ± 171.63		109.63 ± 164.03	
<RNI		69 (63.3)		71 (65.1)
≥RNI		40 (36.7)		38 (34.9)
Vitamin D (ug)	0.70 ± 1.29		0.72 ± 1.11	
<RNI		100 (100.0)		109 (100.0)

4.5.1 Iron intake

Table 4.8 shows the distributions of participants' iron intake by food groups in IRONIC-FFQ. IRONIC-FFQ was used to analyze common food groups consumed where it also measured the total daily dietary iron of participants. The results are presented according to 12 different food groups.

Table 4.8: Distribution of participants' iron intake by food groups in IRONIC-FFQ

Food groups	Mean ± SD
Meat	1.04 ± 0.72
Meat products	0.24 ± 0.05
Eggs	0.40 ± 0.43
Fish	0.12 ± 0.12
Dairy products	0.12 ± 0.14
Cereal products	1.37 ± 2.19
Fruits	0.26 ± 0.30
Vegetables	0.91 ± 0.87
Potatoes	0.07 ± 0.12
Fats	0.18 ± 0.13
Nuts and seeds	0.12 ± 0.31
Cocoa products	0.26 ± 0.33
Average intake of iron	4.53 ± 2.00

Majority of participants in this study did not meet the RNI for iron intake reported by 24HR (89.0%) and DH (94.5%). It was observed that the mean ± SD iron intake of participants during weekend (15.52 ± 11.72 mg) was higher compared to weekdays (14.11 ± 8.35 mg). These findings was found to have a similar trend with a study carried out among university students in Selangor which reported that 90.5% of female students did not meet RNI for iron compared to male (Hakim et al., 2012). The mean ± SD of daily iron intake of participants was 4.53 ± 2.00 mg. The highest food groups was cereal products (1.37 ± 2.19 mg) and the lowest food group was potatoes (0.07 ± 0.12 mg). The participants' mean ± SD of iron intake analyzed from IRONIC-

FFQ (4.53 ± 2.00 mg) was very low compared to mean ± SD iron intake measured by 24HR (14.56 ± 10.17 mg) and DH (14.47 ± 7.85 mg). FFQ estimation are generally prone to overestimating consumption of foods but surprisingly, underreporting was reported in this study when using IRONIC-FFQ. Participants may have under estimated the serving sizes of foods. It was postulated that underreporting occurred in IRONIC-FFQ because both dietary assessment (24HR and DH) had high prevalence of under reporters. Participants may be difficult to estimate the serving size of foods especially from those who were not in the nutrition course background. Researchers also observed that during the interview session, most of the participants did not remember to include and report foods during their dinner time. Additionally, this method of dietary assessment is highly depending on memory and honesty of the participants. The participants might have difficulty in remembering the consumption frequency or the amount of food consumed in a week (Yang et al., 2010). It was also found that DH had a relatively higher validity compared to FFQ (Yang et al., 2010). Other than that, the IRONIC-FFQ did not contain foods that are usually being consumed by Malaysian population including cooked rice. Hence, participants could not report other iron-containing foods that were usually consumed.

4.5.2 Vitamin C

Vitamin C is one of the micronutrients that may affect the hemoglobin concentrations of the study population. It was reported that the most efficient enhancer of iron absorption is ascorbic acid (Teucher et al., 2004). In this study, only 33.9% (24HR) and 34.9% (DH) participants met RNI for Vitamin C. The mean ± SD of Vitamin C intake was approximately similar for both weekday (109.19 ± 171.63 mg)

and weekend (109.63 ± 164.03 mg). A similar trend was also found in a study carried out among university students in Klang where the participants had an inadequate intake of micronutrients including Vitamin C (Koo et al., 2019). Insufficient intake of Vitamin C may be because of low consumption of fruits and vegetables, mainly university students (Koo et al., 2019). Further analysis has been carried out and result are shown in **Appendix A**.

4.5.3 Vitamin D

The study population in this study did not meet the RNI for vitamin D. The mean ± SD of Vitamin D intake was approximately similar for both weekday (0.70 ± 1.29 μg) and weekend (0.72 ± 1.11 μg). A similar trend was found in a study carried out among Malaysian adults where the prevalence of Vitamin D deficiency was 67.4 % (Shafinaz & Moy., 2016). This findings may be due to the low exposure of sunlight for the participants. University students were less exposed to sunlight as all the classes were conducted in a closed room. Other than that, the clothing styles of Malaysian population including wearing veils, long sleeves, using umbrellas and sunblock lotion might also explained this findings (Shafinaz & Moy, 2016). Deficiency of vitamin D may cause decreasing iron status and hence, increase the risk of anemia (Lenczowska et al., 2018).

4.5.4 Analysis of misreporting

Table 4.9 shows the distributions of misreporting among 109 participants in this study. 62.4% (24HR) and 74.3% (DH) of the participants were under reporters,

12.8% (24HR) and 8.3% (DH) were normal or plausible reporters and 24.8% (24HR) and 17.4% (DH) were over reporters.

Table 4.9: Distribution of misreporting of participants

Characteristics	24 hour dietary recall n (%)	Diet History n (%)
Under-reporters	68 (62.4)	81 (74.3)
Normal-reporters	14 (12.8)	9 (8.3)
Over-reporters	27 (24.8)	19 (17.4)

In the present study, it was reported that there were more under reporters in DH. This may be due to the memory of the participants where the participants need to recall their food intake for the past 3 days compared to 24HR which was only recalling for food intake in the past 24 hours. According to Zainuddin et al. (2019), there were 18 to 54%and could be up to 70%prevalence of under reporting in large nutritional surveys in particular groups. Under reporting was high in this study and under reporters was consistent with the increasing of under reporting in EI in Malaysian Adult Nutrition Survey (MANS) from 53% in 2003 to 61% in 2014 (Zainuddin et al, 2019). Under reporters were also found to be higher in women compared to men (Zainuddin et al, 2019). Misreporting may occur due to this dietary assessment method highly depend on the memory of the participants.

4.6 Association between calcium and hemoglobin concentration

The association between calcium and hemoglobin concentrations is presented in **Table 4.10**. No significant association was found between dietary calcium intake recorded and hemoglobin concentration (r= 0.127, p= 0.1888 for 24HR and r=-0.022, p= 0.821 for DH).

Table 4.10: Results of spearman association between calcium and hemoglobin concentrations (n= 109)

Iron	Hemoglobin concentrations	
	r	p-value
24 hour dietary recall	0.127	0.188
3 day diet history	-0.022	0.821

A study carried out among girls age 12-14 years also reported no significant difference between calcium supplementation after one year and iron status (hemoglobin: P= 0.31, SF: P= 0.73, Tfr: p= 0.12) (Mølgard, Kæstel and Michaelsen, 2005). The participants consumed 500 mg of calcium per day for one year whilst blood were obtained to assess various iron status including hemoglobin concentrations, SF concentrations and Trf.

A study carried out among adolescent girls aged 11 to 15 years and women aged 20 to 23 years in six European countries found that dietary calcium intake was weakly, inversely associated with SF level (r= -0.09 and r= -0.07 for adolescent girls and women, respectively, p< 0.05) (Vijver et al., 1999). Serum iron concentration, transferrin level & SF concentration were obtained from 10 ml blood of non- fasting participants for determination of iron status whilst 3 day food record were used to obtained participants' calcium intake (Vijver et al., 1999). A cohort study carried out among adults aged 35 to 60 years in France found that SF reported to be negatively correlated with calcium (Preziosi et al., 1994) (r= -0.10, p< 0.001). Venous blood were obtained to determine hemoglobin concentrations, serum iron, serum transferrin, TIBC and SF concentrations whilst dietary intake were obtained using diet history (Preziosi et al., 1994). A study carried out in Canada among adults age 20 to 35 years also found significant inverse correlation between calcium and iron absorption (r= -0.58, p= 0.037) (Benkhedda, Abbe & Cockell., 2010). However, the study only

focused on low iron status women where SF concentrations were used as iron biomarkers which was obtained by 15 ml of venous blood (Benkhedda, Abbe & Cockell., 2010). The participants consumed 500 mg of calcium carbonate tablet with meal and iron status was assesses after 14 days (Benkhedda, Abbe & Cockell., 2010). These three studies managed to find negative association between calcium and iron status which may be due to iron biomarkers used which was SF concentrations.

4.7 Association between other nutrients and hemoglobin concentration

There are some other nutrients that may affect hemoglobin concentration either by acting as enhancers or inhibitors. In this study, dietary iron, vitamin C and vitamin D were hypothesized to may have affected hemoglobin concentrations and analyzed **Table 4.11** shows the association between iron, vitamin C and vitamin D and hemoglobin concentration. No significant association was found between all nutrients (iron, vitamin C and vitamin D) and hemoglobin concentrations.

Table 4.11: Results of spearman association between iron, vitamin C and vitamin D and hemoglobin concentration

Nutrients	Hemoglobin concentrations	
	r	p-value
Iron		
24 hour dietary recall	0.076	0.431
3 day diet history	1.000	0.097
Vitamin C		
24 hour dietary recall	0.145	0.132
3 day diet history	-0.016	0.867
Vitamin D		
24 hour dietary recall	-0.041	0.669
3 day diet history	0.022	0.819

Previous studies suggested that iron intake was associated with hemoglobin level and other biomarkers of iron. A cross sectional study carried out among adults

aged ≥ 20 years in China found that iron intake was significantly associated with hemoglobin level (r= 0.259, p= 0.001) (Shi et al., 2006). A similar trend was found in a study carried out among non- pregnant women aged 18 to 35 years in Australia where heme iron (*b* = 0.128, p = 0.009) and non-heme iron (*b* = 0.037, p = 0.028) were positively associated with SF (Young et al., 2018). Lopez et al. (2016) mentioned that measurement of SF concentration is the most precise test to determine the total body iron stores.

A study carried out in Pakistan among female university students aged 19 to 25 years reported similar findings where there was no association found between vitamin C and hemoglobin concentrations (p= 0.394) (Safwan & Asar., 2017). However, it was reported that participants with normal hemoglobin level has higher mean consumption of vitamin C rich foods compared to those below normal hemoglobin level (Safwan & Asar., 2017). Inconsistent findings were found in other studies in determining association between vitamin C and other iron biomarkers. A stable isotope study carried out among women aged 18 to 35 years in India reported that iron absorption was significantly increase when ascorbic acid added to a meal at a molar ratio 2:1 in both IDA group and control group (p< 0.001) (Thankanchan et al., 2007). A similar trend was found in a cohort study carried out among adults aged 35 to 60 years to determine the association between dietary fruits, vegetables and juices (FVJ) according to their vitamin C and fiber contents and hemoglobin concentration and SF (Péneau et al., 2008). It was reported that hemoglobin were positively associated with fiber-poor fruits, fiber-poor vitamin C-rich vegetables and juices (FVJ) and fiber-poor FVJ and (p< 0.05) (Péneau et al., 2008). Premenopausal women were found to have an increment from 1 to 2 g/L of hemoglobin concentration in third tertile compared to first tertile (Péneau et al., 2008). Further analysis using categorical variables defining

non anemic and anemic groups with vitamin C shown significant results between groups. Results are shown in Appendix 1.

Liu et al. (2015) mentioned that Vitamin D deficiency was significantly associated with increased the risk of developing anemia ($p< 0.001$). A review study carried out by Smith & Trangpicha (2015) reported that vitamin D status has been positively associated with hemoglobin concentrations and may reduce risk of anemia. Another review study carried out among those aged 17.5 to 68 years old found that there was no significant difference between vitamin D supplementation and hemoglobin levels ($p= 0.76$) (Arabi et al., 2020). However, vitamin D was found to be significant associated with serum iron ($p= 0.01$) (Arabi et al., 2020).

4.8 Association between dietary calcium and dietary iron

A further analysis was carried out to determine the association between dietary calcium intake and dietary iron intake. **Table 4.12** shows the results of spearman association between dietary calcium intake and dietary iron. There were significant strong positive association between calcium and iron measured by both 24 HR ($r= 0.625$, $p= 0.001$) and DH ($r= 0.594$, $p= 0.001$).

Table 4.12: Results of spearman association between dietary calcium and dietary iron

Calcium	Iron	
	r	**p-value**
24 hour dietary recall	0.625	0.001*
3 day diet history	0.594	0.001*

*significant value at $p< 0.001$

Similar trend of findings was found in a study carried out in Sweden among adults age 24 to 65 years where 165 mg of calcium chloride was found to be significantly associated with the absorption of heme iron (Hallberg et al., 1992).

Significant association was found in this study may be due to double isotopic method used to measure the iron status of study population.

However, a study carried out among 33-47 years women where 34 days of 600 mg calcium supplementation (CaCO3) did not affect heme and non-heme iron bioavailability measured by hemoglobin concentrations (p= 0.57) (Castillo et al., 2013). Inconsistent findings were found in a cohort study carried out among women aged 35 to 69 years where calcium supplements were not associated with SF concentrations (p= 0.007) (Cade et al., 2005). A study carried out among women aged 18 to 39 years in Belgium reported that calcium has no significant association with iron biomarkers measured (SF concentrations and sTfr level) (Pynaert et al., 2008). Another study carried out in Japan among dietetic students aged 18 to 25 years also reported no association between dairy products and iron status (Asakura et al., 2008).

A study carried out among 15 to 30 years female mentioned that high intake of calcium was associated with an increase risk to become iron deficient, after controlling other factors (Rangan et al., 1997). A larger population based study is needed to determine the negative association between dietary calcium and iron intake. There were possibly insufficient power to show the negative association between dietary calcium intake and iron intake due to the small sample sizes and majority of the participants were in normal hemoglobin concentrations compared to low hemoglobin concentrations group (Beck et al., 2014). It was suggested that the effects of dietary intake on iron status would have a better results by intervention studies rather than cross-sectional studies (Beck et al., 2014). The intervention studies with duration of 12 to 16 weeks should include women with low iron stores but are not anemic with to recognize time needed for an effect to be seen (Beck et al., 2014). It was also

suggested that single-meal method intervention would emphasise more on the effects of enhancers or inhibitors on the absorption of iron (Lonnerdal, 2010).

However, significant association that was found in this present study indicated that calcium may effect up to iron absorption and not have inhibitory effect to hemoglobin level. Lonnerdal (2010) suggested that the reason why there is no effect on iron status although iron absorption are affected by high calcium intake may be due to mechanisms regulating the absorption of iron will adapt with high calcium intake and try to achieve iron hemostasis. It has been suggested that calcium may become a weak inhibitor of iron absorption if studied in the context of dietary diet (Cade et al., 2005; Hallberg, 2002; Lynch, 2002). Other than that, it has been suggested that in some cases, the displacement of other foods from the diet (e.g., meat), may cause negative associations between calcium or dairy product intake and iron status, rather than inhibitory effect of calcium itself (Beck et al., 2014; Heath et al., 2001)

CHAPTER 5

CONCLUSION, LIMITATIONS AND FUTURE RECOMMENDATIONS

5.1 Conclusion

In this study, the prevalence of anemia observed was 38.5%. The mean ± SD of hemoglobin concentrations among this study population was 12.08 ± 1.30 g/dl with majority of the female students were not anemic (61.5%). The calcium intake of study population in this present study was below the RNI for calcium intake (91.7% recorded by 24HR and 94.5% recorded DR) with mean ± SD of calcium 479.29 ± 430.64 mg (24HR) and 460.74 ± 282.69 mg (DH). There was no association observed between dietary calcium intake and hemoglobin concentrations of female students (r= 0.127, p= 0.1888 for 24HR and r= -0.022, p= 0.821 for DH). Other dietary components including iron, vitamin D and vitamin C intake in this study were also not found to affect hemoglobin concentration. Majority of the female students did not meet RNI for iron (89.0% for 24HR and 94.5% for DH) and vitamin C intake (66.1% for 24HR and 65.1% for DH). However, significant association was found between dietary calcium and dietary iron recorded by 24HR (r= 0.625, p= 0.001) and DH (r= 0.594, p= 0.001) which indicates calcium may have affected iron absorption.

The prevalence of anemia in the present study was quite alarming especially because it was from younger population which is the university students. Students need proper diet intake in order to focus on their study as anemia may cause negative effects on cognitive function, audiovisual reaction time and physical performance of an individual. A proper health education to increase knowledge about anemia among university student could be carried out to reduce these health complications related to anemia. Policy makers should identify strategies to lower the burden of anemia especially among university students population.

In addition to that, calcium is one of the important micronutrients needed especially for women. Despite the non-significant association observed between calcium and Hb concentration in this present study, calcium intake was demonstrated to be associated with iron intake, which may indicate possible connection with iron absorption but not to the extent where it affects hemoglobin concentrations. Low intake of calcium may cause negative effects in later life. Hence, health educators should emphasizes more on intake and importance of consuming calcium to general population and not just university students. University students should also be educated on their dietary intake as it is important to their health. University students need proper dietary intake as they are the posterity of the country in the future.

5.2 Limitation

In this study, there are some limitations that should be taken into consideration. Firstly, this study is a cross sectional study where the determination of causal effects could not be identify due to this study design. Secondly, the type of anemia were not being distinguish whether anemia faced by participants were due to IDA or other type of anemia. Thirdly, other than dietary intake, other factors that may affect the

prevalence of anemia among females such as menstrual history and thalassemia were not being taken account. In addition, two dietary assessment that were used solely depend on honesty and truthfulness of participants. Other than that, although IRONIC-FFQ has been validated to measure average iron intake, it does not include iron-rich foods that were usually being consumed by Malaysian population. Hence, there would be under reporting of iron-rich foods consumed by participants. Another limitation is sample population for this study is from one faculty and one university which may not be representative of other university. Therefore, the findings could not be generalized to other populations.

5.3 Future recommendation

Future research should focus more on the association between calcium and iron intake as calcium may directly affect iron status rather than anemia. SF may be used in future research as a measurement of iron status as it may have better result compared to hemoglobin concentrations. Future research should also take account other factors that may affect anemia such as the menstrual or hereditary diseases. Other than that, dietary habit including breakfast consumption or snacks consumption should also be assessed to explore more on the diet intake of the participants. Moreover, diet diary may be used for determination of dietary calcium intake of a population rather than using 24HR and DH. Under reporting or over reporting issue may be reduced by using diet diary as the participants would report their diet intake on the same day and it does not highly depends on the memory of participants to recall their dietary intake.

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APPENDICES

Appendix A: Results of chi square test of independence association between Vitamin C by two dietary assessment and anemia (n= 109)

Vitamin C (mg)	Anemic (n= 42) n (%)	Non anemic (n= 67) n (%)	χ^2	p-value
24 hour dietary recall			9.097	0.003
<RNI	35 (83.3)	37 (55.2)		
≥RNI	7 (16.7)	30 (44.8)		
3 day diet history			0.022	1.000
<RNI	27 (64.3)	44 (65.7)		
≥RNI	15 (35.7)	23 (34.3)		

ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS
(JKEUPM)
UNIVERSITI PUTRA MALAYSIA

Research title	:	**The Association Between Dietary Vitamin D, Calcium and Polyphenol Intake with Haemoglobin Concentration in Female Staffs and Students in Faculty of Medicines and Health Sciences, Universiti Putra Malaysia.**
Study Site	:	**Faculty of Medicine and Health Sciences, Universiti Putra Malaysia**
JKEUPM Ref No.	:	**JKEUPM-2019-456**
Researcher	:	**Nursyafiqah Aqilah Suhaimi, Fazirah Samah, Noor Aiman Afaf Afiffudden.**
Supervisor	:	**Dr. Salmah Faeza Ahmad Fuzi**

Documents received and reviewed with reference to the above study:

1. Ethics Application Form, Version 1 dated 7/11/2019
2. Respondent Information Sheet & Consent (English), Version 2 dated 20/12/2019
3. Respondent Information Sheet & Consent (Malay), Version 2 dated 20/12/2019
4. Proposal (English), Version 2 dated 6/12/2019
5. Questionnaires/ Interviews (English), Version 1 dated 7/11/2019
6. Curriculum Vitae of:
    a. Dr. Salmah Faeza Ahmad Fuzi

The University Research Ethics Committee, Universiti Putra Malaysia (JKEUPM) operates in accordance to the ICH-GCP Guidelines.

Decision by JKEUPM:

[X] Approved

[X] **Permission MUST BE OBTAINED from the respective hospitals/ institutions before conducting the research**

[ ] Disapproved

Please note that the approval is **VALID UNTIL 20 DECEMBER 2020**

Researchers should comply with the following:

I. Complete a Study Final Report upon study completion (Form 3.2).

II. Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

Appendix C: Information sheet and consent form (English)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

You are being invited to take part in a research study. You need to read Respondent's Information Sheet to understand why the research is being carried out and what it will involve. If you agree to participate in this study, you are required to sign the Respondent's Consent Form in Page 3. Upon completing the respondent's consent form, please return it to the researcher. It will take approximately 30 minutes to complete this study. Your participation in this study is voluntary. You have the right to withdraw from this study anytime without giving any reason and no penalty will be applied upon your withdrawal. Take time to decide whether or not you wish to take part. Do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE :.

The association between dietary vitamin D, calcium and polyphenol intake with hemoglobin concentration in female staffs and students in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

2. INTRODUCTION:

Anemia is a condition where there are lack of red blood cells (RBCs), which serves to carry oxygen in our body, to meet the basic needs of human. The lack of RBCs may be determined by measuring one of the important iron status indicator, which is hemoglobin. Iron deficiency anemia (IDA) was found to be the most significant contributor in anemia. IDA is a condition where the balance between iron consumption from food, stores and loss from the body are not enough to support production of the RBCs. Findings from previous studies have suggested that dietary vitamin D, calcium and polyphenol intake may have effect on how iron is absorbed and used in the body. Therefore, this study aims to determine the association between dietary vitamin D, calcium and polyphenol intake with hemoglobin concentration in female staffs and students in Faculty of Medicine and Health Sciences, UPM.

3. WHAT WILL YOU HAVE TO DO?

You will first be screened to assess your eligibility to take part in the study. Inclusion criteria includes female staffs and students, age between 19 to 49 years old with no history of gastrointestinal disorders and chronic diseases such as diabetes mellitus and diseases of metabolism, do not regularly consume nutritional supplements and not donating blood for the past 6 months. You will then be required to complete a set of questionnaires to obtain information on socio-demographic characteristics. Your body weight and height will be measured by the researcher in a closed room. Your habitual dietary intake will be assessed by recording your foods and beverages consumption from the previous day (24-hour dietary recall) and 3- day diet history (two weekdays and one weekend). A small amount of blood from the finger prick will be collected using a small needle called lancet to test the hemoglobin level.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

Students and staffs who are male, non-Malaysian, pregnant, lactating and menopause women, individuals who use contraceptives, individuals who have celiac disease, gastroesophageal reflux disease, diabetes mellitus and other diseases related to metabolism, regularly consume nutritional supplements, and have donated blood for the past 6 months will be excluded from this study. You have been chosen because you satisfy the inclusion criteria of the study.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

You will be provided with a summary of dietary analysis, body weight status (body weight, height, body mass index) and hemoglobin concentration data. Dietary analysis will be performed by the researcher on completion of the study using dietary analysis software. The assessment will be based on the information you will have already provided us during 24-hour dietary recall and diet history. We will provide you information on your nutrient intake of vitamin D, calcium and polyphenol as well as information on your total energy, fat, carbohydrate and protein intake with a comparison to recommended values. You will also be contributing to the development of knowledge on the association between dietary vitamin D, calcium and polyphenol intake with hemoglobin concentration.

(b) TO THE INVESTIGATOR?

Findings of this study will provide information on socio demographic characteristic, body weight status, dietary vitamin D, calcium, polyphenol and iron intake as well as hemoglobin concentration in female staffs and students in Faculty of Medicine and Health Sciences, UPM. Findings of this study also will help nutritionists and health promotion planners to develop appropriate intervention and health promotion programs in improving nutritional and health status as well as anemia in female staffs and students.

6. WHAT ARE THE POSSIBLE RISKS?

This study has minimal risk where it involves finger prick method to obtain small amount of blood, anthropometry measurements (body weight, and height), 24-hour dietary recall and diet history as well as filling up a questionnaire. If you were found to have low hemoglobin level after the measurement, you will be advised to seek further medical attention from your general practitioner.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All information which is collected about you during the course of the rearch will be kept strictly confidential and anonymised so that only the researcher carrying out the research wil have access to such information. The researcher will also not provide any participant information to any party without the consent of subjects.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

If you have any enquiries, you can contact as follows;

Researcher:
Nursyafiqah Aqilah binti Suhaimi
014-5568978 / sfiqah@gmail.com
Fazirah binti Samah
011-23096752 / fazirahakak@gmail.com
Noor Aiman Afaf Binti Afiffudden
016-9474678 / aimanafaf29@gmail.com

Supervisors
Dr. Salma Faeza binti Ahmad Fuzi
0386092974 / salmafaeza@upm.edu.my
Dr. Nurul Huda binti Mohd Nor
0386092976 / hudamohdnor@upm.edu.my

Thank you for your interest in this research.

*Please initial here if you have read and understood the contents of this page*_____

9. CONSENT

I ……. Identity Card No. ……
address……..
……..hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature …… Signature ……
(Respondent) (Witness)

Date :…… Name :……

I/C No. :……

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date …… Signature ……
(Researcher)

Appendix C (Cont): Information sheet and consent form (Malay)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Anda dijemput untuk mengambil bahagian dalam kajian penyelidikan. Anda perlu membaca Borang 2.4: Penerangan dan Persetujuan Responden untuk memahami tujuan dan proses penyelidikan. Jika anda bersetuju untuk mengambil bahagian dalam kajian ini, anda dikehendaki menandatangani borang persetujuan responden di Halaman 4. Setelah mengisi borang kebenaran responden, sila kemukakan kepada penyelidik. Anda akan mengambil masa kira-kira 30 minit untuk menyelesaikan kajian ini. Penyertaan anda dalam kajian ini adalah secara sukarela. Anda mempunyai hak untuk menarik diri dari kajian ini pada bila-bila masa tanpa sebarang alasan dan tidak akan dikenakan penalti semasa pengeluaran anda. Luangkan masa anda untuk membuat keputusan sama ada anda mahu mengambil bahagian atau tidak. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN

Perkaitan antara pengambilan vitamin D, kalsium, dan polifenol dalam diet dengan kepekatan hemoglobin di kalangan staf dan pelajar wanita di Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia.

2. PENGENALAN

Anemia adalah satu keadaan di mana terdapat kekurangan sel darah merah, yang berfungsi untuk membawa oksigen ke dalam badan kita bagi memenuhi keperluan asas manusia. Kekurangan sel darah merah boleh ditentukan dengan mengukur salah satu penunjuk penting bagi status zat besi iaitu hemoglobin. Anemia kekurangan zat besi didapati sebagai penyumbang paling utama dalam anemia. Anemia kekurangan zat besi adalah keadaan di mana keseimbangan antara pengambilan zat besi dalam makanan, simpanan dan kehilangan daripada tubuh tidak mencukupi untuk menyokong pengeluaran sel darah merah. Penemuan dari kajian terdahulu telah mencadangkan bahawa pengambilan vitamin D, kalsium dan polifenol dalam diet boleh mempengaruhi bagaimana zat besi diserap dan digunakan di dalam badan. Oleh itu, kajian ini bertujuan untuk menentukan perkaitan antara pengambilan vitamin D, kalsium dan polifenol dalam diet dengan kepekatan hemoglobin pada staf dan pelajar wanita di Fakulti Perubatan dan Sains Kesihatan, UPM.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Anda akan menjalani pemeriksaan untuk menilai kelayakan anda untuk mengambil bahagian dalam kajian ini. Kriteria inklusi termasuk staf dan pelajar wanita, berumur antara 19 hingga 49 tahun tanpa sejarah gangguan gastrousus dan penyakit kronik seperti kencing manis dan penyakit metabolisma, tidak mengambil suplemen nutrisi dengan kerap dan tidak menderma darah selama 6 bulan yang lalu. Anda kemudian akan dikehendaki untuk melengkapkan satu set soal selidik untuk mendapatkan maklumat mengenai ciri sosio-demografi. Berat dan ketinggian badan anda akan diukur oleh penyelidik di dalam bilik tertutup. Pengambilan makanan lazim anda akan dinilai dengan merekodkan makanan dan minuman anda dari hari sebelumnya (24 jam peringatan makanan yang lepas) dan 3 hari sejarah pengambilan makanan (dua hari bekerja dan satu hujung minggu). Sebilangan kecil darah dari jari telunjuk akan dikumpulkan menggunakan jarum kecil dikenali sebagai lancet untuk menguji tahap hemoglobin.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Pelajar dan staf lelaki, bukan warganegara, hamil, menyusu dan menopaus, individu yang menggunakan kontraseptif, individu yang mempunyai penyakit seliak, penyakit refluks gastroesophageal, kencing manis dan penyakit lain yang berkaitan dengan metabolisma, kerap mengambil suplemen nutrisi dan telah menyumbang darah selama 6 bulan yang lalu akan dikecualikan daripada kajian ini. Anda telah dipilih kerana anda memenuhi kriteria inklusi kajian ini.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Anda akan diberikan analisis diet, status berat badan (berat badan, ketinggian, indeks jisim badan) dan data kepekatan hemoglobin. Analisis diet akan dilakukan oleh penyelidik setelah kajian ini selesai menggunakan perisian analisis diet. Analisis ini akan berdasarkan kepada maklumat yang anda telah berikan semasa peringatan makanan 24 jam dan sejarah diet selama 3 hari. Kami akan memberi anda maklumat tentang pengambilan nutrien vitamin D, kalsium dan polifenol serta maklumat mengenai jumlah tenaga, lemak, karbohidrat dan pengambilan protein anda dengan perbandingan dengan nilai yang disyorkan. Anda juga akan menyumbang kepada pembangunan pengetahuan mengenai hubungan antara vitamin D, kalsium dan polifenol dalam diet dengan kepekatan hemoglobin.

b) KEPADA PENYELIDIK?

Penemuan kajian ini akan memberi maklumat tentang ciri sosio-demografi, status berat badan, pengambilan vitamin D, kalsium dan polifenol dalam diet serta kepekatan hemoglobin pada staf dan pelajar wanita di Fakulti Perubatan dan Sains Kesihatan, UPM. Penemuan kajian ini juga akan membantu para pakar pemakanan dan perancang promosi kesihatan untuk merancang intervensi yang sesuai dan menjalankan program promosi kesihatan dalam meningkatkan status pemakanan dan kesihatan serta anemia dalam kalangan staf dan pelajar wanita.

6. ADAKAH IA BERISIKO?

Kajian ini mempunyai risiko yang minimum di mana ia hanya melibatkan kaedah cucuk jari untuk mendapatkan sedikit darah, pengukuran antropometri (berat badan, dan ketinggian), peringatan makanan 24 jam dan sejarah diet 3 hari serta mengisi borang soal selidik. Jika anda didapati mempunyai tahap hemoglobin yang rendah selepas pengukuran, anda akan dinasihatkan untuk mendapatkan perhatian perubatan lanjut dari pengamal perubatan umum anda.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Semua maklumat yang dikumpulkan mengenai anda semasa kursus ini akan disimpan secara rahsia dan tidak dikenali supaya hanya penyelidik yang menjalankan penyelidikan untuk mengakses maklumat tersebut. Penyelidik juga tidak akan memberikan sebarang maklumat peserta kepada mana-mana pihak tanpa persetujuan peserta

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Jika anda mempunyai sebarang pertanyaan, anda boleh menghubungi seperti berikut;

Penyelidik:

Nursyafiqah Aqilah binti Suhaimi
014-5568978 / sfiqah@gmail.com
Fazirah binti Samah
01123096752 / fazirahakak@gmail.com
Noor Aiman Afaf Binti Afiffudden
0169474678 /aimanafaf29@gmail.com

Penyelia:

Dr. Salma Faeza binti Ahmad Fuzi
0386092974/ salmafaeza@upm.edu.my

Terima kasih atas kerjasama anda.

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _____

9. PERSETUJUAN

Saya……. No Kad Pengenalan. ……
beralamat…….
……..dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan. Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan ……. Tandatangan …….
(*Responden*) (*Saksi*)

Tarikh :…….. Nama :……
No. K/P: ……

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh …… Tandatangan ……
(*Penyelidik*)

Appendix D: Set of questionnaire

Reference No: ……..……

Fakulti Perubatan dan Sains Kesihatan
Faculty of Medicine and Health Sciences
Jabatan Pemakanan dan Dietetik
Department of Nutrition and Dietetics

PKK 4999
PROJEK ILMIAH TAHUN AKHIR
FINAL YEAR PROJECT

"SULIT"
"CONFIDENTIAL"

Soal Selidik
Questionnaire

Hubungan antara Pengambilan Vitamin D, Kalsium dan Polifenol dengan Kepekatan Hemoglobin dalam Kalangan Staf dan Pelajar Wanita di Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia

The Association between Dietary Vitamin D, Calcium and Polyphenol Intake with Hemoglobin Concentration in Female Staffs and Students in Faculty of Medicines and Health Sciences, Universiti Putra Malaysia

Penyelidik	/*Researcher*	: Nur Syafiqah Aqilah Bt Suhaimi (190004)/ Fazirah Bt Samah (190336)/ Noor Aiman Afaf Bt Afiffudden (189938)
Program	/*Program*	: B. Sc. (Nutrition and Community Health)
Penyelia	/*Supervisor*	: Dr. Salma Faeza Ahmad Fuzi
Tarikh	/*Date*	: / / 2020

Semua maklumat yang diberikan di sini hanya digunakan untuk tujuan akademik sahaja dan akan dirahsiakan. Penyertaan dan kerjasama yang diberikan didahului dengan ucapan terima kasih.

All information given in this questionnaire form are for academic purposes only. It will be kept confidential. Your involvement and cooperation are greatly appreciated. Thank you.

SECTION A: SOCIO-DEMOGRAPHIC CHARACTERISTICS

Instruction: Fill in the blank or tick (√) in the space provided below.

No.	Information	
1.	Age	
2.	Date of birth	__/__/__ (dd/mm/yyyy)
3.	Ethnicity (Please tick 1 only)	☐ Malay ☐ Chinese ☐ Indian ☐ Others:……
4.	Religion	☐ Islam ☐ Buddhism ☐ Hinduism ☐ Christian ☐ Others:.....
5.	Marital status (Please tick 1 only)	☐ Single ☐ Married ☐ Divorce ☐ Widower
6.	Education level (Please tick 1 only)	☐ No formal education ☐ Primary School ☐ Secondary School/STPM/Matriculation ☐ Diploma ☐ Bachelor ☐ Master/PhD
7.	Staffs information a) Occupation b) Monthly household income	_____ RM _____
8.	Students information a) Year of study and program b) Monthly allowance	_____ RM _____

SECTION B: ANTHROPOMETRIC MEASUREMENT

Measurement	Reading 1	Reading 2	Average
Weight (kg)			
Height (m)			
BMI (kg/m^2)			

SECTION C: HEMOGLOBIN LEVEL

Measurement	Reading 1	Reading 2	Average
Hemoglobin level (g/dL)			

SECTION D: DIETARY ASSESSMENT

I. 24 Hour Dietary Recall

Date of interview:

(circle the recall day)

Recall day 1. Monday 2. Tuesday 3. Wednesday 4. Thursday
5. Friday 6. Saturday 7. Sunday

Time/Location	Food/Drink	Quantity	Details of food and drink

II. IRONIC-FFQ

Group of products	Products	Serving size	Number of servings
Meat	Liver (pork, beef, calf, poultry), pork kidney	100 g (palm of small hand)	
	Other pork offal, poultry stomach	100 g (palm of small hand)	
	Beef, calf, lamb, horse, goose, duck meat	100 g (palm of small hand)	
	Pork meat	100 g (palm of small hand)	
	Poultry meat	100 g (palm of small hand)	
	Broth	250 g (1 glass)	
Meat products	Blood pudding sausage	25 g (e.g. 1/2 of wiener, medium slice of ham, 5 slices of sausage)	
	Other offal cold cuts	25 g (e.g. 1/2 of wiener, medium slice of ham, 5 slices of sausage)	
	Loin cold cuts, ham, poultry sausages	25 g (e.g. 1/2 of wiener, medium slice of ham, 5 slices of sausage)	
	Other sausages, wiener, smoked gammon, spam, pate, salami, brawn cold cut, bacon	25 g (e.g. 1/2 of wiener, medium slice of ham, 5 slices of sausage)	
Eggs		50 g (1 egg)	
Fish	Sardines	50 g (deck of cards)	
	Other fish and fish products	50 g (deck of cards)	
Dairy products	Milk and milk beverages (yoghurt, kefir, buttermilk, cream)	250 g (1 glass)	
	Cottage cheese	50 g (1 thick slice, 2 tablespoons)	
	Rennet and processed cheese	25 g (1 slice, 1 triangle serving)	
Cereal products	White wheat and rye bread, bakery wares	35 g (1 slice, small roll)	
	Dark bread, wholemeal, with grains, graham bread, pumpernickel bread	35 g (1 slice, small roll)	
	Crispbread	10 g (1 slice)	
	Wheat bran, wheat germs	10 g (1 spoon)	
	Iron-fortified corn flakes and cereals	35 g (1 glass)	
	Other cereal products (uncooked)	100 g (e.g. 1 glass of pasta or oatmeal, 1/2 glass of rice or groats)	
Fruits	Fresh fruits	100 g (1 medium piece, 1 glass)	
	Dried fruits	50 g (handful)	
Vegetables	Dry legumes	100 g (1/2 of glass)	
	Other vegetables	100 g (1 medium piece, 1 glass)	
Potatoes		100 g (1 large piece)	
Fats		10 g (1 spoon)	
Nuts and seeds	Poppy, pumpkin and flaxseed	30 g (handful, 3 spoons of seeds)	
	Other nuts and seeds	30 g (handful, 3 spoons of seeds)	
Cocoa products	Cocoa	10 g (1 spoon)	
	Chocolate	20 g (1/5 of bar)	

III. Diet History (Day 1)

Date of interview:			
Time/Location	**Food/Drink**	**Quantity**	**Details of food and drink**

IV. Diet History (Day 2)

Date of interview:			
Time/Location	**Food/Drink**	**Quantity**	**Details of food and drink**

V. Diet History (Day 3)

Date of interview:			
Time/Location	**Food/Drink**	**Quantity**	**Details of food and drink**

SECTION E: SUN EXPOSURE

Participant were required to answer every question by writing a number of minutes or hours in each space on the table given.

Write an answer for each time of day:	7-9am	9-11am	11am-1pm	1-3pm	3-5pm	5-7pm
1. How much time did you spend outdoors between these periods?						
2. Where would you be during this time?						
3. How would you describe the weather conditions?						
4. How much time did you spend in the shade (e.g. cloud, tree or sunshade)?						
5. How much time was your head covered (e.g. by a brimmed hat or hijab)?						
6. What areas of your body were exposed to sunlight?	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________	☐Face ☐Hands ☐Full arms ☐Half arms ☐Full legs ☐Half legs ☐Other (please specify) ________
7. How much time did you wear sunscreen?						
8. Did the sunscreen you used have SPF and UVB protection? ☐ SPF ☐ UVB *state the sun protective factor (SPF): ____________						