ANGLIA RUSKIN UNIVERSITY

FACULTY OF HEALTH, EDUCATION, MEDICINE, AND SOCIAL CARE

DIETARY NUTRIENT DENSITY AND BODY FAT PERCENTAGE

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A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

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DECLARATION OF AUTHORSHIP

I, Osinachi Ekeagwu, confirm that the work presented in this thesis is my own. Where information was derived from other sources, I confirm that this has been indicated in the work.

Signed:  Date: January 2020.

ANGLIA RUSKIN UNIVERSITY

ABSTRACT

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Background: The intake of nutrient-dense foods is recommended for overall health improvement and maintaining healthy body fat. However, there is a dearth of evidence on the relationship between the dietary nutrient density and body fat percentage. Investigating this relationship depends on the availability of valid dietary assessment methods for estimating micronutrients and the bioavailability of micronutrients, considering that nutrient-dense foods are rich in phytate which may affect the bioavailability of micronutrients.

Aims: This thesis primarily investigated the association between change in dietary nutrient density and change in body fat percentage using a prospective cohort study design. It also validated a 4-day food photography method for estimating micronutrients in the diet and conducted a systematic review on the influence of dietary phytates on the bioavailability of micronutrients.

Methods: This research was conducted in three stages. Firstly, a 4-day food photography method was validated against the weighed food record method as a reference. Secondly, a systematic review was conducted on the influence of dietary phytate on the bioavailability of micronutrients. Finally, the association between change in dietary nutrient density and change in body fat was investigated in a 6-month prospective cohort study involving 108 adults. For the validation study, measures obtained from both methods were compared using Student's t-test, and the agreement between both measures was assessed using Bland-Altman analysis. In the systematic review, specific databases were searched, and the results were reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement. The relationship between dietary nutrient density and body fat percentage was investigated using linear mixed model regression. For all stages, the statistical significance was considered for p-values less than 0.05.

Results: In the validation study, the difference between the measures obtained by the food photography method and the weighed food record was not statistically significant ($p > 0.05$). Bland-Altman plots showed a good agreement for the nutrient estimates obtained by both methods. The bias for each nutrient estimate was less than 20%. The systematic review concluded that phytate negatively influenced the bioavailability of iron, magnesium and, zinc. In the cohort study, an increase in the dietary nutrient density of vitamins A, E, K and C, folate, iron, calcium, magnesium, potassium, selenium, zinc and, phosphorus were each found to correspond to a decrease in body fat percentage ($p < 0.05$) after adjusting for dietary phytate and covariates.

Conclusions: The food photography method is suitable for assessing dietary micronutrients; dietary phytate reduces the bioavailability of iron, magnesium and, zinc; an increase in dietary nutrient density of vitamins A, C, E and K, folate, iron, calcium, magnesium, selenium and phosphorus was associated with a decrease in body fat percentage in adults.

***Keywords*:** Dietary nutrient density, dietary assessment, micronutrients, body fat percentage, phytate.

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FREQUENTLY USED ABBREVIATIONS

AHEI	Alternative Healthy Eating Index
BF%	Body fat percentage
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CDC	Centre of Disease Control and Prevention
CT	Computerised tomography
DASH	Dietary Approaches to Stop Hypertension (DASH)
DQI	Diet Quality Index
DXA	Dual-energy X-ray absorptiometry
FFM	Fat-free mass
FFQ	Food frequency questionnaire
FM	Fat mass
HC	Hip circumference
HDI	Healthy Diet Indicator
HEI	Healthy Eating Index
MDS	Mediterranean Diet Score
MRI	Magnetic resonance imaging
NDNS	National Diet and Nutritional Survey
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
PDA	Personal digital assistant
RFS	Recommended Food Score
RMR	Resting metabolic rate
SD	Standard deviation
SEM	Standard error of the mean
WHO	World Health Organisation
WHR	Waist-to-hip ratio
GPAQ	Global Physical Activity Questionnaire

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STRUCTURE OF THE THESIS

This thesis is structured in chapters as follows:

Chapter 1 introduces obesity as a form of malnutrition, highlights the role of energy and micronutrient intake in energy balance. It also presents the research aim and objectives, research question, hypothesis, justification, and significance, and provides a conceptual framework for the relationship between dietary nutrient density and body fat percentage.

Chapter 2 presents a background to adipose tissue (body fat) biology, methods of estimating body fat and consequences of excess body fat. This chapter critically reviews studies associating dietary intake with body fat, discusses measures of dietary assessment, highlights nutrient density as a nutrient profiling model and explores dietary factors influencing the bioavailability of micronutrients with a focus on phytate.

Chapter 3 achieves the first objective of the thesis by reporting a systematic review on the influence of phytate on the bioavailability of minerals. It also discusses and makes conclusions on the findings of the systematic review.

Chapter 4 meets the second objective of the thesis by investigating the validity of a method of dietary assessment known as the food photography record for assessing dietary micronutrients. The findings of the investigation are also discussed and concluded upon in this chapter.

Chapter 5 describes a prospective cohort study for investigating the association between change in dietary nutrient density and change in body fat percentage. It also states the results of the cohort study, discusses the findings and concludes.

Chapter 6 presents the general discussion of the findings in the various studies (chapters 4, 5 and 6).

Chapter 7 states the overall conclusion of the thesis and highlights the implications for research and practice of the findings. This chapter also indicates the strengths and limitations of the thesis and proposes recommendations for future research.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Background

1.1.1. Body fat composition and nutrition

Excess body fat accumulation in the form of obesity and overweight constitute a growing global public health problem affecting an estimated 1.9 billion adults and 340 million children and adolescents (World Health Organisation (WHO), 2018). In the United Kingdom, the rate of obesity has increased since the 1980s and is projected to continue rising. Health statistics show that the rate of adult obesity increased by 14% between 1993 and 2017 (National Health Service (NHS), 2018), and predicted to affect half of the UK population by 2030 (Wang, et al., 2011). Obesity amounts to 9000 premature deaths and 8.7% of all deaths yearly in the UK (Kelly, et al., 2009) in addition to increasing health conditions including but not limited to musculoskeletal disorders, diabetes, cancers and cardiovascular diseases (Villareal, et al., 2005). Also, managing obesity-related morbidity accrues to increased economic burden in the form of increased health costs up to 30% higher than those of normal weight, and loss of productivity (Withrow and Alter, 2011). An economic analysis of the impact of obesity shows that obesity-related health costs amount to approximately £6 billion a year (5% of the entire National Health Service (NHS) budget) and is expected to rise to £10-12 billion per year by 2030 (Dobbs, et al., 2014).

Obesity and overweight are considered as a form of malnutrition (WHO, 2015), but the actual nutrient imbalance involved remains controversial. While it is predominantly associated with increased intake of energy-dense foods and limited energy expenditure (Wright and Aronne, 2012), some researchers have suggested otherwise after observing that altering energy intake and expenditure failed to achieve meaningful changes in body fat (Griffith, Lluberas and

Luhrmann, 2013; Hafekost, et al., 2013; Ladabaum, et al., 2014). Other researchers have suggested the role of micronutrients, given their involvement as co-enzymes and cofactors, structural components of enzymes and cytochromes in energy metabolism (Institute of Medicine, 1997; 2001; Major, et al., 2008), the co-occurrence of certain obese states with suboptimal micronutrient status (Nead, et al., 2004; Garcia, et al., 2009; Via, 2012; Zavala, et al., 2012; Payahoo, et al., 2013; Azab, et al., 2014), and the relationship between the intake of nutrient-dense foods and body mass (Mozaffarian, et al., 2011; Castellanos-Guiterrez, et al., 2018). Although these observations are important, they seem inadequate regarding evidence on the influence of nutrient intake on body fat mass for various reasons. Firstly, most of the studies have only focussed on specific micronutrients and failed to consider energy intake simultaneously (Nead, et al., 2004; Azab, et al., 2014; Gonzalez-Reimers., et al., 2014). Secondly, most studies have applied cross-sectional research design to investigate the association between micronutrients and body fat mass, which makes it unclear whether the low micronutrient status is a consequence of metabolic changes related to increased body fat, or that increased body fat occurs due to inadequate dietary intake of micronutrients. Thirdly, studies which applied a study design other than cross-sectional design have used surrogate measures for body fat (body mass and BMI) (Mozaffarian, et al., 2011; Castellanos-Guiterrez, et al., 2018).

Micronutrient and energy intakes can influence body fat. However, there is a dearth of research evidence indicating the nature of this association. Therefore, this thesis primarily aims to contribute to the literature by investigating the association between change in dietary micronutrient and energy intakes (nutrient density) and change in body fat percentage using a prospective cohort study design that permits tracking participants' dietary intake and body fat percentage over time.

1.1.2. Nutrient density

Nutrient density, also known as micronutrient density, is an application of nutrient profiling, which is the science of ranking foods based on nutritional composition (Drewnowski, 2005; WHO, 2010). The nutrient density of foods is calculated in terms of the index micronutrients per reference amount of energy (100 g, 1000 kcal, or per serving) (Drewnowski, 2014). The concept of nutrient density can equally be applied to individual foods, composite meals, and the total diet. Also, it may be applied in various ways such as; in assessing the environmental impact of foods where it is defined as nutrient density in relation to greenhouse gas emissions, or food affordability metrics, in which case it is defined as nutrient density in relation to monetary cost. In the present thesis, however, dietary nutrient density is considered in relation to body fat percentage.

The nutrient density of foods is a crucial element of preventive nutrition. It has been shown that consuming nutrient-dense foods is associated with a decreased risk of cardiovascular disease and diabetes (Chiuve, Sampson and Willet, 2010), and is inversely correlated with all-cause mortality (Chiuve, Sampson and Willet, 2010; Streppel, et al., 2014). The increased intake of foods with high nutrient density is also considered necessary to end the intergenerational cycle of obesity (Troesch, et al., 2015). Since scientific evidence indicating the inverse relationship between the intake of nutrient-dense foods or diets and body fat percentage is as yet unavailable, the recommendation made by Troesch, et al. (2015) seems premised on the supposed role of energy-dense foods in the obesity epidemic. Nutrient-dense foods include but are not limited to leafy-greens, whole grains, legumes, oilseeds, fruits and vegetables, lean meat and oily fish rich in omega-3-fatty acids. The current UK diet recommendation states that individuals aged 11 years and over should consume at least 5 portions of fruit and vegetables per day (NHS, 2019), while the World Health Organisation recommends the consumption of more than 400 grams of fruits and vegetables per day (WHO,

2004). Also, the US Department of Health and Human Services and Agriculture (USDA) recommends the consumption of grains (up to 170 grams daily, half of which are whole grains), legumes, seeds and soy products (HHS and USDA, 2015).

The recommendations for the increased intake of nutrient-dense foods are geared towards overall health improvement and reducing the risk of chronic diseases. However, nutrient-dense foods can only influence health outcomes if they are optimally absorbed after consumption. An important characteristic of most nutrient-dense foods is the high phytate content which has the propensity to influence dietary micronutrient bioavailability (Bohn, et al., 2004; Phillippy, 2006) and becomes an issue for any research with outcomes dependent on the influence of dietary nutrient density.

1.1.3. Micronutrient bioavailability and dietary phytate

Phytate (the salt form of phytic acid) is a widely distributed compound in plants, occurring as the main storage form of phosphorus in oilseeds, legumes and cereals, and accrues up to 5% by weight (Vats and Banerjee, 2004). During plant germination, it is hydrolysed to release relevant minerals for the development of the seedlings. Chemically, it has a small molecular size and strong negative charge under physiological conditions and is found in nature bound to multivalent minerals (Barrientos and Murthy, 1996). Its chemical characteristics reduce its likelihood of passage through the bi-lipid layer of plasma membranes, especially as no transport mechanisms have yet been found. Also, due to its strong negative charge, it tends to complex multivalent minerals especially iron, calcium, magnesium, and zinc when present in the diet, thereby limiting their bioavailability (Cosgrove, 1980; Schlemmer, et al., 2009). As such, the consumption of plant-based foods high in phytates, particularly cereals, oilseeds, and legumes remains concerning especially in populations at risk of micronutrient deficiency. The preponderance of research in agreement with this view (McCance and Widdowson, 1942; Hallberg, et al., 1989; Reddy, et al., 1996; Bohn, et al., 2004; Phillippy, 2006) has since lent

support for refining techniques towards its removal from food (dephytinisation) (Nout, 1993). Nevertheless, considering that phytates are also associated with health benefits (Schlemmer, et al., 2009), the rationale for dephytinisation remains questionable. Given that this thesis investigated the association between change in dietary nutrient density and change in body fat percentage, it was necessary to clarify whether dietary phytate influences the bioavailability of micronutrients.

1.2. Aim and objectives of the thesis

The overall aim of this thesis is to investigate the association between change in dietary nutrient density and change in body fat percentage. This aim is achieved through the following specific objectives:

1. To evaluate the influence of phytate on the bioavailability of micronutrients through a systematic review of the literature. This objective is necessary considering that dietary phytate may confound the association between change in dietary nutrient density and change in body fat percentage.
2. To validate a food photography method for estimating dietary micronutrients against the weighed food record method as a reference. The essence of this objective is to ensure that the proposed dietary assessment method (food photography method) to be used can validly and reliably assess dietary micronutrients required for calculating nutrient density.
3. To assess the dietary nutrient density and body fat percentage of the research participants and examine the relationship between change in dietary nutrient density and change in body fat percentage using a prospective cohort study design.

4. To highlight implications for research and practice based on the conclusions drawn on the association between change in dietary nutrient density and change in body fat percentage.

1.3. Research question and hypothesis

The overall aim of this thesis is focussed on answering the research question: What is the association between change in dietary nutrient density and change in body fat percentage? Given this question, it is hypothesised that an increase in dietary nutrient density is associated with a decrease in body fat percentage, provided dietary phytate does not influence the relationship.

1.4. Research rationale and significance

Research suggests that energy intake (Romieu, et al., 2017; Hutchison, et al., 2019) and micronutrient intake (Institute of Medicine (IOM), 2001; Government Office for Science, 2009) can influence energy imbalance. Nevertheless, there is a dearth of research indicating the association between the energy and micronutrient intakes in the diet and body fat percentage. Furthermore, with several studies reporting weight gain among university students (Lloyd-Richardson, et al., 2009; Kapinos, Yakusheva and Rosenberg, 2014; Finlayson, et al., 2012; Vadeboncoeur, Foster and Townsend, 2015; Vadeboncoeur, Foster and Townsend, 2016), the university is known to be a critical period for weight gain. This has been associated with poor dietary habits (Crombie, et al., 2009; Vella-Zarb and Elgar, 2009) and influenced by factors such as a decrease in parental influence on the diet (Pliner and Saunders, 2008; Deliens, et al., 2014), poor cooking skills and inadequate knowledge to make healthy food choices (Cluskey and Grobe, 2009), poor time management and self-control (Deliens, et al., 2014), and living in an environment with many inexpensive catering facilities (Vadeboncoeur, Foster and

Townsend, 2015). A study of 345 British university students found that 81% of them did not have healthy eating behaviours (Tanton, et al., 2015). Hence, this thesis aims to contribute to the literature by investigating the association between the changes in the proportion of micronutrient-to-energy intake in the diet (dietary nutrient density) and body fat percentage among university students. The findings of this thesis will contribute to the literature by providing evidence of the association. Also, through the findings, weight change will be better understood and can inform the design of nutrition and health interventions for bodyweight management especially for university students.

A review of weight-loss interventions shows that several interventions have solely relied on achieving weight loss by reducing energy intake and increasing physical activity while neglecting other factors that can influence energy balance (Hafekost, et al., 2013). This highlights the need for alternative weight management approaches. Effective weight management approaches can reduce the rates of obesity and comorbidities in the United Kingdom (NHS, 2018) and globally (WHO, 2018). According to research on the health and economic burden of the projected obesity trends in the United Kingdom, an effective weight loss intervention that can reduce body fat by 1% over 20 years would amount to a gain of 3 million quality-adjusted life years (QALYs) and reduce incident cases of diabetes, cancer and cardiovascular disease by 202,000, 32,000, and 122,000, respectively (Wang, et al., 2011).

Presently, nutrient density (micronutrient per energy (calorie)) is an accepted nutrient profiling model more commonly used for ranking foods, single meals and diets based on their nutritional value (Drewnowski, 2005; WHO, 2010). The findings of this thesis can extend the implication of nutrient density beyond its use for rating diet quality, to reflect implications for metabolic advantage. As such, its inclusion in nutrient labels can inform consumers of the nutrient density of their diet as well as how their body fat composition may be influenced.

1.5. A conceptual framework for the association between change in dietary nutrient density and change in body fat percentage

A conceptual framework is a written or visual explanation of the key variables, concepts or factors of a specific research and the presumed association among them (Miles and Huberman, 1994). The conceptual framework in Figure 1.1 has guided the research methods used in this thesis. The development of this framework is underpinned by the Geometric Framework for Nutrition (Raubenheimer and Simpson, 2012), which posits that changes in nutrient intake can result in measurable physiological changes. In keeping with the Geometric Framework for Nutrition, two variables are primarily considered in this conceptual framework; dietary nutrient density and body fat percentage. The conceptualised relationship between both variables is such that an increase in dietary nutrient density is associated with a decrease in body fat percentage, provided that dietary phytate does not influence the relationship. Since the variables are quantitative, a quantitative method is used to assess the association between them.

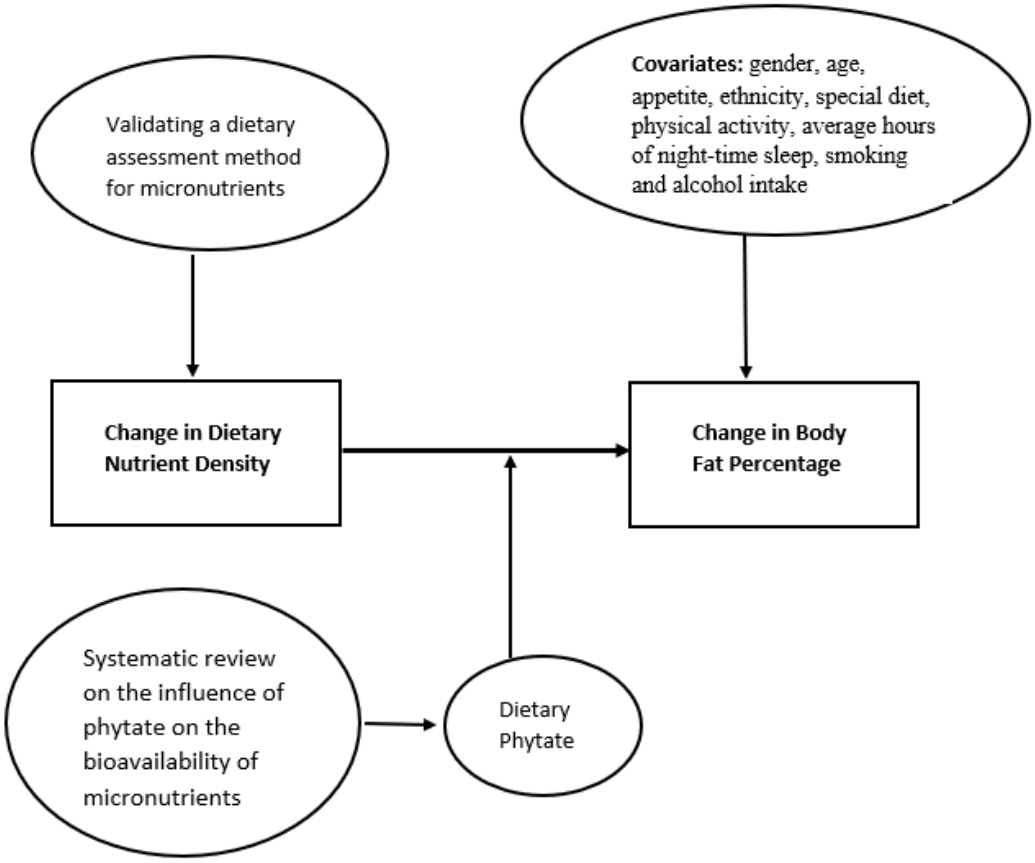


Figure 1.1: A conceptual framework for the relationship between change in dietary nutrient density and change in body fat percentage.

In addition to the primary variables of this conceptual framework and the presumed relationship between them, the framework considers covariates and a moderator, dietary phytate, which can influence the relationship between the primary variables. Previous literature shows that some important covariates include gender, age, appetite, ethnicity, special diet, physical activity, average hours of night-time sleep, smoking and alcohol intake. These variables have been discussed in the literature review aspect of this thesis and will be assessed quantitatively. Further, the framework addresses two critical questions necessary for assessing the relationship between the primary variables. Firstly, how can dietary micronutrients be assessed? Secondly, should dietary phytate be considered as a moderator? To resolve these questions respectively,

the framework includes the validation of a dietary assessment method for micronutrients and a systematic review of evidence on the influence of dietary phytate on the bioavailability of micronutrients. These components are necessary for assessing the association between change in dietary nutrient density and change in body fat percentage. For instance, without a valid method of assessing micronutrients, dietary nutrient density cannot be calculated, and the relationship cannot be assessed. Also, since the influence of dietary micronutrient density on body fat is only feasible with the bioavailability of the micronutrients consumed, there is the need to clarify on how phytate might influence the relationship. Among the factors with the propensity to influence the bioavailability of micronutrients, the role of phytate remains controversial.

CHAPTER 2. GENERAL LITERATURE REVIEW

2.1. Introduction to literature review

In keeping with the objectives of this thesis, this chapter aims to present a background to adipose tissue (body fat) biology, methods of estimating body fat and dietary assessment techniques, as well as critically review studies associating dietary intake with body fat.

2.2 Overview of the body fat tissue

2.2.1 Adipose tissue biology

The body fat tissue, otherwise known as the adipose tissue, body fat or simply, fat, is a loose connective tissue composed mainly of fat cells known as adipocytes. These cells primarily arise from preadipocytes, cells that differentiate into mature adipocytes (Charriere, 2003). Body fat plays various roles including serving as a central nexus of metabolic communication and control, an arbiter of thermoregulation, a buffer against trauma and the cold, and a regulator of reproduction and satiety. It is also recognised as an important energy-storing depot in the body, able to store excess energy and release in time of need (Berry, et al., 2013). More so, it is considered the body's largest endocrine organ, regulating many aspects of the systemic physiology and biological processes such as appetite, insulin sensitivity, ageing, glucose homeostasis, fertility and fecundity, and body temperature (Spiegelman and Flier, 2001; Nawrocki and Scherer, 2004; Gesta, et al., 2007).

There are two histologically, and functionally distinct classes of adipose tissue, known as the white and brown adipose tissue. The white adipose tissue serves for energy storage and coordinates systemic metabolism and has two divisions known as the visceral and

subcutaneous fat. The visceral fat occurs around the organs, while subcutaneous fat lies under the skin (Cypress, et al., 2013). The amount of visceral and subcutaneous fat depot in an individual is suggested to have some metabolic and health implications. For instance, increased subcutaneous fat deposition is thought to be protective against certain forms of metabolic dysfunctions (Snijder, et al., 2003a; 2003b), while an increased visceral fat depot is considered to be associated with metabolic complications and increase the risk of diabetes, cardiovascular disease and hyperlipidaemia (Grauer, et al., 1984). As a result of these associations, subcutaneous and visceral fat are often termed "good" and "bad" fat, respectively. The reasons for these associations are unclear, but studies have suggested differences in histological characteristics (Bjorntorp, et al., 1971; Salans, et al., 1973; Weyer, et al., 2000), lipolytic rates (Fischer, et al., 2002), and blood supply (Berry, et al., 2013). Aspects of regional fat deposition are, to a large extent, hereditary, whereas extrinsic or environmental factors appear to have more influence on the tendency to gain or lose body fat (Shi and Clegg, 2009).

The brown adipose tissue primarily converts chemical energy derived from nutrients into heat (Kajimura, et al., 2010). Akin to the white adipose tissue, the brown fat is present at several body locations and can be increased or decreased in response to environmental cues such as temperature changes and energy deficiency or overload (Wu, et al., 2012).

2.2.2 Body fat distribution and turnover

From birth, boys and girls tend to be lean and similar with regards to body fat. During and after puberty, girls tend to accumulate relatively large amounts of fat, particularly in the peri-pelvic and thigh region, unlike boys. Boys rather accumulate a relatively large amount of bone and muscle (lean body mass), not fat mass. With ageing, both sexes tend to accumulate body fat in the upper body aspects (truncal region), also associated with an increase in visceral fat. In

women, it is influenced by a decrease in oestrogen/testosterone, which occurs during menopause (Lemieux, et al., 1996; Toth, et al., 2000).

Fat tissue may respond to expansion through hyperplasia and hypertrophy. However, the extent of contribution of these two responses is influenced by the genetic background, modifier effects, hormones, depot preference for fat storage and diet (Berry, et al., 2013). Body fat tissue maintains the ability to expand with age, hence ageing being associated with an increased body fat percentage (Yanovski, et al., 2000). About one-tenth of the total fat cell pool is renewed every year through adipogenesis and adipocyte death (Spadling, et al., 2008).

2.3. Assessing body fat

The first published attempt to define body fat levels was in the 19th century when the Life Insurance industry published data expressing body weight adjusted for height as an independent determinant of life expectancy (Rogers, 1901). Subsequently, tables of average body weights for heights (Wt/Ht) by sex for different ages were also published by the Metropolitan Life Insurance Company in 1959. Based on this standard (Wt/Ht), one with Wt/Ht of 20% above or below the mean for an age category was considered overweight or underweight respectively (Metropolitan Life Insurance Company, 1959). This index was, however, shown to be biased when it was recognised that one's bone mass, height and leg length could affect the results (Eknoyan, 2008). Presently, there are many methods of estimating body fat composition used in clinical practice and for epidemiological research (Cornier, et al., 2011; Fosbol and Zerahn, 2014). In epidemiological research, the commonly used methods for assessing body fat include criterion and indirect methods (Duren, et al., 2008). The methods in both categories are summarised in table 2.1 below and discussed further in this review.

Table 2.1. Methods for estimating body fat

Criterion Methods	Indirect methods
Dual-energy X-ray absorptiometry (DXA)	Body mass index (BMI)
Imaging methods	Waist and hip circumference
Hydrodensitometry	Skinfold thickness
Air displacement plethysmography	Bioelectric impedance analysis (BIA)

2.3.1. Criterion methods

The criterion methods estimate body fat directly based on its density or using magnetic imaging or X-ray techniques. Criterion methods include dual X-ray absorptiometry (DXA), magnetic resonance imaging (MRI), computed tomography (CT) and densitometry (Roche, 1996).

2.3.1.1. Dual-energy X-ray absorptiometry (DXA)

The dual-energy x-ray absorptiometry operates by the principle of detecting the differential attenuation of two x-ray beams by bone, soft tissue, fat-free and fat tissue when passed through the body. The procedure is simple, quick and rapidly becoming frequently used in estimating body fat composition for the whole body and specific regions in clinical studies. Some of the studies in which the technique has been applied include the NHANES (1999- 2000) (Centres for Disease Control and Prevention, 2000) and Health ABC study (Snijder, et al., 2002). It produces highly accurate and reproducible measures and is mostly accepted as a reference method (Hu, 2008; Bacchi, et al., 2017). Kiebzak, et al. (2000), for example, recorded a coefficient of variation (CV %) of 1.89% for total body fat percentage after scanning 20 participants once daily for 4 consecutive days. Regional fat estimates for arms, legs, trunk, and

pelvis, though, were less precise and resulted in an approximate coefficient of variation of 1%-5%. The short duration of the study (Kiebzak, et al., 2000) does not seem to have had any significant influence on the findings, as 3-month reproducibility estimates had a coefficient of variation of 1.3% (Cordero-MacIntyre, et al., 2002).

Body fat estimates obtained by DXA have also been validated against other reference methods. A comparison with hydrodensitometry indicated a correlation of 0.92 for body fat percentage estimates (Norcross, and Van Loan, 2004). Similarly, a comparison of body fat estimates obtained by DXA and computed tomography in the Health ABC study also showed a correlation of 0.98 (Snidjer, et al., 2002).

Furthermore, unlike an imaging technique such as a CT scan, DXA exposes individuals to low levels of radiation which makes it suitable for a wide range of populations. Notwithstanding, due to its immobility and high cost, its use is limited, especially in large epidemiological studies (Hu, 2008).

2.3.1.2. Imaging methods

Two imaging methods or techniques for measuring body composition include computed tomography (CT) and magnetic resonance imaging (MRI). CT scans, also referred to as CAT scans use x-ray and a computer to create detailed images of the body components. In contrast, MRI uses strong magnetic fields, radio waves, and an electric field gradient to generate images of body components. Measurement of volume and distribution of subcutaneous versus visceral fat, muscle mass and organ composition can be carried out using these techniques (Hu, 2008). Due to the high accuracy of imaging methods, they may be used as a reference for validating other methods for estimating body fat. Nevertheless, reduced accessibility and high expense in acquiring them are notable limitations to their use. In clinical and epidemiological studies, imaging methods have demonstrated significant correlation with visceral and subcutaneous adipose tissue mass (Kvist, Sjostrom and Tylen, 1986; Abate, et al., 1995), and metabolic

diseases (Labovitz and Banerji, 2005), although estimates have not been entirely consistent (Miles and Jensen, 2005).

2.3.1.3. Hydrodensitometry

This method estimates body fat composition based on changes in the density of the body when immersed in the water. Hydrodensitometry is based on the principle that body fat is less dense in water (Hu, 2008); hence, individuals with more fat will be less dense than those with less. The method requires the measurement of body mass while submerged in water using a sensitive electronic scale. In this manner, body volume, body density, and percentage of body fat are calculated based on established formulas (Hu, 2008). While using this method, inaccurate correction for residual lung volume occurs as a source of error (Heymsfield, Shen and Wang, 1998).

2.3.1.4. Air displacement plethysmography

The air displacement plethysmography is another method that estimates body fat based on the principle of density but uses air rather than water (Hu, 2008). Body fat is estimated using body volume and mass, while one sits in a testing chamber. Total body volume is estimated by applying the basic gas laws using the variation in air pressure in the testing chamber with and without the individual involved being assessed (Going, 2005). Estimates of body volume are also corrected for the average amount of air present in the lungs, unlike in hydrodensitometry. The BodPod Body Composition system is presently the most common application of air-displacement plethysmography (Demster and Aitkens, 1995).

Air-displacement plethysmography has a between-day test-retest reliability higher than 0.90 (Going, 2005), and shows a correlation of 0.94 with body fat percentage measured by hydrodensitometry (Fields, Goran and McCrory, 2002). It also has a correlation of 0.91 with dual x-ray absorptiometry estimates. In a study among overweight and obese individuals, Ginde, et al. (2005) demonstrated its accuracy in measuring body fat in severe obesity (BMI ≥

40 kg/m^2). Its accuracy has also been demonstrated among children (Buchholz, et al., 2004; Fields, Higgins, and Radley, 2005). Notwithstanding its use as a criterion method for several validation studies since its introduction in 1995, some studies claim that it tends to underestimate percentage body fat by approximately 2-3% (Fields, Goran and McCrory, 2002), due to increased body heat and moisture (Fields, Higgins and Hunter, 2004), facial hair, scalp hair (Higgins, et al., 2001), and clothing (Fields, Hunter and Goran, 2000).

2.3.2. Indirect methods

The indirect methods estimate body fat through measuring proportions of the human body or based on the biological interrelationships among body tissues and their distributions among healthy individuals (Roche, 1996). Some commonly used indirect methods in epidemiological studies include BMI, waist and hip circumferences, skinfold thickness and bioelectric impedance analysis **(**BIA).

2.3.2.1. Body mass index (BMI)

BMI is obtained by dividing body mass by a square of the height. Keys, et al. (1972) claimed that the equation reduced the contribution of leg length and tended to reduce the influence of variance in height. Although the authors (Keys, et al., 1972) admitted that the equation poorly represented body fat percentage, it was considered an important calculation since most of the body fat is in the trunk. Eventually, it was adopted by the WHO as an estimate of body fat and published by the WHO Expert Committee in 1995 (WHO, 1995) after which it became widely accepted (Flegal, et al., 1998). BMI is mathematically expressed as mass (in kilograms) per square height (in metres) and is the most widely used measure for assessing the risk of obesity. It has been used for over two decades in a myriad of epidemiological studies and is recommended for use in clinical practice to guide recommendations for weight management in children and adults (WHO, 2018).

Initially, when the BMI classification was published by the World Health Organisation, the National Institute of Health in the United States classed individuals having a BMI of 27.8 kg/m^2 (for men) and 27.3 kg/m^2 (for women) or greater as overweight. And if one was of BMI below this value, they were considered "normal," according to an 85% cut off point of people examined in the National Health and Nutrition Examination Survey (NHANES) II (Najjar and Rowland, 1987; Kuczmarski, et al., 1994). When the cut-off point was reduced to 25kg/m^2 in agreement with the WHO guidelines (WHO, 1995; NIH, 1998), parenthetically, the number of Americans considered overweight was increased. In 1997, the International Obesity Task Force (IOTF) created more BMI categories to include various degrees of obesity. With the new classification, a BMI below 18 kg/m^2, ranging from 18.5- 24.9 kg/m^2, and 25 kg/m^2 to 29.9 kg/m^2 were considered underweight, normal weight and "pre-obesity," respectively. While BMI of 30 to 34.9, 35.0 to 39.9, and 40 kg/m^2 and over were referred as class I obesity, class II obesity, and class III obesity, respectively.

A scheme for the classification of individuals based on their body fat mass is necessary for managing obesity. However, the use of the BMI classification system has been criticised as being presumptuous and careless, given that BMI is not a direct reflection of an individual's fat mass, and that the use of the term "pre-obesity" implies a phase of transition between being of normal weight and being obese (Nuttal, 2015). Also, it has been argued that the use of "pre-obesity" in the classification could mean that those who are underweight may be referred to as "pre-normal," and that "pre-obesity" could easily be misconstrued for "not yet obese, hence still healthy" thereby encouraging having BMI in that range despite the health risks associated.

According to the WHO definition, "obesity" implies a state of accumulated body fat stores (WHO, 2019). Based on this premise, the use of BMI for estimating body fat has been debated (Keys, et al., 1972; Strain and Zumoff, et al., 1992; Wellens, et al., 1996; Heitman, et al., 2000;

Flagal, et al., 2009). Body mass, a component of BMI is made up of both lean body mass and fat mass and tends to vary with factors that affect relative compositions of both such as sex, age and ethnicity (Garn, et al., 1986). Variation in lean body mass and fat mass with sex is well documented, given that for the same BMI, women have more body fat and less lean body mass and vice versa. For instance, in population-based studies, women are shown to have a lower BMI compared to men, yet they have approximately 20- 45% more fat mass (Norgan, 1994; Wang, et al., 1994). Among individuals of the same sex, BMI has also shown a weak correlation with percentage body fat. In an early study assessing the accuracy of the BMI estimate, Smalley, et al. (1990) indicates that participants of 27 kg/m^2 BMI had percentage body fat ranging from 10%- 32%. In the study, based on the BMI criterion, the participants were "overweight" or "pre-obese" whereas some of them were below the cut-off limit for body fat percentage categorised as obese (NIH criterion for obesity based on percentage body fat for men is 25%, and for women is ≥ 35%) (National Institute of Health, 1990). Results from the National Health and Nutrition Examination Survey (NHANES) III have also shown a weak correlation between percentage body fat and BMI (Romero-Corral, et al., 2008); participants' percentage body fat varied from 14% and 35% in men, and 26% and 43% in women where BMI was 25 kg/m^2. More recent NHANES findings similarly suggest a weak correlation between BMI and percentage body fat (Flegal, et al., 2010). Interestingly, the BMI of the entire NHANES III cohort correlated better with the lean body mass in men (Romero-Corral, et al., 2008).

A significant limitation of the BMI estimate for body fat is the difficulty in differentiating the relative contributions of lean and fat body mass. Age-associated changes in lean body mass and fat mass composition may reduce the validity of BMI estimates of body fat mass. Data from 4115 US adults (Flegal, et al., 2002), for instance, shows a gradual increase in mean BMI during young and middle-aged adult life, peaking at 5^{th} to 6^{th} decade of life, and gradually

declining afterwards (Villareal, et al., 2005), despite the known increase in body fat mass and decrease in lean body mass with ageing (Gallagher, et al., 1996). Micozzi and Harris (1990) have similarly found weaker correlations between BMI and body fat with ageing in men and women. However, a stronger association between BMI and lean body mass was noted. Observing similarly in a study of body weight changes and health implications in the elderly, Seidell and Visscher (2000) concluded that BMI better-reflected changes in lean body mass than fat mass in the elderly.

Additionally, the validity of BMI as a measure of body fat may be affected by ethnic variations in body composition. In a comparative review on the measures of body composition in white people and black people, Wagner and Heyward (2000) noted that black people had higher estimates of lean body mass as measured using dual x-ray absorptiometry. Due to this difference, black people tend to have higher BMI estimates compared to white people, but without significant differences in body fat percentage (Kleerekoper, et al., 1994; Galagher, et al., 1996). Evidence also abounds on the higher percentage body fat in Asians than in white people given the same BMI (Deuranberg, Deurenberg-Yap, 1998). Deuranberg, Deurenberg, and Guricci (2002) found that the body fat percentage was 3%- 5% higher in Asians than in white people of the same BMI. Hu (2008) suggests that the higher body fat in Asians is related to their characteristic short legs, smaller frame, and lower muscularity. However, these features do not seem representative of all Asians as differences in BMI and body fat relations are also found within the Asian population (Deurenberg-Yap, et al., 2000). In recognition of the differences in the association between BMI and body fat percentage in Asian populations, a WHO expert consultation has indicated BMI measures of under 18.5 kg/m2, 18.5–23 kg/m2, 23–27.5 kg/m2, and 27.5 kg/m2 and over for underweight, normal, overweight and obesity categories, respectively (WHO Expert Consultation, 2004).

2.3.2.2. Waist and hip circumference: Due to different body shapes either present within the same gender or associated with sexual dimorphism, giving rise to “android” and “gynaecoid” body shapes for men and women respectively, waist and hip circumference measurements have become necessary (Klein, et al., 2007). An individual’s waist circumference measures their natural waist, which typically occurs midway between the iliac crest and the lower rib margin. In some obese individuals, the level of the umbilicus is used to reduce variations in measurement due to difficulty locating the waist (Heymsfield, Shen and Wang, 1998). Waist circumference is frequently used for determining central obesity.

The hip circumference (HC) measures the maximum circumference of the gluteal region. When the ratio of the waist circumference to hip circumference is taken, it yields another measure known as the waist-to-hip ratio (WHR). Whereas the waist circumference is a well-accepted measure of abdominal obesity, the implication of the hip circumference is unclear, particularly as either high subcutaneous fat, a large pelvic bone or a well-developed gluteal muscle can be reflective of hip size (Willet, 1998). Consequently, interpreting the WHR as an index of both measures (waist and hip circumference) may be complicated. Also, considering that each measure (waist circumference or HC) may have conflicting effects on metabolic risk factors, the use of WHR becomes difficult. For instance, Seidell, et al. (2001) in a Quebec family study demonstrated that while waist circumference (adjusted for BMI and HC) was significantly associated with low levels of high-density lipoprotein cholesterol levels, high fasting triglycerides, glucose and insulin concentration, HC (adjusted for BMI and waist circumference) was inversely linked with these factors.

Moreover, changes in waist circumference and HC may correlate with different levels of disease risk. This was noted in a prospective study indicating 1.7 times the risk of diabetes due to increased waist circumference of 14.6cm or more when compared with individuals of stable waist circumference, while a decrease in HC beyond 4.1cm showed a 1.5-time risk of diabetes

compared to men with stable HC, respectively (Koh-Banerjee, et al., 2004). Although both measures (waist circumference and HC) are valuable in providing additional information on disease risk, more merits accrue to the use of waist circumference in practice due to its simplicity, and ease in explaining disease risk, and its strength of association with the disease risk (Wang, et al., 2005).

The recommended cut-off for waist circumference and WHR is 102cm for men and 88cm for women; 0.95 for men and 0.88 for women, respectively. In large epidemiological studies, self-reported measures are frequently used (Hu, 2008) given the high correlation between self-reported and measured values (Rimm, et al., 1990; Bigaard, et al., 2005). Both measures (waist circumference and WHR ratio) have been observed to be equally correlated with intra-abdominal fat using magnetic resonance imaging (Kamel, et al., 2000). In a study of 51 obese women, WHR offered a valid prediction of intra-abdominal fat measured with computed tomography (Ferland, et al., 1989). Owens, et al. (1999) similarly demonstrates WHR as a strong predictor of magnetic resonance imaging-derived intra-abdominal adipose tissue estimates.

Some studies have however shown that waist circumference is a better predictor of abdominal visceral fat compared to WHR, particularly in women (Pouliot, et al., 1994; Ross, Raissanen and Hudson, 1996; Snehalatha, 1997), owing to the high likelihood of the outcome being affected by variations in the HC (Rankinen, et al., 1999). As a result of differences in pelvic bone structure, and the amount of subcutaneous tissue and muscle mass, variability in HC is usually higher in women than in men. Rankinen, et al. (1999) for instance in their study observed that although the waist circumference of women was slightly higher than in men, the standard deviation in HC was almost twice higher in women compared to men. Implying that there is a more significant impact of HC on the WHR in women compared to men, thereby complicating its interpretation. Experimental evidence also indicated that weight loss

frequently occurred with a decrease in waist circumference and HC, but WHR remained the same or only changed slightly (Zamboni, et al., 1993; Ross, Raissanen and Hudson, 1996). Furthermore, WHR may result in erroneous estimates in health conditions such as type-2 diabetes associated with peripheral muscle wasting leading to smaller HC based on age (Seidell, et al., 1997).

Despite that obesity and overweight are conventionally defined and classified by BMI, waist circumference and WHR remain more practical indices and stronger predictors of regional adiposity (Rankinen, et al., 1999).

2.3.1.3. Skinfold thickness: This technique involves the indirect measurement of body fat distribution using special callipers to estimate the thickness of double-layer of skin and fat underlying predetermined sites (Lohman, Roche and Martorell, 1998). Measurement is carried out at specific standard body sites such as biceps, triceps, subscapular, abdomen and thigh, and preferably by a single individual to increase reproducibility and reduce inter-observer variations (Marks, Habicht and Mueller, 1989; Lohman, Roche and Martorell, 1998). In taking skinfold measures, skinfold in the specific body aspect is pinched between the thumb and forefinger and pulled away from the underlying muscle, and the callipers are used from the ridge of the skin formed. At approximately three seconds after applying the callipers, the readings are taken. It is recommended that three readings should be taken, and the average calculated (Durnin and Wormersley, 1974).

Skinfolds from the four aspects are summed and the percentage body fat calculated through application of population-specific equations either based on a two- (Durnin and Womersley, 1974; Jackson and Pollock, 1980) or four-compartment model (Peterson, Czerwinski and Siervogel, 2003). A correlation exceeding 0.9 was recorded between predicted skinfold measures and hydrostatically determined body fat percentage using the two-compartment

model among a group of young and middle-aged women (Jackson and Pollock, 1980). A comparison between the two models among men and women indicated more accurate predictions obtained by the four-compartment model (Peterson, Czerwinski and Siervogel, 2003). Nonetheless, both measures were stated to underestimate body fat percentage. More recent equations have been derived using skinfold thickness in combination with other anthropometric measures such as waist circumference, HC and bone breadth measurements. The estimates of body fat mass obtained using these equations show a high correlation with estimates measured by dual x-ray absorptiometry and a negligible tendency to underestimate body fat mass compared to the two- and four-compartment models. These equations are, however, population-specific (Garcia, et al., 2005).

Skinfolds in various body sites are associated with some health risks but may not sufficiently serve as independent predictions. For instance, subscapular skinfold is claimed to be predictive of all-cause mortality in women, while fore-arm triceps and biceps, and subscapular skinfolds predict fatal coronary heart disease (Kim, Meade and Haines, 2006). Rather than depend on the values of single anatomical sites, Tanne, Medalie, and Goldbourt (2005) combined skinfold measures from multiple sites. In their study, they indicated that trunk versus peripheral fat distribution was a more predictive measure of stroke mortality compared to either measure from a single site. In contrast, other studies posited that skinfold measures were not independent predictors of cardiovascular disease or mortality (Spataro, et al., 1996; Menotti, et al., 2005).

The fundamental limitation of using skinfold measures lies in the callipers which have an upper measurement limit of 45 to 55mm, thereby restricting use to moderately overweight individuals. Despite that some skinfold callipers are reported to allow large measurement, this improvement does not seem significant due to the difficulty associated with grasping a large skinfold while taking readings on the calliper (Duren, et al., 2008). Further, most national

reference values available for skinfold are limited to biceps, triceps and subscapular regions. Since skinfold in these regions, especially at the triceps varies by gender and reflects changes in underlying muscle rather than body fat, its measures become doubtful.

Additionally, due to the tendency of the calliper readings to decline after the initial application of the calliper to the skinfold (Fletcher, 1962; Becque, et al., 1986; Hakori and Okamoto, 1993), coupled with the variability in skinfold compressibility at various body sites (Clegg and Kent, 1967; Martin, et al., 1992), the accuracy of skinfold estimates remains in question. Hakori and Okamoto (1993) observed sex differences with skinfold compression; women having higher skinfold compression in the trunk area and less in the limbs compared to men. Although the authors claimed it was owing to sex differences in skin thickness and skin tension, studies are not in consensus on these differences (Dao and Kazin, 2007). Skinfold estimates may also be affected by the physical characteristics of the callipers used. In a comparative study of callipers used in Brazil and USA, Edilson, et al. (2003) showed that body fat estimates obtained from skinfold estimates for nine anatomical sites significantly varied between both callipers (up to 31%). Earlier studies (Lohman, et al., 1984; Gruber, et al., 1990) have likewise highlighted the limited reliability of skinfold estimates due to the use of different callipers. This limitation is related to differences in the design and mechanics of callipers and can be minimised by developing correction equations among the different callipers for various anatomical sites. An adequate correction equation may also consider the time course of skin compression at the time of measurement necessary to ensure reliability. Despite that, some researchers (Becque, Katch and Moffatt, 1986) have recommended that readings should be taken within 4 seconds of calliper application, the duration may rather be dependent on the pressure exerted on the skinfold by the callipers in use. Edilson, et al. (2003) clarified on the variation in pressure exerted by different callipers, stating that increasing pressure resulted in a decrease of skinfold

thickness due to squeezing of subcutaneous interstitial water. Hence, increasing the time of compression given high pressure will result in false low skinfold thickness estimates.

Notwithstanding the challenges associated with the use of skinfold to estimate percentage body fat, Yeung and Hui (2008) in a validation study showed an internal consistency of at least 0.988 for skinfold thickness estimates from triceps, biceps, subscapular, suprailiac, thigh, and calf, respectively) of skinfold estimates while using air-displacement plethysmography as a reference method. Earlier studies (Ferrario, et al., 1995; Mueller and Malina, 2005) reported similarly. Skinfold is especially useful in monitoring changes in body fat in children due to their small body size, and since most of the fat is subcutaneous (Brambilla, et al., 1994).

2.3.2.4. Bioelectric impedance analysis (BIA)

The BIA is a minimally invasive approach that assesses body composition by measuring the resistance to a small alternating electric current (about 800μA, 50 kHz) passed through body tissues. It is based on the principle that the resistance to the flow of alternating current is proportional to the body fat composition (Chumlea and Sun, 2005). Biological tissues have unique electrical properties by which they produce distinguishable responses to alternating current signals. Body fat is composed of adipocytes, cells of low electric conductivity and high impedance, while lean tissues contain electrolytes and extracellular and intracellular fluid which are of high conductivity and low impedance. Impedance refers to the resistance to the flow of electricity, and conductivity is the measure of the ability of a material to allow the flow of electricity. When the body is subject to alternating current as in the case of using BIA, the current signal is conducted through the body components which contain more water and electrolytes. In this process, the impedance or resistance to the flow of electric current changes depending on the amount of water or conductive material present in the body. Impedance varies for body water, body fat and muscle tissue; the greater the lean body mass or water

composition, the lower the resistance to the flow of current, while the more the fat content, the greater the resistance (Chumlea and Sun, 2005; Tushar, 2014). The measure of the body fat composition obtained using the BIA is obtained by assuming that the body is a conducting cylinder, from which the fat-free mass is directly determined through the equation below (Equation 1):

Equation 1: $FFM = 0.475 \times [(height\ (cm))^2/Resistance\ (ohms)] + 0.295 \times weight + 5.49$

In Equation 1, the FFM obtained is subtracted from total body mass (TBM) to obtain the body fat mass.

BIA may either apply single or multiple frequency current in measurement (Lukaski, et al., 1985). It is argued that the use of the former only guarantees the passage of current through the extracellular fluid, whereas the use of the latter allows the passage of current through both intracellular and extracellular fluid (Cole and Cole, 1941). A study (Gaba, et al., 2015) comparing the single and multiple frequency BIA indicated that for the same group of subjects, the estimated fat mass was lower for the former and higher for the latter. The researchers also observed that the estimates from the multi-frequency BIA were better and showed narrower limits of agreement with the reference method (DXA).

Several studies have compared measures of body fat obtained by BIA with other devices. A comparative study of BIA and DXA estimates of body fat among 591 healthy subjects (Sun, et al., 2005) found a correlation coefficient of 0.88. The authors concluded that BIA only offered a good alternative for measuring body fat for normal-weight individuals. Several studies have similarly recommended BIA for estimating body fat (Ling et al., 2011; Anderson et al., 2012). After examining body fat mass in 50 participants including lean, normal weight and obese men and women using BIA and DXA, both studies concluded that BIA was a valid estimator of body fat mass (Ling, et al., 2011; Anderson et al., 2012). Hu (2008) however stated the need to

control for sources of bias such as body posture, hydration states, ambient air, skin temperature, recent physical activity, and consumption of food and beverage when using BIA.

Some advantages of the BIA include its portability, low cost, and ease of use, which make it suitable for use in large epidemiologic and clinical studies. For instance, in the NHANES (III), BIA measures were included for 17000 individuals aged 12 years or older (USDH, 1996). BIA was also used in the Swedish cohort of 10,902 men and 16,814 women aged 45 to 73 years (Lahman, et al., 2002) and a Danish study involving 57,053 participants (Bigaard, et al., 2004). Developing accurate and reliable measures of body fat percentage is critical to nutrition and obesity research.

The criterion methods for assessing body fat are more accurate than the indirect methods. Nevertheless, both categories have strengths and limitations which can influence their use in epidemiological research. The choice of a method may be tailored to individual needs, the population under study and available resources. For instance, field research carried out in a remote location would require the use of anthropometric techniques such as skinfold thickness, waist circumference and WHR techniques involving highly portable equipment. Whereas in research centres or clinic settings, body fat estimates may be obtained using more sophisticated equipment such as DXA and BIA. Since anthropometric methods are relatively cheaper, occasions of limited resources may warrant their use. However, it should be noted that they are indirect measures of body fat and may vary with factors such as age, sex and ethnicity. Furthermore, multiple assessment techniques may improve accuracy and reliability (Duren, et al., 2008). Although, there remains limited evidence of its feasibility, particularly regarding interpretation in clinical practice.

2.4. Implications of excess body fat

According to the World Health Organisation (2018), overweight and obesity refer to the abnormal accumulation of body fat to the extent that it constitutes health risks. Although this

definition makes no mention of overeating, obesity is considered the consequence of a form of malnutrition associated with the intake of nutrients beyond the body's requirements-overnutrition (NHS, 2016). Obesity prevalence worldwide has tripled since 1975, presently affecting over 650 million adults, and approximately 340 million adolescents and children aged 5-19 years (WHO, 2018). In the United Kingdom, it affects 26% of adults and 20% of children aged 10-11 years (NHS, 2018). Affected individuals are at increased risk of chronic diseases, not limited to type-2 diabetes, coronary heart disease, breast cancer, bowel cancer, stroke and psychological problems negatively impacting their quality of life. These amount to substantial economic cost through increased healthcare costs and loss of productivity. Statistics indicate an obesity healthcare expenditure of approximately £6 billion a year, which is expected to rise to 10-12 billion pounds per year by 2030 (Dobbs, et al., 2014).

Being overweight and obese has also been noted to result in stigma and associated with laziness, sloppiness, emotional and physical weakness (Puhl and Heuer, 2010). Some studies have documented the pervasiveness of fat-bias across various domains including workplace, educational institution, mass media, and healthcare, particularly in westernised societies (Puhl and Brownell, 2001; Brownell, et al., 2005). According to the reports, weight stigma has increased by 66% over the past decade (Andreyeva and Brownell, 2008), comparable with the rates of racial discrimination in a country such as the USA (Puhl, et al., 2009). Weight stigma may be triggered by the perception of the cause of body fatness (Puhl, et al., 2009; Ekeagwu, 2017) or by cultural norms (Scot, et al., 2013).

The social and medical implications of being overweight and obese emphasise the need to maintain body fat within healthy levels. Arguably, the maintenance of healthy body fat may be challenging with an inadequate understanding of the process of body fat regulation and the factors influencing body fat composition. These are discussed next in this chapter.

2.5. Body fat regulation and factors influencing body fat composition

Body fat is fundamentally regulated through the balance of fat synthesis (lipogenesis) and fat breakdown (lipolysis). These processes occur in response to metabolic energy demands, enabling the conservation and release of energy, respectively. In vivo research shows that both processes differ between the sexes. Women, for instance, tend to store a higher percentage of meal-derived fatty acids in the subcutaneous adipose tissue (38%) (Romank, Nekson and Jensen, 2000) compared to men (24%) (Uranga, Levine and Jensen, 2005), while men store more meal-derived free fatty acids in the visceral adipose tissue than in women. Regarding lipolysis, at rest, the rate is 40% higher in women than in men (Nielsen, et al., 2003). Lipolysis and lipogenesis are influenced by hormonal and nutritional signals, which are regulated by the central nervous system. This is accomplished when the brain receives and feeds back on continuous impulses related to energy stores and fluxes due to ingested food, absorbed nutrients and basal and situational energy needs of critical organs (Schwarts, et al., 2000; Woods, 2005).

The factors influencing body fat can be broadly categorised into factors over which the individual has no control (unmodifiable factors) and those over which the individual has potential control (modifiable factors). Factors in the former group include genes, age, ethnicity, and physiological influences, while those in the latter include dietary intake, physical activity, sleep, smoking and alcohol intake (IOM, 2004). All listed factors are discussed in this review, with more emphasis on the diet which embodies the primary focus of the thesis.

2.5.1 Genes

Differences in the way the individual body responds to changes in the energy balance are well-established to have a genetic component. Estimates of the contribution to genetic variation to observed differences in body fatness are considered to range from 30 to 70% (Comuzzie, et al., 1993; 1994; 1996). Although little is known about the specific causes of the heterogeneity

(Perusse and Bouchard, 1999), genes are thought to influence body fat by altering energy metabolism and neural control of appetite (Bogardus, et al., 1986). The genetic influence on body fat has been used to explore familial aggregation of the risk of obesity. Stunkdard and colleagues (1986) observed that children who were raised away from their biological parents from 3 months of age had similar body weight to those of their biological parents than those of their adoptive parents. The authors estimated that 70% of the variance in the occurrence of obesity was linked to genetic factors.

While some researchers suggest that genetic factors account for approximately 30 to 50% of the variance in the occurrence of obesity (Bouchard, 1997; Chagnom, et al., 2000), more recent studies, have argued that its impact can be overridden by environmental factors. For instance, a study of 17,058 Danes published in 2008 observed that individuals who had the obesity-promoting gene and were physically inactive had higher BMI compared to those who had the obesity-promoting gene but were physically active (Andreasen, et al., 2008). The study also found that those who had the obesity-promoting gene and were active had similar BMI to those who did not have the obesity-promoting gene (Andreasen, et al., 2008). Although the findings of the researchers are noteworthy, subsequent studies have reported contrasting findings (Rampersaud, et al., 2008; Jonsson, et al., 2009; Ruiz, et al., 2010). To arrive at a definitive conclusion, a meta-analysis of 54 studies conducted among adults and children (Kilpeläinen, et al., 2011) found that individuals who had the obesity-promoting gene had a 23% higher risk of being obese compared to those who did not have the gene. Nevertheless, among those who had the gene and were physically active, the risk of obesity reduced by 30% in comparison to those who had the gene but were not physically active.

Genes can influence body fatness; nonetheless, the influence may be overridden by environmental factors. Hence the need to account for such factors while investigating the impact of genes on body fat. The reviewed studies on the influence of physical activity on the

impact of genes on body fat are an important contribution to the literature. However, the studies (Rampersaud, et al., 2008; Jonsson, et al., 2009; Ruiz, et al., 2010) seem to limited by failing to account for other environmental factors which have the propensity to influence body fat such as diet, sleep, smoking and other lifestyle factors. Also, considering that all the studies use of BMI, an indirect measure of body fat makes it difficult to ascertain that there was an actual impact on body fat. There is a consensus on the bias associated with using BMI as a measure of body fat (Romero-Corral, et al., 2008; Flegal, et al., 2010).

2.5.2. Physiological factors

The physiological factors influencing body fat mass act through altering non-volitional components of energy expenditure, resting metabolic rate (RMR), thermic effect of feeding (TEF) and non-exercise activity thermogenesis (NEAT). The RMR is the rate of energy expended at rest in a post-absorptive state and under thermos-neutral conditions, and it accounts for approximately 60 to 75% of the total energy expenditure in most individuals. The amount of energy expended in this component is primarily related to the maintenance of lean body mass, nutrient synthesis and breakdown, cellular homeostasis, temperature regulation, and nervous system, pulmonary and cardiovascular function (Gallagher, 1998). The RMR is higher in men than in women owing to the higher lean tissue mass composition in men, and a low resting metabolic rate relative to body size has been observed to predict body fat increases in men and women (Ravussin, et al., 1988). Also, the resting metabolic rate decreases with age, beginning from the middle of the fourth decade (IOM, 2003), but can be maintained by engaging in physical activity, particularly for middle-aged women (Gilliat-Wimberly, et al., 2001).

The thermic effect of feeding describes the incremental increase in energy expenditure after a meal is consumed due to the energy cost of absorption, assimilation, nutrient synthesis,

transport, and storage. It accounts for approximately 5-10% of energy expenditure and varies with fat-free mass (Astrup, 1996). Non-exercise activity thermogenesis (NEAT) refers to energy expended during involuntary movements such as fidgeting, postural control, shivering or other spontaneous physical activity. Some studies have demonstrated that differences in the non-exercise physical activity may be responsible for inter-individual variation in energy expenditure and body fat mass consequently (Zurlo, et al., 1992; Levine, et al., 1999; De Groot, et al., 2014). Von-Loeffelholz (2014) states that NEAT shows a wide range of variation and in some cases can vary up to 2000 kcal per day between individuals of similar size.

2.5.3. Age

The influence of age on body fat composition is well documented. Studies have demonstrated that body fat mass increases with age even after adjusting for the influences of variation in physical activity and body mass (Gallaghter, et al., 1996; Caso, et al., 2012; Gaba and Pridalova, 2013). Gallaghter, et al. (1996) for instance observed that among younger non-exercising women of the same BMI ($25 kgm^{-2}$), the mean body fat for those aged 40 years and over was 6% higher than for those aged 17-20 years. Gaba and Pridalova (2013) similarly observed that the occurrence of a significant increase in body fat mass with increasing age in a sample of 1,970 healthy subjects. Body fat mass usually increases throughout life, generally peaking between the fifth and seventh decades and then either plateaus or declines slightly (Coin, et al., 2008; Kuk, et al., 2009; Borrud, et al., 2010). The increase in body fat with age observed by the various studies may be linked to the changes in resting metabolic rate which occurs with age (Krems, et al., 2005), and given that the resting metabolic rate constitutes the most significant component of energy expenditure (Hall, et al., 2012).

Age is also associated with the redistribution of body fat. With ageing, there is a shift of body fat towards a more central fat deposition (the viscera) from the periphery (arms, legs, and face).

Not much is known regarding the mechanism or reason for this shift, but it is known to be influenced by diet and lifestyle factors (Larson, et al., 1996; Hunter, Gower and Kane, 2010).

2.5.4 Ethnicity

Independent of gender, BMI and sexual maturation, ethnicity is considered a significant correlate of body fat. Daniels, Khoury and Morrison (1997) demonstrated this evidence in a study of 201 white and African American children aged 7-17 years. In another study, the influence of ethnic differences was noted on the total body fat of 8-10-year olds (Lui, et al., 2011). Liu, et al. (2011) found that the participants' total body fat varied with their country of origin. Data reported from the NHANES (1999- 2000) also indicate the ethnic variation in the prevalence of overweight and obesity (Flegal, et al., 2002). Flegal, et al. (2002) found that, in men aged 20 years and over, the prevalence of overweight was 6.7% higher for non-Hispanic whites than non-Hispanic blacks and 14% higher for Mexican-Americans compared to non-Hispanic blacks. A British study similarly found significant ethnic differences in body fat among schoolchildren (Shaw, et al., 2012). The researchers observed that South-Asian boys and girls had the highest percentage of body fat. Body fat distribution has also been demonstrated to be influenced by ethnicity. Results of a Multicultural Community Health Assessment Trial (M-CHAT) indicated that men and women of South-Asian origin of a given body mass index have a higher amount of visceral adipose tissue compared to Europeans (Lear, et al., 2007). Furthermore, black women are noted to have lower visceral adipose than white women, for a given BMI, WHR and waist circumference (Albu, et al., 1997; Perry, et al., 2000). Black women were also shown to have less visceral adipose tissue than their Hispanic counterparts (Carroll, et al., 2008). Fernandez et al. (2003) had a similar finding after analysing 11 cross-sectional studies involving African-Americans, European-American and Hispanic-Americans. The causes of the variance in body fat characteristics between ethnic groups are thought to be related to a combination of behaviour, culture, and physiology.

Several studies have explored the possible reasons for these ethnic differences. In early research examining the differences in resting energy expenditure between white and African American women, Foster et al. (1997) found that the resting energy expenditure was closely related to the body weight and that the former had a higher resting energy expenditure. Melby and colleagues investigated the physiological and behavioural characteristics of young, sedentary, non-obese Caucasian and African-American women of similar age and anthropometric characteristics (Melby, et al., 2000). The authors found that the resting and physical activity energy expenditures were higher in Caucasian-Americans than in African-Caucasians. Other studies have highlighted that social and behavioural factors may contribute towards the ethnic differences in body fat between African and American-Caucasians (Kumanyika, et al., 1993; Stevens, et al., 1994). The researchers stated that attitudinal and behavioural factors might influence the ability to lose weight and maintain weight loss. Regardless of their body weight, African-Caucasian women are half as likely to consider themselves overweight compared to Caucasian women due to differences in body image perceptions and tolerance for overweight. In a weight reduction program, for instance, Glass, et al. (2002) observed that although African-American women responded as well as American-Caucasian women, their drop-out rate doubled that of their Caucasian counterparts. More so, socio-economic factors may explain the ethnic disparities. Residing in deprived social environments with limited access to fresh fruits and vegetables and whole-grain, but with cheap and readily available highly processed foods may influence dietary behaviour and body composition outcomes eventually (Dutko, Ver Ploeg and Farrigan, 2012). In England for instance, ethnic minorities are more likely than the white British to live in the most deprived 10% of neighbourhoods (Ministry of Housing, Communities and Local Government, 2018), and childhood obesity is strongly associated with deprivation (Conrad and Capewell, 2012).

Additionally, most deprived neighbourhoods are marked by the poor quality of built environments with limited access to facilities to encourage physical activity to reduced obesity (Feng, et al., 2010). In some of such neighbourhoods, due to either perception of crime or high levels of crime, the residents are discouraged from using available recreational facilities (Hood, 2005). A study found that increased access to physical activity facilities for residents of deprived neighbourhoods was related to reduced odds of being overweight (Gordon-Larsen, et al., 2006).

Most studies agree that ethnicity influences body fat and is important when comparing body fat between subject groups and when considering the appropriateness of levels of body fat in multi-ethnic populations. However, based on the suggested reasons for its influence, it appears that its influence is uncertain and dependent on several other factors which are not always constant. For instance, in the studies by Carroll, et al. (2008) and Fernandez, et al. (2003) described earlier, it was concluded that Black women had less visceral adipose tissue than their Non-Black counterparts. While acknowledging the researchers' contributions to the literature, since they failed to account for the influence of relevant factors such as the levels physical activity of the participants, dietary intake, alcohol intake and smoking, it is unclear how the participants' ethnicity influenced their body fat. It is essential to consider the influence of the stated factors since it may be erroneous to assume that individuals of a particular ethnicity necessarily have peculiar social or behavioural characteristics.

2.5.5. Sleep

There is increasing evidence on the association between sleep duration and obesity. In some cross-sectional studies, short sleeping duration (< 7hours per night) was linked to increased body fat and a higher risk of obesity than normal sleep duration (7-8 hours per night) (Chen, Beydoun and Wang, 2008; Patel and Hu, 2008). Longitudinal studies have likewise

demonstrated that short sleep duration is associated with higher weight gain than normal sleep duration. The Nurses' Health Study showed that weight gain increased with decreasing hours of night sleep, from ≤ 5 hours to 6 hours, to 9 hours over a 16-year follow-up period (Patel, et al., 2006). It was also reported that those who reported sleeping for 7 and 8 hours per night had the lowest weight gain (Patel, et al., 2006). In the same study, short sleepers were also at a higher risk of developing obesity than the normal sleepers. The Zurich Cohort Study data also showed that sleep duration strongly predicted obesity in longitudinal models (Hassler, et al., 2004). Corresponding findings were obtained in a Spanish cohort of older adults, as women who reported sleeping for ≤ 5 hours per night had more weight gain (≥15kg) and a higher risk of developing obesity compared to normal sleepers after a 2-year follow-up period (Lopez-Garcia, 2008). Chaput and colleagues in their 6-year Quebec Family Study similarly found that short sleepers were at increased risk of weight gain relative to normal sleepers (Chaput, et al., 2008).

Some studies have shown contrasting results. Watanabe, et al. (2010) after a one-year follow-up of a cohort of working Japanese adults, found that a short sleeping duration did not increase the odds of developing obesity in women. Also, Lopez-Garcia (2008), in their Spanish study, failed to find an association between sleep duration and weight gain in men. The reason for the mixed findings in the research associating sleep and body fat is unclear; however, might be related to the variation in sample size, poor control of confounding variables in some studies and the use of self-reported measure for assessing sleep duration. Studies have highlighted inherent errors in self-reported measures of sleep, especially due to difficulty integrating sleep information over long periods with high night-to-night variability in sleep time (Lauderdale, et al., 2009; Appelhans, et al., 2013). Notwithstanding, short sleep duration remains commonly accepted to be associated with an increase in body fat. This is perhaps consequent on the influence of the Nurses Health Study considering that it involved a large cohort (68,183

participants) and a long follow-up period (16 years), coupled with the fact that most other studies which have reported contrasting findings have only observed it only among some of their participants. For instance, in the Watanabe, et al. (2010) study, it was observed that although a short sleeping duration did not increase the odds of developing obesity in women, it did in men. Similarly, Lopez-Garcia (2008) failed to observe an association between sleep duration and weight gain in men but did in the women who participated in the study.

Despite the prevailing epidemiological evidence on the relationship between sleep and body fat, the mechanism involved is unclear. However, some suggest that the regulators of energy balance are affected. Spiegel (2004) described a short sleep duration as associated with a decrease in leptin (signalling satiety) and an increase in ghrelin (signalling hunger). The proposed process seemed promising mechanistically until subsequent research emerged with conflicting findings; either an increase (Omisade, Buxton and Rusak, 2010; van Leeuwen, 2010) or no change (Nedeltcheva, et al., 2009; St-Onge, et al., 2012) in leptin after sleep restriction. Also, ghrelin findings were inconsistent (Nedeltcheva, et al., 2009; St-Onge, et al., 2012). The differences in energy balance states and feeding protocol between the studies might be the reasons for the inconsistent findings. St-Onge (2013) clarifies that these factors can influence hormone secretion and appetite. Additionally, variations in the timing of the sleep-wake schedule and the differences in the structural organisation of normal sleep (sleep architecture) might be responsible for the discrepant results.

2.5.6. Cigarette smoking, alcohol intake, pharmacological agents

Cigarette smoking may result in loss of body fat mass by increasing the metabolic rate owing to the nicotine content. Thus, the observed decrease in energy expenditure with smoking cessation (Dalloso and James, 1984; Chiolero, et al., 2008; Parsons, et al., 2009). Hofstetter, et al. (1986) reported that 24-hr energy expenditure in smokers increased by 140- 200 kcal/day

on a day with smoking in comparison to a day without smoking. Similarly, other researchers found that the resting metabolic rate increased by 6% (Walker, et al., 1992) and 3% (Dalloso and James, 1984) after smoking. Although some studies have failed to observe the corresponding increase in basal metabolic rate after smoking a cigarette (Burse, et al., 1975; Stanford, et al., 1986), there remains a consensus on the increased energy expenditure with smoking. Furthermore, cigarette smoking is also posited to influence body fat mass by altering energy intake through suppressing appetite (Chiolero, et al., 2008). In earlier studies, this influence has been suggested to be due to the nicotine content of cigarettes (Perkins, et al., 1991; Jensen, et al., 2005).

Alcohol intake serves as a means of energy intake, thus favouring a positive energy balance (Tremblay, et al., 1995; Tremblay and St-Pierre, 1996). A 5-year prospective study of middle-aged men observed that regardless of the type of alcohol consumed, heavy alcohol intake (defined as ≥ 30g/day of alcohol) was positively associated with increased body fat (Wannamethee and Shapper, 2003).

Changes in body fat may also occur due to the use of some pharmacological agents such as anti-depressants, anti-hypertensives and hypoglycaemic agents such as insulin (Vendelbo, et al., 2018).

2.5.7. Physical activity

Physical activity refers to any bodily movement caused by skeletal muscle requiring the investment of energy (Caspersen, Powell and Christenson, 1985). Energy expenditure rises above resting or basal levels during physical activity and may vary with the muscles involved, the intensity, duration, and frequency at which the activity is performed (van-Baak, 1999). Body fat oxidation also tends to increase with physical activity, up to approximately 55-65% of the maximum oxygen consumption (VO_2 max). Considering that physical activity favours

energy expenditure, it is frequently considered an important requirement for the regulation of body fat. Indeed, a predominant body of literature asserts the negative relationship between body fat mass and physical activity. In a review on the alterations in energy balance with exercise, Westerterp (1998) noted that a significant decrease in fat mass accompanied an increase in the total energy expenditure varying between 286 kcal and 669 kcal/day during an exercise training intervention lasting up to 4 weeks. Similarly, Wilmore, et al. (1999) in the HERITAGE Family Study observed that among 557 men and women of black and white races, total body fat decreased by 0.7% and 0.9% respectively after 6 weeks of endurance training. Other more recent studies have found otherwise; an inverse relationship between physical activity and body fat mass (Nikolaidis, 2012; 2013; Bradbury, et al., 2017).

Generally, a decrease in body fat after physical activity is expected given that physical activity is a means of energy expenditure. However, some evidence suggests that physical activity may be insufficient for achieving a sustained loss in body fat mass (Hafekost, et al., 2013). In the review, the authors evaluated 27 interventions assessing methods for weight loss or preventing weight gain, many of which acknowledged the complexity of the body's energy balance mechanism. Health statistics from Public Health England (2015) further indicate that physical activity may not necessarily influence body fat mass. In the United Kingdom, despite showing that regular exercise increased by 7% in men and 8% in women from 1977 to 2008, obesity rates during that same period increased by 5% (Public Health England, 2015). A 10-year study on the prevalence of physical activity and obesity in US counties also showed that an increase in physical activity matched an increase in obesity rate during the same period (Dwyer-Lindgren, et al., 2013). Several factors may be responsible for the inconsistency of findings, such as the differences in the intensity, duration and mode of physical activity, including other factors such as adherence, and the means of assessing physical activity between studies. For instance, in the study by Dwyer-Lindgren, et al. (2013), physical activity was self-reported,

while in the HERITAGE Family Study, Wilmore, et al. (1999) monitored physical activity using ergometers. Nevertheless, physical activity is accepted as a means of energy expenditure.

Besides resulting in a negative energy balance, physical activity may also interact with energy intake. Compared to relatively sedentary individuals, those who are physically active tend to consume less or delay eating after exercise. Most researchers have explained this tendency as due to reduced perceived hunger among those who are more physically active (Elder and Roberts, 2007). In agreement with this view, King et al. (1994) clarified that the influence of exercise on hunger was dependent on the intensity of exercise performed. Based on their observation, appetite was suppressed after high-intensity exercise but not after short-term low-intensity exercise. In a subsequent study, the researchers (King et al., 1997) observed that suppressed hunger or reduced appetite occurred within 48-hours of exercise. Melzer, et al. (2005) posited that the perceived hunger resulted from increased glucose free-fatty acids and plasma lactate-acid levels.

2.5.8. Diet

Diet describes the sum of energy and nutrients derived from the consumption of foods and beverages (Aragon, et al., 2017). Evidence highlighting the critical role of diet in body fat abounds and will be discussed in more depth in this review. However, it is noteworthy that the other factors which have been discussed previously are worth considering especially in epidemiological and clinical research concerned with isolating a single factor or group of factors predicting body fat, to enable efficient control of confounding.

2.6. Characterising dietary intake

Food intake represents a critical aspect of nutrition, a process through which nutrients are ingested for sustenance. Food intake may be described with regard to the individuals' eating

pattern or eating event, based on the regularity, timing and frequency, format and context of food intake (Lee et al., 2015). Kearney, Hulshof and Gibney (2001) for instance, explained food intake in terms of the temporal distribution of consumption of food groups, and the intake of energy and nutrients within a meal, claiming it is necessary to provide a specific picture of cultural practices regarding food intake in the development of food-based dietary guidelines. Some researchers (de Castro and Elmore, 1998; Jakubowicz, et al., 2012; Morgan, et al., 2012) agreed with this construct, whereas others have characterised food intake patterns based on the mean number of meals or snacks, the regularity with which they are consumed (Albertson, et al., 2007; Sierra-Johnson, et al., 2008; Popkin and Duffey, 2010), and location (Laska, et al., 2011).

Events of food intake may be participant-identified, considered based on the time-of-the-day, the quantitative and qualitative characteristics of the food consumed (food-based), or simply as eating events. Considering the poor generalisability of the participant-identified, time-of-the-day and the food-based approaches, especially when applied to individuals of varying cultural backgrounds, the use of "eating event" as a neutral term describing an occasion of food intake in epidemiological studies is supported (Makela, et al., 1999). By using this approach, empirical data can be collected and standardised by a criterion suitable to describe the data, which allows for cross-population comparison.

2.7. Estimating dietary intake

Since the examination of diet and disease relationship in the early Framingham and Tecumseh community, diet has been researched as a major lifestyle-related risk linked to a wide range of chronic diseases, based on which both dietary pattern- and nutrient-based recommendations have emerged (Tapsell, et al., 2016; Mozaffarian, Rosenberg and Ricardo, 2018). Unlike other lifestyle risk factors, such as smoking and alcohol use, assessing dietary exposure can be

challenging since it is a common denominator for all individuals, varies in amount and kind consumed between individuals, and can be perceived differently between individuals with regards to quality and quantity (Willet, 1998). Hence, methods used should be able to limit errors arising from these factors to achieve valid and reliable measures of dietary intake, especially as inaccurate dietary assessment may pose challenges to understanding the influence of diet on disease. Dietary assessment describes a process of collation and interpretation of qualitative or quantitative measures of diet obtained from biochemical, anthropometric or clinical studies for survey purposes, screening or surveillance (Gibson, 2005). The measures obtainable in this manner capture the amount made available to the body without details of absorption or assimilation. Broadly, a dietary assessment may be carried out using biological markers, objective observation, or subjective report.

2.7.1. Dietary assessment using biological markers

"Biological markers" are quantifiable markers used in the measurement of a parameter of biological relevance. In dietary assessments, they are used as a substitute measure for long- and short-term dietary intake of specific nutrients or dietary components in epidemiological studies (Kim, et al., 2012; Lim, et al., 2012). Examples of biological markers include doubly labelled water, 24-hour urine nitrogen, and 24-hour urine potassium. Studies correlating biological markers with dietary intake levels have emphasised the absence of challenges regarding subjects' ability to describe the type and quantity of food taken, impacts of social desirability bias and recall challenges (Potischman, 2003). These merits have encouraged the use of biological marker nutritional research.

Notwithstanding the advantages associated with using biological markers, some limitations have been identified. Firstly, considering that some nutritional biomarkers are subject to homeostatic control, challenges in ascertaining the validity of outcome measures abound as a

variation between administered and measured amounts may exist (Kaaks, et al., 2002). Secondly, given that most biomarkers require the collection of body fluid or other body products, they are considered burdensome and expensive to collect and analyse. Furthermore, the specificity of biomarkers to some nutrients limits the assessment of multiple aspects of dietary intake (Lamps, 2008).

2.7.2. Dietary assessment by objective observation

Dietary assessment may also be performed by objective observation, using the duplicate diet method or the food consumption method. The duplicate diet approach involves the collection of duplicate samples of one's diet, which are analysed and then used to estimate their potential dietary intake (Shim, Oh, and Kim, 2014). This method can be used in the measurement of individuals' exposure to environmental, chemical contaminants in food and beverages (Fromme, et al., 2007) but may be unsuitable for large-scale studies due to increased waste involvement.

The food consumption record approach entails the collection of details of one's food preparation and consumption in their home by objective observation of trained research staff. This approach is usually valuable in populations with low literacy rate, or among individuals who prepare most of their food at home. It has been applied in a study of South-Korean households in the National Nutritional Survey (1969-1995) (South Korean Ministry of Health and Welfare, 1997; Kim, Moon and Popkin, 2000), where trained research staff observed and recorded foods consumed in the households concerned for two consecutive days, based on the age, sex and number of household members. Apparently, due to the use of skilled field workers, considerable accuracy of estimates would be expected. However, it may be difficult if applied at an individual level in large population studies. The use of this method can also pose challenges when used among individuals who frequently eat outside the home. More so, the

employment of research staff especially in large epidemiological studies can amount to considerable research cost.

2.7.3. Dietary assessment by subjective report

Dietary assessment by subjective report provides an opportunity for the individual involved to play a key role in assessing their diet. It may occur in the form of a 24-hr food recall, food frequency questionnaire (FFQ), dietary history or dietary record.

2.7.3.1 Food frequency questionnaire

FFQ assesses details of dietary intakes, such as the frequency of intake and portion size over a long period (Thompson and Subar, 2008). Since it is self-administered, it is economical to the researcher and may constitute much less burden in cases where the responses are registered electronically. Nevertheless, significant limitations of this method include being insensitive to portion size (Smith, Job and Mingay, 1991), limiting participants reports by the use of closed-ended responses and the exclusion of other relevant dietary details such as the time interval between eating occasions which could provide more comprehensive information (Shim, e al., Subar, 2014).

2.7.3.2. 24-hour dietary recall

The 24-hour dietary recall is a dietary assessment method which is administered by a trained interviewer, which requires individuals to remember and register dietary details (food and beverages) consumed the previous day (Thompson and Subar, 2008). This technique is usually associated with difficulty distinguishing between meals which one had the previous day and that which they usually have (Guin, et al., 2008), thereby increasing the likelihood of omissions and a tendency to report foods which were not consumed.

2.7.3.3. Dietary assessment by dietary history

Developed by Burke (1947) for assessing long-term dietary intake, this method involves obtaining details of specific foods eaten regularly and those eaten less regularly and using the data obtained to describe food and nutrient intake over periods such as a month or a year. Van-Staveren and colleagues in the Survey in Europe on Nutrition and the Elderly Concerted Action (SENECA) study used this method to assess the diet of elderly adults during one month (van Staveren, de Groot and Havemen-Nies, 2002). Trained professionals usually participate in collecting information on the participant's usual diet through an in-depth interview, often taking over an hour to complete (Shin, Oh and Kim, 2014).

The strengths of dietary history method include the fact that it reveals details of individual foods, covers habitual dietary intake such that a single interview can serve for a specific period under consideration, provides details of food which is not consumed regularly, and the participants from whom data is collected need not be literate. The limitations, on the other hand, include that the interview technique can result in observer bias, participants may have difficulty ascertaining food portion sizes, and accuracy of the data obtained depends heavily on the interviewer’s skill. Also, it may pose challenges for those with irregular eating patterns and is subject to recall bias due to difficulty remembering food consumed in the past. Furthermore, the method is time-consuming and associated with increased participant burden (Shin, Oh and Kim, 2014). Hence, it may not be suitable for use in epidemiological studies.

2.7.3.4. Dietary assessment by dietary record

A dietary record refers to a prospective dietary assessment technique in which respondents are required to record all foods and beverages consumed over a given period. Respondents may also include details of their diets such as information regarding the method of food preparation used, the brand name of commercial food products, and ingredients of mixed dishes, to enhance the accuracy of the assessment. The respondent usually provides dietary data collected in

dietary records while the food is being consumed, hence reducing the reliance on their ability to remember what they ate. Due to the need for the participation of respondents, they need to be trained to provide accurate data. Also, the respondents should be highly motivated since a considerable burden is passed onto them (Thompson and Byers, 1994; Shim, Oh and Kim, 2014).

Dietary records may be taken using free open forms with a structured format, with additional questions requesting participants to provide details of the eating occasion such as the name of the meal (breakfast, lunch, dinner), and time of consumption and location. Structured forms for dietary records may take any format; however, necessarily provide adequate space for participants to records all dietary data required (Ortega, et al., 2015). A pocket diary or notebook can also be provided to enable the recording food consumed away from home (Ortega, Requejo and Lopez-Sobaler, 2009). Close-ended forms may also be used for taking the dietary record. Close-ended forms usually include a list from which the participant indicates the specific food consumed (Thompson and Subar, 2013; Lillegaard, Loken and Andersen, 2007). Portion size estimates can also be requested, either using categories or in an open-ended style (Ortega, et al., 2013; Thompson and Subar, 2013).

In some cases, like when children or individuals are incapacitated, the dietary record can be completed by someone else (Thompson and Subar, 2013). The food recorded is, however, taken during each eating occasion. Records can either be taken on paper or dictaphones, particularly for individuals of low literacy (Thompson and Byers, 1994).

As earlier stated, there is considerable participant burden associated with the dietary record; hence there is a need for training of respondents before obtaining records. Respondents should receive adequate training to enable them appropriately to describe the foods consumed, portion sizes, recipes, and method of cooking. Training may also be reinforced by contacting the

respondent after the first day of taking records, to resolve ambivalence arising. Contact with the respondent can also be made after the duration of the study to clarify the dietary details supplied (Thompson and Subar, 2013).

Precise portion size estimation in dietary records is necessary for accurate estimation of food consumed by the respondent. Portion sizes can be estimated by various methods such as using a kitchen weighing scale or household measures such as bowls, cups, and spoons. The use of household measure for estimating food portion size may increase the likelihood of bias due to variation in the sizes and designs of household wares and difficulty gauging some foods with household measures (Gibson, et al., 2016). For instance, a slice of pizza cannot be accurately measured using a cup or spoon. Alternatively, portion size estimates can be made based on standard two- or three-dimensional photographic aids (Thompson and Subar, 2013). When standard measures are used by the participants to describe the measures of food consumed, training is also necessary for the coders or recipients of the data to enable the transformation of the measures received into grams of food consumed. A Software which incorporates a comprehensive database with information on the weight of standard portion sizes or household measures can be used to process dietary records. DIAL (Ortega, et al., 2013) is a typical example of a software which can facilitate dietary record processing. Notwithstanding the need for such software, it may be challenging to find a single software package which has a comprehensive database of foods, especially in multi-ethnic populations.

Another important aspect of dietary records is the length of time for collecting dietary data from the respondents. It is important to establish a suitable duration for dietary records, as well as whether data should be collected consecutively or not. Ideally, dietary records should only last for some days, long enough to provide valid and reliable information of respondents' usual diet. At the same time, this period should be balanced against the risk of losing motivation leading to poor compliance (Ortega, Requejo and Lopez-Sobaler, 2009; Ortega and Requejo,

2000). Typically, dietary records are taken for seven consecutive days to minimise error related to differences in food intake during the days of the week and capture information on foods consumed less frequently (Ortega, et al., 2015). However, research indicates that recording dietary information for more than four consecutive days provides unsatisfactory results as food intake decreases due to respondents becoming tired. Also, when dietary records are taken over a long period, respondents who comply may be markedly different from those who do not (Ortega and Requejo, 2000; Ortega, Requejo and Lopez-Sobaler, 2009).

Furthermore, with prolonged duration for dietary records, respondents have been noted to complete the records retrospectively instead of simultaneously with dietary intake towards the latter days of recording (Thompson and Subar, 2013). Hence, the reliability of dietary records collected for seven days tends to decrease in the latter days compared to data collected in the earlier days (Ortega and Requejo, 2000; Thompson and Subar, 2013). The 4-day food record has been demonstrated to be valid for dietary assessment (Liberato, Bressan and Hills, 2009) and as a reference in validation research (Steinemann, et., 2017). Although studies have argued that decreasing the number of days does not necessarily guarantee valid dietary data and that the optimal number of days for obtaining valid dietary record depends on the size of the sample under study and the nutrient of interest (Basiotis, et al. 1987; Ortega, et al., 2015), some investigators (Ortega, et al., 2015) have failed to clarify on a minimum or maximum duration corresponding to specific sample sizes or nutrients. Others (Basiotis, et al., 1987) have stated, for instance, that less extensive dietary records are required to estimate energy intake compared to vitamin A intake. In a study of the dietary records of 29 adults, Basiotis and colleagues concluded that an average of 31 and 433 days was required to predict an individual's usual energy and vitamin A intakes, respectively. They also indicated an average of 3 and 41 days for predicting the group's energy and vitamin A intakes, respectively (Basiotis, et al., 1987). The estimates were determined based on dietary records taken for 365 consecutive days from

adults who participated in another study (Mertz and Kelsay, 1984). Considering that dietary data taken over a long period increases the risk of compromising participants' compliance and the validity of dietary data provided (Lopez-Sobaler, 2009; Thompson and Subar, 2013), the feasibility of applying such duration in practice as recommended by Basiotis, et al. (1987), especially for a large cohort is questionable.

The use of consecutive days in traditional dietary records has been argued to be limited in reflecting one's usual diet, since foods and amounts eaten on consecutive days may be related (Ortega, et al., 2015). Hence, the use of non-consecutive day records to reliably reflect one's usual diet has been recommended (Thompson and Subar, 2013). Additionally, controlling for working and weekend days is also necessary to reflect overall diet (Thompson and Subar, 2013). Collection of dietary records in different seasons has also been highlighted in the existing literature to control for influences of variation of food availability and preparation with the season. Therefore, recording specific dates of the dietary record is encouraged (Ortega and Requejo, 2000; Ortega, Requejo and Lopez-Sobaler, 2013; Thompson and Subar, 2013).

Dietary records are applicable in diverse groups of people with a range of eating habits (Thompson and Subar, 2013; Shim, Oh and Kim, 2014), and in intervention studies to gain knowledge of dietary habits (Glanz, et al., 2006; Thompson and Subar, 2013). Thompson and Subar (2013) have also claimed that engaging in the recording is a useful measure for weight loss. Perhaps this claim is related to keeping dietary records as a means of self-monitoring, as it can make one more conscious of their dieting behaviour. Self-monitoring has been identified as the centrepiece of behavioural weight loss intervention, and significantly associated with weight loss (Burke, Wang and Sevick, 2011).

Regardless of the advantages associated with using dietary records, critics have underscored some limitations. For instance, the limited number of days applied may only reflect one's

current dietary intake rather than their usual dietary intake (Ortega, et al., 2015). Also, due to the level of required participation of the respondents, they should be both literate and motivated (Ortega, et al., 2015). More so, it has been argued that although training of respondents can reduce the possible difficulties associated with using dietary records, it can result in increased cost and may not be feasible in low-budget research. Furthermore, since motivation is highly necessary for compliance of the participants through the entire course of recording, it may pose a selective influence on the participants thereby limiting the generalisability of the findings (Thompson and Subar, 2013). Besides, the use of dietary record techniques such as weighed food record may be complex and unfeasible in situations where meals are consumed outside the home.

Another aspect of the burden posed by the dietary record is that which is experienced by the researcher who is charged with the responsibility of collecting and analysing the data. Unless aided by electronic devices, coding of dietary records with an open-ended format, data entry and analysis may pose significant challenges. Also, where portion-size has been identified in terms of house-hold measures, conversion to actual weight must be done, before matching with the food in a food composition database. These processes, though feasible, can be time-consuming and laborious to implement (Shim, Oh and Kim, 2014), except if automated with the use of computer software (Thompson and Subar, 2013). Due to these limitations, using a dietary record may not be practical for large population studies.

The sources of error in the use of dietary records may either be due to the respondent or the field worker. Those due to the respondent include poor literacy skills, lack of motivation, poor perception of the type and amount of food consumed, and personal characteristics (sex, age, being obese). Supporting evidence indicates that an individual's characteristics can influence their dietary records. For instance, underestimation of energy intake tends to increase with weight (Ortega, et al., 1995; 1997). And individuals who intend or wish to lose weight have a

higher tendency to underestimate their energy intake compared to their counterparts who do not want to lose weight (Ortega, et al., 1996; 1997). The sources of error due to the field worker include inadequate training necessary to instruct respondents undertaking the dietary record, errors in coding and entering dietary data, poor checking of dietary details received from respondents. Other errors which are neither due to the field worker nor the respondent may also arise due to errors in the food composition database used regarding the equivalent weight of portion sizes and nutritional composition of foods (Ortega, et al., 2015). Despite these limitations, dietary records are generally considered the "gold standard" against which other dietary assessment methods can be validated (Thompson and Byers, 1994; Willet, 1998; Thompson and Subar, 2001).

2.7.4. New technologies for dietary assessment

New technologies developed for dietary assessment have improved on some of the drawbacks of traditional methods by; reducing respondents' burden, making multiple self-administrations feasible and improving the accuracy and increasing feasibility of dietary records in large epidemiological studies (Schatzkin, et al., 2001; Ekman and Litton, 2007; Schatzkin, et al., 2009). Some of these new technologies include personal digital assistant (PDA), mobile phone-based technologies and interactive computer-based technologies (Illner, et al., 2012).

2.7.4.1. Personal digital assistant (PDA) technologies

The first application of personal digital assistants for dietary assessment was in the mid-1990s (Kos and Battig, 1996). Since then, although progress in the development of the equipment and software has continued, the concept of its application remains similar. Using the PDA, participants are first taken through some face-to-face training after which they are instructed to record foods and beverages which they have consumed by selecting the specific foods on an integrated menu (Kos and Battig, 1996). PDAs offer respondents options of food selections

over 4000 items (McClung, et al., 2009). The devices also provide the participants with some portion-size estimation aids to enhance quantifying the amounts of foods which they have consumed. Some may instruct users to quantify the foods consumed using traditional food models and portion-size aids (Fowles and Gentry, 2008; McClung, et al., 2009). While others may display photographs and equivalent amount (in grams) of foods selected and allow the user to adjust the displayed portion size to the amount consumed (Fukuo, et al., 2009). Devices also provide an opportunity for other assessments such as recording food labels with the nutritional information of purchased foods (Beasley, Riley and Jean-Mary, 2005), or qualitative dietary information related to dietary habits (Fukuo, et al., 2009). Also, devices often integrate personalised feedback and recording alerts (Illner, et al., 2012).

2.7.4.2. Mobile phone-based technologies.

Mobile phone technologies allow for short-term dietary assessment by capturing "real-time" eating events. They differ from PDAs in that the eating events are not based on a manual selection from a pre-defined listing of food items. The Japanese "Wellnavi" is a variant in this category which directs respondents to take photographs of their foods and beverages before and after eating. The captured images are then sent through a mobile phone network service to the study dietitians (Wang, Kogashiwa and Kira, 2006). Also, while capturing images, the respondents are advised to include an object of known size in the photograph to help the dietitian in estimating the portion sizes of foods consumed.

The American Mobile-phone food record developed in 2010 applies a similar procedure (Six, et al., 2010). When the dietitian receives photographs, food identification and portion-size estimation are based on automated visualisation and digital image and segmentation analysis using custom software (Yang, et al., 2008). More so, "spoken dietary record" can also be provided by respondents in the form of recorded verbal dietary information, including portion-size descriptions and recipe ingredients (Lacson and Long, 2006).

2.7.4.3. Interactive computer-based technologies

Interactive computer-based technologies are used to record food and beverage intake either recently or in the past. They have built-in comprehensive systems for probing, coding and analysing food intake through multimedia features and electronic data transmission. Digital photographs and corresponding frequency categories of single foods and dishes are displayed using standard portion sizes, and a food frequency questionnaire reads aloud any reminders, question and answers on the screen (Wong, et al., 2008). Interactive technologies based on the Diet History Questionnaire (DHQ) technique are also available as a self-interviewing tool (Edwards, et al., 2007). Similar technology has also been developed for 24-hour dietary records for low-literacy populations (Zoellner, Anderson and Gould, 2005). The device functions by displaying a brief introductory instruction and allows respondents to listen and select their answers by choosing the appropriate selection from the available options. The particular merit of this technology is that the digital photographs of portion-sizes displayed can be interactively adopted by the respondent (Zoellner, Anderson and Gould, 2005).

2.7.5. Strengths and weaknesses of innovative technologies

2.7.5.1. Dietary food records versus The PDA and mobile phone-based technologies

Compared to the weighed food records, the PDA and mobile phone-based technologies provide a means for facilitated real-time dietary data collection, entry and coding. They can also foster high motivation and reasonable compliance using automatic reminders. Also, there is better standardisation and quality control of records. However, the requirement for training and involvement of high technical development efforts can be expensive. The safety of security infrastructure may also become an issue since digital data transfer is involved (Beasley, Riley and Jean-Mary, 2005; Yon, et al., 2006; Arab, 2010; Chen, et al., 2010). Furthermore, in addition to the advantages accruing to the use of PDAs and mobile phone technologies, the

camera- and tape machine-based devices can be used for assisted dietary assessment in children or specialised groups (Swanson, 2008).

Studies evaluating the validity of a PDA having a camera and mobile phone card against one-day weighed food records found that, of energy and 32 nutrients intakes, there was no difference between the nutrient estimates of both methods, except for manganese, zinc, vitamin E, dietary fibre, saturated fatty acid and polyunsaturated fatty acid (Wang, Kogashiwa and Kira, 2006; Kikunaga, Tin and Ishibashi, 2007). Also, in those studies, 33 nutrient estimates obtained by both methods were observed to be positively correlated, with a mean Spearman's correlation coefficient of 0.62 (Spearman's correlation coefficient range: 0.21 to 0.86). Research involving 75 normal weight and obese adults concluded that although the nutrient intake estimates of the PDA having a camera and mobile phone card were positively correlated to those obtained using a five-day weighed food record (Spearman correlations coefficient: range=0·32–0·77; mean=0·47), they were significantly higher by an average of 17% (Kikunaga, Tin and Ishibashi, 2007).

2.7.5.2. 24-hour dietary recall versus interactive computer and web-based innovative technologies

The traditional methods for dietary assessment have some advantages (Section 2.7.3), however, their drawbacks such as the literacy requirement of respondents, reliance on memory, a limited number of days necessary to reflect usual dietary intake, increased likelihood of interviewer bias and high probability of incomplete data and measurement error, have reduced their desirability for use in epidemiological studies (Illner, et al., 2012; Shim, Oh and Kim, 2014; Rodrigo, et al., 2015). The interactive computer- and web-based technologies improve on these weaknesses and may increase the motivation of participants by providing an interactive platform with audio and visual guides. However, the technologies are limited in that they require literacy and a computer for efficient use. Also, the use of these technologies may result

in altered dietary behaviour and result in erroneous records (Arab, et al., 2010; Six, et al., 2010). The security of data transferred using the web-based system may also become a concern (Wang, Kogashiwa and Kira, 2006; Apovian, et al., 2010; Arab, et al., 2010; Six, et al., 2010). Moreover, more studies are needed on the feasibility of these approaches in various populations (Illner, et al., 2012).

A comparative study of 63 pregnant women evaluating dietary records obtained by a web-based dietary assessment tool and those obtained using a four-day food diary concluded that there was a correlation between the nutrient estimates of both methods (Spearman's correlation coefficient >0.80) for energy and macronutrient intake (Benedik, et al., 2015). Furthermore, in an earlier study, after comparing 48 nutrient estimates obtained by paper and web-based dietary records among 16 pregnant women, the authors found that except for four nutrients (free sugars $p< 0.001$, α-linolenic acid $p= 0.041$, folate $p= 0.036$, and pantothenic acid $p= 0.023$), there were no significant statistical differences among 44 nutrients (Benedik, et al., 2014). They concluded that nutrient estimates from both methods were comparable across a range of nutritional parameters, with a few exceptions (Benedik, et al., 2014).

Since there are different methods of dietary assessment, the preference for a specific method may depend on its validity for the purpose for which it is required (Shim, Oh and Kim, 2014). The practice of administering a combination of dietary assessment methods to address methodological limitations of specific techniques is becoming an increasingly popular practice (Illner, et al., 2011; Carroll, et al., 2012). If the methods are demonstrated to be valid and reliable, using a combination of methods can allow the strengths of one to make up for the weaknesses of the other. Also, the practice can provide more comprehensive information and illustrate hidden relationships between dietary intake characteristics. For instance, in nutrition surveillance surveys, the administration of multiple 24-hour dietary recalls with a non-quantitative food frequency questionnaire and the application of statistical modelling can reveal

the relationship between the probability of consuming a specific food (deduced from the non-quantitative food frequency questionnaire) and the quantity consumed on a particular day (deduced from the multiple 24-hour dietary recalls) (van Klaveren, et al., 2012). Despite the benefit of combining dietary assessment methods, the concomitant increase in respondent burden and research cost should be considered.

Dietary intake can be assessed through various methods and have been reviewed in this thesis because they represent a prerequisite for investigating the relationship between dietary intake and health. Further on in this thesis, the association between dietary intake and body fat composition will be discussed.

2.8 Dietary intake and adiposity

2.8.1 Dietary patterns

The consumption of an optimal diet for the maintenance of health and disease prevention remains a public health priority. Historically, nutrition research investigating the relationship between diet and body composition has focussed on specific nutrients (Garcia, et al., 2009; Mikhail, 2009; Kelishada, et al., 2010; Azab, et al., 2014). Although this approach has provided some epidemiological insight, it seems to have some limitations: First, it fails to recognise that food is consumed in combination and that inter-nutrient interactions and synergies could occur (Cespedes and Hu, 2015; Tapsell, et al., 2016). Also, despite how clear a public health recommendation proposed using the approach may seem, it may not be easily translatable into eating behaviours since it does not provide a composite measure of dietary quality. Hence, in recent years, nutritional epidemiology has shifted focus towards exploring dietary patterns and health associations to reflect the complexity of overall diet consumed in a population and dietary preferences available in the "real world" (Hu, 2002; Schulze and Hoffmann, 2006). Prominent approaches developed to assess dietary quality include theoretically oriented

approaches which are *a priori*, and data-driven or empirically derived approaches which are *a posteriori* (Moeller, et al., 2006; Schulze and Hoffman, 2006). Further on in this review, each dietary pattern will be discussed in more detail, especially in relation to adiposity.

2.8.1.1. A priori dietary patterns

A priori dietary patterns are theoretically derived approaches represented by pre-defined dietary scores based on dietary guidelines, which are developed using previous scientific evidence. Various *a priori* dietary indexes are developed to assess adherence to established dietary patterns. Frequently, the indexes describe differences in the same dietary pattern, such as the Mediterranean diet, or use various scoring schemes, such as population-specific intakes (Dietary Approaches to Stop Hypertension (DASH)), or specific cut-offs for recommended intakes (Alternate Healthy Eating Index) (Kant, 2004; Gibson, 2005; Waijers, Feskens and Ocke, 2007; Fransen and Ocke, 2008; Bhupathiraju and Tucker, 2011).

2.8.1.1.1 Mediterranean Diet Score

The Mediterranean diet was first defined by Ancel Keys and colleagues, after noting a lower incidence of cardiovascular disease in some Mediterranean countries (Keys, et al., 1985). The Mediterranean diet refers to the dietary pattern characteristic of some countries in the Mediterranean Basin in the early 1960s, such as Spain, Greece and Italy (Willet, et al., 1995). The diet is characterised by a significant consumption olive oil, fruits, cereals, vegetables, nuts and legumes, a moderate to low intake of dairy, fish and wine (consumed at mealtime), and a low intake of meat and meat products (Willet, et al., 1995). The Mediterranean Diet Score was first developed in 1995 (Trichopoulou, et al., 1995) and revised later to include fish intake and is known to be the most commonly investigated pre-defined dietary pattern (Trichopoulou, et al., 2003). The score for adherence to this diet ranges from minimal adherence to maximal

adherence, represented by 0 and 9, respectively. For individuals who consume beneficial dietary components, such as fruits, vegetables, legumes, nuts, cereals, monounsaturated and -saturated lipid, and fish, below the sex-specific median are assigned a value of 0, otherwise, 1. Those who consume "detrimental" dietary components such as dairy and meat below the sex-specific median value are assigned a value of 1; otherwise, 0. Also, within this scoring system, the consumption of ethanol between 10g and 50g, and 5g and 25g per day for men and women, respectively, are assigned a score of 1.

Since the development of the Mediterranean Diet Score, several modifications have emerged (Bach, et al., 2006). However, despite the variations, the association with body fat has been somewhat consistent. Boghossian et al. (2013) in a cross-sectional analysis, found that adhering to the Mediterranean dietary pattern was significantly related to lower measures of regional and total body fat assessed by anthropometry and dual X-ray absorptiometry. This association remained even after adjusting for potential confounders such as physical activity, age, education, ethnicity, and energy intake. Other previous observational studies, despite varying in terms of study design, study population and measures of adiposity, have achieved similar results. Three cohort studies, with follow-up periods ranging from 5- 9 years observed that individuals who had poorer adherence to the Mediterranean diet pattern were more likely to gain weight or become overweight and obese than those with better adherence (Woo, et al., 2008; Beunza, et al., 2010; Romaguera, et al., 2010). More so, in nine cross-sectional studies which examined BMI and waist circumference as measures of adiposity, adherence to the Mediterranean dietary pattern was inversely associated with the adiposity outcomes (Schroder, et al., 2004; Fung, et al., 2005; Schubair, McColl and Hanning, 2005; Trichopoulou, et al., 2005; Panagiotakos, et al., 2006; Panagiotakos, et al., 2007; Rossi, et al., 2008; Romaguera, et al., 2009; Schroder, et al., 2010). Two studies, however, have contrasting findings. In the study of a Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-

Spain), although the authors stated that adhering to the Mediterranean diet pattern could in part be beneficial in combatting overweight and obesity, they failed to find any significant association between adherence to the diet and weight gain (Mendez, et al., 2006). Also, Sanchez and colleagues found no relationship between adherence to the Mediterranean diet pattern and weight gain after adjusting for the relevant confounders (Sanchez, et al., 2006).

Based on the characteristics of Mediterranean diet scores reviewed in various studies (Bach, et al., 2006; Hernandez-Ruiz, et al., 2015), the heterogeneity of Mediterranean Diet adherence scores increases the likelihood for differences in analyses and findings. In other words, it might be challenging to compare the results between studies that have not applied the exact Mediterranean score. Also, the availability of different scores raises the potential for confusion on choosing a specific score. Studies (Terwee, et al., 2007; Carbenero, et al., 2008) have emphasised that the knowledge of the quality criteria offered by the score is necessary for proper use. In a systematic review evaluating the conceptual suitability, applicability, as well as the psychometric properties of scores developed internationally for measuring adherence to the Mediterranean diet, Zaragoza-Martí and colleagues observed that very few scores fulfilled the applicability parameters and psychometric quality (Zaragoza-Marti, et al., 2017). Notwithstanding, the authors stated that the use of scores to measure adherence to the Mediterranean diet was a valuable tool for identifying the dietary patterns of the population.

The potential protective role of the Mediterranean diet on adiposity is considered to lie in the overall anti-oxidant and anti-inflammatory impact of its components (olive oil, fruits, vegetables, cereals, whole grains and fish) (Schroder, 2007; Dai, et al., 2008; Gaskins, et al., 2010).

2.8.1.1.2. Healthy Eating Index

The Healthy Eating Index is a 10-component *a priori* dietary pattern consisting of five food groups (fruit, vegetable, grains, meat and milk) and four nutrients (saturated fatty acids, total fat, cholesterol and sodium), with scores ranging from 0 to 100. It was initially proposed by the United States Department of Agriculture as a measure of adherence to the Dietary Guidelines for Americans and the Food Guide Pyramid (Kennedy, et al., 1995), and subsequently modified into the Alternative Healthy Eating Index (AHEI). The AHEI was developed to assess the intake of food groups and macronutrient associated with a reduced risk of chronic disease (McCullough, et al., 2002). In comparison with the Healthy Eating Index that was developed initially, it constitutes 9 components (vegetables, fruit, nuts and soy protein, white meat to red meat ratio, cereal fibre, trans-fat, polyunsaturated-to-saturated fat ratio, duration of multivitamin use, and alcohol) with overall scoring range of 2.5–87.5 (McCullough, et al., 2002).

Several studies have examined the relationship between the HEI and measures of adiposity. Guo and colleagues used a cross-sectional analysis of data from 10,930 participants who took part in NHANES III to investigate the relationship between HEI and obesity (Guo, et al., 2000). The researchers found that a low HEI score was linked to overweight and obesity. In another study, individuals on the highest HEI quartile were less likely to be overweight or obese (34%) or have increased waist circumference (35%) when compared with those on the lowest quartile (Nicklas, O'Neil and Fulgoni, 2013). Tande, Magel and Strand (2010) similarly found that, in both men and women, the risk of abdominal obesity significantly reduced by 3.1% and 2.6% respectively with an increase in HEI score. Others have reported likewise (Guo, et al., 2004; Gao, et al., 2008; Lassale, et al., 2013; Erfani, et al., 2017), among which two studies (Lassale, et al., 2013; Erfani, et al., 2017) used the most recent version of the Healthy Eating Index (HEI-2010). In contrast, some researchers have not found an association between the Healthy Eating

Index and adiposity measures. In a prospective cohort study involving 1474 participants in Tehran, Asghari, et al. (2012), found no association between the HEI-2005 and obesity or abdominal obesity.

Research has also indicated the relationship between AHEI and adiposity. In a cross-sectional study involving 1,480 adults recruited from seven European countries, it was found that the AHEI diet-quality score was significantly negatively associated with measures of adiposity such as the BMI and waist circumference (Fallaize, et al., 2018). In contrast, another study found that the AHEI was not associated with BMI (Khakpouri, et al., 2019). Khakpouri and colleagues in their cross-sectional study of 748 Iranian men observed that there was no difference between the AHEI dietary scores in obese and normal-weight men. The variation in the results between both studies might be related to the demography involved. Since men have been observed to have a lower AHEI diet-score compared to women (Fallaize, et al., 2018; Pestoni, et al., 2019), perhaps this factor might have influenced the Iranian study (Khakpouri, et al., 2019). The differences between the findings of both studies may also be due to the cultural differences in dietary behaviour.

2.8.1.1.3 Dietary Approaches to Stop Hypertension Diet Score

The Dietary Approaches to Stop Hypertension (DASH) diet score is a dietary pattern which emphasises the intake of vegetables, fruits, low-fat dairy foods, poultry, nut, fish, legumes and whole grains, and limits the intake of red meat, added fats and sugar-sweetened foods. It is an established dietary pattern in the prevention and control of hypertension (Siervo, et al., 2014). A cross-sectional study of 2,830 of Iranian adolescents and children concluded that there was an inverse non-significant relationship between adherence to a Dietary Approaches to Stop Hypertension diet and obesity, after adjusting for potential confounders. Furthermore, two American cross-sectional studies among diabetic youth investigating the influence of adherence to the DASH diet on adiposity among diabetic young people found no association

(Gunther, et al., 2009; Liese, et al., 2011). Two cross-sectional studies on 1,441 Korean pre-school children (Shin, Oh and Park, 2007) and Iranian children and adolescents (Golpour-Hamedani, et al., 2017) also found no relationship between the DASH diet and weight status. On the contrary, other studies have demonstrated a protective effect of the DASH diet on obesity. In two studies involving diabetics and patients with metabolic syndrome, Azadbakht, et al. (2005; 2011) reported that adherence to the DASH diet was associated with a lower risk of obesity. Folsom, et al. (2007) as well as Barak, et al. (2015) likewise found that the DASH diet was inversely related to central obesity among 293 nurses, even after controlling for potential confounders. The different findings might b related to factors such as sample size, the validity of the dietary assessment measure used, variation in the definition of the DASH diet among studies, and the study population.

The biological mechanism for the influence of the DASH diet on adiposity is unclear. However, it is known that the DASH diet stimulates satiety as a low-energy-density and a low glycaemic index diet (Schwingshackl and Hoffmann, 2013). Also, the high amounts of calcium, dairy products and fibre of the DASH diet are inversely associated with obesity (Azadbakht, et al., 2005; Lindstrom, et al., 2006).

2.8.1.1.4 Other a priori dietary patterns

In addition to the previously mentioned dietary patterns, others include the Healthy Diet Indicator (HDI), Diet Quality Index (DQI), and Recommended Food Score (RFS). The HDI was developed by Huijbregts, et al. (1997) based on adherence to the dietary guidelines for nutrients and food components given by the World Health Organisation (World Health Organisation, 2003). It constitutes nine components (protein, total carbohydrates, monosaccharides and disaccharides, dietary fibre, cholesterol, saturated fatty acids and poly-unsaturated fatty acids, seeds and nuts, pulses, fruits and vegetables), and the scores range from 0 to 9. Where one meets the dietary guideline, they score 1, otherwise, 0.

The DQI consists of eight components and is based on the food group and nutrient recommendations from the American Food and Nutrition Board (cholesterol, total fats, saturated fatty acids, sodium, calcium, proteins, bread, cereal and legumes) (Patterson, Haines and Popkin, 1994). The RFS was developed by Kant and colleagues based on the recommendation of the Dietary Guidelines for Americans and includes low-fat dairy products, cereal products, fish, fruits and vegetable) (Kant, et al., 2000).

2.8.1.2. A posteriori dietary patterns

A posteriori dietary patterns are assessed through exploratory or data-driven approaches and include factor analysis and cluster analysis (Kant, 2004; Waijers, Feskens and Ocke, 2007; Bhupathiraju and Tucker, 2011). The factor analysis approach (also known as principal component analysis) involves the identification of food items and groups that are frequently consumed together, based on the degree of correlation with one another (Schulze and Hoffmann, 2006; Moeller, et al., 2007), while cluster analysis involves the derivation of dietary patterns based on the variation in dietary intake between individuals separated into mutually exclusive groups (Hu, 2002; Moeller, et al., 2007). In cluster analysis, individuals are assigned to a single cluster, whereas in factor analysis, individuals are scored based on their level of adherence to a specified dietary pattern. Both data-driven approaches, when used to generate dietary patterns, tend to limit bias related to making prior assumptions while using existing data to characterise diet, which results in meaningful, interpretable and reproducible results across populations (Moeller, et al., 2007).

Several studies have investigated the relationship between *a posteriori* dietary patterns and adiposity. Tucker et al. (2015) using a cross-sectional design, examined the extent to which independent dietary patterns identified by factor analysis accounted for differences in BMI and

body fat percentage among 281 healthy smokers. Each dietary pattern identified ("Prudent" (high in fruits, vegetables, legumes, fish, and poultry), Low-fat milk, and Meat dietary patterns) was significantly correlated with the measures of adiposity; higher intake of the "prudent" dietary pattern corresponded with lower BMI ($F= 4.4$, $p= 0.0363$) and body fat percentage; the Low-fat milk pattern was inversely associated with body fat percentage and BMI; increased intake of the Meat pattern was associated with higher levels of BMI and body fat percentage. Likewise, another study of 291 post-menopausal women found that those who had high scores of the "prudent" pattern were less likely to be obese (Paradis, et al., 2009). Although other studies have similarly used the factor-analysis approach, a variety of dietary patterns have been researched (Okubo, et al., 2006; Shin, Oh and Park, 2007; Lioret, et al., 2008; Paradis, et al., 2009; Nkondjock and Bizome, 2010; Cho, Shin and Kim, 2011; Denova-Gutierrez, et al., 2011; Hamer, et al., 2013; Chan, et al., 2014). Due to the variety of dietary patterns employed in different studies and consequent disparity in the categorisation of factor scores, it becomes difficult to compare the findings obtained between studies regarding the relationship between dietary patterns and adiposity. A systematic review of 18 studies also highlights this challenge (Mu, et al., 2017). Although the authors concluded that there was an inverse relationship between a "prudent" or healthy dietary pattern and the risk of overweight or obesity, and a positive association between a Western or unhealthy diet and the risk of overweight or obesity, they acknowledged excluding some studies in the meta-analysis due to inconsistencies in factor score categorisation.

The use of dietary patterns constitutes some merits which include permitting examination of the combined influence of the consumption of a variety of foods and nutrients, and being able to reflect the complex and multidimensional nature of diets and "real world" diet preferences (Hu, 2002; Schulze and Hoffmann, 2006). Also, they are more amenable to public health practice (Cespedes and Hu, 2015) and when applied carefully, they can result in meaningful,

interpretable and reproducible findings across populations (Moeller, et al., 2007). Nonetheless, they have some limitations. First, the effects of specific nutrients, though crucial, may be obscured by the influence of the overall dietary pattern (Hu, 2002; Kant, 2004; Schulze and Hoffmann, 2006). Hence it becomes difficult to understand and appreciate the specific roles of the constituent nutrients in the diet when applying overall dietary patterns. Although previously it has been argued that studying nutrients rather than dietary patterns does not give a realistic picture of what people eat (Cespedes and Hu, 2015), it is important to note that regardless of the dietary pattern or approach used in deriving dietary patterns, the basic elements- nutrients- are the drivers of the observed effects. Another limitation is that the use of dietary patterns in long term trials may be associated with poor dietary compliance and high prohibitive cost (Cespedes and Hu, 2015).

Furthermore, due to the availability of various dietary patterns and approaches employed in designating and categorising dietary scores, it becomes challenging reaching a consensus on the specific score to be applied (Zaragoza-Marti, et al., 2017). More so, some adherence scores despite being used to dietary patterns are of questionable validity and reliability. A recent systematic review evaluating Mediterranean diet adherence scores showed that of 27 studies which yielded 28 adherence scores, only a few scores met the quality criteria given by the Scientific Advisory Committee (SAC) of the Medical Outcomes Trust based on; applicability (demands on the respondent and administrator, interpretability), conceptual suitability (cultural and linguistic adaptation, conceptual and measurement model) and psychometric properties (responsiveness, validity and reliability) (Zaragoza-Marti, et al., 2018). Among the adherence scores considered by authors (Zaragoza-Marti, et al., 2018), those stated to meet the applicability parameters and psychometric quality standards include scores developed by Sotos-Prieto, et al. (2015), Panagiotakos, et al. (2006), and Buckland, et al. (2009). Also, in a study of the validity and reliability of the Diet Quality Index (DQI) for assessing the dietary

pattern of Australian preschool children, it was shown that the DQI was reliable but of weak validity (Kunaratlam, et al., 2018). These limitations of dietary patterns put together do not imply that dietary patterns are contraindicated for use in nutrition and epidemiological research. However, they indicate that the seeming epidemiological shift from nutrient-focussed research to dietary patterns appears more to be influenced by public health research needs. For instance, the epidemiological transition from an era of undernutrition and nutritional deficiencies to that of chronic diseases observed in most high-income countries and some low and middle-income countries (Global Burden of Disease, 2013), and the need for creating dietary recommendations which are more amenable to translation and public health practice are posited to be some of the influential factors (Tapsell, et al., 2016).

Nutrient-focussed research continues to be important, serving to identify specific interactive patterns and to promote a mechanistic understanding of diet effects by dismantling knowledge regarding the essential nutrients and quantities needed from foods (Tapsell, et al., 2016). The mechanisms by which the various dietary patterns perpetuate their benefits are only understood through nutrient-focussed research. Also, without nutrient-focussed research, it may be challenging to address suboptimal nutrient intake arising from poor dietary choices. Furthermore, although nutrient-based recommendations are generally considered less amenable to translation and public health practice than dietary patterns, they may become necessary when a specific nutrient poses a disease risk or is etiologically relevant with regard to a particular disease. For instance, the association between trans-fatty acids derived from partially hydrogenated oils and the risk of heart disease (Mozaffarian, Aro and Willet, 2009), and the role of folate in preventing neural tube defects (Willet, 2013).

Additionally, a critical benefit of the nutrient-focused approach is that it emphasises that nutrients are responsible for specific metabolic processes that can be compromised in the event of inadequacy. Over the past 40 years, nutritional epidemiological research has highlighted a

variety of influences of nutrients on health, including the underlying mechanisms. The roles of specific nutrients in adiposity are reviewed in further sections of this thesis.

2.9 Nutrient intake and adiposity

Since the characterisation of obesity and overweight by the presence of excess energy storage in the form of fat, considerable research has investigated the link between energy intake and body fat composition. Nevertheless, whether obese people consume more energy compared to lean people remains a source of controversy (Hafekost, et al., 2013; Ladabaum, et al., 2014). Considering that energy is mainly derived from macronutrients in the diet (carbohydrates, proteins and fats) (Prentice, 2005), the potential link between their intake and body fat has been reported in the existing literature.

2.9.1 Dietary fat

A high-fat diet may promote the development of excess body fat (Bahceci, et al., 1999; Cheverud, et al., 1999; Astrup, et al., 2000; Blundell and Cooling, 2000; Maffeis, et al., 2001) based on the premises that it contains more energy than other macronutrients (up to 2.25 times more than carbohydrates and proteins), and is prone to overconsumption as it contributes to the flavour and palatability of foods and has a low satiety value (Rolls and Hammer, 1995; Rolls, et al., 1999). Also, when studied under metabolic conditions, dietary fat tends to have a low thermogenic effect compared to carbohydrates, which favours its efficient utilisation and accumulation (Donato and Hegsted, 1985; Astrup, 1993). Donato and Hegsted (1985) highlighted that when energy intake from fat exceeded expenditure, only 3% of the energy consumed was required to store the excess energy. Indeed, research shows that dietary fat intake may promote body weight gain. For instance, an intervention trial found that individuals gained weight when overfed with varying proportions of energy derived from fat (40% to 53%

kcal as fat) (Sims, et al., 1973). Also, in a 6-month intervention study, Westerterp and colleagues found that participants showed changes in body fat with dietary fat intake. A meta-analysis of experimental and epidemiological studies also found that there was a change in body fat with dietary fat intake. Dietary fat intake was positively associated with body fat, resulting in a 5% variation in body fatness ($p< 0.001$) (Westerterp, et al., 1996). Despite these pieces of evidence, some studies have recorded mixed findings.

In a review of cross-sectional studies examining the correlation between dietary fat and body fatness, Lissner and Heitmann (1995) observed that while some studies showed either a positive association (Lissner, et al., 1987) or no association (Tremblay, et al., 1989; George, et al., 1990; Slattery, et al., 1992; Klesges, et al., 1992; Lissner and Lindroos, 1994), other researchers have observed a positive but transient correlation between dietary fat and body fat (Sheppard, Kristal and Kushi, 1991). Sheppard, Kristal and Kushi (1991) noted this transient correlation in the Women's Health Trial, where they observed that after fat was reduced by approximately 18% of energy, individuals who were in the low-fat group lost 3.2kg of body weight by 6-months but regained some body fat by 24 months. Perhaps the disparities in the findings of the stated studies may be related to methodological differences due to the participants under study and each study design. For instance, in the Women's' Health Trial (Sheppard, Kristal and Kushi, 1991), the duration of the study was of critical implication, such that the weight regain was only noted with an extended period of the research up to 2 years but was not observed by 6 months of the study. Hence one may argue that the conclusion of the intervention study by Westerterp and colleagues was influenced by the length of the study. Westerterp et al. (1996), in their 6-month study, concluded that increased dietary fat corresponded with an increase in body fat.

The observation of a higher prevalence of overweight in affluent countries with higher fat intake than in poorer countries with low fat intake has lent some support for the positive relationship between dietary fat and body fat composition (Willet 1998a). While this is noteworthy, there remains a high likelihood of bias related to variables unaccounted for such as food availability, cultural attitudes towards body fat, smoking and the level of physical activity.

2.9.2 Dietary protein

High protein diets have been demonstrated to have the potential to influence body composition (Halton and Hu, 2004; Dominik and Varman, 2014). High protein diets may occur in various forms, each containing high, low or adequate amounts of other macronutrients. Some of these include Atkins diet (containing carbohydrates as low as 30g/day, but high protein and high fat) (Atkins, 2002), the Stillman diet (containing low carbohydrate, low fat and high protein), the South Beach diet (containing low carbohydrates and high protein), and the Zone diet (containing low carbohydrates and high protein) (Jeor, et al., 2001). These diets are considered to induce a negative energy balance or reduce body fat through influencing satiety, increasing energy expenditure, increasing the concentration of anorexigenic hormones (such as glucagon-like peptide-1, pancreatic polypeptide and cholecystokinin (CCK)) and amino acids, and altering gluconeogenesis (Dominik and Varman, 2014). These mechanisms ensure decreased food intake while promoting energy output.

Studies have shown that the intake of high protein diets increases energy expenditure by increasing diet-induced thermogenesis, otherwise known as the thermic effect of food. Diet-induced thermogenesis arises from various processes of digestion, absorption and transport, metabolism and storage of nutrients, resulting in a percentage increase in the energy output beyond the basal metabolic rate. Diet-induced thermogenesis is highest for proteins (15- 30%),

followed by carbohydrates (5- 10%) and fat (0- 3%) (Acheson, 1993; Westerterp, 2004). A meta-analysis of experimental and epidemiological data indicated that the thermic effect of food increased by about 6.9kcal per 1000kcal of digested food when 10% of the energy is derived from protein (Eiseinstein, et al., 2012). A study of the effect of protein intake on 24-hr energy expenditure observed that individuals on a high protein diet (deriving 36% of energy from protein) had a 71kcal per day higher energy expenditure ($p< 0.05$) than those who had a low protein diet (15% of energy derived from protein) (Whitehead, McNeil and Smith, 1996). Mikkelsen, et al. (2000) similarly found that the intake of diets containing 29% of protein had a 212.9kcal per day increase in basal metabolic rate compared to those whose diet contained only 1% of protein. Although the reviewed studies agree on the effect on protein intake on energy expenditure which can influence body fat, they failed to investigate if changes in body fat percentage of the participants occurred with a change in energy expenditure when diets with varying protein amounts were consumed. Also, given that they involved small sample sizes (Mikkelsen, et al. (2000)- 12; Whitehead, McNeil and Smith (1996)- 8), their findings are not generalisable.

Furthermore, the intake of high protein diet has been suggested to increase satiety and subsequent food intake. The mechanism is thought to be related to the increase in oxygen demand required for protein metabolism (Westerterp-Plantenga, et al., 1999), similar to the phenomenon of appetite suppression observed in areas of high altitude characterised by lowered amounts of oxygen (Lippl, et al., 2010). The high oxygen demand for protein metabolism occurs due to high postprandial amino-acid oxidation rate (Dominik and Pesta, 2014). The satiation impact associated with the intake of dietary protein has also been linked to the stimulation of CCK release due to protein hydrolysis during digestion (Liddle, et al., 1985). CCK is a hormone found in the brain and gastrointestinal tract, which induces satiety

(Moran and Kinzig, 2004). Brenan, et al. (2012) noted that among obese and lean subjects, energy intake was suppressed after a high protein diet.

A high protein diet is also found to influence satiety through altering gluconeogenesis (Portier, Darcel and Tome, 2009), especially when containing low amounts of carbohydrates. Experimental research shows that the intake of a high protein diet upregulates two enzymes involved in gluconeogenesis (glucose-6-phosphatase and phosphoenol-pyruvate carboxylase) which increase satiation (Azzout, et al., 2007). Veldhorst, Westerterp-Plantega and Westerterp (2009) observed an increase in gluconeogenesis in healthy men after a high protein diet compared to a normal protein diet. In a subsequent study, the authors also observed that increased gluconeogenesis occurred, along with increased production of ketones, after a high protein diet. The ketone generated, beta-hydroxy-butyric acid was associated with a positive satiating influence or effect (Veldhorst, Westerterp-Plantega and Westerterp, 2012).

Additionally, high protein diets are also known to promote a satiety response through increasing serum concentration of amino acids. Nefti, et al. (2007) posit that the intake of a high protein diet induces vagal feedback to the satiety centre in the brain stem and hypothalamus, which suppresses hunger. Based on their study on the short-term effects of macronutrient preload, high protein preload induced a significantly higher satiating effect compared to preloads containing isoenergetic amounts of fats or carbohydrates (Poppitt, McComack and Buffenstein, 1998). Westerterp-Platenga, et al. (1999) likewise found higher satiety among subjects after consuming a high protein diet than after consuming a high-fat diet.

Although existing evidence shows that the intake of a high protein diet can influence body fat through altering energy expenditure and satiety, more research is necessary to demonstrate the changes in body fat due to increased protein intake. Notwithstanding, high protein intake may not be worth recommending for successful management of body fat since metabolomics studies

have revealed that high protein intake is associated with the increased risk of metabolic diseases (Reddy, et al., 2002; Newgard, 2012). In a study of renal haemodynamics and clinical variables in healthy young men, Frank, et al. (2009) observed significant alterations in the glomerular filtration rate, filtration fraction and urinary pH values in subjects who consumed a high protein diet. Similarly, other researchers have reported the risk of renal stones formation in the urinary tract with a high protein diet (Robertson, et al., 1979; Reddy, et al., 2002).

2.9.3 Dietary carbohydrates

Since it was observed that there was an increase in obesity despite the decrease in the percentage energy derived from dietary fat intake, concern has arisen regarding the influence of carbohydrate diets on body fat (Atkins, 1998; Willet, 2001). An important premise for this is based on the insulin response elicited by carbohydrates measured in terms of glycaemic index (Crapo, Reaven and Olefsky, 1976). The glycaemic index serves as a standard for comparing how quickly carbohydrate increases blood sugar (Jenkins, 1981). The intake of foods with high glycaemic index are posited to increase blood glucose levels which can lead to hyperinsulinemia, insulin resistance (Liu, 2002) and increasing hunger (Ludwig, 2002).

A positive association between high glycaemic index and body fat storage has been demonstrated in short-term observational research (Ludwig, et al., 1999). Also, using longitudinal data from the Seasonal Variation of Blood Cholesterol Study, Ma, et al. (2005) concluded that BMI was positively correlated with glycaemic index, but not with glycaemic load, daily carbohydrate intake or percentage of calories obtained from carbohydrates. Several experimental studies have similarly demonstrated a positive association between the glycaemic index and body fat (Slabber, et al., 1994; Bouch, et al., 2002). Notwithstanding these findings, it may be argued that the influence on body fat observed might have been due to factors other than carbohydrates, especially as challenges are associated with ascertaining the glycaemic

response of mixed meals (Pi-Sunyer, 2003; Aziz, 2009; Aziz, Dunamis and Barber, 2013). Especially as the International Scientific Consensus Summit from the International Carbohydrate Consortium (ICQC) maintains that the calculated glycaemic index of mixed meals may not necessarily predict their glycaemic response since the glycaemic response of other nutrients in the diet may also have an impact (Augustin, et al., 2015).

2.9.4 Calorie intake and body fat

The potential influence of macronutrients (proteins, fats and carbohydrates) on body composition is mostly premised upon the caloric content. Logically, since body fat is a product of stored energy, regulating calorie intake seems crucial, and indeed has become the fundamental principle underpinning the predominant body of obesity research and anti-obesity campaigns. For instance, the United Kingdom policy on obesity and healthy eating suggests "putting calorie information on menus" and "helping people eat fewer calories by changing the portion sizes or recipe of a product" (Benton and Young, 2017). Similarly, the United States government dietary guidelines suggest the avoidance of oversized food portions and promote the indication and availability of lower-calorie food options in supermarkets (Benton and Young, 2017). If only regulating caloric intake was the solution, these measures should suffice, except that they seem to undermine other nutritional factors influencing the homeostatic mechanism of energy regulation.

Biological evidence proposes that energy intake and expenditure are balanced through feedback mechanisms which function by monitoring blood metabolites (Kennedy, 1953; Hall, et al., 2012; Lam and Ravussin, 2016). Due to these feedback mechanisms, a reduction in caloric intake results in hormonal changes that stimulate appetite (Lean and Malkova, 2016), lower metabolic rate (Dulloo and Jacquet, 1998), and the stimulation of food intake (Benton,

2005). To elucidate on these homeostatic feedback mechanisms, how the body responds to caloric intake is discussed below.

2.9.5 Metabolic response to caloric intake

The assumption that a reduction in caloric intake will necessarily precipitate body fat loss reflects an implicit assumption that physiological mechanisms do not influence energy balance. Contrary to this, extant reviews have highlighted the phenomenon of energy compensation, concluding that, once there is a reduction in body fat, the body mass loss is regained through an altered physiological mechanism which tends to conserve energy and influence the nature of food consumed (Poppit and Prentice, 1996; Drewnowatz, 2015). In a single-blinded 14-day experimental trial, individuals were observed to completely compensate for the calories lost (Foltin, et al., 1988). Other studies have similarly reported some degree of energy compensation up to 100%. For instance, Reid and Hammersley (1998) and Larvin, French and Read (1997) observed 100% energy compensation, while Foltin, et al. (1990) and Porikos, Hesser and van Itallie (1982) noted a 70% to 80% energy compensation. In other studies (Naismith and Rhodes, 1995; Porikos, Booth and van Itallie, 1997), 40% to 50% energy compensation was recorded.

Furthermore, metabolic changes may also occur to aid energy compensation. Levitsky and DeRosino (2010) in a 24-hr food restriction study during which young women either fasted, ate normally, or consumed 1200kcal after which they ate freely chosen meals, found that the loss in body mass in the subjects was regained, but without any increase in the amount of food eaten. Hence, suggesting the influence of psychological mechanisms in energy compensation. Likewise, Rosenbaum, et al. (2008) reported adaptive changes in thermogenesis in individuals maintaining a reduction in body mass. In their study, subjects who lost 10% of their body mass experienced a decrease in their total energy expenditure which was sustained for the entire

study which lasted for one year. These findings suggest that energy intake and expenditure, which are components of energy balance, are related or dependent on each other.

In the event of reduced energy intake, changes in metabolites, such as leptin, peptides YY, insulin, ghrelin, cholecystokinin, a gastric inhibitory polypeptide from baseline values may occur (Sumithran, et al., 2011). Based on a review (Lean and Malkova, 2016), the changes occurring in the levels of metabolites occur in favour of increased appetite and body weight gain. Dulloo and Jacquet (1998) in their study observed similar physiological changes with body fat loss and concluded that the changes facilitate compensation for the weight loss to cause a return to one's original weight. Research has shown that although the changes may be subtle, they are persistent. After measuring food intake for a few days, de Castro (1996) found that although the feedback mechanisms occurred, it was not apparent for at least a day. Also, in keeping with the findings of an earlier study of military trainees (Edholm, et al., 1955), there was no relationship between energy intake and expenditure on a day-to-day basis. However, after two days, a relationship was observed. Again, Saris (1997) found that there was a more significant association between energy intake and expenditure after 3-5 days, rather than in 1-2 days. Corresponding findings have been noted in research involving individuals with normal levels of physical activity (Bray, et al., 2008).

Macronutrients play a crucial role in the maintenance of healthy body fat. However, the influence of diet on body fat cannot be adequately understood with the exclusive focus on the association of macronutrients. It is worth considering micronutrients since several studies have posited that they can influence energy metabolism and body fat (Lee, et al., 2009; Major, et al., 2009; Padmavathi, et al., 2010; Payahoo, et al., 2013; Azab, et al., 2014).

2.9.6 Dietary micronutrients and body fat

Micronutrients are substances required only in minuscule amounts for the maintenance of health and promoting of growth and development (WHO, 2019). Dietary intake can also influence body fat through the intake of micronutrients which include vitamins and minerals. Various studies have examined the potential association between micronutrients and adiposity, especially, given the roles micronutrients play in energy metabolism and the occurrence of obese and overweight states with low levels of some micronutrients (Payahoo, et al., 2013; Azab, et al., 2014).

2.9.6.1 Vitamins

Vitamins can influence body fat through the action of leptin and insulin, and by regulating appetite and energy metabolism (Paolisso, et al., 1995; Major, et al., 2009). Vitamins A, C, and E, for instance, have been demonstrated to limit the risk of insulin resistance by reducing the formation of reactive oxygen species and downregulating C-Jun N-terminal kinase, which increases the threshold of insulin signal receptivity (Hirosumi, et al., 2002). Through these processes, the risk for fat accumulation leading to obesity is reduced. A study of 61 healthy young adults aged 18-22 years also found that vitamin A intake was negatively correlated with several measures of adiposity such as body weight ($r= -0.316$, $p= 0.014$), body mass index ($r=-0.338$, $p=0.008$), waist circumference ($r= -0.315$, $p=0.014$) and waist to hip ratio ($r= -0.309$, $p=0.016$) (Zulet, et al., 2008). In another study, low serum concentration of vitamin E was associated with reduced probability of being overweight (Odds ratio: 0.56, $p<0.05$, 95% CI: 0.37, 0.86) and obese (odds ratio: 0.38, $p<0.01$, 95% CI: 0.24, 0.60) in Mexican-American children from the 2001-2004 NHANES (Gunanti, et al., 2014). Other studies have similarly shown that the absence of vitamin A in the diet (Esteban-Pretel, et al., 2010), and low serum retinol concentration is associated with obesity (Neuhouser, et al., 2001; Sarni, et al., 2005).

Furthermore, lower vitamin D in individuals with excess body fat is a consistent finding across age, ethnicity, and geography (Walsh, Bowles and Evans, 2017). Some studies have reported a relationship between low serum vitamin D (both 25-hydroxyvitamin D and 1,25-dihydroxy vitamin D) and obesity (Bell, et al., 1985; Liel, et al., 1988; Parikh, et al., 2004; Lagunova, et al., 2009), while other researchers have emphasised on the direction of the relationship. A randomised trial involving 77 overweight and obese women, found that vitamin D supplementation caused a significant decrease in body fat mass (Salehpour, 2012). In earlier and later studies, although the effect of vitamin D on body fat was not refuted, the relationship between body fat content and vitamin D was observed to be stronger than that between BMI and vitamin D (Arunabh, et al., 2003; Vimeswaran et al., 2013). Vimeswaran and colleagues in their genetic study demonstrated that higher BMI was correlated with lower vitamin D (25-hydroxyvitamin D), but the effect of lower vitamin D (25-hydroxyvitamin D) on BMI was relatively smaller.

The strength of the relationship between adiposity and vitamin D may be dependent on the measure used for assessing body fat. For instance, the inverse association between body fat content and serum vitamin D (25-hydroxyvitamin D) concentration is reportedly stronger than that between vitamin D (25-hydroxyvitamin D) and body mass or BMI (Arunabh, et al., 2003). Also, Salehpour (2012) in a randomised trial of overweight and obese women, found that although vitamin D supplementation caused a significant decrease in body fat mass, there were no changes in waist circumference and body mass. The mechanism for lower vitamin D levels in increased adiposity is not well understood. However, the presence of nuclear and membrane vitamin D receptors in adipocytes suggests that the adipose tissue is responsive to vitamin D (Ding, et al., 2012).

A low serum concentration of vitamin B-12 has also been linked to increased body fat. Supporting evidence includes a cross-sectional primary care-based study which found that

obese individuals had lower serum vitamin B-12 in comparison with their non-obese counterparts (Baltaci, et al., 2013).

The studies reviewed provide evidence to suggest that vitamin intake influences a decrease in body fat. While this is supported by the evidence on the specific physiological mechanisms involved, it is necessary to acknowledge that some of the studies used a cross-sectional design (Arunabh, et al., 2003; Gunanti, et al., 2004; Zulet, et al., 2008; Baltaci, et al., 2013), hence the direction of the association cannot be presumed and causality cannot be established. Also, where a different study design was used, the researchers either estimated BMI as a surrogate estimate of body fat (Parikh, et al., 2004) or failed to adequately control for confounding (Salehpour, et al., 2012). In Salehpour, et al. (2012) for instance, while the effect of vitamin D supplementation was investigated, the authors failed to evaluate the sun exposure of the participants, which is a confounding factor. Furthermore, most of the studies reviewed are limited having only focussed on specific vitamin rather than considering vitamins and energy intake simultaneously in association with body fat (Arunabh, et al., 2003; Gunanti, et al., 2004; Zulet, et al., 2008; Salehpour, et al., 2012; Baltaci, et al., 2013; Vimaleswaran, et al., 2013). Since both energy and vitamin intake can influence body fat, the pieces of evidence provided by the studies reviewed are important but inadequate in explaining the relationship vitamin intake and body fat.

2.9.6.2 Minerals

a. **Iron**: The first association between low iron levels and increased body fat in humans was cited in the 1960s (Wenzel, Stults and Meyer, 1962). Since then, other researchers have related low blood iron concentration with increased body fat in adults (Azab, et al., 2014) and children (Nead, et al., 2004). The prevalence of iron deficiency in obese individuals may

suggest that the obese generally have an unhealthy diet leading to low iron levels, or that they have reduced absorption of iron and suffer sequestration of iron due to chronic inflammation. The latter was suggested by Zimmerman, et al. (2008) after noting reduced iron absorption in obese women despite contrasting findings by other researchers (Menzie, et al., 2008). The mechanism by which iron influences body fat composition is yet unclear but is thought to be through increasing insulin stimulation which increases adipogenesis.

b. **Zinc**: Among other micronutrients, zinc is one of those demonstrated to have a significant impact on energy homeostasis. It is involved in the synthesis and storage of insulin (Payahoo, et al., 2013). At a low concentration in the blood, it is associated with glucose intolerance and insulin resistance which has been strongly correlated with obesity (Mikhail, 2009; Kelishada, et al., 2010). Studies showing lower plasma zinc levels in obese individuals compared to their non-obese counterparts are in support of this view (Zavala, et al., 2012; Azab, et al., 2014).

c. **Calcium**: Calcium, an essential nutrient, plays a role in various physiological processes involving enzymes and hormones. A dose-response relationship was noted between the amount of calcium in the blood and fat deposition (Garcia, et al., 2009). At low levels of calcium, a cascade of reactions involving calcitriol-mediated increased intracellular calcium concentrations occurs, leading to poor sensitivity to intracellular calcium fluxes in cells responsible for glucose transport, which increases the risk of insulin resistance (Zemel, 2004). Other mechanisms involved include reducing body fat by increasing faecal fat excretion (Heaney, 2011) and suppressing "calcitriol-mediated inhibition" of adipocyte uncoupling binding protein 2 (UCP2) known to regulate body weight through thermogenesis (Shi, Dirienzo and Zemel, 2001). More so, a systematic review (Heaney and Rafferty, 2009) and meta-analysis (Onakpoya, et al., 2011) concluded that calcium helps to reduce body fat. In the meta-

analysis, there was a small mean difference in body fat (0.93kg) between the placebo group and the test group (calcium), however, it was statistically significant.

d. **Magnesium**: Low magnesium concentration in obesity has been reported in several studies, forming a basis for its association with metabolic diseases (Neilson, 2010). For instance, research carried out over two decades ago reported lower plasma magnesium levels in people with obesity and diabetes compared to the non-obese (Corcia et al., 1997). Other studies in adults (Lee, et al., 2009) and children (Huerta, et al., 2005) have reported similar findings. Magnesium is the main co-factor in many enzymatic reactions (over 300 enzymatic reactions including all the enzymes of glycolysis) and involved in; the regulation of insulin signalling, the post-receptorial action of insulin, the phosphorylation of insulin receptor kinase, and insulin-mediated cellular glucose uptake (Saris, et al., 2000; Gunther, 2011). The clinical consequence of a chronic magnesium deficit is known to be post-receptorial insulin resistance and reduced glucose use in the cells, which increase adipogenesis. In patients with type-2 diabetes, hypomagnesaemia is observed to worsen the reduced insulin sensitivity present (Barbagallo, et al., 2003; Lopez-Ridaura, et al., 2004).

e. **Selenium**: In a case-control study of Egyptian children, serum selenium levels were lower in obese children as compared to controls (Azab, et al., 2014). An Italian study of obese school children also observed lower selenium levels than normal-weight children (Ortega, et al., 2012). Even more recently, a cross-sectional study concluded that dietary selenium intake accounts for 9-27% variation in body fat percentage (Wang, et al., 2016). Selenium is suggested to influence body fat through its antioxidant effect.

f. **Chromium**: Chromium is known for its role in carbohydrate and fat metabolism (Padmavathi, et al., 2010). Although more evidence regarding its influence on body fat has been demonstrated in animal studies, an early study notes the reduction of body weight in

people with diabetes, alongside increased high lipoprotein density cholesterol, and decreased total cholesterol and triglycerides with the supplementation of chromium (Mert, 1969). A similar conclusion has been reached in a systematic review and meta-analysis, although the authors clarified that the magnitude of the effect was small (Onakpoya, Posadzki, and Ernst, 2013).

g. **Other minerals**: The relationship between copper deficiency and obesity was initially suggested by Reiling, et al. (2007), before subsequent studies observed lower levels of serum copper in obese subjects compared to their non-obese counterparts in adults (Gonzalez-Reimers., et al., 2014) and children (Azab, et al., 2014). Low serum manganese levels are likewise associated with increased body fat, given its role in the regulation of cellular energy, carbohydrate, protein and lipid metabolism (Aschner, et al., 2007).

Given the roles played by minerals, the relationship with body fat indicated by the studies reviewed is plausible. Nevertheless, there remains a lack of evidence on a causal relationship where cross-sectional associations were evaluated (Nead, et al., 2004; Ortega, et al., 2012; Zavala, et al., 2013; Wang, et al., 2016). In studies where a design other than a cross-sectional study design was used, the findings were not generalisable due to the study participants involved (Onakpoya, et al., 2011; Azab, et al., 2014). For instance, Azab, et al. (2014) recruited children (aged 5.5- 10 years), while all but one of 7 studies included in the meta-analysis by Onakpoya, et al. (2011) involved only women. Also, as a result of the use of BMI as a proxy for the percentage of body fat by some studies (Nead, et al., 2004; Huerta, et al., 2005; Ortega, et al., 2012; Azab, et al., 2014), it is difficult to ascertain if the observed estimate is due to body fat or lean body mass, especially as minerals can also influence lean body mass (van Dronkelaar, et al., 2017). Furthermore, as indicated earlier for vitamins, considering that energy intake was not simultaneously considered with mineral intake in the studies reviewed is a

limitation. A useful index which allows for the simultaneous consideration of both micronutrient and energy intake is Nutrient density (Willet, 1998).

2.10 Nutrient density

The term nutrient density may have various connotations depending on the context in which it is used. In developmental nutrition, nutrient density can be considered based on the adequacy of a nutritional package (food or diet) to sustain life (Rao, 2002). Adequacy, in this context, is considered based on the availability and amounts of selected nutrients (Briend, 2001). In another context, nutrient density can be considered in terms of the absence of some food classes such as sugar and oil (Kant, 2000), used especially in disease prevention literature (Demark-Wahnefried and Rock, 2003). An epidemiological perspective, however, implies that nutrient-density is the ratio of crude nutrient intake to total energy intake (Willet, 1998). Regardless of the context, nutrient density is an application of nutrient profiling; the science of classifying or grouping individual food products or diet for health promotion and disease prevention (World Health Organisation, 2017). Through this process, numerical scores are generated against each food or diet by which consumers can make healthier choices (Katz, et al., 2009; USDA, 2014), and regulatory policy decisions can be made (Drewnowski, 2007; Tetens, et al., 2007). More than 40 different nutrient profiling schemes have been identified (FSA, 2007), each with its unique algorithms and criteria for classification based on the purpose for which it is required (Scarborough and Lobstein, 2009). In the present research, nutrient density is used in the epidemiological context, implying the ratio of crude intakes of micronutrients to total energy intake. By using this ratio, the nutrient density of a food or diet can be estimated. However, it does not identify specific foods or diet as “nutrient-dense” except in comparison to another concerning a specific nutrient (Nicklas, Drewnowski and O’Neil, 2014).

The Dietary Guidelines Advisory Committee of 2005 highlights that the nutrient density of food or diet is considered to be based on the vitamins and minerals supplied versus the caloric composition (US Department of Health and Human Services, 2005). Extant literature posits that this explanation is ambiguous as it fails to highlight the unit to be used, the nutrients to be included in the algorithm and those nutrients to be limited (Pennington, et al., 2007; Scarborough, Rayner and Stockley, 2007). More recent literature also identified these challenges; however, concluded that there is an absence of a universal standard for nutrient density. It stated that the approach for calculating nutrient density and the nutrients to be included or limited would depend on the needs of the scientific literature, consumer document, marketing strategy or policy document being considered (Nicklas, Drewnowski and O'Neil, 2014).

An epidemiological perspective to nutrient density considers the crude nutrient intake ratio to the total energy intake. As such, the nutrients to be considered are those present in the diet consumed. Although some models used for determining nutrient density scores have only included specific nutrients, which have been identified as shortfall, or under-consumed nutrients, (such as vitamins, A, C, E, and calcium) (USDA and Health Services, 2005; USDA, 2010) and nutrients to be limited (such as cholesterol, total fat, trans-fat, saturated fatty acid, sodium and added sugars) (Drewnowski, 2005; Scheidt and Daniel, 2005; Fulgoni, Keast and Drewnowski, 2009; Arsenault, et al., 2012), the selective inclusion of nutrients may imply that the excluded nutrients do not contribute to health promotion and disease prevention (Nicklas, Drewnowski and O'Neil, 2014). For instance, potassium is frequently not included in some nutrient profiling models, although its inverse association with blood pressure has been established (USDA, 2011). Also, some algorithms for determining nutrient density have traditionally excluded saturated fatty acids (SFAs) due to previous literature positing that SFAs increase the risk of coronary heart disease (Hooper, et al., 2011). However, the rationale for

limiting SFAs may be questionable, given recent research stating that it is not associated with cardiovascular disease (Huth and Park, 2012; de Souza, et al., 2015). Hence, in epidemiological research, the exclusion of a nutrient might result in a false negative relationship or null finding, whereas it does have an influence.

Also, in calculating nutrient density, various standards have been used as a unit for measuring total energy intake, such as the Reference Amount Customarily Consumed (RACC), 100g, and 1000 kcal. Some authors have urged for the use of the most common; 100g (Scarborough, Rayner and Stockely, 2007), while others have argued that this might result in challenges if the food concerned is not customarily consumed in that amount (Nicklas, Drewnowski and O'Neil, 2014). Presently, a standard unit for measuring nutrient density is arbitrary. However, the United States Department of Agriculture emphasises the need to use a unit which is easier for consumers to understand (USDA, 2013).

Furthermore, it is noteworthy that nutrient density only reflects the nutritional quality of specific foods or diets (as represented by a score) but does not reflect the bioavailability of the nutrients considered. Bioavailability refers to the proportion of a nutrient that is absorbed from the food or diet consumed and used to meet bodily needs. Whether or not nutrient density should reflect the bioavailability of the nutrients concerned has constituted some challenges with defining nutrient density (Nicklas, Drewnowski and O'Neil, 2014). Nicklas and colleagues have clarified that determining the effect of nutrient density on the bioavailability of nutrients is "nearly impossible," as food may be nutrient-dense regarding a specific nutrient but only allows the absorption of a small proportion of the nutrient (Nicklas, Drewnowski and O'Neil, 2014). The bioavailability of a nutrient depends on several factors, especially relating to the various steps in the metabolic pathway which it undergoes, such as the release of the nutrient from the dietary matrix, influence of digestive enzymes, binding and uptake in the

intestine, transmission of nutrient into the lymphatic or blood circulation, uptake of nutrient for metabolic use, storage and excretion (Aggett, 2010). The characteristic physicochemical matrix of the food can influence bioavailability by allowing or restricting the release of the nutrient concerned. Other stated factors are physiological factors that can be influenced by age, gender, life stage and nutrient status. Generally, the bioavailability of macronutrients (proteins, carbohydrates and fats) is usually more than 90% of the amount ingested, while that for micronutrients (vitamins and minerals) can vary widely (European Food Information Council, 2010). The following section of this thesis will discuss the influence of physicochemical dietary characteristics on the bioavailability of micronutrients.

2.11 Physicochemical dietary characteristics and the bioavailability of micronutrients

The physicochemical properties of food may either increase or decrease the bioavailability of the constituent micronutrient. Those which increase the bioavailability are known as enhancers while those which reduce the bioavailability are referred to as inhibitors. Enhancers can act in various ways to promote micronutrient bioavailability, either by protecting the nutrient from inhibitors or increasing its solubility. For instance, the solubility of carotenoids is enhanced in the presence of fats or oil (van Het, et al., 2000), and iron absorption is increased up to three times in the presence of vitamin C (Teucher, Olivares and Cori, 2004). On the other hand, inhibitors act by binding nutrients and rendering them less available for absorption. For absorption involving carrier-mediated transport of nutrients through the mucosal cell, the affinity of the chelating compound for the nutrient is a determinant of the bioavailability. If the affinity of the chelating compound for the nutrient is more than the affinity the specific carrier molecule for the nutrient, then it results in a decrease in bioavailability (Davidsson, 2013). Inhibition can also occur due to competition between the nutrient concerned and another nutrient for the uptake system. It usually occurs when the uptake system has an affinity for both

nutrients. A typical example is the interaction which occurs between calcium and non-haem iron and the transporter on the surface of the intestinal cell. While non-haem iron binds to the transporter and gets absorbed through the process, calcium merely binds to the transporter and prevents non-haem iron from binding (Gibson, 2007). More so, inhibition can occur when the nutrient in question is available in a form which is not absorbable by the uptake system.

Inhibition can also be due to dietary constituents such as oxalates and phenolics can limit the bioavailability of micronutrients, particularly trace minerals. Oxalates are found in many plants and known to bind and limit the absorption of calcium. Phenolic compounds such as tannic acid, flavonoids, and phenolic acid are particularly of high concentration in beverages (tea, coffee, cocoa, and red wine). Early research found that a meal consumed with tea resulted in a four-fold decrease in iron absorption (Disler, et al. 1975). Coffee was also found to reduce iron absorption (Morck, et al., 1983) due to the constituent chlorogenic and phenolic compounds (Brune, Rossander and Hallberg, 1989). In a trial of 33 participants, Cook, et al. (1995) observed that red wine reduced iron absorption from a bread meal by 75%. Other studies have stated similar findings (Hallberg and Rossander., 1982; Gillooly, et al., 1983; Tuntawiroon, et al., 1991).

Furthermore, phytate (the salt form of phytic acid), a widely distributed compound in plants (oilseeds, legumes, and cereals) (Vats and Banerjee, 2004) has been suggested by some studies to inhibit mineral absorption and reduce the bioavailability consequently. Chemically, phytate has a small molecular size and strong negative charge under physiological conditions and is found in nature bound to multivalent minerals (Barrientos and Murthy, 1996). Its chemical characteristics reduce its likelihood of passage through the bi-lipid layer of plasma membranes, especially as no transport mechanisms have yet been identified. Also, due to its strong negative charge, research posits that it complexes multivalent minerals especially iron, calcium,

magnesium, and zinc in the diet forming chelates (compounds formed by the bonding of molecules to a metal atom) with limited absorbability (McCance and Widdowson, 1942; Hallberg, et al., 1989; Reddy, et al., 1996; Bohn, et al., 2004; Phillippy, 2006). Contrary to this, other studies have claimed that the presence of metabolic effects of phytate indicates their absorbability (Graf, Empson and Eaton, 1987; Shamsuddin, 1995). The reason for the inconsistency is unknown but may be linked to the study duration. Since most studies suggesting that phytate reduces micronutrient bioavailability lasted for less than four weeks, it may be argued that they failed to allow enough time for mineral absorption before concluding, especially as some lasting for more than 4 weeks (Mazariegos, et al., 2006 (10 weeks); and Kennedy, Hambidge and Manary, 2010 (5.7 weeks)) found no influence of phytate on mineral bioavailability. The form of phytate present in the diet may also be a source variation in the studies since the different of phytate have different affinity for minerals (Sandberg, et al., 1999). Evidence on the influence of phytate on mineral bioavailability, however, remains inconclusive.

2.12 Why consider the bioavailability of micronutrients with nutrient density?

A high nutrient density may not necessarily translate into high nutrient bioavailability (Nicklas, Drewnowski and O'Neil, 2013). For instance, considering varying amounts of milk and spinach, despite having similar calcium density, only 5% of calcium is absorbed from spinach compared to 27% of calcium from milk (Heaney, Weaver and Recker, 1988). Similarly, despite having a similar iron density, iron found in meat (haem-iron) is more bioavailable compared to non-haem iron in spinach (Nicklas, Drewnowski and O'Neil, 2013). Hence, it becomes necessary to understand the dietary sources of nutrients and how component dietary factors can influence nutrient bioavailability, particularly when the influence of dietary nutrient density is dependent on the bioavailability of the nutrients in question.

2.13 Summary of literature review

Body fat is a healthy body component which is distributed through the body in various proportions and locations and is involved in critical biological functions to promote health and sustain life. Various methods can be applied to estimate body fat, which can be broadly grouped into anthropometric and non-anthropometric methods. The methods of assessing body fat are particularly important for serving as a criterion for classifying an individual as being; underweight, of normal weight, overweight or obese. Body fat is regulated within healthy limits, however, can be influenced by various factors including genes, physiological factors, age, ethnicity, sleep, drugs, cigarette smoking, alcohol intake, physical activity and diet. Excess body fat may occur in the form of overweight or obesity, both of which have adverse health and social implications.

Diet is an important factor influencing body fat and may be characterised based on the individual, the time of the day when it occurs, the qualitative and quantitative characteristics, or as an eating event. Dietary intake can also be estimated by various methods including, subjective and objective methods. More recent methods have applied mobile technology (mobile phones, personal digital assistant and web-based technologies) to increase usability and reduce the burden associated with use. However, despite the improvements they offer compared to more traditional techniques, they have drawbacks associated with an increased cost of acquisition, the requirement of technical training and a considerable level of literacy to operate. Mobile technologies which maintain the merits while improving on these weaknesses would be preferable for dietary assessment.

The influence of diet on body fat can be considered based on specific nutrients or dietary patterns. While the latter is mostly considered of more merit since it focusses on a combination of nutrients consumed, recognises inter-nutrient interactions and synergies and is easily

translatable into eating behaviours, the former provides a foundation for understanding the latter.

More predominantly, existing literature has cited the association between caloric intake and body fat. This association is justified by the presence of excess energy storage in overweight and obesity. However, whether obese individuals consume more energy as compared to their lean counterparts remains controversial. Perhaps this controversy has persisted owing to the exclusive consideration of macronutrients or calorie intake while neglecting micronutrients, which play a critical role in energy homeostasis.

The role of micronutrients in the energy balance is recognised, and various studies have explored the potential association between micronutrients and body fat. Nevertheless, the exclusive focus on the influence of specific micronutrients on energy balance remains a drawback and may be related to prevailing inconsistent findings. Given that both caloric intake and micronutrient intake have a role to play in maintaining body fat, simultaneous consideration of both groups seems necessary for more balanced and reliable evidence of the influence of diet on body fat.

The term nutrient density or micronutrient density epidemiologically has been used in the literature to describe the ratio of micronutrient to calorie intake. Previous studies have however used this index in ranking foods and diet based on healthiness, and not in relation to body fat. The present research hence seeks to contribute to the literature by investigating the relationship between change in dietary nutrient density and change in body fat percentage.

Dietary nutrient density can only influence body fat when the nutrients consumed are absorbed. Research posits that under healthy body metabolic conditions, dietary factors may influence the absorption of micronutrients in the diet. Consequently, the intake of nutrient-dense foods may not necessarily translate into high nutrient bioavailability. Phytate is an important dietary

factor which can influence the bioavailability of micronutrients. However, the nature of its influence has remained controversial in the literature.

The literature review has highlighted some important gaps. First, although micronutrients and energy intake are important dietary factors with the propensity to influence body fat percentage, there is a dearth of research on how changes in micronutrient and energy intakes influence body fat. Secondly, dietary assessment is crucial for investigating the association between diet and health. However, there is a scarcity of research on valid and reliable methods for assessing dietary micronutrient intake in free-living adults. Third, phytate is an important dietary factor which has the propensity to influence the bioavailability of micronutrients in the diet, but there is inconsistent evidence on the nature of its influence. This thesis aims to address these gaps in the literature through specific objectives which include: To conduct a systematic review on the influence of phytate on the bioavailability of micronutrients, to validate a food photography method for the assessment of micronutrients in the diet and to investigate the association between change in dietary nutrient density and change in body fat percentage.

CHAPTER 3. A SYSTEMATIC REVIEW ON THE INFLUENCE OF PHYTATE ON THE BIOAVAILABILITY OF MICRONUTRIENTS

3.1 Abstract

Aim: This systematic review critically appraised research evidence regarding the influence of phytate intake on the bioavailability of micronutrients in humans.

Methods: Searches were conducted within databases: PubMed/MEDLINE, CINAHL Plus, Google Scholar, Scopus, Web of Science, ScienceDirect for papers presenting original data published from 1940 to 2017. Electronic searches were supplemented by hand-searching in relevant journals. Quality assessment of the studies was carried out based on the Critical Appraisal Skills Programme (CASP) checklist, and a structured form was used to extract data from the studies. The results were reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement.

Results: Of the 24,182 study records identified through database and hand-searching, thirty-three full-text articles met the inclusion criteria. These studies were of a variety of study designs; non-randomised trial (5), randomised controlled trial (4), cross-sectional, cross-over trial (23) and cohort study designs (1). The studies involved a total of 793 participants and the minerals studied included iron, zinc, magnesium, copper, calcium and potassium. The bioavailability of zinc, magnesium and iron were negatively influenced by dietary phytate, whereas calcium, copper and potassium were not affected.

Conclusion: Dietary phytate intake reduced the bioavailability of three minerals; zinc, magnesium and iron. Further research investigating the dose-response relationship and the

possible moderating factors such as the dietary source of phytate and habitual intake is recommended.

3.2 Background

Chemically, phytate occurs as phytic acid, which is myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. It is the major storage form of phosphorous comprising 1–5 % by weight in cereals, legumes, oilseeds and nuts and represents 50–85 % of total phosphorus in plants (Vats and Banerjee 2004). Food sources of phytic acid include cereals, legumes, oilseeds and nuts (Schlemmer, et al., 2009). Phytate and its influence on mineral bioavailability have already been discussed in the literature review section of this thesis (section 2.11).

3.3 Method and design

The concept and scientific rationale for this systematic review has been included in the general methods found in the appendix section of this thesis (Appendix 1). The protocol for the systematic review was prepared and registered in the PROSPERO- database of prospectively registered systematic reviews in April 2017 (www.crd.york.ac.uk/PROSPERO/index.php CRD42017062158). Before registration, a search was conducted within the PROSPERO database to ensure the same systematic review was not already being undertaken. A search was also conducted within databases (Cochrane Database of Systematic Reviews, MEDLINE, CINAHL Plus, Google Scholar, PubMed, Scopus, Web of Science, ScienceDirect) and randomly within search engines to ensure that there was no existing systematic review on the influence of phytate intake on the bioavailability of micronutrients. This was a systematic review, so did not require ethics approval from the ethics committee.

3.3.1. Study selection based on inclusion and exclusion criteria

Studies gathered from the search were included or excluded from the review based on the PICOS (Participant–Intervention–Comparator–Outcomes–Study design) format (Liberati, et al., 2009).

Table 3.1. Inclusion and exclusion criteria

Criteria	Inclusion	Exclusion
Participants	Human subjects, regardless of their age and race.	Animals, pregnant women, individuals with eating disorders or those using medication altering the gastrointestinal function or nutrient absorption were excluded. Individuals with renal or hepatic diseases where nutrient excretion was assessed.
Intervention	Objective or subjective assessment of phytate content in the diet.	No assessment of phytate and micronutrient in the diet.
Comparison	Bioavailability of micronutrients in phytate-free or low-phytate versus high phytate containing diet.	No comparison of the bioavailability of micronutrients in phytate-free or low-phytate versus that in high phytate containing diet.
Outcome	Estimates of micronutrient in the plasma or serum, or micronutrient excretion in faeces or urine.	No account of phytate and micronutrient intake, and no estimates phytate and micronutrients in the blood or excretion.
Study design	Cohort studies, randomised controlled trials, intervention trials, case-control studies, cross-over trials, and cross-sectional studies.	Study designs other than randomised controlled trials, intervention trials, case-control studies, cross-over trials, cohort studies and cross-sectional studies.
Publication type	Original published primary studies.	Abstracts, reviews, books, book chapters, letters, conference abstracts, short surveys, research protocols, dissertations, conference

		proceedings, reports and comment articles.
Date	1940 to 2017.	
Language	Studies published in English.	Studies published in any language other than English.

Titles and abstracts obtained through the electronic and hand searches were screened based on the inclusion and exclusion criteria stated. Where it was unclear if the title and abstract of the article retrieved met inclusion criteria, the full text was retrieved and reviewed.

3.3.2. Data sources and search strategy

Three search strategies were adopted. The first strategy included dietary minerals, while second and third pertained to phytate, and bioavailability, respectively. The guiding subject headings and keywords used based on the scope of the review are indicated as follows:

Table 3.2. Subject heading and keywords

Subject heading	Keywords
Phytate	Phytate
	Phytic acid
	Inositol phosphate
	Myo-inositol hexakisphosphate
	Phytinise
Micronutrient	Nutrient
	Element
	Micronutrients
	Metal
	Microelement
	Mineral
Bioavailability	Bioavailability
	Absorption
	Biological availability

These keywords were derived based on descriptions of the subject headings in the research literature with the assistance of an expert librarian. The search was undertaken for original studies conducted from 1940 to 2017 within Databases such as PubMed/MEDLINE, CINAHL Plus, Google Scholar, Scopus, Web of Science, ScienceDirect.

To supplement electronic searches, hand searching of selected journals were undertaken in relevant journals including Journal of Nutrition, Journal of American Dietetic Association, American Journal of Clinical Nutrition, Diabetes Care, European Journal of Clinical Nutrition and British Journal of Nutrition. Hand-searching was necessary to find published studies which were not indexed by the electronic databases searched. Reference lists of relevant published primary studies and systematic reviews were also cross-checked against searches obtained through searching electronic databases.

Searches were refined to find studies published in English from 1940 to 2017.

3.3.3. Quality assessment

Quality assessment of studies was carried out by the researcher only, based on the appropriate Critical Appraisals Skills Programme (CASP) checklist for the study design of each study.

3.3.4. Data extraction and synthesis

A structured form for data extraction was prepared, tailored to the review question. The form design made provision for general information (citation and record number), study characteristics (study name and design, recruitment method, micronutrient estimated), participant characteristics (mean age, gender and number), setting, and length of follow-up and outcome data (outcome measure assessed, and level of significance indicated). Papers which recorded two or more independent trials were recorded as a single study but differentiated by

suffix symbols such as “a” and “b” and so on, while studies which were conducted by the same author(s) and in the same year were differentiated by numerical suffixes such as “1” and “2” and so on. Data extraction was undertaken by the researcher alone.

A narrative synthesis of the data from the included studies was presented. A meta-analysis was not performed due to the differences across the studies, including diversity of protocols and inconsistency of reporting of outcomes.

3.4 Results

The flow in the selection of studies carried out in this systematic review is described in Figure 4.1. As shown in Figure 5.1., a total of 24,182 study records were identified. After screening and removal of duplicates, 16,927 studies were considered potentially relevant. From these studies, 9,256 studies were excluded after the titles and abstracts were screened. Of the remaining 7,671 studies, 7,638 studies were excluded after applying the full measure of inclusion and exclusion criteria regarding language, setting, participants, intervention, and outcome (Table A2.1-Appendix 2). This resulted in 33 full-text articles which were included in the review. Quality assessment of included studies using the CASP checklist based on the study design is reported in Tables A2.2 (a and b) and Appendix 2.

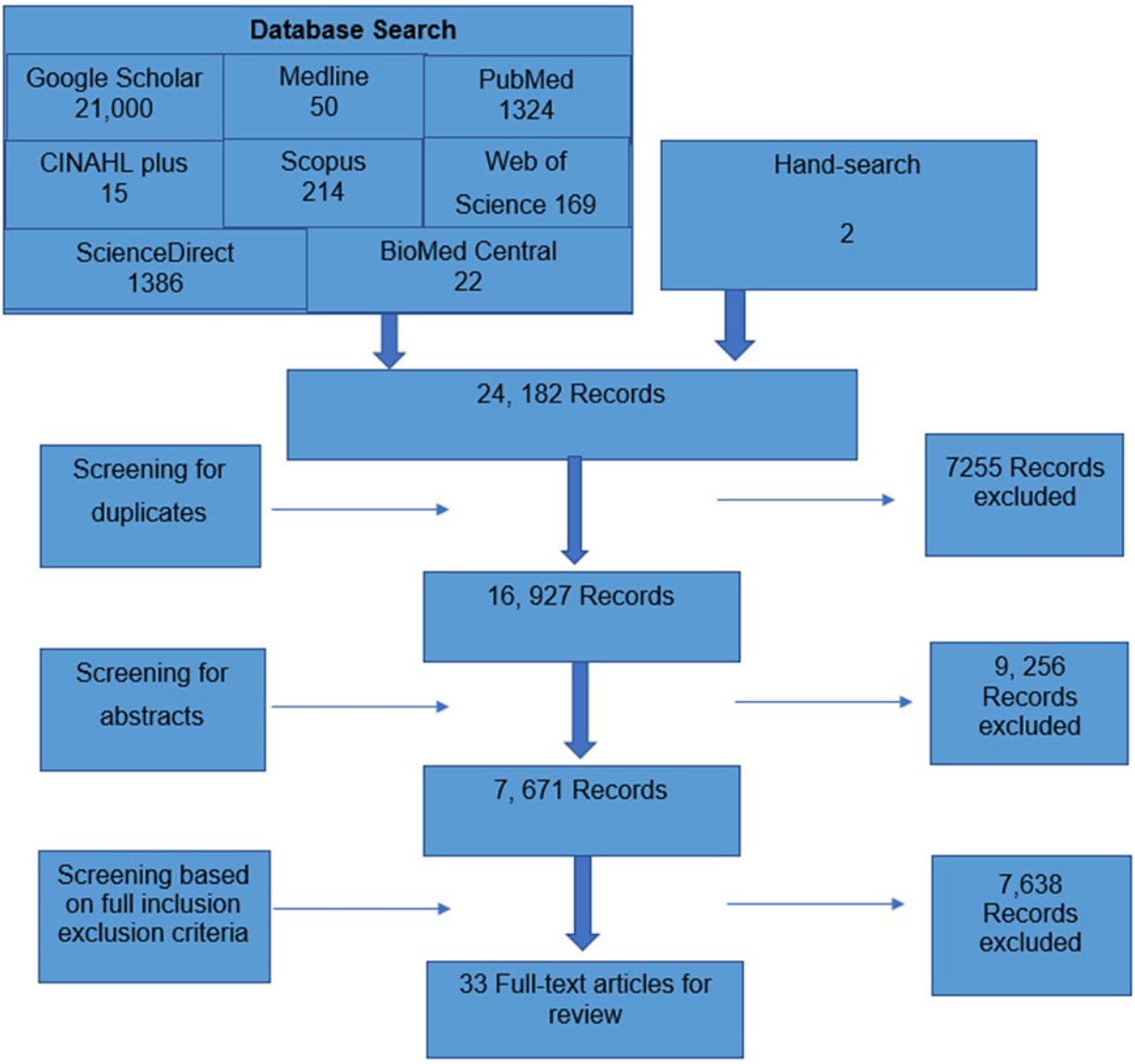


Figure 3.1. Flow diagram of the search results and selected studies.

3.4.1. Study characteristics

The included studies involved a total of 793 participants. **Table 3.3** shows the characteristics of the studies. The participants studied were from North America (Hurrell, et al., 1992; Mendoza, et al., 1998; Hurrell, et al., 2003; Hambidge, et al., 2004; Hanson, et al., 2006; Mazariegos, et al., 2006; DellaValle, et al., 2015), South America (Jaramillo, et al., 2015), Africa (Gillooly, et al., 1984; Thatcher, et al., 2009; Kennedy, Hambidge and Manary, 2010; Manary, et al., 2014; Petry, et al., 2014), Europe (McCance and Widdowson, 1942(1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Lonnerdal, et al., 1984; Navert, Sandstrom and Cederblad, 1985; Sandstrom, et al., 1987; Hall, et al., 1989; Hallberg, Brune and Rossander, 1989; Hallberg, Rossander and Skanberg, 1989; Sandstrom and Sandberg, 1992; Couzy, et al., 1993; Couzy, et al., 1998 (a, and b); Sandberg, et al., 1999; Sandstrom, et al., 2000; Bohn, et al., 2004; Egli, et al., 2006; Fredlung, et al., 2006; Brnic, et al., 2014) and Asia (Kim, et al., 2007), including adults (young and elderly) and children of both gender.

Studies were either randomised control trials (Lonnerdal, et al., 1984; Navert, Sandstrom and Cederblad, 1985; Manary, et al., 2000; Hanson, et al., 2006; Mazariegos, et al., 2006), non-randomised trials (Couzy, et al., 1998 (a and b); Sandberg, et al., 1999; Fredlung, et al., 2006; Brnic, et al., 2014), cross-over (McCance and Widdownson, 1942(1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Hallberg, Rossander and Skanberg, 1987; Sandstrom, et al., 1987; Brune, Rossander and Hallberg, 1989; Hallberg, Brune and Rossander, 1989; Hall, et al., 1989; Hurrel, et al., 1992 (a, b, c, d); Sandstrom and Sandberg, 1992; Couzy, et al., 1993; Mendoza, et al., 1998; Sandstrom, et al., 2000; Egli, et al., 2003; Bohn, et al., 2004; Hambidge, et al., 2004; Kim, et al., 2007; Petry, et al., 2014; Brnic, et al., 2014; Jaramillo, et al., 2015) or prospective cohort studies (Kennedy, Hambridge and Manary, 2010), with most considering a

single dietary mineral (Mg, Zn, Cu, Fe, Ca, K). Only five studies (McCance and Widdowson, 1942(1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Egli, et al., 2003; Thatcher, et al., 2009) considered more than one trace element. The earliest (McCance and Widdowson, 1942(1)) and the latest studies (DellaValle, et al., 2015; Jaramillo, et al., 2015) were conducted in 1942 and 2015, respectively.

Table 3.3. Study characteristics

Study Year of publication Country	Study design Length of study	Number, age, and characteristics of participants	Micronutrient estimated, and Measured outcome	Main findings
Bohn, et al. (2004) Switzerland	Randomised Cross-over study 8 days	17 participants (males and females). Adults (Mean age = 27±12 years).	Magnesium (^{25}Mg and ^{26}Mg) Faecal monitoring.	Addition of phytic acid significantly lowered the fractional apparent magnesium absorption from 32.5± 6.9% (no added phytic acid) to 13.0 ± 6.9% (1.49 mmol added phytic acid; $p< 0.0005$), and from 32.2± 12.0% (no added phytic acid) to 24.0± 12.9% (0.75 mmol added phytic acid; $p< 0.01$). The inhibiting effect of phytic acid was dose-dependent.
Brnic, et al. (2014) Switzerland	Randomised cross-over design. 59 days	10-participants (4- men and 6 women) Mean age: 22.6 ± 2.3 years)	Zinc ($ZnSO_4$) Fractional zinc absorption.	The fractional absorption of zinc was significantly increased by over 80% after dephitinisation of maize porridge ($p< 0.001$).
Brune, Rossander and Hallberg (1989)	Cross-over study. 32 days	19 particpants: Test group: 13 (4 men and 9 women). Mean age= 61 years	Iron (^{55}Fe or ^{59}Fe). Non-haem iron. Serum iron	The addition of bran containing phytate in the diet of both vegetarians and non-vegetarians (control group) significantly decreased iron absorption by 92% ($p< 0.001$ and 93 $p<0.002$) respectively.

Sweden		Control group: 6 Participants (3 men and 3 women). Mean age= 54 years (24- 70 years).		There was no significant difference in iron absorption between both groups.
Couzy, et al. (1993) Switzerland	Cross-over trial. 6 weeks	17 males: 9 young men, and 8 elderly men. Young men aged 24-40 years. Elderly men aged 70- 83	Zinc (^{70}Zn). Fractional absorption of zinc.	Zinc absorption from the test meal containing higher amounts of phytic acid showed lower zinc bioavailability. Zinc absorption was 40% lower in the young (23.4±10.2%) and 43% low in the elderly (19.8± 6.1%) when a meal with more phytic acid (538mg) was consumed compared to that containing no phytic acid. No significant effect due to age was found between the young and elderly participants ($p > 0.05$).
Couzy, et al. (1998) (a) Switzerland	Intervention study 3 hours	19 Participants (9 elderly and 10 young). Elderly participants aged (74.3±1.8); Young participants (33.9±4.8).	Zinc Serum zinc	Phytic acid significantly decreased zinc absorption. $p < 0.05$

Couzy, et al. (1998) (b) Switzerland	Intervention trial. 3 hours	20 Participants (10 young and 10 elderly participants). Participants of both gender Elderly participants aged (73.7±2), Young participants (31.4± 5.9)	Zinc Serum zinc	Phytic acid significantly decreased zinc absorption. $p< 0.05$
DellaValle, et al. (2015) USA	Randomised Cross over study design. 2 weeks	19 Non-pregnant women grouped into two: Group 1: 6 Anaemic women (mean age 23.4 ± 4.13) Group 2: 13 Non-anaemic women (mean age= 23.7± 4.63).	Iron Serum ferritin (difference in blood iron levels when the test (Dal) and control foods were consumed.	There was significantly lower absorption from the test meal prepared from lentils compared to ferrous sulfate. ($p<0.001$). Results were considered significant if $p< 0.05$. Absorption of non-heme iron from a test meal prepared from lentils was inversely associated with serum ferritin ($r = 20.50$, $p = 0.05$)
Egli, et al. (2003) Sweden	Randomised Crossover study. 32 days.	9 Adults 26- years Caucasian Menopausal women.	Zinc and Copper Faecal excretion of non-absorbed zinc.	Apparent zinc absorption was significantly higher ($p=0.005$) from dephytinised complementary food (34.6 ± 8.0%) than from the complementary food with native phytic acid (22.8 ± 8.8%). Apparent copper absorption did not change after intake of dephytinised complementary food (19.7 ± 5.1%) compared to that with native phytic acid (23.7 ± 8.1%).

Fredlund, et al. (2006) Sweden	Intervention study 28 days	36 participants (28 Females and 8 males). Age range: 19-55 years	Zinc Serum zinc concentration	The addition of 50mg phytate or more significantly decreased zinc absorption (p= 0.01) as compared to absorption from test meals with no added phytate
Gillooly, et al. (1984) South Africa	Intervention trial. 14 days	16- Parous women. Over 18 (unclear)	Non-haem Fe. Serum ferritin	The presence of phytate significantly reduced iron absorption by 20%. p< 0.0005
Hall, et al. (1989) United Kingdom	Randomised Cross-over design. 12hrs	26 Participants 19-26 years. (Gender not defined).	50mg of elemental Zinc (equivalent to 765µmol). Plasma zinc	There was a significant increase in the area under the plasma zinc time curve (AUC) from -5.9± 2.7% to 18.5 ± 4.2 µmol.h/l (mean ± SD, p< 0.001) after reducing the phytate content of bran from 2.92g/100g to 1.13g/100g of wheat bran
Hallberg, Brune and Rossander (1989) Sweden	Cross over study. 4 days	63 Participants (18 men and 45 women). Age range: 19-47 years.	Iron (^{59}Fe and ^{55}Fe) in the form of ferrous sulphate. Plasma iron	The inhibition of iron was significantly related to the amount of phytate added: 2mg inhibited iron absorption by 18% (p< 0.001), 25mg by 64% (p< 0.001), and 250mg by 82% (p< 0.001). The addition of ascorbic acid significantly counteracted the inhibitory effect of phytate.

Hallberg, Rossander and Skanberg (1987) Sweden	Cross-over trial 32 days	10 Participants: 4 men and 6 women. Age range: 19- 58 years.	Iron (^{55}Fe or ^{59}Fe) Serum iron	There was significantly increased iron absorption with dephytinised bran (6.2%) compared to wheat flour with bran (2.6%) ($p< 0.005$).
Hambidge, et al. (2004) USA	Cross-over trial 2 days	10 participants (6 men and 4 women). Mean age: 26 years.	Zinc Fractional absorption of zinc.	A negative relationship was found between the fractional absorption of zinc and dietary phytate ($p< 0.001$).
Hanson, et al. (2006) USA	Randomised intervention trial 42 days	55 Postmenopausal females. (47 -72 years age range) Mean age = 58 years	Iron Serum iron	Serum iron was significantly reduced with treatment meals containing native phytate compared to those containing low amounts. $p= 0.04$
Hurrel, et al. (1992) (a) USA	Cross over study 31 days.	8 Participants: 6 men and 2 women. Mean age 24 years.	Non-haem Iron Serum ferritin	Iron absorption significantly increased from 1.50% to 3.15% with reduction of phytate by 0.2mg/g ($p< 0.05$). Iron absorption significantly increased by four to five-fold when phytic acid was reduced from its native amount of 4.9- 8.4 to less than 0.01 mg/g of soy protein isolate. ($p= 0.01$.

Hurrel, et al. (1992) (b) USA	Cross over study 31 days.	9 Participants 5 men and 4 women. Mean age 23 years.	Non-haem Iron. Serum ferritin	Iron absorption significantly increased from 0.92% to 1.91% ($p< 0.02$) with reduction of phytate from 7.2mg/g to 1.0mg/g of isolate When phytic acid was added back, the absorption of iron reduced to almost its original value.
Hurrel, et al. (1992) (c) USA	Cross over study 31 days	8 Participants: 7 men and 1 female. Mean age 23 years.	Non-haem Iron. Serum ferritin	There was a significant increase in iron absorption with a decrease in phytic acid from 6.5 to ≤ 0.01mg/g soy-protein isolate ($p< 0.001$).
Hurrel, et al. (1992) (d). USA	Cross over study 31 days	7 Participants: 3 men and 4 women. Mean age 22 years	Non-haem Iron. Serum ferritin.	There was a significant increase in iron bioavailability with dephitinisation from 0.53% to 2.50% ($p< 0.001$) with a reduction of phytic acid from 6.5mg/g to ≤ 0.01mg/g of soy protein isolate.
Hurrell, et al. (2003) Kansas, USA	Intervention trial. 32 days	30 participants (14 males and 16 females). Mean age: 25 years	Iron Serum ferritin	There was a significant increase in iron absorption with dephytinisation. When wheat porridge was reconstituted with water, dephytinization increased iron absorption from rice porridge from 1.73% to 5.34% ($p < 0.001$), from oat from 0.33% to 2.79% ($p< 0.0001$), from maize from 1.80% to 8.92% ($p< 0.0001$), from

				wheat from 0.99% to 11.54% ($p< 0.0001$) and from the wheat-soy blend without ascorbic acid from 1.15% to 3.75% ($p< 0.005$)
Jaramillo, et al. (2015). Chile	Cross-over study 2 days	15 Multiparous women. Mean age = 39 ±4	Iron (as FeSO4). Serum ferritin	No significant difference in iron bioavailability was found with or without the addition of phytic acid. The addition of phytic acid, however, reduced iron bioavailability from 25.0% to 18.9%.
Kennedy, Hambidge and Manary, 2010 Malawi	Prospective (observational) 40 days	10 Children 2- 5years	Zinc Endogenous faecal zinc (EFZ).	Endogenous faecal zinc was similar before and after dietary phytate reduction, 1.15 ± 0.33mg/day and 1.17 ± 0.16mg/day, respectively. Endogenous faecal zinc was not affected by dietary phytate.
Kim, et al., 2007. South Korea	Cross-over study 28 days	17 women 7(young) participants Mean age: 23± 1 years	Zinc (radiolabelled isotope ^{70}Zn). Fractional zinc absorption	There was a significant decrease in fractional and total zinc absorption with the intake of a high phytate diet (43 versus 22% in young women; 34% versus 20% in elderly women). $p< 0.001$

		10(elderly) participants aged (70+- 3) years.		
Lonnerdal. (1984). Sweden	Randomised control trial. 14 days	36 Participants (Gender not defined). Age range: 20- 30 years old	Zinc (^{65}Zn). Fractional zinc absorption.	Phytate was found to have a significant effect on zinc absorption; the addition of 360µM of phytate to cows' milk formula (to yield a phytate concentration similar to that for soy formula) resulted in a decrease in zinc absorption from 31% to 16% ($p< 0.05$).
Manary, et al. (2000) (a). Malawi	Randomised controlled trial. 8 days	14 Children (6 boys and 8 girls) recovering from tuberculosis. The age range of children: 3-13 years.	Zinc Fractional absorption of zinc	There was a significant increase in FAZ and total zinc absorption with dephytinisation. Dietary phytate reduction resulted in higher Fractional absorption (0.41 ± 0.14 versus 0.24 ± 0.09, mean ± SD, $p<0.05$) and total zinc absorption (169 ±55 versus 100 ± 46µg/(kg.d),($p< 0.05$).
Manary, et al. (2000) (b) Malawi	Randomised controlled trial. 8 days	9 children (5 boys and 4 girls) free of tuberculosis or well. The age range of all the children: 3-13 years.	Zinc Fractional absorption of zinc	There was no significant effect of phytate reduction on fractional or total zinc absorption of zinc was seen in healthy children. Fractional and total zinc absorption after intake of high and reduced phytate diets were; 0.24 ±0.03 versus 0.24 ±0.02, and 2.17 ± 0.41 versus 1.59 ± 0.47, respectively.
Mazariegos, et al. (2006).	Randomised Control Trial.	60 children (29 boys and 31 girls).	Zinc	There was no significant effect of phytate on fractional and total zinc absorption. The mean (±SD) phytate intake for

Guatemala	70 days	GRP-1 Mean age: 7.9±1.2 years GRP 2 (Mean age: 8.3±1.3 years, GRP-3 Mean age: 8.6±1.4 years.	Fractional and total absorption of Zinc.	low-phytate, wild-type, and local maize groups were 1536 ±563, 2056 ± 517, and 2253 ± 687mg/d. While corresponding fractional zinc absorption were 0.32 ± 0.07, 0.28 ±0.07 and 0.29 ±0.06. Corresponding total absorption values were 2.72 ± 0.88, 2.30 ± 0.96, and 2.78 ± 1.04 mg/d
McCance and Widdowson, 1942 (1) United Kingdom	Cross-over trial 21 days (Mg), 20 days (Ca), 35 days (K).	8 Participants: 4 men and 4 women. Mean age: 29.75 years	Magnesium, calcium, potassium Mineral balance: Measurement of the amount in diet and that excreted in the urine (Mg and Ca), and faeces (K).	There was a 33.15% (104mg/day) average decrease in magnesium absorption with the intake of bread baked with the addition of sodium phytate. There was 38.95% (51mg/day) average decrease in calcium absorption with the intake of bread baked with the addition of sodium phytate. There was no change in potassium absorption with the addition of sodium phytate
McCance and Widdowson, 1942 (2) United Kingdom	Cross-over trial 14 days, with 3 days of washout	6-participants: 3 men and 3 women Mean age: 26.3 years	Magnesium and calcium Mineral balance: Measurement of the amount in diet and	There was a 9.5% average increase in the absorption of magnesium with the intake of dephytinised brown bread (containing 13mg/100g of phytic acid) compared to brown bread containing 99mg/100g of phytic acid.

	period before each week.		that excreted in the faeces (Mg and Ca)	There was a 20.8% average increase in the absorption of calcium with the intake of dephytinised brown bread (containing 13mg/100g of phytic acid) compared to brown bread containing 99mg/100g of phytic acid.
Mendoza, et al., 1998. USA	Cross-over study. 28 days	13 non-anaemic men. Mean age = 28.3±7.3 years	Iron (111kBq ^{55}Fe or 55.5kBq ^{59}Fe). Fractional absorption of iron	Iron absorption was significantly higher (49%) in a maize strain with less phytic acid (8.2%) compared to the wild strain (containing more phytic acid) 5.5% of intake ($p< 0.001$).
Navert, Sandstrom, Cederblad, 1985. Sweden	Randomised control trial. 1 day.	42 students: 17 men and 25 women. Median age: 23 years	Zinc (^{65}Zn). Serum zinc	Zinc absorption significantly decreased with increased phytate/ zinc ratio ($p< 0.05$).
Petry, et al., 2014. Rwanda	Randomised cross-over study design. 84 days	22 Rwandese women with low iron status. Adults aged 18- 30 years.	Iron (Fe) Plasma ferritin	There was a significant increase in iron bioavailability with approximately 50% and 95% dephytinisation from biofortified bean meal compared to that which contained its native phytate amount by 37% ($p< 0.005$) and 51% ($p< 0.0001$).
Sandberg, et al. (1999). Sweden	Cross-over study 4days	48 Participants Participants were men and women	Iron Wholebody iron retention and erythrocyte uptake of	There was significant inhibition of iron absorption by 39% with the independent addition of inositol phosphate 5 (IP5).

		Age range= 18- 54 years.	isotopes 55Fe and 59Fe	Phytate in the form of inositol -3-phosphate and inositol-4-phosphate when added independently showed no significant inhibitory effect on iron absorption However, when phytate in the form of inositol -3-phosphate and inositol -4-phosphate were added together with inositol-5 phosphate and inositol-6-phosphate, iron absorption was significantly inhibited by 54% ($p< 0.001$) and 64% ($p< 0.005$)
Sandberg, Hasselblad and Hasselblad, 1982. Sweden	Cross over design. 14 days	8 participants (6-men, 2 women). Mean age for men: 38 years Women (52- 67yrs).	Zinc, iron, calcium, and magnesium Apparent absorption of the minerals was measured by the Difference between dietary intake and ileostomy content.	The addition of wheat bran containing phytate significantly impaired zinc absorption ($p< 0.01$), while significantly increasing iron absorption ($p< 0.02$). Calcium and Magnesium absorption remained constant.
Sandstrom and Sandberg, 1992. Sweden	Randomised crossover 48 days.	18 healthy non-pregnant women. 22-37 years	Zinc (^{65}Zn). Wholebody zinc counting	Inositol-6-phosphate and inositol-5 phosphate significantly depressed zinc absorption ($p< 0.05$). The absorption of zinc from whte bread with no detectable phytate content was 43.3 ± 17.9% (mean ± SD) and from breads with added 400 µmol of inositol-6-phosphate: 14.3 ± 3.2%; 200µmol of inositol-5-phosphate; 27.1 ± 5.3%; 400µmol of inositol-5-phosphate: 18.1 ± 4.2%; and 400µmol of inositol-4-phosphate: 41.5 ± 11.3%.

Sandstrom, et al. (2000). Denmark	Crossover trial Two metabolic periods of 21 days each	12 participants (6-males, 6- females). Non-pregnant females aged 22-30 years	Zinc Fractional absorption of zinc.	Oat bran containing phytate had no significant effect on zinc absorption. The fractional absorptions (means ± SD) of zinc from oat bran diets containing phytate were 0.48 ± 0.11 and 0.40 ± 0.15 ($p = 0.07$) respectively.
Sandstrom, et al., 1987. Sweden	Randomised cross over study. 1 day	29 men and 11 non-pregnant women. Age range: 19-52 years	Zinc (^{65}Zn). Serum zinc	The absorption of zinc was negatively correlated with the phytic acid content of the meal. This yielded a significant negative correlation ($r= -0.5$, $p< 0.01$).
Thacher, et al., 2009 Nigeria	Intervention trial. 6 days	34 Children. 52 Months (52 ± 24) months for the rachitic and 57± 24 months for the controls (non-rachitic).	Calcium and Zinc Calcium absorption from 24 hr urine collection (for Ca), and 72-hr spot urine collection for zinc	The presence of phytate increased Ca absorption although not significantly. Enzymatic dephitinisation significantly increased relative zinc absorption from a meal by 101 ± 81% ($p< 0.001$)

3.4.2. Participants

Only two studies (Manary, et al., 2000; Thatcher, et al., 2009) included unhealthy participants; rachitic children and children recovering from tuberculosis, respectively. Two European papers, a cross-over study (Hall, et al., 1989) and randomised controlled trial (Lonnerdal, et al., 1984) which studied zinc absorption in adults failed to indicate the gender of their participants. Twelve studies (Lonnerdal, et al., 1984; Navert, Sandstrom and Cederblad, 1985; Sandstrom, et al., 1987; Hall, et al., 1989; Sandstrom and Sandberg, 1992; Egli, et al., 2003; Bohn, et al., 2004; Hanson, et al., 2006; Mazariegos, et al., 2006; Brnic et al., 2014; Petry, et al., 2014; DellaValle, et al., 2015) indicated to have applied randomisation in recruiting or assigning participants to study groups of which one was a randomised controlled trial in children (Mazariegos, et al., 2006), while the rest were in adults- of either cross-over (Sandstrom, et al., 1987; Hall, et al., 1989; Sandstrom and Sandberg, 1992; Egli, et al., 2003; Bohn, et al., 2004; Brnic, et al., 2014; Petry, et al., 2014; DellaValle, et al., 2015) or randomised controlled trial design (Lonnerdal, et al., 1984; Navert, Sandstrom and Cederblad, 1985; Manary, et al., 2000). The highest and least number of participants were recruited in a Swedish (Hallberg, Brune and Rossander, 1989) and an American (Hurrel, et al., 1992d) cross-over trials, respectively.

3.4.3. Quality of included studies

Based on the quality assessment conducted using the Critical Appraisal Skills Programme (CASP) checklists for cohort studies, randomised controlled and cross-over trials (Tables A2.1 (a and b)- Appendix 2), the following observations were made.

3.4.3.1. Cohort study

The cohort study included in the systematic review (Table A2.2a- Appendix 2) addressed a focussed issue and recruited the participants in an acceptable way. Also, the authors identified important confounders and sources of bias which could affect the study design and analysis of the results and indicated the follow-up period for the cohort and the results obtained. The authors, however, failed to clarify the implications of the study for practice and the steps that were taken to ensure that the exposure and outcome assessed were measured accurately to minimise bias.

3.4.3.2. Randomised controlled and cross-over trials

All the studies included in this category (Table A2.2b- Appendix 2) addressed a clearly focussed issue, considered important outcomes and treated all participant groups alike aside from the experimental intervention. Similarly, in all the studies, all the participants who entered the trial were accounted for at the end. 3.1% and 6.3% of the studies included unequal groups at baseline and failed to clarify the extent of the treatment effect, respectively. Also, 56.3% and 59.4% of studies did neither blinded the participants nor study personnel involved and did not randomise the assignment of participants, respectively. Only 12.5% of the studies included indicated the precision of the treatment effect.

3.4.4. The influence of dietary phytate on micronutrient bioavailability

3.4.4.1. Magnesium

Of the four studies included which investigated the influence of dietary phytate on magnesium bioavailability (McCance and Widdowson, 1942(1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Bohn, et al., 2004), three of them (McCance and Widdowson, 1942(1; 2); Bohn, et al., 2004) found that phytate reduced the bioavailability of magnesium. One study reported no influence of phytate on the bioavailability of magnesium (Sandberg, Hasselblad, and

Hasselblad, 1982). In a randomised cross-over trial involving 17 participants, Bohn, et al. (2004) found that fractional absorption of magnesium from white-wheat bread was significantly impaired by the addition of phytic acid in a dose-dependent manner. In their study, the addition of 1.49 and 0.75mmol of phytic acid lowered the apparent fractional absorption of magnesium from 32.5 ± 6.9% to 13.0 ± 6.9%, and from 32.2 ± 12.0% to 24.0 ± 12.9% respectively. McCance and Widdowson in two cross-over trials (1942(1; 2) observed a 33.15% and 9.5% average decrease in magnesium absorption with the addition of phytate respectively. In a Swedish study, Sandberg, Hasselblad, and Hasselblad (1982) reported that magnesium absorption remained constant with the addition of phytate contained in 16g of bran. Their finding was, however restricted to absorption in the small intestine since their participants were ileostomy patients.

3.4.4.2. Iron

Thirteen studies investigated the influence of phytate on iron bioavailability (Sandberg, Hasselblad and Hasselblad, 1982; Gillooly, et al., 1984; Della; Hallberg, Rossander and Skanberg, 1987; Brune, Rossander and Hallberg, 1989; Hallberg, Brune and Rossander, 1989; DellaValle, et al., 2015; Hurrel., 1992 (a, b, c, d); Mendoza, et al., 1998 Sandberg, et al., 1999; Hurrel, et al., 2003; Hanson, et al., 2006; Petry, et al., 2014; Jaramillo, et al., 2015). All except two (Skanberg, Hasselblad and Hasselblad, 1982; Jaramillo, et al., 2015) found that phytate reduced the bioavailability of iron. Specifically, Hanson, et al. (2006) in a double-blind randomised controlled trial involving 55 post-menopausal females found that serum iron was significantly reduced after having a meal containing a higher amount of phytate compared to that containing a lower amount. Also, in a randomised trial, although of cross-over design, Gillooly, et al. (1984) observed that iron was significantly less well absorbed from highly iron-bioavailable broccoli (*Brassica oleracea*) with the addition of sodium phytate; absorption reduced by 20%. Petry, et al. (2014) reported that in Rwandese women, phytic acid

significantly reduced iron bioavailability from iron-fortified beans. Among 30 American adults, Hurrel, et al. (2003) in an intervention trial involving 32 participants found that dephitinisation significantly increased iron absorption from rice porridge (1.73% to 5.34%, $p<0.001$), from oat (from 0.33% to 2.79%, $p<0.0001$), from maize (from 1.80% to 8.92%, $p<0.0001$), and from wheat (from 0.99% to 11.54%. $p<0.0001$), and wheat soy (from 1.15% to 3.75%, $p<0.005$). Hurrel and colleagues also noted that the influence of dephitinisation was reduced when meals were reconstituted with milk rather than with water but remained significant ($p<0.005$), reducing absorption from 1.15% to 3.75% in wheat-soy when blended with an enhancer (ascorbic acid). They also found that the positive influence of dephitinisation on iron absorption was reduced when reconstituted with milk rather than with water. In an 18-day cross-over Swedish trial, Sandberg, et al. (1999) observed that the influence of phytate on iron absorption depended on the form of phytate involved. In their research, inositol-5-phosphate and inositol-6-phosphate when isolated had an inhibitory influence on iron absorption, while inositol-3-phosphate and inositol-4-phosphate when isolated had no influence. DellaValle, et al. (2015) in a randomised cross-over study involving 19 non-pregnant women reported that absorption of non-haem iron from a lentil meal (2.20% ± 3.40%) was significantly lower than that observed from the same iron load given as ferrous sulphate (23.6 ± 13.2%). The authors also noted that women who were anaemic absorbed more iron from either source compared to those who were iron-replete.

In a sample of 32 adults, Hurrel, et al. (1992) found that iron absorption increased four to five-fold when phytic acid was reduced from its native amount of 4.9mg/g- 8.4mg/g to less than 0.01mg/g of soy protein isolate. In the study, it was also observed that a relatively small amount of residual phytate was significantly inhibitory until the phytic acid level was reduced below 0.3mg/g of soy-protein isolate before a meaningful increase in iron absorption was recorded. In a 4-day cross-over Swedish study, Hallberg, Brune and Rossander (1989) noted that phytate

significantly reduced iron bioavailability and that its influence was dose-dependent. 2mg, 25mg and 250mg of phytate inhibited iron absorption by 18%, 64% and 82% respectively. Also, the influence of phytate was observed to be significantly counteracted by the addition of ascorbic acid. Mendoza, et al. (1998) in a cross-over trial of 13 non-anaemic men comparing the iron absorption from tortillas prepared with low-phytic acid than wild-type strains of maize, found that iron absorption was significantly higher (4.9%) from the low phytic acid strain than from the wild-type. In another cross-over study, Hallberg, Rossander and Skankberg (1987) found that dephitinisation of wheat bran by endogenous phytase and hydrochloric acid significantly increased iron absorption. In the same study, when the phytate level was reconstituted, inhibition of iron absorption was almost restored. In a cross-sectional study of 19 participants, Brune, Rossander and Hallberg (1989) showed that the addition of phytate in bran resulted in 92% to 93% individual average decrease in iron absorption. The authors noted that the inhibitory influence was not related to the habitual intake of high phytate diet. Sandberg, Hasselblad and Hasselblad (1982) in a Swedish cross-over study, found that iron absorption significantly increased with the addition of phytate contained in 16g of bran. Finally, using 15 Chilean multiparous women in a cross-over study, Jaramillo, et al. (2015) observed no difference in iron bioavailability with the administration of phytic acid. This observation remained despite the addition of 800mg of calcium.

3.4.4.3. Zinc

Eighteen studies investigated the influence of phytate on the bioavailability of zinc (Couzy, et al., 1998 (a and b); Sandberg, Hasselblad and Hasselblad, 1982; Lonnerdal, et al., 1984; Navert, Sandstrom and Cerderblad, 1985; Sandstrom, et al., 1987; Hall, et al., 1989; Sandstrom and Sandberg, 1992; Couzy, et al., 1993; Sandstrom, et al., 2000; Manary, et al., 2000 (a and b); Egli, et al., 2003; Hambidge, et al., 2004; Fredlung, et al., 2006; Mazariegos, et al., 2006; Kim, et al., 2007; Thatcher, et al., 2009; Kennedy, Hambidge and manary, 2010; Brnic, et al., 2014),

of which fifteen of them observed that phytate reduced the bioavailability of zinc (Brnic, et al., 2014; Couzy, et al., 1993; Couzy, et al., 1998 (a and b); Egli, et al., 2003; Fredlung, et al., 2006; Hall, et al., 1989; Hambidge, et al., 2004; Kim, et al., 2007; Lonnerdal, et al., 1984; Manary, et al., 2000 (a); Navert, Sandstrom and Cerderblad, 1985; Sandberg, Hasselblad and Hasselblad, 1982; Sandstrom and Sandberg, 1992; Sandstrom, et al., 1987; Thatcher, et al., 2009) while four studies found otherwise (Manary, et al., 2000(b); Sandstrom, et al., 2000; Mazariegos, et al., 2006; Kennedy, Hambidge and Manary, 2010).

Fredlung, et al. (2006) conducted a 4-week intervention trial in which they found that adding 50mg of phytate or more to a test meal significantly decreased zinc absorption as compared to when no phytate was present. In a Swedish randomised crossover trial, Egli, et al. (2003) observed that among Caucasian menopausal women, the apparent zinc absorption was significantly higher from a dephitinised cereal-based complementary food (34.6 ± 8.0%) than from a cereal-based complementary food with native phytic acid concentration (22.8 ± 8.8%). Using a 4-week crossover trial, Kim, et al. (2007) showed that there was a significant decrease in fractional and total zinc absorption with a high phytate diet. They also indicated that the finding did not differ between young (22-24 years) and elderly women (65-75 years).

In an intervention study, Couzy, et al. (1998a and b) found that phytic acid significantly depressed zinc absorption. They also observed that the influence of phytic acid increased with a higher dose (from 0.13/200ml to 0.26g/200ml of zinc-enriched soya milk) and that the age of the participants had no influence on the findings. Thatcher and colleagues (Thatcher, et al., 2009) found that zinc absorption in enzymatically dephitinised maize porridge significantly increased by 101 ± 81% compared to that from a non-dephitinised meal. Brnic, et al. (2014) in a randomised crossover Swiss study indicated that the removal of phytate by adding phytase to maize porridge before consumption significantly increases the fractional absorption of zinc by over 80%. In a Swedish cross-over study of ileostomy patients, Sandberg and Hasselblad and

Hasselblad (1982) noted that the addition of phytate in 16g of bran to the diet significantly reduced the absorption of zinc in the small intestine. Hambidge, et al. (2004) in an American crossover study, found a significant negative relationship between dietary phytate content and the fractional absorption of zinc from maize tortillas. In an 8-day randomised controlled trial, Manary, et al. (2000b) observed that among 14 Malawian children recovering from tuberculosis, dietary phytate reduction resulted in significantly increased fractional and total absorption of zinc. Sandstrom, et al. (1987) in Swedish randomised crossover study of 40 participants observed that a significant negative correlation between zinc absorption and the phytic acid content of a meal based on 60g of rye, barley, oatmeal, triticale and whole wheat ($r= -0.5$, $p< 0.01$).

In another Swedish study of randomised control design, Navert, Sandstrom, and Cederblad (1985) observed that leavening bread reduced the phytate content of bran and resulted in increasing amount and percentage of the zinc absorbed as fermentation was prolonged. Couzy, et al. (1993) in a crossover trial found that zinc absorption from a test meal containing no phytic acid was significantly more than when it contained 538mg of phytic acid in both young (38.9 ± 9.8% versus 23.4 ± 10.2%) and elderly women (35.0 ± 10.9% versus 23.4 ± 10.2%). The authors, however, highlighted that the difference in absorption between both groups of participants (young and elderly women) was not significant. In healthy non-pregnant women aged 22-37 years, Sandstrom and Sandberg (1992) noted that compared to white bread with no detectable phytate content, zinc absorption significantly decreased from 43.3± 17.9% to 18.1 ± 4.2% and 14.3 ± 3.2% with the addition of either 400µmol inositol-5-phosphate and 400µmol inositol-6-phosphate respectively. Hall, et al., (1989) found that that plasma zinc concentration significantly decreased from 74.6 ± 7.4 µmol.h/l to 18.5 ± 4.2 µmol.h/l when reduced phytate wheat bran was given after standard wheat bran was administered. In a randomised controlled trial involving 36 Swedish adults, Lonnderdal, et al. (1984) found that the addition of phytate

to cow's milk formula to 6:1 phytate/zinc ratio reduced zinc absorption by 49.1% (from 32.2 ± 1.4% to 15.8 ± 0.8%). Kennedy, Hambidge and Manary (2010) in a study of Malawian children who habitually consumed a high phytate diet, observed that their endogenous faecal zinc (which varies with absorbed zinc) was similar before and after phytate was reduced from a maize diet. The endogenous faecal zinc before and after the removal of phytate from the diet was 1.15 ± 0.33mg/day and 1.17 ± 0.16mg/day, respectively. In a 10-week Guatemalan randomised controlled trial, Mazariegos, et al. (2006) recorded no significant differences in fractional and total zinc absorption between children who consumed low and high phytate maize meals. In another study, Sandstrom, et al. (2000) observed that the fractional zinc absorption did not significantly differ between a low fibre meal and a low fibre meal with oat bran meal containing 0.5mmol and 4.0mmol of phytic acid, respectively. In an 8-day randomised controlled trial involving 9 children aged 3-13 years, Manary, et al. (2000) recorded no change in the fractional absorption of zinc between participant groups who consumed high-phytate (121mg) and low-phytate diets (29mg).

3.4.4.4. Copper

Only one study investigated the influence of phytate on the bioavailability of copper (Egli, et al., 2003). Using a randomised cross-over study design, the authors found that the apparent fractional absorption of copper did not significantly differ among nine premenopausal Caucasian women who consumed a dephytinised cereal-based meal and a cereal-based meal containing native amounts of phytate. The fractional absorption of copper from the former and latter were 23.7 ± 8.1% and 19.7 ± 5.1% respectively.

3.4.4.5. Calcium

Of the four studies which studied the influence of phytate on the bioavailability of calcium (Thatcher, et al., 2009; Sandberg, Hasselblad and Hasselblad, 1982; McCance and Widdowson, 1942(1; 2)), two studies observed that phytate had no influence, while both studies by McCance

and Widdowson, 1942(1; 2) concluded otherwise. Thatcher, et al. (2009) conducted a 6-day intervention trial involving 34 rachitic and non-rachitic children and found that enzymatic dephitinisation of maize porridge decreased calcium absorption, although not significantly. They also observed that calcium absorption was not affected by the presence of rickets. In a Swedish crossover trial, Sandberg, Hasselblad, and Hasselblad (1982) noted that in ileostomy patients, calcium absorption remained constant on the addition of phytate contained in 16g of raw bran to a low-fibre test meal. McCance and Widdowson in one of their cross-over trials which lasted for 21 days, observed that the calcium absorption decreased by an average of 66.2% when bread baked from flour containing 214mg of phytic acid per 100g was administered after bread baked from flour containing 56mg of phytic acid per 100g was given (McCance and Widdowson, 1942(1)). Also, in another 21-day cross-over trial, the authors found that there was a 20.8% average increase in the absorption of calcium with the intake of dephytinised brown bread (containing 13mg/100g of phytic acid) compared to brown bread containing 99mg/100g of phytic acid (McCance and Widdowson, 1942(2)).

3.4.4.6. Potassium

The influence of phytate on the bioavailability of potassium was investigated only by McCance and Widdowson (1942(1)) using a crossover study design involving 8 adult participants. It was observed that the percentage of potassium absorbed from the diet (white bread) (16%) did not change after the addition of sodium phytate.

3.5. Discussion

This systematic review aimed to identify, appraise and synthesise evidence on the influence of phytate in the diet on the bioavailability of micronutrients. The evidence in this review indicates that phytate in the diet negatively influences the bioavailability of zinc, magnesium and iron.

In this chapter, the included studies have been grouped into 6, based on the trace mineral investigated; Mg, Zn, Cu, Fe, Ca and K. Due to the heterogeneity in the study protocols and outcome measures in each group, a meta-analysis was not carried out. A narrative summary of the results was rather presented.

3.5.1. The influence of phytate on magnesium bioavailability

Four studies included in the review investigated the influence of phytate on magnesium absorption (McCance and Widdowson, 1942(1; 2); Bohn, et al., 2004; Sandberg, Hasselblad, and Hasselblad, 1982. In three of them (Bohn, et al., 2004; McCance and Widdowson, 1942(1; 2)), phytate reduced the bioavailability of magnesium, while Sandberg, Hasselblad and Hasselblad (1982) otherwise observed that magnesium absorption remained constant despite the dietary phytate content administered. In their study (Sandberg, Hasselblad, and Hasselblad, 1982), participants were ileostomy patients; hence, it was not possible to account for magnesium absorption in the colon. Although the authors argue that the principal absorption of the mineral occurs in the small intestine, previous research suggests increased phytate-magnesium interaction at lower pH levels as occurs in the colon (Schleimer, et al., 2005). The role of the distal part of the digestive tract for magnesium absorption is well documented (Karabach and Rummel, 1990). As food products containing fermentable complex carbohydrate (resistant starch, pentosans, and fructans) are present in the colon, they are fermented by gut microbes producing acids which cause a reduced pH that lessens the inhibitory effect of phytate on magnesium (Rayssiguier and Remesy, 1977; Lopez, et al., 1998). Perhaps, the finding in the study (Sandberg, Hasselblad, and Hasselblad, 1982) was due to the unaccounted colonic absorption of magnesium in the participants. It is unlikely other dietary factors would have interfered with the magnesium absorption since it was ensured that no other meal was given alongside the test meal.

Furthermore, some methodological errors appear to have occurred in the study (Sandberg, Hasselblad, and Hasselblad, 1982). First, wheat bran was used as a source of phytate, but its trace mineral content, especially for magnesium, was not stated despite being known to be a rich source of minerals. Since magnesium absorption is dose-dependent (Coudray, et al., 2002), the inhibition of magnesium absorption could have occurred during the metabolic phase of the study when wheat bran was administered but was insignificant compared to the corresponding increased absorption. Also, it seems both metabolic periods of the cross-over were poorly controlled, as the phytate content of the meals administered during the wheat bran-free study period was not accounted for. Some of the foods provided, such as rice and white bread, might have contained phytate and interfered with the findings. In addition, the research failed to apply randomisation in the assignment of patients to treatments and blinding of either the study participants or personnel. Given these factors, it appears that the results of the research (Sandberg, Hasselblad, and Hasselblad, 1982) do not provide reliable evidence on the influence of phytate on the bioavailability of magnesium.

3.5.2. The influence of phytate on iron bioavailability

For all 13 studies (Sandberg, Hasselblad and Hasselblad, 1982; Gilloly, et al., 1984; Hurrel, et al., 1992 (a, b, c, d); Hallberg, Rossander, Skanberg, 1987; Brune, Rossander and Skanberg, 1989; Hallberg, Brune and Rossander, 1989; Mendoza, et al., 1998; Sandberg, et al., 1999; Hurrel, et al., 2003; Hanson, et al., 2006; Petry, et al., 2014; DellaValle, et al., 2015; Jaramillo, et al., 2015) which investigated the influence of phytate on iron absorption, 11 of them concluded that phytate negatively influenced the bioavailability of iron (Gilloly, et al., 1984; Hallberg, Brune and Rossander, 1989; Mendoza, et al., 1998; Hallberg, Rossander, Skanberg, 1987; Brune, Rossander and Skanberg, 1989; Hurrel, et al., 1992 (a, b, c, d); Sandberg, et al., 1999; Hurrel, et al., 2003; Hanson, et al., 2006; Petry, et al., 2014; DellaValle, et al., 2015). Sandberg, et al. (1999), however, clarified that the negative influence of phytate noted in their

study did not occur for some phytate forms (inositol 3-phosphate, or inositol 4-phosphate). Contrastingly, Sandberg, Hasselblad, and Hasselblad (1982) found that phytate significantly increased iron absorption, while Jaramillo, et al. (2015) found no influence of phytate on the bioavailability of iron. Considering that all studies in the group administered phytate sources containing inositol 6-phosphate (known to bind iron readily), the findings of Sandberg, Hasselblad and Hasselblad (1982) and Jaramillo, et al. (2015) seem unexpected. Especially as neither reported to have provided any meal together with the test meal, which could interfere with iron absorption. Jaramillo, et al. (2015) speculate that the null influence of phytate on iron could have been due to the dose of phytate administered (10mg), that a significant influence on bioavailability might occur with higher amounts. Perhaps this is likely since the influence of inhibitors of iron absorption may be dose-dependent (Hallberg, et al., 1989). However, their finding seems to be more likely biased by the iron status of the participants (multiparous women of childbearing age) involved in the study (Jaramillo, et al., 2015). Women of childbearing age are known to be at risk for the negative iron balance due to increased iron loss through menstruation, and high parity (Crompton and Nesheim, 2002; Harvey, et al., 2005). Although the study (Jaramillo, et al., 2015) stated that only two participants were iron deficient and the whole group had a mean serum ferritin level of 24µg/L at baseline, the authors failed to indicate whether some or all the participants were on their menstrual period during the study. This might have contributed by eliciting increased absorption despite the inhibitory influence of phytate present. Evidence on the inverse relationship between iron status and absorption from the diet is well documented (Thankachan, et al., 2008; Kalasuramath, Kupad and Thankachan, 2013).

Furthermore, in the other study, Sandberg, Hasselblad, and Hasselblad (1982) reported administering the test meal with 16g of wheat bran (containing an average of 4.2mmol of phytate) in one metabolic phase of their study. Given the phytate content of the supplement, a

decrease in iron absorption would have been expected in the test phase of the study. Perhaps their findings were related to the serum iron levels of the participants and intestinal pH of the ileostomy patients, details of which were not reported. Although existing literature clarifies that the small intestine pH ranges between 6 and 8, and may not vary significantly in ileostomy patients, providing the details would have contributed in reducing the likelihood of methodological bias, particularly as the formation of the iron-phytate complex is pH-dependent.

Another factor which limits the validity of the findings of those with contrasting findings (Sandberg, Hasselblad, and Hasselblad, 1982; Jaramillo, et al., 2015) includes the absence of blinding of either the study participants. Since the participants in Jaramillo, et al. (2015) were aware of the treatment phase, they might have modified their intake of the iron dose administered through distilled and deionized water due to the variation in taste.

3.5.3. The influence of phytate on zinc bioavailability

Fourteen out of 18 studies concluded that phytate negatively influenced the bioavailability of zinc (Sanberg, Hasselblad and Hasselblad, 1982; Lonnerdal, 1984; Navert, Sandstrom and Cederblad, 1985; Sandstrom, et al., 1987; Hall, et al., 1989; Sandstrom and Sandberg, 1992; Couzy, et al., 1993; Couzy, et al., 1998 (a and b); Manary, et al., 2000; Sandstrom, et al., 2000; Egli, et al., 2003; Hambidge, et al., 2004; Fredlund, et al., 2006; Mazariegos, et al., 2006; Kim, et al., 2007; Thatcher, et al., 2009; Brnic, et al., 2014). Only four studies (Mazariegos, et al., 2006; Manary, et al., 2000; Sandstrom, et al., 2000; Kennedy, Hambidge and Manary, 2010) found otherwise, three (Manary, et al., 2000; Mazariegos, et al., 2006; Kennedy, Hambidge and Manary, 2010) of which involved children: 3-5 years (Kennedy, Hambidge and Manary, 2010); 6.7-10 years (Mazariegos, et al., 2006); 3-13 years (Manary, et al., 2000). Their findings, however, do not appear linked to the age of the participants since there is a dearth of evidence

suggesting a variation in the influence of phytate on zinc bioavailability due to age. Especially as the study by Manary et al. (2000a) which similarly involved children, observed that phytate influenced zinc bioavailability. Hence, the findings of the studies (Manary, et al., 2000b; Sandstrom, et al., 2000; Mazariegos, et al., 2006; Kennedy, Hambidge, and Manary, 2010) seems unexpected.

Kennedy, Hambidge, and Manary (2010) allowed 40-days for the children in their study to habituate to the phytate diet. Also, every child had their exogenous faecal zinc measured before and after the dietary intervention to control for possible physiological determinants such as individual genetic background and the influence of microbiota. Although these strengthen the validity of the finding, the authors admitted administering two snacks without providing details on the type of snacks. It could be argued that this could have interfered their result in the event of containing other zinc inhibitors resulting in no significant increase in zinc absorption despite phytate reduction in the diet. The presence of another trace mineral such as calcium could have also influenced their finding. Early research (Fordyce, et al., 1987) clarifies that calcium in the diet may exacerbate the inhibitory influence of phytic acid on zinc absorption by forming Ca-Zn-phytic acid complexes in the intestine which is even less soluble than phytate-zinc complexes.

Other studies (Manary, et al., 2000b; Sandstrom, et al., 2000; Mazariegos, et al., 2006) either indicated the meal supplied alongside the dietary intervention (Sandstrom, et al., 2000; Mazariegos, et al., 2006) or provided none (Manary, et al., 2000b). However, two of them were carried out within a short duration (21-days (Sandstrom, et al., 2000), and 8-days (Manary, et al., 2000b)), which is insufficient time to reflect zinc balance. Schwartz, et al. (1986) has suggested an adaptation period of at least 4-weeks with a constant intake of the element of interest to reflect a reliable balance. Perhaps this affected the findings of both studies (Manary, et al., 2000b; Sandstrom, et al., 2000).

Acceptably, enough time was allowed in the other two studies which found no influence of phytates on the bioavailability of zinc; 5 weeks and 5 days (Kennedy, Hambidge and Manary, 2010), and 10 weeks (Mazariegos, et al., 2006). Nevertheless, the findings of the studies might have been biased by the fact that the participants habitually consumed high-phytate foods as staples; Guatemalan children (Mazariegos, et al., 2006) and Malawian children (Kennedy, Hambidge and Manary, 2010). Research shows that habitual intake of high phytate diet may reduce the inhibitory effect of phytate, although the mechanism by which this occurs is yet unknown (Schlemmer, et al., 2009).

Among the studies with contrasting findings (Mazariegos, et al., 2006; Manary, et al., 2000; Sandstrom, et al., 2000; Kennedy, Hambidge and Manary, 2010), the participation of children in Manary, et al. (2000) and Mazariegos, et al. (2006) limits the applicability and comparability of the results. Nevertheless, it is unlikely to have influenced the results. Other noteworthy limitations include bias attributable to the poor estimation of the exposure variables (Kennedy, Hambidge and Manary, 2010), the lack of randomisation of research participants to study groups and the absence of blinding of either the research participants or study personnel (Sandstrom, et al., 2000).

An important implication of the negative influence of phytate on zinc bioavailability is that individuals who are at risk of zinc deficiency due to inadequate dietary supply may exacerbate this risk by phytate intake. Gibson, et al. (1997) indicates that molar ratios of phytate-to-zinc in the diet may predict the inhibitory effect of phytate; molar ratios in excess of 15:1 progressively inhibits zinc absorption and is associated with suboptimal zinc status.

3.5.4. The influence of phytate on copper bioavailability

Only one study investigated the association between dietary phytate intake and copper bioavailability (Egli, et al., 2003). In the study, phytate intake in the diet did not influence the

bioavailability of copper. During the investigation, the participants were randomly allocated to the different study phases, and no food or drink was allowed except water to be consumed along with the phytate-rich meal to limit the risk of interfering effects. Also, a washout time of 2-weeks was allowed between the study periods of the cross-over trial to avoid a carry-over effect of the dietary intervention. Although the observation of these precautions strengthens the validity of the result, the fact that the participants were not blinded to the intervention might have influenced their intake of the test meals especially as it is unclear if the participants' consumption was supervised. Also, considering that the participants are all menopausal women, more research involving both genders may be required to generate more generalisable findings.

It is noteworthy that the finding does not necessarily imply that phytate does not bind copper, as in-vitro experiments show that phytate has a high affinity for copper (Persson, et al., 1998). Perhaps in the study reviewed (Egli, et al., 2003), phytate-copper complexes were formed but were soluble over a wide pH range, thereby allowing for absorption in the intestinal mucosa. Regardless of this speculation, in-vitro studies seem to prove otherwise. It was found that phytate-copper complex at a molar ratio of 1:1 (1 phytate: 1 copper) and above, dissolved at pH 3.5 (Nolan, et al., 1987), but remained in solution at pH 7 (Champagne and Fisher, 1990). Hence, copper absorption may only be expected to occur in the stomach and colon, where pH tends to be lower. Presently, the role of the colon in copper absorption is not well recognised, but the stomach is known to be the site of primary copper absorption (van den Berghe and Klomo, 2009).

3.5.5. The influence of phytate on calcium bioavailability

Four studies investigated the influence of phytate intake calcium bioavailability (McCance and Widdowson, 1942 (1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Thatcher, et al., 2009)

two of which found no influence of phytate intake on calcium bioavailability (Sandberg, Hasselblad and Hasselblad, 1982; Thatcher, et al., 2009). In the studies by McCance and Widdowson (1942 (1; 2)), calcium bioavailability decreased with phytate intake up to 61% and 20.8% respectively. Although no measure of significance was considered, the authors concluded that phytate had a negative influence on the bioavailability of calcium (McCance and Widdowson, 1942 (1; 2)). In all the studies, phytate was supplemented in the diet; maize (Thatcher, et al., 2009), wheat bran (Sandberg, Hasselblad, and Hasselblad, 1982), bread (McCance and Widdowson, 1942 (1; 2)), and additional meals were provided alongside the test meal in each case except in McCance and Widdowson, (1942 (1; 2)). Although the studies did not report that providing a meal alongside the test meal confounded the results, it seems to have biased the finding in Thatcher, et al. (2009) where some orange juice was provided. The presence of organic acids may reduce the pH favouring the increased solubility of the calcium-phytate complex. Experimental evidence shows that the solubility of calcium-phytate complex increases at pH levels of 4 and below (Grynspan and Cheryan, 1983). Furthermore, three of the studies (McCance and Widdowson, 1942(1; 2); Sandberg, Hasselblad, and Hasselblad, 1982) lasted for less than 4 weeks, which may be argued to be insufficient time to achieve mineral balance (Schwartz, et al., 1986). In addition to these potential sources of biases, the absence of randomisation of the participants to the study groupings (McCance and Widdowson, 1942 (1; 2); Sandberg, Hasselblad and Hasselblad, 1982; Thatcher, et al., 2009) and lack of concealment of the participants to the interventions received (McCance and Widdowson, 1942 (1; 2); Sandberg, Hasselblad and Hasselblad, 1982) raise some doubts on the validity of the results. Hence, the findings may be considered inconclusive.

Phytate-rich meals are presently encouraged as a risk-reducing factor for renal lithiasis, given that it inhibits the crystallisation of calcium salts through maintaining adequate urinary calcium (Grasses, et al., 2000). Based on epidemiological evidence, renal stones are more prevalent in

developed countries where more refined flour is consumed, compared to developing nations where cereals and legumes, which are known to be phytate-rich, are consumed. This systematic review is, however, not conclusive regarding the influence of phytate-rich meals on calcium bioavailability. More research excluding possible confounders is required to investigate the relationship between phytate intake and calcium bioavailability.

3.5.6. The influence of phytate on potassium bioavailability

McCance and Widdowson (1942(1)) did not observe any influence of phytate intake on the absorption of potassium. In the study, potassium absorption remained at approximately 16% during the intervention and control phases of the experiment. Between both phases of the study, the participants were allowed a washout period of 3 days and were not provided with other meals besides those prepared for the study. Evidently, both measures were taken to ensure that there was no interference with the treatment. Nevertheless, the lack of blinding of participants and failure to randomise the assignment of participants to the treatment remain factors which might have influenced the findings. There is a paucity of research on the influence of phytate on the bioavailability of potassium, possibly owing to evidence on the affinity of phytate for polyvalent rather than monovalent cations (Schlemmer, et al., 2009).

It is necessary to note that despite the differences in study design, the form of phytate administered and the measure of micronutrient bioavailability, all the papers included in this systematic review highlighted the influence of phytate in the diet on the bioavailability of at least one mineral (magnesium, iron, zinc, copper, calcium and potassium).

3.6. Conclusion

Based on the synthesised findings of included studies, this systematic review concludes that dietary phytate intake reduces the bioavailability of zinc, magnesium and iron. Further research

investigating the dose-response relationship and the influence of factors such as the dietary source of phytate and habitual intake is recommended.

CHAPTER 4. VALIDATION OF A FOOD PHOTOGRAPH RECORD METHOD FOR DIETARY ASSESSMENT OF MICRONUTRIENTS

4.1 Abstract

Objective: The validity of the food photography record technique for dietary assessment has been established in assessing macronutrients, but not much is known of its validity for assessing micronutrients in free-living conditions. This study, therefore, aimed to establish that the food photography record (test method) can validly and reliably estimate the dietary micronutrients in free-living adults while using the weighed food record as a reference method.

Methods: Thirty-six adult university students undertook a 4-day diet record using the test method (food photography record) and the reference method (weighed food record) simultaneously. Before this, both methods were piloted among 10 students to assess the feasibility and identify possible limitations associated with undertaking the validation study among the students. After students undertook the validation study, the measures obtained from both methods were compared by paired Student's t-test, and Pearson's correlation analysis was conducted to investigate the linear correlation between both measures. Bland-Altman analysis was used to measure the level of agreement between both methods, and the intra-rater reliability of the test method was measured using the intra-class correlation coefficient.

Results: Although not consistently, the food photography method tended to underestimate some nutrients. Nevertheless, the difference was not statistically significant compared to the weighed food record ($p > 0.05$). Bland-Altman analysis showed that the measure of bias for each nutrient did not significantly differ from zero and occurred less than ±20% of the mean measure of the reference method. Furthermore, the trend line (line of regression) plotted upon the Bland-Altman plot indicated no slope of statistical significance ($p > 0.05$). Pearson's correlation analysis showed a significant direct correlation between both measures ($p < 0.05$),

while the reliability test of the test method yielded a minimum intra-class correlation coefficient of 0.75.

Conclusion: The food photograph diary can provide accurate and reliable estimates of dietary micronutrients when used with university students in the United Kingdom.

4.2 Background

As stated in Section 2.7, dietary records are generally accepted as the "gold standard" for assessing dietary intake (Williamson, et al., 2003; Frank-Stromborg and Olsen, 2004). Food photography is a dietary record technique which requires a camera for capturing images of food consumed rather than recording in a booklet as in weighed food record. Several studies have applied the food photography technique for dietary assessment and found it valid for estimating dietary intake in various populations, including adults and children (Williamson et al., 2002; Williamson et al., 2003; Williamson et al., 2004; Martin et al., 2007; Williamson et al., 2007; Williamson et al., 2008; Nicklas et al., 2012). For instance, portion size estimates for food intake obtained using a food-photograph technique showed a high correlation with weighed portion sizes ($r = 0.92$, $p < 0.0001$) while the mean difference between both estimates was 5.2 ± 0.95 g (mean ± SEM), with no systematic bias over levels of food intake (Williamson, et al., 2003). Furthermore, studies have indicated a consistently high inter-rater agreement with the use of food photography techniques. Martin and colleagues observed an intra-class correlation coefficient for energy intake of 0.92 (95% confidence intervals, $p < 0.0001$) (Martin, et al., 2009) and 0.93 (Martin, et al., 2007), which indicated that multiple raters can rate food intake without significant variation in the estimates obtained. The raters in these studies include dietitians, research associates, individuals with college degrees and student workers attending college, which suggests that individuals with at least some college experience can be trained to estimate food intake accurately using this method.

The food photography method being validated in this chapter is similar to those used in previous studies (Williamson et al. 2007; 2008; Martin, et al., 2012). However, it differs by using a secure website for collecting dietary records rather than automated or semi-automated data management software. Although the use of a food photography data management software

helps to reduce the task associated with managing large numbers of digital records (Martin, et al., 2012), it is expensive to design. Hence it has not been used in the present validation study. More so, despite the availability of other variants of food photography for dietary assessment in previous studies (Lassen, et al., 2010; Lazarte, et al., 2012; Martin, et al., 2012; Martin, et al., 2014; Olafsdottir, et al., 2016), there is an apparent lack of evidence of the validity for estimating micronutrient intake of free-living individuals. Hence, this study aimed to investigate the validity of food photography for assessing dietary micronutrients among free-living adults.

4.3. Method

4.3.1. Research question and hypotheses for validating the food-photograph method

Based on the aim of the study; to investigate the validity of the food photograph method for assessing dietary micronutrients among free-living adults, the resulting research question was whether the food photograph method was valid for assessing dietary micronutrients among free-living adults. The following null (H_0) and alternative (H_1) hypotheses were therefore postulated thus:

H_0: The food photograph method is not valid for assessing dietary micronutrients among free-living adults.

H_1: The food photograph method is valid for assessing dietary micronutrients among free-living adults.

4.3.2. Research design for validating the food-photograph record

A quantitative research design was applied to validate a food photography record for students of Anglia Ruskin University, United Kingdom. Concurrent validation method was applied using the weighed food record as a reference by collecting dietary details using both methods and then comparing the results. Concurrent validity is a type of criterion validity which seeks to measure the performance of an approach against a standard by applying both methods (the test and reference methods) in measuring a single parameter (Mokkink, et al., 2010). It is usually used when the test method is intended to be used as a replacement for the reference technique. Of other methods of criterion validation, (content, predictive, convergent and discriminant validity), concurrent validity was applied since it is in keeping with the objective set out to be achieved at this research.

4.3.3. Ethical approval for the validation study

Before commencing the validation study, ethical approval was sought and gained from the Faculty of Medical Sciences Research Ethics Panel (FREP), Anglia Ruskin University (FREP Number: 15/16082 (see Appendix 3).

4.3.4. Participants

Participants of both the pilot study and the larger validation study were students of Anglia Ruskin University based in the Cambridge campus.

4.3.5. Instrumentation for the validation study

The materials used in the study included a 5kg Precision kitchen scale (SAVISTO Glass Platform Scale: SV-KITC-Z039) and two secure, user-friendly WordPress websites (WordPress 4.5 Beta 1) that were designed by the researcher. The websites were designed before the study began. One website was designed for carrying out a pilot, while the other was

designed for the larger validation study. Both websites were prioritised for the collection of meal photographs consumed by the participants during the period of the research. The log-in details for both websites were programmed to provide each participant with a unique space on the website were their dietary data was stored.

All the kitchen scales provided for the research were exactly the same (5kg Precision kitchen scale- SAVISTO Glass Platform Scale: SV-KITC-Z039), purchased at the same time and first installed in each participant's kitchen or other eating areas for the research. After installation, each scale was calibrated. Before calibration, the surface of the scale was wiped clean to ensure that there was no wetness or debris. After cleaning, reference was made to the user manual for the manufacturer's specific instructions. The calibration instructions provided by the user manual was followed. Accordingly, a smooth and flat surface was first located. The flatness of the surface was ensured using a 0.2- metre bubble (spirit) level (Stanley Spirit level). The kitchen scale was turned on, and the "ZERO" button pressed to zero out the scale and cleared any pre-programmed data. After the scale was zeroed, standard weights (9.5g, 47.5g, 90.5g, 190g, 665g, 1kg and 5kg) were carefully cleaned and placed on the scale. For each standard weight placed on the scale, the scale indicated when it was ready to calibrate and required that the "ENTER" button be pressed. When this stage of the procedure was concluded, the device was turned off as recommended by the user manual.

Each participant was allowed access to a kitchen scale. Where participants shared the kitchen or living space, a single kitchen scale was supplied and placed in the common area for use. Sharing of living space was common, especially for students who lived in shared housing. A record booklet and pen were also provided for each participant to enable them to record the weighed food estimates and other necessary dietary data. The materials provided for the research were in accordance with the aim of the study.

Before the validation procedure, a pilot study was carried out to assess the feasibility and identify possible limitations associated with undertaking the validation study among the students. Details of the pilot study are reported below.

4.3.6. Pilot study

4.3.6.1. Sample size determination and participant recruitment

In determining the appropriate sample size for the pilot study, the researcher adopted a 95% confidence level and a probability level of 0.5. This probability level of 0.5, represents the minimum likelihood of the occurrence of an unforeseen event which could affect the validation study. A probability of 0 would imply that there is no likelihood that an unforeseen event which could affect the validation study would occur. On the other hand, a probability of 1 suggests that an unforeseen event which could affect the validation study would certainly occur. Since at the point of conducting the pilot study, neither the nature nor frequency of possible events which could affect the main validation study was known to the researcher, a probability of 0.5 was considered an appropriate choice. The choice of the probability level was made by the researcher and guided by a study on the calculating sample size in pilot studies (Viechtbauer, et al., 2015). When the confidence level and assumed probability were used to calculate the sample size, in the NCSS PASS statistical software, the minimum sample size obtained was four participants.

Ten students of Anglia Ruskin University were recruited by convenience sampling from within 5 different student halls of residence (Peter Taylor House, YMCA Queen Anne, Anastasia House, Swinhoe Halls and Collier Road Shared Housing). Due to the risk of losing participants to follow-up, it was necessary to recruit more than the minimum required number.

4.3.6.2. Data collection for the pilot study

From each student approached, informed consent was sought, and for anyone who accepted to participate, they indicated by signing the consent form provided to them. It was ensured that each participant had access to a functioning smartphone. To each participant, the details of the research were explained by the researcher before the procedure.

a. Weighed food record: Since each student resided in some form of shared accommodation with a common cooking and dining area, weighing scales were made available at such central point for participants' use. All ten subjects were provided with record booklets for documenting weighed measures of their meals and other necessary details. The recording booklet was designed such that a section on the recording sheet was dedicated to recording food weight and other dietary details, while another section was designed to assess participants' feedback regarding the perceived ease of use and satisfaction with both the test and reference methods.

b. Food photograph record: It was ensured that all participants had access to a smartphone and each one was shown appropriate positions to capture images of their meal and how to upload the images to the website prepared for the study. They were also instructed to take pictures before eating, and after having their meal (meal remnant) if they had some leftover. For ease in the estimation of portion sizes from the photographs, all participants were directed to capture images from different positions (at least two positions including from the top and side- about 45^{o}). Additionally, they were asked to attach a description of the food which they consumed for all captured images. Participants were required to take photographs of the nutrient labels of pre-packaged foods and post to the website.

Each participant provided a 2-day food record using both methods simultaneously for the same meal. The 2-day record was taken on two non-consecutive days.

4.3.6.3. Data analysis for the pilot study

The "perceived ease of use" was assessed by seeking participants' opinion on the statement: "I found the food photography method/weighed food record easy to use" based on a five-point scale rating (1- "Strongly disagree", 2- "Disagree", 3- "Neutral", 4- "Agree", 5- "Strongly agree"). Similarly, "satisfaction" was assessed by requesting participants' opinion on the statement: "I am satisfied with the procedure of food photography method/weighed food record for assessing my diet." The responses were also rated on a five-point scale; 1- "Strongly disagree", 2- "Disagree", 3- "Neutral", 4- "Agree", 5- "Strongly agree." At the end of the recording sheet, participants were requested to state any other comments relating their experience of either of the procedures undertaken. The details on "perceived ease of use" and "satisfaction" were adapted from the Global Satisfaction Scale (Aletras, et al., 2010) and the Health Information Technology Usability Evaluation Scale (Schnall, Cho and Liu, 2018), respectively.

4.3.6.4. Findings of the pilot study

All participants carried out both procedures simultaneously for each meal recorded and reported only being able to undertake the procedures for meals consumed within their accommodation. Although some of the captured images were not clear and of disproportionate sizes, all subjects reported considerable ease of use (Figure 4.1) and satisfaction (Figure 4.2) with the food photograph method. Of all the participants, only 2 (20%) claimed using the kitchen weighing scale was difficult and unfavourable for taking dietary records during occasions of eating out, while all indicated they would not mind undertaking both methods requested on a later date. Also, some disparity in the spelling and naming of foods was noted between specific meals recorded by both methods. However, in each case, the descriptions made on the booklet corresponded to the images uploaded.

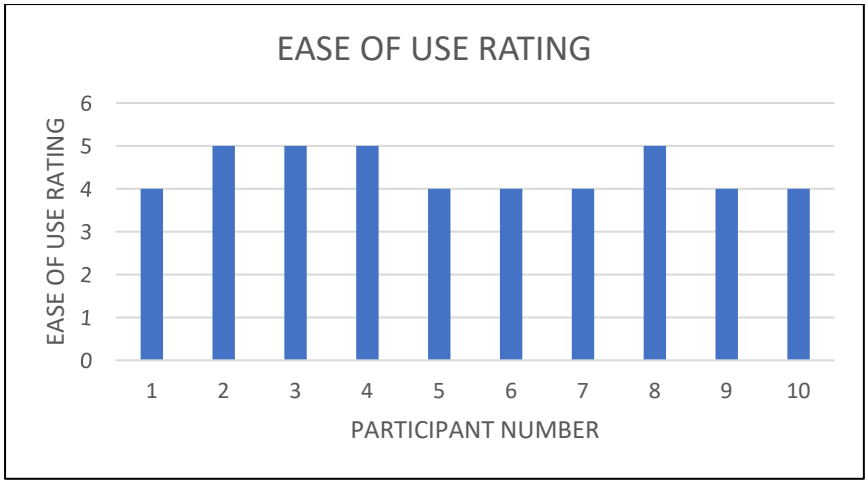


Figure 4.1. A bar chart of ease of use ratings based on the use of the food photograph method for dietary assessment (Pilot study).

The vertical axis represents a five-point scale of "Ease of use" ratings: 1- "Strongly disagree", 2- "Disagree", 3- "Neutral", 4- "Agree", 5- "Strongly agree."

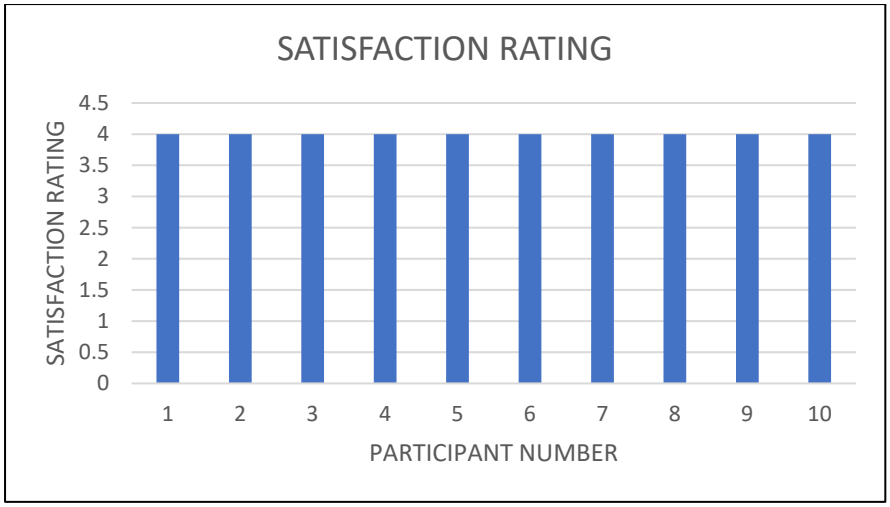


Figure 4.2. A bar chart of satisfaction ratings based on the use of the food photograph method for dietary assessment (Pilot study)

The vertical axis represents a five-point scale of "Satisfaction" ratings; 1- "Strongly disagree", 2- "Disagree", 3- "Neutral", 4- "Agree", 5- "Strongly agree."

Based on the findings, it was deduced that the students were adaptable and comfortable with taking dietary records using the food photograph method and weighed food record. Nevertheless, they require guidance through the procedure for capturing images to ensure uniformity in capturing photographs and recording dietary data. By noting these details, the aim of the pilot study was satisfied, and measures were taken to avoid the observed challenges in the main validation study. The measures which were taken are discussed below (section 4.3.6.5).

4.3.6.5 Modifications based on the pilot study findings

Due to the observations recorded in the pilot study, some steps were taken to limit the factors with the likelihood of introducing bias during the main validation study. Some of the steps included the design of a scaled table mat with a colour code to enhance the easy and accurate estimation of portion sizes of meal photographs (Figure 4.3- below) and provision of adequate

instruction on how to use kitchen scales and record measurements. As a support for the latter, illustrative charts and samples of completed weighed food records created by the researcher were prepared to guide respondents through recording the weighed measurements when using the reference technique. Also, sample images showing the required positions for taking photographs of the meal were prepared for the respondents.

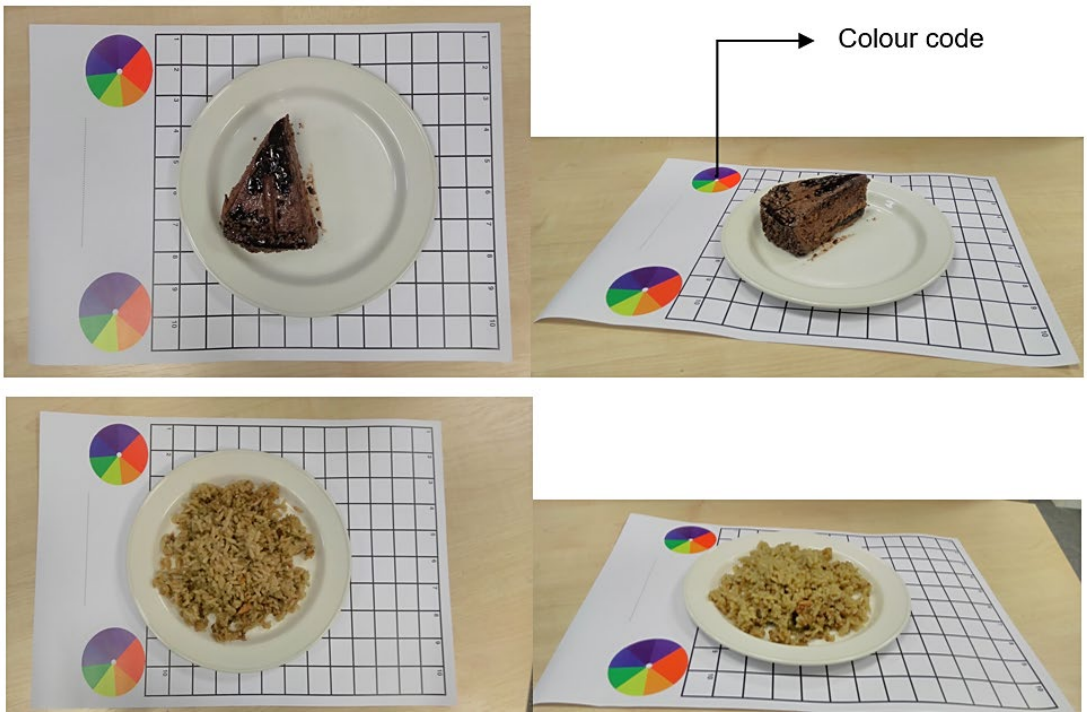


Figure 4.3. Scaled table mat for the food photography method.

4.3.7. Sample calculation for the validation study

In determining an appropriate sample size necessary to detect a difference between dietary data estimated by the test method (food photograph method), and the reference method (weighed food record method), a standard deviation of 2 units (±2SD) and an effect size of 1.42 were adopted, with a 5% level of significance and a power of 80%. These yielded a sample size of

31 when calculated using the PASS Statistical Software (2016). The estimates used for estimating the suitable sample size for the study were based on previous studies. The standard deviation and effect size estimates were deduced from a previous similar study of 19 participants evaluating the validity of a digital photography method for dietary assessment against the weighed food record (Lassen, et al., 2010). While the expected effect size was estimated based on those used in previous studies (Lazarte, et al., 2012; Olafsdottir, et al., 2016).

Furthermore, based on a previous study on food photograph method in free-living subjects (Martin, et al., 2012), an anticipated loss to follow-up of participants of 16% was considered, which resulted in the recruitment of 5 additional participants.

4.3.8. Defining sample selection for the validation study

Thirty-six participants of the study were recruited using a convenience sampling method. This technique was used due to its related advantages of being relatively fast, easy and inexpensive (Antony, 2002; Kate, 2007). These merits were of utmost consideration given the resources and time allotted to the project, especially as it was required for the next stage (the cohort study) to proceed. Although only 31 participants were required based on the calculated sample size, a risk of 16% attrition was anticipated based on a previous similar study (Martin, et al., 2012), which led to the recruitment of thirty-six subjects. All participants were students of Anglia Ruskin University based at the Cambridge campus and recruited by the researcher on weekdays.

4.3.9. Data collection for the validation study

Data collection for the validation study began on 24th July 2016 given that the pilot study results indicated that students were satisfied with the protocol, and the potential challenges had

been improved upon through the modifications made. Each participant was approached within the halls of residence, briefed about the research and the nature of their participation in the research. Those who agreed to participate were then handed a Participant Information Sheet (see Appendix 4) for clarity on the research details. Interested participants signed a consent form agreeing to participate in the study before being handed a record booklet. Kitchen scales were placed in central kitchens and dining areas except for subjects who had private kitchens. At each weighing point, a description of how to use the kitchen scale was posted to enhance use. Those with private kitchens were handed a printed copy of the description. This step was taken having noted that in the pilot study, participants had difficulty using the kitchen scales even after the researcher had verbally described the procedure to them. Every participant was given a record booklet, a pen and scaled table mat, regardless of sharing their cooking space. Consent to use the data was implied by the respondent handing the signed consent form back to the researcher.

4.3.10. Weighed food record (Reference method)

The weighed food record entailed that the participants weighed their meal before eating, and plate waste after eating using a kitchen scale and recorded the readings in a record booklet. Due to the challenges experienced by participants which were noted during the pilot study, adequate instruction was provided on how to use the kitchen scales at the cooking and dining areas. Samples of completed weighed records were also shown to each respondent to illustrate the exercise and highlight the requirements of the research.

4.3.11. Food photography method (Test method)

The researcher verified participants' access to a smartphone, and each participant was shown appropriate positions to capture images of their meal and how to upload the images to the

website. They were also instructed to take pictures before eating, and after having their meal (meal remnant) if they had some leftover. For ease in the estimation of portion sizes from the pictures, all participants were directed to capture images from different positions (at least two positions including from the top and side- about 45^{o}). Also, to ensure the appropriate scaling of the photographs, participants were required to place their meal on the table mat designed for the research without obscuring the colour code (Figure 4.3) before taking photographs. The researcher also recommended additional descriptions of food consumed for all captured images such as the name of the food, the method by which it was prepared (steaming, boiling, frying). Considering that some foods consumed could be pre-packaged, participants were required to take photographs of the nutrient labels as well and post to the website.

Each participant provided a 4-day food record using both methods simultaneously for the same meal. The 4-day record was to be taken for four non-consecutive days (including one weekend day) and during a two-week period. Data collection was concluded on 5th October 2016. An illustration of the steps carried out for validating the food photography method using weighed food record as a reference is shown in Figure 4.4 below.

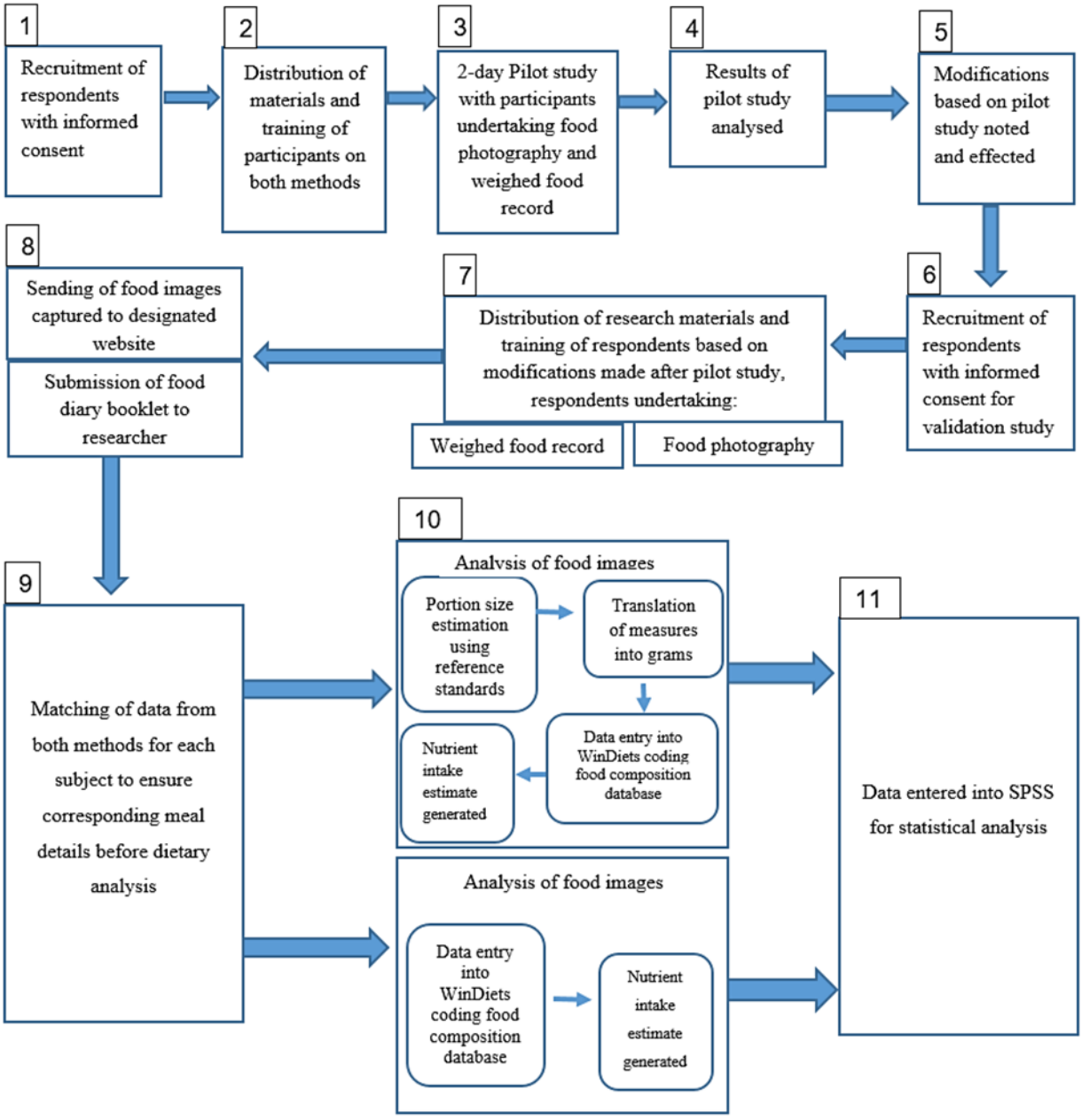


Figure 4.4. A schematic diagram describing the steps carried out for validating the food photograph method using weighed food record as a reference

4.3.12. Data analysis for the validation study

After data collection was concluded, food photographs from the website were exported into specific folders and differentiated based on the participant and time of collection (baseline and 6th month period). Using Canva, a graphic design interface (https://www.canva.com/), each food image was pasted and aligned to 1280 x 720 pixels and overlaid with a translucent layer of the scaled table mat designed for the research to identify the area of the serving plate and food contained. This was repeated for images of the plate waste were available. Food images were translated to the weight equivalents following the guidelines provided by the food portion size directory provided by the Food Standard Agency (2008) and Safefood (2016), and the difference between the weight measures of the served meal and plate waste was calculated to obtain the measure of food consumed by the participant. Where the food consumed was pre-packaged, scaling using Canva as described above was not necessary since information regarding the weight was present on the pack included by the participants. The weight equivalents in each case were keyed into the WinDiets Research Package (version 2015), and the nutrient components generated were exported into Microsoft Excel spreadsheets. Within the spreadsheets, nutrient components for each participant's meals were summed for the 4 days recorded and averaged to obtain the mean daily intake estimate. The mean daily intake estimate was calculated separately for data collected at baseline and at 6-months of the study.

The WinDiets Research is a nutritional analysis tool or coding programme which uses the 2015 version of McCance and Widdowson's food tables and other international food tables. Through the food tables, specific food items can be found by name, the portion sizes and corresponding amount of nutrients contained in grams. Dr Alan Wise originally designed the analysis tool for teaching nutrition (Robert Gordon University, 2017). Since then it has been further developed into a research tool for nutrition analysis and is largely recommended for nutritional analysis (McGeoch, et al., 2011; Howard, Adams and White, 2012; Astell, et al., 2014).

Dietary assessment using both methods was undertaken simultaneously. Where food consumed was pre-packaged, the nutrient label was used to provide estimates of nutrient content. In cases where there was some food remnant, the amount consumed was obtained by subtracting the remnant portion from the original portion. Where no image of food remnant was captured, it was considered as non-existent. Nutrient data were imported into the Statistical Package for Social Sciences (SPSS) for analysis. Means and standard deviations for measures obtained by both methods where calculated. Paired t-tests were conducted to compare the mean measures obtained by both methods, and results which were of p-value less than 0.05 were considered statistically significant.

Pearson's correlation analysis was conducted to investigate the linear correlation between the measures obtained by both methods, which was necessary before investigating the agreement between them.

Bland-Altman (1986) analysis was used to measure the level of agreement between both methods for each nutrient. The difference between both methods on the y-axis was plotted against the mean of both methods on the x-axis, and the zero-bias line, 95% upper and lower confidence limits (mean difference ±2SDs of the differences) was overlaid on the plot. A mean difference of 20% was considered as the criterion for an agreement based on a previous relevant study (Olafsdottir, et al., 2016). A trend line was also overlaid on the same plot, its slope was calculated, and the level of significance determined. P-values which were less than 0.05 in each case was considered statistically significant. The Bland-Altman analysis was introduced by Bland and Altman to evaluate the agreement between two quantitative measures by creating a scatter plot of the difference between both measurements against their average (Bland and Altman, 1999). Through this plot, the standard deviation and the mean difference between both measures are used to calculate statistical limits. The mean difference between both measures is known as the bias. If one measure is sometimes higher and other times lower than the other

measure, then the bias occurs nearer to zero on the plot. The further away the bias occurs from zero indicates that the measures are different. The statistical significance of the bias can be calculated. However, its acceptance is determined a priori, either based on research or clinical goals (Giavarina, 2015). The acceptable level of bias in the present study was given a priori at 20% based on previous research on the development and validation of a photographic method for dietary assessment (Olafsdottir, et al., 2016). The plot may also include a trend line which evaluates the tendency of one measure to either occur higher or lower than the other through the statistical significance of its gradient (Giavarina, 2015).

4.3.13. Reproducibility of food photography method for dietary assessment

After the data obtained was analysed, food photographs (both original image before eating and plate waste) obtained from the participants were re-analysed a month later, and the results compared with the first set of results by the same technique. This was done to check how similar repeated measures obtained by the test method are. Given that both were conducted by the same rater, the intra-rater reliability was measured using the intra-class correlation coefficient.

4.4. Results

The characteristics of the participants are represented in Table 4.1 below. All 36 participants who took part in the research followed through the study with complete records. Although not consistently, the test method tended to underestimate most nutrients (Table 4.2 and Figure 4.5). Nevertheless, this did not amount to any significant difference compared with the reference method as measured using the paired t-test.

Table 4.1. The number (N) and age (mean±SD) of participants based on gender.

	N	Age (years)
Males	22	26.3 ± 5.5
Females	14	23.1 ± 3.4
Total	36	25.1 ± 4.9

Table 4.2. Nutrient estimates (mean± SD) obtained by the test method (food photography method) and the reference method (weighed food record).

Dietary Component	Food photograph record	Weighed food record	p-value (t-test)
Vitamin A (µg)	480.7± 136.3	501.1 ± 143.7	0.07
Riboflavin (B_2) (mg)	1.3 ± 0.2	1.5 ± 0.2	0.10
Folate (µg)	224.4 ± 47.0	236.0 ± 47.0	0.34
Vitamin D (µg)	7.5 ± 4.0	6.6 ± 4.6	0.37
Iron (mg)	9.4 ± 3.0	8.4 ± 3.3	0.30
Calcium (mg)	784.8 ± 187.7	815.3 ± 127.8	0.26
Vitamin E (mg)	11.7 ± 3.0	13.7 ± 2.7	0.09
Magnesium (mg)	225.2 ± 44.5	248.9 ± 36.4	0.09
Potassium (mg)	2629.7 ± 517.0	2809.3 ± 492.9	0.31
Iodine (µg)	108.3 ± 40.5	118.7 ± 42.7	0.10
Selenium (µg)	53.8 ± 20.0	64.0 ± 37.3	0.12
Zinc (mg)	9.1 ± 3.3	10.1 ± 3.4	0.40
Vitamin C (mg)	113.8 ± 28.2	105.4 ± 30.5	0.08
Manganese (mg)	3.4 ± 1.7	3.0 ± 1.7	0.17
Copper (mg)	1.3 ± 0.62	1.16 ± 0.5	0.13
Phosphorus (mg)	453.8 ± 136.3	496.5 ± 175.2	0.07
Niacin (B_3) (mg)	1.8 ± 0.9	1.6 ± 0.7	0.20
Cobalamin (B_{12}) (µg)	4.2 ± 1.1	4.6 ± 1.8	0.22
Thiamine (B_1) (mg)	0.5 ± 0.7	0.6 ± 0.6	0.53
Pantothenate (mg)	2.8 ± 1.5	3.0 ± 1.5	0.45
Pyridoxal phosphate (B_5) (mg)	1.2 ± 0.6	1.3 ± 0.5	0.19
Biotin (µg)	24.6 ± 9.1	27.3 ± 8.5	0.12
Folate (µg)	302.7 ± 40.5	283.3 ± 55.6	0.08
Vitamin K (mg)	63.3 ± 21.8	55.4 ± 18.7	0.07

The Bland Altman analysis showed that there was good agreement between measures obtained by both methods; the measure of bias for any of the nutrients assessed did not occur more or less than 20% of the reference method (Figure 4.5). This measure of agreement was determined *a priori* as a standard for acceptance of nutrient estimates in dietary assessment (Olafsdottir, et al., 2016). Furthermore, the trend line (line of regression) plotted between the average and the difference of estimates obtained by both methods (test and criterion methods) indicated a slope. However, this was not statistically significant for any of the nutrients estimated ($p>0.05$) (Figure 4.6(a and b) in the text below and others in Appendix 5).

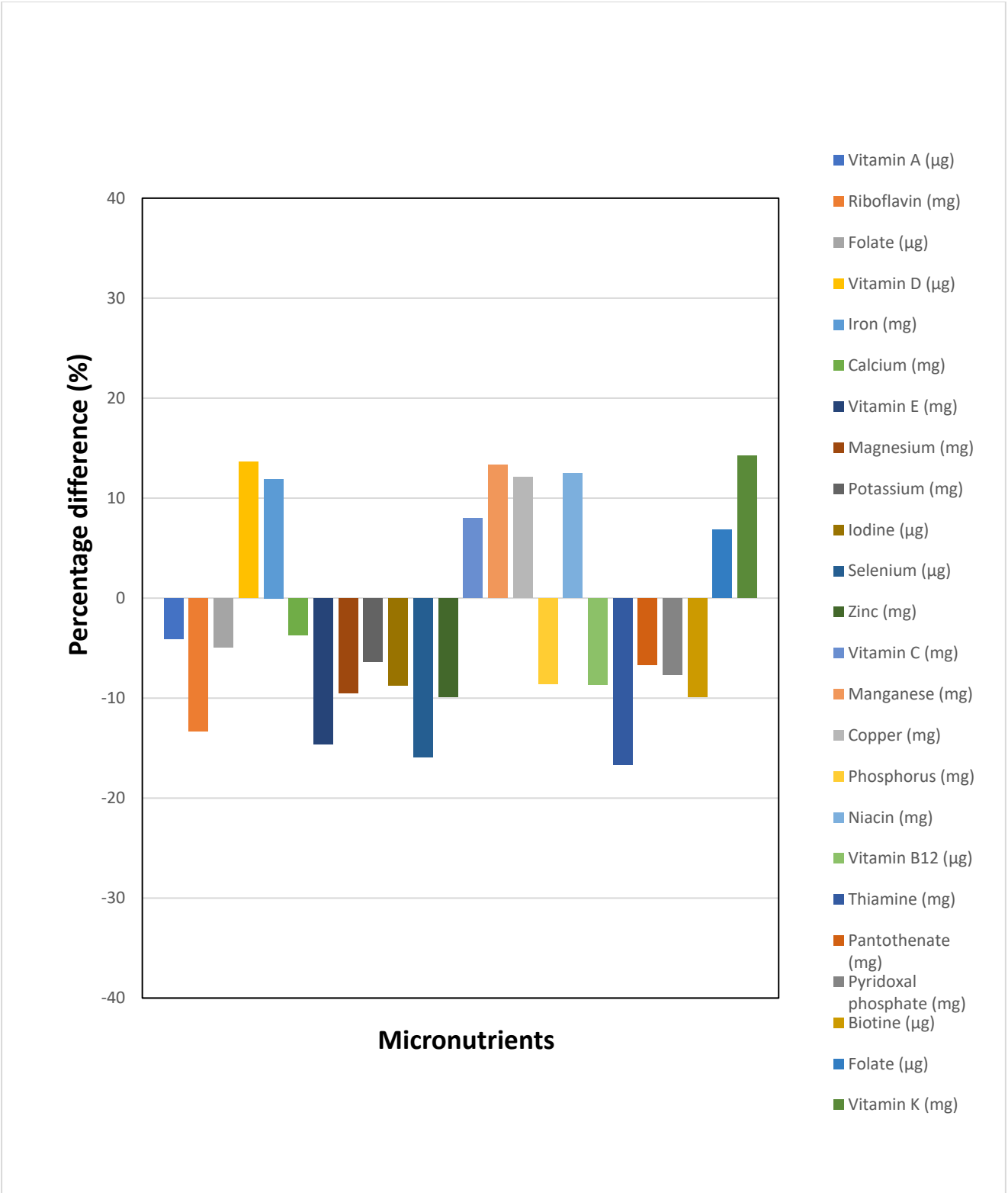


Figure 4.5 Percentage Difference between the Weighed Food Record and Food Photography Method

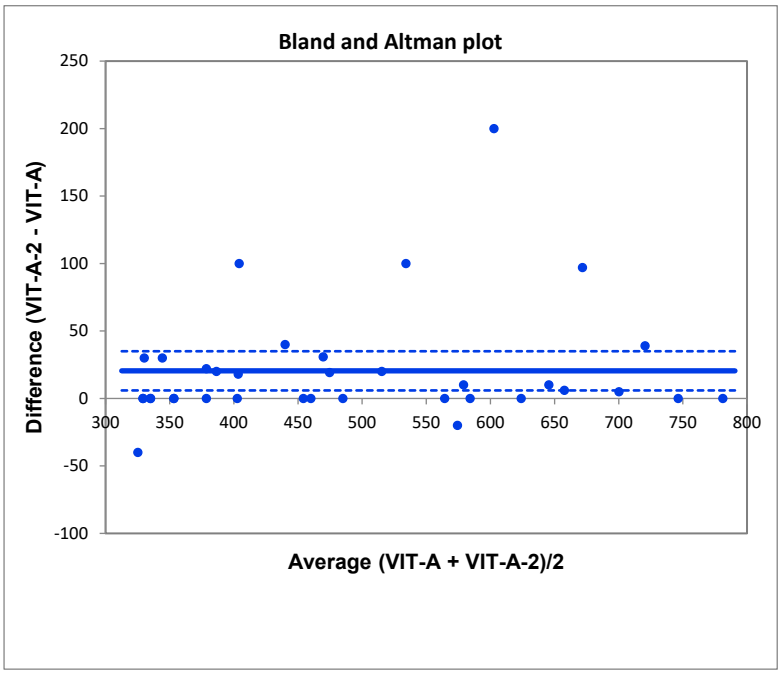


Figure 4.6a. Bland Altman plot for food photograph and weighed estimates of vitamin A in participants' diets.

Figure 4.6a: Bland and Altman plot for the food photograph (VIT-A) and weighed estimates (VIT-A2) of vitamin A in participants' diet showing the bias and confidence interval (CI). Both axes are in microgram (µg) unit.

= Limits of agreement; a range within which 95% of the differences between both measurements are included.

= Bias; the difference between the estimates by both methods.

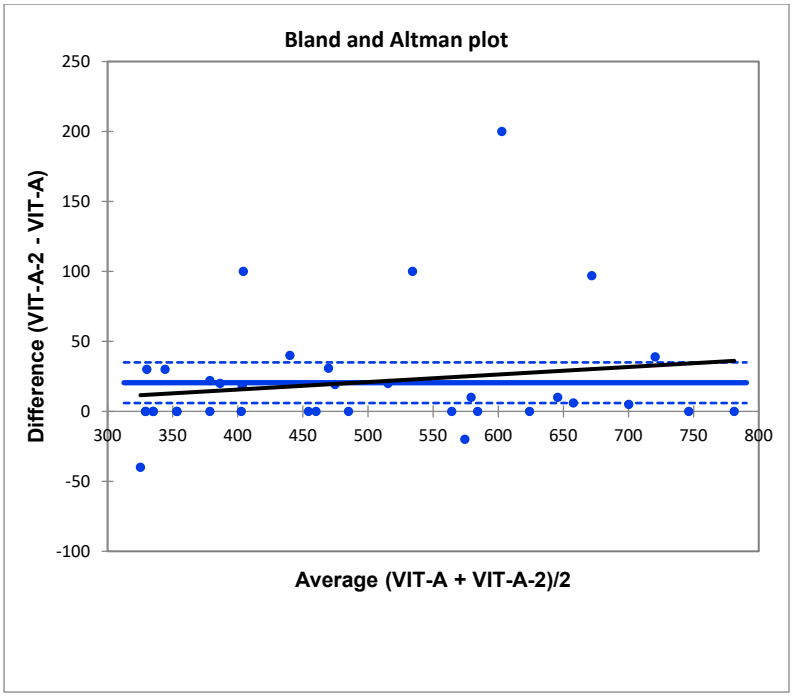


Figure 4.6b. Bland Altman plot for food photograph and weighed estimates of vitamin A in participants' diets with a line of regression

Figure 4.6b: Bland and Altman plot for the food photograph (VIT-A) and weighed estimates (VIT-A2) of vitamin A in participants' diet showing the bias and confidence interval (CI). Both axes are in microgram (µg) unit. This graph also indicates the trend line showing the tendency of the test method to overestimate or underestimate the reference measure. (——— Linear (OK))

- - - - - = Limits of agreement; a range within which 95% of the differences between both measurements are included.

——— = Bias; the difference between the estimates by both methods.

The correlation analysis conducted showed that the measures obtained by both methods were significantly directly correlated ($p< 0.05$) (Table 4.3).

Table 4.3. Pearson correlation coefficients and the corresponding p-values between dietary estimates obtained by weighed food records and food photograph method.

Nutrient	Correlation coefficient (r)	p-value (p< 0.05*)
Energy (kcal)	0.9920	<0.0001*
Vitamin A (µg)	0.9994	<0.0001*
Riboflavin (mg)	0.7767	<0.0001*
Folate (µg)	0.9423	<0.0001*
Vitamin D (µg)	0.9824	<0.0001*
Iron (mg)	0.7163	<0.0001*
Calcium (mg)	0.9998	<0.0001*
Vitamin E (mg)	0.9779	<0.0001*
Magnesium (mg)	0.9829	<0.0001*
Potassium (mg)	0.9717	<0.0001*
Iodine (µg)	0.9969	<0.0001*
Selenium (µg)	0.9716	<0.0001*
Zinc (mg)	0.9379	<0.0001*
Vitamin C (mg)	0.9965	<0.0001*
Manganese (mg)	0.9844	<0.0001*
Copper (mg)	0.9844	<0.0001*
Phosphorus (mg)	0.8155	<0.0001*
Niacin (mg)	0.9921	<0.0001*
Vitamin B12 (µg)	0.9243	<0.0001*
Thiamine (mg)	0.9142	<0.0001*
Pantothenate (mg)	0.9977	<0.0001*
Pyridoxal phosphate (mg)	0.9865	<0.0001*
Biotin (µg)	0.9976	<0.0001*
Folate (µg)	0.9176	<0.0001*
Vitamin K (mg)	0.9376	<0.0001*

In table 4.3, the p-values marked with an asterisk are statistically significant.

When the food photograph diet records were re-analysed and compared with the measures obtained previously using the same method, a minimum intra-class correlation coefficient (coefficient of reliability) of 0.75 was obtained.

Table 4.4. Mean measures of micronutrients obtained with repeated photograph method and reliability coefficient

Dietary component	Food photograph method- 1	Food photograph method- 2	Reliability coefficient
Vitamin A (µg)	480.7± 136.3	494.8 ± 146	0.95
Riboflavin (mg)	1.3 ± 0.2	1.4 ± 0.2	0.86
Folate (µg)	224.4 ± 47.0	233.7 ± 41.5	0.76
Vitamin D (µg)	7.5 ± 4.0	7.4 ± 4.7	0.90
Iron (mg)	9.4 ± 3.0	9.1 ± 3.5	0.80
Calcium (mg)	784.8 ± 187.7	800 ± 124.2	0.78
Vitamin E (mg)	11.7 ± 3.0	12.8 ± 2.8	0.75
Magnesium (mg)	225.2 ± 44.5	227.7 ± 42.9	0.75
Potassium (mg)	2629.7 ± 517.0	2722.8 ± 527.1	0.77
Iodine (µg)	108.3 ± 40.5	114.8 ± 35.6	0.75
Selenium (µg)	53.8 ± 20.0	55.1 ± 18.9	0.76
Zinc (mg)	9.1 ± 3.3	9.6 ± 2.9	0.74
Vitamin C (mg)	113.8 ± 28.2	112.3 ± 24.2	0.85
Manganese (mg)	3.4 ± 1.7	3.6 ± 2.0	0.75
Copper (mg)	1.3 ± 0.62	1.2 ± 0.5	0.75
Phosphorus (mg)	453.8 ± 136.3	496.5 ± 173.8	0.82
Niacin (mg)	1.8 ± 0.9	1.35 ± 0.7	0.76
Vitamin B12 (µg)	4.2 ± 1.1	4.6 ± 1.2	0.78
Thiamine (mg)	0.5 ± 0.7	0.7 ± 0.6	0.86
Pantothenate (mg)	2.8 ± 1.5	3.2 ± 1.5	0.84
Pyridoxal phosphate (mg)	1.2 ± 0.6	1.3 ± 0.4	0.80
Biotin (µg)	24.6 ± 9.1	26.5 ± 7.7	0.80
Folate (µg)	302.7 ± 40.5	277.4 ± 54.7	0.78
Vitamin K (mg)	63.3 ± 21.8	58.6 ± 20.7	0.77

4.5. Discussion

This study aimed to establish that the food photography method (test method) can validly and reliably estimate the dietary micronutrients when compared to the weighed food record (reference method) in free-living adults. The results showed that although the food photography record tended to underestimate some micronutrients, the difference was not statistically significant compared to the weighed food record ($p > 0.05$). Pearson's correlation analysis showed a significant direct correlation between both measures ($p < 0.05$), and the Bland-Altman analysis showed that the measure of bias for each micronutrient estimated was less than ±20% of the mean measure of the reference method. Based on these findings, the null hypothesis was rejected. Furthermore, the trend line plotted upon the Bland-Altman plot indicated no slope of statistical significance ($p > 0.05$), while the reliability test of the test method yielded a minimum intra-rater intra-class correlation coefficient of 0.75.

In previous research, the use of food photography methods for dietary assessment has been demonstrated to provide valid nutrient estimates. In a Bolivian study involving 45 women, a comparison between dietary estimates obtained using a food photography 24-hr recall method and the weighed food record showed non-statistically significant mean differences (from -14.4g to 4.5g) (Lazarte, et al., 2012). On the contrary, other studies have found that although food photography method resulted in a small difference in portion size estimates (from -9.1g to 18.3g) (Williamson, et al., 2003), and energy intake (-6.6%) compared to the weighed food record (Martin, et al., 2009), the differences were statistically significant. Also, a positive correlation (0.93 to 0.95) has been observed between energy intake estimates for food photography method and weighed food record (Martin, et al., 2009). In the present study, the mean difference between micronutrient intake estimates of the food photography method and weighed food record were not statistically significant ($p > 0.05$) and the estimates were directly correlated (from 0.71 to 0.99).

The Bland Altman analysis carried out in the present study showed that over 95% of the mean difference between the food photograph method and the weighed food record were distributed along the bias line and near to the line of equality (bias= 0). Previous research has reported a similar distribution in the Bland Altman plot (Williamson, et al., 2003; Martin, et al., 2009; Lazarte, et al., 2012). Although the authors of these studies claimed that the Bland Altman plot showed a good agreement between the methods, unlike the present research (20%), they did not refer to an *a priori* criterion for agreement.

Furthermore, in the present research, the line of regression plotted upon the Bland-Altman plot indicated no slope of statistical significance ($p > 0.05$), implying that the mean difference between the estimates obtained by the food photograph method and the weighed food record were random and not due to systematic bias. Lazarte and colleagues observed a similar trend for energy intake estimates obtained by food photography and weighed food record (Lazarte, et al., 2012).

In the current study, when the dietary assessment was repeated using the food photography method, the reliability test yielded a minimum intra-rater intra-class correlation coefficient of 0.75. According to the guideline for selecting and reporting intraclass correlation coefficient (Koo and Li, 2015), this estimate indicates “good” intra-rater reliability. It implies an acceptable margin of error when the same assessor repeats the method. Other researchers have likewise demonstrated good reliability for photography methods for dietary assessment. For instance, an Australian study on the reliability of a photograph assessment of the lunches of 176 children indicated an intra-rater intraclass correlation coefficient of 0.78 - 1 (Mitchell, et al., 2010).

Unlike traditional methods such as 24-hour recall, estimated food records and diet history, the food photography method has advantages of being less time-consuming and convenient, which

is important for participants to ensure they stay motivated through the study (Olafsdottir, et al., 2016). Also, it is tailored to the growing trend of photographing and posting images on social media (Spence, 2016). Hence it is unlikely to result in changes in habitual dietary intake during the study. Changes in dietary habits during nutritional assessment has been highlighted as an important cause of inaccuracy (Shim, Oh and Kim, 2014). Another merit associated with using the food photograph method is that participants are not given the task of rating their portion sizes, which is noted in previous studies to be a source of error leading to over- or under-reporting (Shim, Oh and Kim, 2014).

Besides serving as a quick, unobtrusive, convenient and valid method for obtaining dietary data, the food photography method used in this study also is of relatively low cost, compared to other digital methods which employ either a digital camera for capturing photographs (Williamson, 2003; 2004) or data management software (Martin, et al., 2012). Furthermore, the use of smartphones offers more flexibility for individuals, making it easy to use in free-living conditions. The Remote Food Photograph technique validated by Martin et al. (2009; 2012) is claimed to be of similar merit, except that the authors stated to have provided the food consumed by participants; which implies that the condition of the participants was not purely free-living.

Some potential limitations with the validation study are acknowledged. For instance, while using the food photograph method, in the event of participants forgetting to capture food images, losing their phones before sending dietary data to the website, challenges in collecting data could result since there are no back-up measures provided. Martin, et al. (2012) have recommended the use of a food record as a back-up when participants are unable to capture food images; however, it may result in some participant burden. Also, this measure can become burdensome for the assessors in large epidemiological studies and result in prolonging the time

for analysis and feedback. Additionally, the feasibility and acceptability of the method to participants may be dependent on participants' demographics.

The present study has some limitations. Although the paired t-test showed no statistical significance when the mean estimates obtained using the test and reference methods were compared, it is admitted that large differences may exist between individual estimates of both methods. Also, the paired t-test was used with the assumption of independence of the methods compared, a normal distribution and the absence of outliers from the distribution (Kim and Park, 2019). Hence, caution may be applied in interpreting the results. Another limitation includes the use of convenience sampling technique used. Convenience sampling technique, which involves the recruitment of participants who were accessible to the researcher (Bornstein, et al., 2013) was used because it was cheap, quick and simple to implement. However, this might have been a source of bias by favouring the recruitment of participants who are either more nutrition or health-conscious, have similar diets or apt to capture diet using the smartphones thereby resulting in unclear generalisability. As such, the estimates obtained might be biased and not reflective of the actual validity and reliability of the dietary assessment method used. Hence, caution may be applied in interpreting the results.

It is necessary to highlight that the validity of the food photograph method in this research was evaluated based on the statistical significance of the difference between its nutrient estimates and those of the weighed food record, and the acceptable level of agreement between both methods. The statistical significance, however, does not imply clinical significance. The clinical significance was not discussed due to the context and scope of the study. It is acknowledged that the difference between the nutrient estimates obtained by both methods might be clinically important while remaining non-statistically significant.

4.6. Conclusion

The food photography method is valuable in research for obtaining reasonably valid and reliable estimates of micronutrient intake when used with an adult student population.

CHAPTER 5. A COHORT STUDY ON THE ASSOCIATION BETWEEN CHANGE IN DIETARY NUTRIENT DENSITY AND CHANGE IN BODY FAT PERCENTAGE

5.1. Abstract

Aims: To investigate the association between change in dietary nutrient density and change in body fat percentage using a prospective cohort study design.

Methods: A cohort of 108 adult university students were followed for 6 months, during which their dietary intake and body fat percentage were assessed at baseline and in the 6th month, at the end of the study. A validated 4-day food photograph diary was used to assess the dietary intake, and body fat percentage was estimated by bioelectric impedance analysis. Data were analysed using SPSS V.24. Student's t-test was used to compare the mean anthropometric and nutrient intake measures obtained from participants based on age, gender and being on a special diet, while one-way analysis of variance (ANOVA) was applied for comparing measures from participants based on ethnicity. The level of significance was considered for p-values less than 0.05.

Results: Overall, the dietary nutrient density of vitamins A, E, K and C, folate, iron, calcium, magnesium, potassium, selenium, zinc and phosphorus were each significantly inversely associated with body fat percentage ($p< 0.05$) after adjusting for dietary phytate and covariates.

Conclusions: An increase in the dietary nutrient density of vitamins A, C, E and K, folate, iron, calcium, magnesium, potassium, selenium and phosphorus is associated with a decrease in body fat percentage in university students over 6 months. The dietary nutrient density of specific micronutrients is a critical consideration for bodyweight management.

5.2. Methods and Materials

5.2.1 Introduction

Research suggests that dietary nutrient density is a crucial element of preventive nutrition and that the intake of nutrient-dense foods is necessary to risk of obesity (Troesch, et al., 2015). Also, studies have shown that the dietary factors which determine nutrient density, micronutrients and energy intake, have the propensity to influence body fat (Major, et al., 2008; Wright and Aronne, 2012). However, evidence indicating the relationship between dietary nutrient density and body fat is still lacking. Therefore, this research aims to contribute to the literature by investigating the association between change in dietary nutrient density and change in body fat percentage using a prospective cohort study.

5.2.2. Research question and hypotheses

The aim of this cohort study was directed toward resolving the research question, whether there is an association between change in dietary nutrient density and change in body fat percentage. The following null (H_0) and alternative (H_1) hypotheses were therefore postulated thus:

H_0: There is no association between change in dietary nutrient density and change in body fat percentage, provided dietary phytate does not influence the relationship.

H_1: An increase in dietary nutrient density is associated with a decrease in body fat percentage, provided dietary phytate does not influence the relationship.

5.2.3. Study Design

The present study is a 6-month prospective cohort study design which involved the collection of dietary and body fat percentage data from research participants at the baseline and 6 months

of the study. The study design was used due to its potential to demonstrate the long-term relationship between change in nutrient density and change in body fat percentage. Although it is known that the study design cannot prove causality, it can show strong relationships (Everitt and Palmer, 2005). Also, the study design was chosen because it permits the follow-up and observation of participants without any attempt to change the variables assessed. Especially as the health and ethical implications of altering the nutrient density of the participants while observing for changes in body fat percentage as would be the case in an intervention study, are unclear.

Admittedly, the prospective cohort study design may have limitations such as being time-consuming, costly and prone to bias associated with altering participant behaviour and losing participants to follow-up (Song and Chung, 2010). Nevertheless, the present research has taken steps to limit these weaknesses. These steps are discussed in section 5.3.12 of the thesis.

5.2.4. Sample size determination

A sample of at least 73 participants was required for the study. This sample size was calculated using the PASS Statistical Software (2016), by applying a 95% confidence level and a power of 80% based on the guideline for estimating the desired level of power (Murphy, Myors and Wolach, 2014). Also, an expected effect size of 2.0 and standard deviation of 4.5 were applied, based on a previous study on dietary nutrient intake as predictors of body composition (BMI, waist-hip-ratio and waist circumference) (Toeller, et al., 2001). By adjusting for the possible loss of participants to follow-up at 16%, 12 more participants were required to be recruited, bringing the minimum required sample size to 85 participants. The adjustment for an anticipated loss to follow-up of participants was made based on a previous study on dietary assessment using food photography in free-living individuals (Martin, et al., 2012). It was

important to consider the adjustment. However, since there was a possibility of losing more than 16%, more than the minimum number of participants required was recruited.

5.2.5. Recruitment of participants.

One hundred and thirty-eight students at Anglia Ruskin University were recruited for the research regardless of their year of study using the convenience sampling technique. Although this sampling technique was non-randomised and liable to selection bias (Antony, 2002; Kate, 2007), it was used in this research for its associated merits such as ease and speed, given that the project was only for a limited period. The cohort was chosen since weight change among university students is well documented (Vadeboncoeur, Foster and Townsend, 2015). Although most studies have focussed on first-year university students (Hajhosseini, et al., 2006; Levitsky, et al., 2006; Finlayson, et al., 2012; Kapinos, Yakusheva and Rosenberg, 2014; Vadeboncoeur, Foster and Townsend, 2016), some have also reported weight changes after the first year (Racette, et al., 2005; Lloyd-Richardson, et al., 2009). Hence, even though the cohort in the present study did not report seeking to lose or gain weight, a change in body weight was anticipated by the researcher considering the trend indicated by previous studies. Also, given that previous research has associated the weight changes with students' dietary habits (Crombie, et al., 2009; Vella-Zarb and Elgar, 2009), university students were considered an appropriate cohort to test the research hypothesis.

All participants were verified to have access to a smartphone (needed for the food photography method for dietary assessment) and not be restricted by any social or religious beliefs prohibiting the use of media or relay of dietary information in the form of images before recruitment. Students under the age of 18 were restricted from participating as they are minors, prohibited from providing sole informed consent for themselves (Economic and Social Research Council, 2017). More so, pregnant females were not recruited as they might

experience body fat changes due to foetal development rather than dietary intake (NHS, 2017). Individuals who had diagnosed conditions associated with body fat changes such as Cushing's syndrome, type-2 diabetes, hypothyroidism, polycystic ovary syndrome, as well as those who used pace-makers or metallic implants were also considered ineligible for the study. Those with metallic implants or pace-makers were not recruited to avoid erroneous body fat percentage estimation while using BIA. The researcher conducted no medical examination as a basis for excluding any individual from recruitment. Every participant was asked if they had once been diagnosed with any of the stated conditions or had metallic implants. Each one was either was recruited or excluded based on their response.

5.2.6. Recruitment setting

Participants were recruited from within the premises of the Anglia Ruskin University campus in Cambridge. This setting was chosen due to its proximity and accessibility to the researcher, making it feasible for easy recruitment of participants.

5.2.7. Materials

Nutrient intake was collected using the food photography method validated in chapter 5, while body fat percentage was measured using bioelectrical impedance analysis (BIA). In addition to demographic data of participants, information regarding dietary phytate and covariates such as the appetite score, physical activity, average sleep time during the night, perceived stress, alcohol intake, and cigarette smoking was collected.

Appetite was measured using the Simplified Nutritional Appetite Questionnaire (Wilson, et al., 2005) (see Appendix 6), while physical activity was measured using the Global Physical Activity Questionnaire (GPAQ) (see Appendix 7). The Perceived Stress Scale (Lee, 2012) was

used to measure perceived stress (see Appendix 8). All measures were taken to achieve the objectives as well as provide relevant data on the participant characteristics.

a. The Simplified Nutritional Appetite Questionnaire

The Simplified Nutritional Appetite Questionnaire (Wilson, et al., 2005) is a self-reported appetite assessment tool comprising of four questions and a total score of 20. Higher scores indicate a good appetite, while lower scores indicate a poor appetite.

b. The Global Physical Activity Questionnaire: The Global Physical Activity Questionnaire was developed by the WHO for physical activity surveillance. It is a self-reported questionnaire which collects data on physical activity participation in three domains; at work, while travelling to and from places and during recreational activities. Higher scores indicate more physical activity, while lower scores indicate less physical activity.

c. The Perceived Stress Scale: This was initially developed by Cohen, Kamarch and Mermelstein (1983), and subsequently reviewed by Lee (2012). It is a self-reported questionnaire which was designed to measure the extent to which individuals consider situations in their lives as stressful. Higher scores on the scale indicate more perceived stress, while lower scores indicate less perceived stress.

5.2.8. Ethical Considerations

Before the commencement of the cohort study, ethical approval was granted by the Anglia Ruskin University Faculty of Medical Science Research Ethics Panel (Ethics approval letter in Appendix 9).

5.2.9. Collection of Data on Nutrient Intake

Participants' access to a smartphone was verified by the researcher after which participants were shown appropriate camera positions to capture images of their meal and how to upload images to a website using the password assigned to them. The website was prepared specially for the research by the researcher. Participants were required to take pictures of their meal, before eating and after having their meal if they had some leftover (meal remnant). For ease in the estimation of portion sizes from the pictures, all participants were directed to place their food on the scaled table mat provided and capture images from different positions (from the top and side-about 45°). Additional description of food consumed was also recommended by the researcher for proper identification and characterisation of the diet before inputting data into the WinDiets software. Diet records were collected for four non-consecutive days over two weeks (capturing weekdays and weekends) to control for nutrient intake variability, at the start of the study, and at the end (at 6 months).

Considering that factors such as variation in day-to-day activities and the day of the week can contribute to intra-individual variability in nutrient intake, the use of at least two or more non-consecutive days spread across the week (including weekdays and weekends) to reflect usual dietary intake is recommended (Morimoto, et al. (2011). Although from a statistical point of view, day to day dietary variability can be eliminated by obtaining daily measures of dietary intake until the measures obtained are equal the maximum period of interest (Hoffman, et al., 2002). However, this not feasible in practice, especially in a long-term study (Morimoto, et al., 2011). Hence, in the present research, the scheduled days and period for collecting dietary data were chosen to ensure the achievement of valid estimates of dietary intake while limiting the influence of day-to-day dietary variability and the risk of losing participants due to loss of motivation to complete the research.

In addition to posting photographs of meals consumed, participants were requested to include the eating occasion, whether "at-home" or "out-of-home eating." Also, due to the tendency for atypical consumption on special occasions, participants were requested to indicate if the eating occasion occurred during a celebration or festive event. The need to obtain estimates of participants' usual diet is increasingly important in the present research, as it ensures that any association observed between diet and body fat is reliable, and the recommendations therefrom. Hence the need to note the occurrence of atypical consumption patterns which are likely to occur during special occasions (Craig, et al., 2002).

In this study, "meal" is used to describe an eating event (event of food intake); food consumed in a general sense including drink, snacks and traditional meal, irrespective of when or where it is eaten (Makela, et al., 1999). Participants were made aware of this definition before being recruited to guide dietary records submitted, regardless of what they knew previously.

5.2.10. Estimating body fat.

The percentage of body fat was estimated using BIA (TANITA DC-360 S-portable). The principle applied by this technique is that different tissues of the body act as conductors of electricity, while others act as insulators. Hence, when a small amount of alternating current of a constant frequency (≈ 50 kHz) is passed through the body, the conductivity measured indicates the proportion of lean tissues which are highly conductive because they contain large amounts of water. Whereas the fat tissue constitutes impedance to electricity as it is anhydrous and a poor conductor. The technique estimates the total body water, from which the lean body mass is calculated. This calculation follows from the assumption that 73% of the fat-free mass constitutes of water, and it remains constant over time. The body fat mass is calculated by subtracting the fat-free mass from the total body mass (Dehghan and Merchant, 2008). More details regarding the BIA have already been discussed in section 2.3.2.7 of this thesis.

The BIA was chosen because it was accessible, non-invasive and easy to use. Also, careful considerations regarding its validity and reliability were made. Given the likelihood of individual variability indicated by previous studies using the BIA method, it was ensured that the specific device used could limit such bias. According to the manufacturer, the TANITA DC-360 S-portable device has a reliability of 99%, and a validity of 98% when compared with the DXA, provided specific precautions are observed. These precautions and the steps taken to observe them in the present research have been described in the following section.

5.2.11. Precautions observed to valid and reliable BIA readings

Given that other factors could affect readings obtained by the BIA, necessary precautions were observed to limit bias and standardise the factors for all participants. For instance, a test circuit (dummy) that simulates the bioelectrical properties of a human body (with known mass and impedance as would be generated by an individual with a known body fat measure) which was supplied along with the BIA device was used as a tool to ensure that the electronic components of the device were of optimum function before use. Also, a specific room (Nurses' theatre, ARU) was used for all the measurements taken and was at room temperature (21.7- 23.9 °C) to minimize the influence of environmental temperature fluctuations on BIA readings. The participants had to sit for 5 minutes upon arrival before being measured, as walking down to the theatre could have constituted some physical activity (irrespective of how seemingly insignificant) which could lead to heat generation and reduce impedance readings. Participants also confirmed not to have been involved in moderate to intensive exercise less than two hours, or drunk fluids 30 minutes before being measured. Female participants confirmed not to be in their menstrual period before they were measured. It was ensured that participants took off metallic pieces in contact with their skin, such as metallic wristwatches, bracelets, belts,

necklaces, and earrings. This was necessary as metals in contact with the skin can increase conductance, thereby reducing impedance (Dehghan and Merchant, 2008).

Also, as a way of avoiding bias, participants were observed to note those who were sweating. Any such individual was not measured to avoid the risk of a false increase in conductance during measurement. Measurements were taken with participants standing in an upright position, with both arms stretched forwards while firmly holding the electrode hand bars of the BIA device. Each participant stood bare-footed placing their feet against the foot-electrodes. By standing in this position, there was no contact between limbs and trunk.

Before using the BIA, its weighing scale was checked using standard weights (8.5kg, 12kg, 50kg, 90kg and 100kg) to ensure it was accurate for each day it was being used. Also, the electrodes were wiped using a dry absorbent piece of cloth between measurements to reduce the risk of transferring bias between participants. Body fat percentage readings were taken three times for every participant to ensure accuracy, and all the device storage and power use instructions indicated by the manufacturer were strictly followed. These precautionary measures observed have also been recommended in the research literature (Buchholz, Bartok and Schoeller, 2004; Dehghan and Merchant, 2008) to limit factors which could result in misleading results.

5.2.12. Managing covariates

Based on evidence from previous research, some covariates may include physical activity, the use of pharmacological agents affecting adiposity (cortisol or insulin therapy), certain medical conditions (type-2 diabetes, hypothyroidism, polycystic ovary syndrome, Cushing's syndrome), smoking status, alcohol intake, and the average hours of night sleep. It was ensured that participants verified that they neither used medication which could affect their body fat

nor had been diagnosed with medical conditions which could affect their adiposity. Factors such as physical activity, smoking status, alcohol intake, and the average hours of night sleep were estimated among the participants and controlled for to avoid the occurrence of a spurious relationship and have been discussed in section 2.5.

In consideration of the findings of the systematic review reported in Chapter 3, dietary phytate was assessed as a moderator, a factor with the propensity to change the magnitude or direction of the exposure's effect on the exposure on the outcome (Field-Fote, 2019). Since data on the phytate contents of foods were not available in the food composition tables used, the dietary phytate values (grams per 100g of dry weight) were derived from published literature (Chen, 2004; Harland, Smikle-Williams and Oberleas, 2004; Joung, et al., 2004; Lestienne, et al., 2005; Shen, et al., 2005; Venkatachalam and Sathe, 2006) for the main dietary sources of phytate (cereals, legumes, nuts and oilseeds) Schlemmer, et al. (2009). Where there was variability in phytate values, the mean was calculated and used to estimate participants' total dietary phytate content. Daily dietary phytate intake estimates were obtained by dividing the total dietary phytate intake estimate by four since a 4-day dietary record was used.

Estimates of dietary phytate and measures of the covariates and in the research were also collected at baseline and at 6 months- the end of the study.

5.2.13. Data analysis

At the end of the data collection process, the nutrient assessment was carried out using WinDiets Software (2015 edition). The portion sizes of participants' meals were first translated into measures in grams according to the Food Standards Agency (1998) guidelines and using pictorial aids from Nutritics 5.0 (available at nutritics.com)- a diet and recipe analysis software which displays pictorial representations of meals with corresponding portion size estimate in

grams. The measures in grams were then entered into the food databases within WinDiets software, which then generated the nutrient composition for foods selected. Based on the assessed nutrient intake measures, the nutrient density was calculated for each micronutrient (amount of micronutrient per 1000kcal).

All data were analysed using IBM SPSS Statistics 26 software. To determine the statistical method to be used, normality of the dependent variable (body fat percentage) was considered based on the skewness and kurtosis z-values (Cramer, 1998; Cramer and Howitt, 2004; Doane and Seward, 2011), the Shapiro-Wilk test p-value (Shapiro and Wilk, 1965; Razali and Wah, 2011), and a visual inspection of the histograms, normal Q-Q and box plot.

The relationship between dietary nutrient density and body fat percentage observed at baseline and at 6 months was investigated separately using simple linear regression after adjusting for covariates (alcohol intake, physical activity, night-time sleep and cigarette smoking) and dietary phytate. While the relationship between change in dietary nutrient density and change in body fat percentage observed throughout the study was investigated using linear mixed model regression over all the participants and participant categories based on age, gender, and ethnicity. The relationship was also investigated after adjusting for dietary phytate and covariates using linear mixed model regression. For this analysis, the mean of the data obtained from baseline and at 6 months was not used, rather, the data was arranged in long format, with repeated measures of each parameter recorded in sequence on a single column for each participant. The linear mixed model regression used allows the analysis of data in this arrangement when there is repeated estimation of the same parameters at different time points. Student's t-test was used to compare the mean body fat percentage and nutrient intake measures obtained from participants based on age, gender and being on a special diet, while one-way analysis of variance (ANOVA) was applied to compare the same estimates from participants

based on ethnicity, after which a Bonferroni correction (Bonferroni post hoc test) was applied for any group which showed a significant difference. The Bonferroni post hoc test is a series of t-tests which compares each pair of groups (Lee and Lee, 2018).

Supplementary analysis of data was carried out to describe various sub-groups within the sample further. The mean body fat percentages and nutrient intakes for participants whose body fat increased by 1% or more, and those whose body fat increased by less than 1% were calculated, and both were compared using the Welch t-test. Also, the mean body fat percentages and nutrient intakes for participants whose body fat increased and those whose body fat decreased were calculated and compared using the Welch t-test. The Welch t-test in each case was used since the groups were of unequal sample size.

The influence of each covariate (physical activity, cigarette smoking, alcohol intake, being on a special diet and sleep) on the relationship between dietary nutrient density and body fat percentage was also investigated. Physical activity was estimated using the GPAQ in sedentary behaviour and three domains; at work, during travel, and recreational activities. For each domain, each participant's physical activity in the last week was estimated as total physical activity MET minutes per week. MET refers to metabolic equivalents, the ratio of work metabolic rate to resting metabolic rate or the energy cost of an activity. One MET is defined as the energy cost of sitting quietly and equates to 1kcal/kg/hour. Different MET values were assigned to moderate and vigorous activities in the different domains. For instance, in the work domain, moderate and vigorous activities are given MET values of 4 and 8, respectively, according to the GPAQ. To calculate the amount of energy expended during an activity, the associated MET value was multiplied by the duration of the activity. For example, engaging in a moderate activity at work for 30 minutes resulted in 120 MET-minutes. A pictorial illustration of various activities and the associated intensities (moderate and vigorous) were indicated on

the GPAQ to assist the participants with their responses. The relationship between dietary nutrient density and body fat percentage was investigated in participants grouped into low, moderate and high physical activity as classified by the GPAQ. Low, moderate and high levels of physical activity refer to the metabolic equivalent minutes < 600, $\leq 600 < 1500$, and ≥ 1500 MET-minutes, respectively.

With regards to cigarette smoking, alcohol intake, being on a special diet and sleep participants were grouped into; smokers and non-smokers, alcohol drinkers and non-drinkers, vegetarians and omnivores, and having night-time sleep for < 7 hours and ≥ 7 hours, respectively. The relationship between dietary nutrient density and body fat percentage was investigated in each group using linear mixed model regression.

Furthermore, the strength of the linear relationship between physical activity and energy and micronutrient intakes was investigated using Pearson's correlation. The level of statistical significance in each case was considered for a p-value less than 0.05.

5.2.14. Other precautions observed

1. Since the study was of prospective cohort type, participants were followed-up and sent monthly messages (via email) to remind them of the follow-up data collection and to request any concerns which they had about participating. This was a way of keeping in touch with the study participants to reduce the risk of loss to follow up.
2. The scaled table mat provided to each participant, as in the validation study (chapter 3), served two main purposes; scaling the food images to ease portion size identification and serving as a standard background for the images to be accepted for analysis. The latter implies that images submitted without the table mat were not be analysed. This step was taken to reduce the risk of copying and to paste pictures from other sources.

Considering that the table mat design is unique to this research, it was meant to limit any such bias. The colour code on the table mat also helped for easy identification of the food colour. Where colour code appeared clear and distinguishable in the image captured, the colour of the food was easy to identify.

3. It was ensured that participants did not have access to others' images or food posts despite being able to post pictures on the same website (using the "contributor log-in" setting on the website). This design was ensured by the researcher to ensure privacy and limit the risk of copying others' posts. Each participant had access only to their own private space within the website
4. The website for the research was set under privacy (not open for public viewing) to protect participants' data.

5.3. Results

5.3.1. Main Findings

The tables below summarise the participants' demographic characteristics, daily nutrient intake, body fat percentage, and the relationship between change in dietary nutrient density and change in body fat percentage.

At baseline, 138 participants were recruited, but dietary records from only 108 participants were included eventually for the analysis. Of the 30 records which were not included, 29 were lost to follow-up after the baseline sampling due to failing to attend the second stage of data collection, while 1 participant withdrew from the study for unknown reasons. As shown in Table 5.1, of 108 individuals who participated in the study, most of them were men, aged 18-29 years, of African ethnicity, and were on a special diet. The ages of the participants ranged from 19-38 years, and more men were older (over 30 years) compared to the women. Also,

more women (9) compared to men (8) indicated to be on a special diet, and all who were on a special diet were vegetarian.

Table 5.1. Demographic characteristics of respondents.

Characteristics	N	%
Gender		
Male	60	55.6
Female	48	44.4
Age (years)		
18-29	81	75.0
30- 39	27	25.0
Ethnicity		
White British	11	10.2
White Other	21	19.4
African	44	40.7
Asian	32	29.7
Special diet		
Yes	17	15.7
No special diet	91	84.3
Total	108	

A Shapiro-Wilk's test and a visual inspection of the histogram, normal Q-Q plots and box plots showed that the body fat percentage estimates were approximately normally distributed. The skewness and kurtosis were 1.71 (standard error = 0.18) and 1.32 (standard error = 0.358), respectively. The Shapiro-Wilk's test gave a non-significant value, p= 0.08 (considering that the p-value was higher than 0.05) (See Figures A10-1, A10-2 and A10-3 in Appendix 10).

Table 5.2 shows that there was a statistically significant difference between the dietary intake of some nutrients (vitamin A, folate, vitamin D, iron, calcium, vitamin E, magnesium,

potassium, selenium, zinc, vitamin C, phosphorus and vitamin K) at baseline and after 6 months of the study.

Table 5.2. The daily dietary nutrient intake (mean ± SD) of participants at baseline and 6 months.

Dietary nutrient	Baseline	After 6-months	p-value
Energy (kcal)	2327.6 ± 224.6	2135.0 ± 223.8	0.09
Vitamin A (µg)	524.2 ± 135.1	568.2 ± 137.1	0.04*
Riboflavin (mg)	1.2 ± 0.2	1.6 ± 0.2	0.05
Folate (µg)	219.5 ± 34.6	259.5 ± 36.9	0.03*
Vitamin D (µg)	6.2 ± 4.1	6.8 ± 4.0	0.04*
Iron (mg)	9.0 ± 2.8	9.0 ± 2.9.0	0.01*
Calcium (mg)	857.9 ± 120.8	855.7 ±120.0	0.04*
Vitamin E (mg)	12.3 ± 3.4	13.3 ± 3.4	0.03*
Magnesium (mg)	239.0 ± 34.8	245.0 ± 34.8	0.04*
Potassium (mg)	2788.1 ±415.5	2796.7 ± 415.9	0.01*
Iodine (µg)	124.1 ± 32.8	123.5 ± 32.8	0.07
Selenium (µg)	62.0 ± 21.2	68.0 ± 21.2	0.03*
Zinc (mg)	10.9 ± 3.0	12.3 ± 3.0	0.02*
Vitamin C (mg)	103.0 ± 27.8	108.6 ± 27.8	0.04*
Manganese (mg)	3.2 ± 1.8	3.2 ± 1.8	0.06
Copper (mg)	1.1 ± 0.5	1.1 ± 0.5	0.09
Phosphorus (mg)	501.0 ± 124.0	511.0 ± 126.9	0.01*
Niacine (B_3) (mg)	1.3 ± 0.1	1.3 ± 0.5	0.09
Cobalamin (B_{12}) (µg)	4.5 ± 1.2	4.1 ± 1.3	0.07
Thiamine (B_1) (mg)	0.6 ± 0.7	0.4 ± 0.7	0.05
Pantothenate (B_5) (mg)	3.4 ± 1.3	2.4 ±1.3	0.08
Pyridoxal phosphate (B_6) (mg)	1.2 ± 0.4	1.2 ± 0.4	0.05
Biotine (µg)	28.2 ± 7.2	28.2 ± 7.2	0.09
Vitamin K (mg)	60.2 ± 15.6	61.6 ± 15.7	0.02*
P-values marked with an asterisk (*) indicate that the estimates are statistically significant.			

As indicated in Table 5.3 below, women had a higher total body fat percentage compared to the men at 6 months ($p= 0.003$) and for the total duration of the study ($p= 0.006$). Africans had more body fat percentage compared to the White-British ($p< 0.0001$) and participants of mixed race ($p= 0.001$), while Asians had less body fat percentage than the mixed-race and White-British participants ($p< 0.0001$) at the end of the study. The body fat percentage for the participants ranged from 14.8% - 38%.

Table 5.3. Participants' body fat percentage (average for baseline, 6-month estimates and both) based on their demographic characteristics.

Demographic Characteristics		Body fat percentage (%)		
		Baseline	**6-month**	**Overall**
Gender	Male	31.4 ± 7.4	29.6 ± 8.5*	30.3 ± 8.0*
	Female	30.3 ± 8.0	36.7 ± 8.2*	33.5 ± 8.6*
Age (years)	18-29	33.6 ± 9.1	29.6 ± 8.6	31.5 ± 9.3
	30-39	31.4 ± 4.5	32.5 ± 6.3	32.0 ± 5.1
Ethnicity	African	35.9 ± 3.9*	39.2 ± 4.7*	37.5 ± 4.4*
	White British	29.7 ± 7.0*	31.3 ± 8.5*	30.5 ± 8.4*
	Mixed	26.6 ± 7.3*	30.8 ± 7.1*	28.7 ± 7.0*
	Asian	27.8 ± 7.6*	27.2 ± 7.6*	27.5 ±7.6*
Special diet	Vegetarian	29.7 ± 5.2	31.5 ± 6.0	30.6 ± 5.6
	No special diet	33.4 ± 8.2	30.2 ± 8.6	31.8 ± 8.8
All participants		31.1 ± 8.5	31.5 ± 8.8	31.6 ± 8.4

The dietary nutrient intake estimates indicated in Table 5.4 shows that men consumed more vitamin A ($p< 0.0001$), iron ($p= 0.02$), calcium ($p= 0.02$), zinc ($p< 0.0001$) and vitamin C ($p= 0.01$) in the diet than women. Women only consumed more manganese in their diet compared to men ($p= 0.03$). The older participants (aged 30-39 years) regardless of their gender consumed more vitamin D ($p= 0.02$), zinc ($p= 0.004$) and manganese ($p= 0.02$) in their diet than their younger counterparts, while the younger participants (aged 18-29 years) consumed more of vitamin B_6 (pyridoxal phosphate) ($p= 0.03$). Based on ethnicity, participants' thiamine, manganese and zinc intake differed significantly ($p< 0.05$); the white-British participants consumed more thiamine than Africans ($p=0.002$) but less zinc compared to their mixed-race counterparts ($p= 0.002$). Asians consumed more manganese than Africans ($p= 0.004$). Those who were on a special diet consumed more vitamin C ($p= 0.002$), zinc ($p= 0.01$), selenium ($p= 0.01$) and iron ($p= 0.01$) in the diet in contrast to those who were not a special diet, whose consumed diet more thiamine ($p= 0.001$) and magnesium ($p= 0.02$).

Table 5.4. Average daily dietary nutrient composition of participants' diet (mean ± SD).

Dietary nutrient composition	**Gender**		**Age (years)**		**Ethnicity**				**Special diet**		**All participants**
	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Energy (kcal)	2420.2 ± 273.8	2192.9 ± 230.8	2234.3 ± 228.2	2222.2 ± 208.7	2251.9 ± 221.0	2225.25 ± 227.4	2169.1 ±203.5	2241.3 ±234.0	2229.5 ± 229.8	2240.4 ± 185.0	2231.3 ± 223.0
Vitamin A (µg)	585.4 ±120.0*	494.3 ± 141.0*	539.7 ±138.8	565.8 ± 129.9	540.0 ±139.0	541.7 ±133.5	558.6 ±122.3	549.0 ±145.8	545.1 ±138.9	551.9 ±126.8	546.2 ± 136.8
Riboflavin (B_2) (mg)	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Folate (µg)	240.5 ± 31.0	238.2 ±43.4	239.0 ± 38.5	241.0 ± 31.3	244.6 ± 39.3	229.8 ± 35.8	244.3 ± 37.8	233.6 ± 31.9	238.0 ± 37.4	247.5 ± 32.6	239.5 ± 36.8
Vitamin D (µg)	6.9 ± 4.3	6.1 ±3.7	6.1 ± 3.8*	7.8 ± 4.5*	6.5 ± 4.4	5.3 ± 3.1	8.0 ± 4.4	6.4 ± 3.6	6.2 ± 3.9*	8.6 ± 4.6*	6.5 ± 4.1

Iron (mg)	9.3 ±2.9*	8.5 ± 2.7	8.9 ± 2.9	9.1 ± 2.5	9.1 ± 3.0	8.2 ± 2.4	9.3 ± 2.9	9.0 ± 2.7	8.8 ± 2.9	10.0 ± 2.5*	9.0 ± 2.8
Calcium (mg)	873.9 ±107.5*	834.2 ±133.2*	849.8 ± 123.4	877.6 ± 110.0	855.5 ± 122.2	871. 0 ± 109.6	879.7 ± 110.6	841.2 ±128.2	851.0 ± 121.1	887.6 ± 114.3	856.8 ± 120.5
Vitamin E (mg)	12.6 ± 3.5	13.0 ± 3.2	12.9 ± 3.3	12.3 ±.3.3	12.5 ± 3.3	12.7 ± 4.1	13.5 ± 3.6	12.9 ± 2.8	12.8 ± 3.4	12.8 ± 3.3	12.8 ± 3.3
Magnesium (mg)	242.5 ± 35.3	241.3 ±34.0	242.1 ±34.2	241.8 ± 36.3	245.2 ± 38.1	241.0 ± 32.3	235.5 ± 32.7	241.1 ± 32.0	244.6 ± 33.9*	228.1 ± 35.7*	242.0 ± 34.7
Potassium (mg)	2773.8 ± 419.3	2817.0 ±410.2	2771.2 ±426.4	2856 ± 375.1	2796.1 ± 399.3*	2883.4 ± 419.8*	2910 ±399.7*	2677.6 ±423.2*	2778.5 ± 418.5	2866.5 ± 392.9	2792.4 ± 415.0
Iodine (µg)	122.9 ± 29.4	125.0 ± 36.8	128.7 ± 31.0	109.1 ± 33.6	126.4 ± 33.6*	123.4 ± 39*	125.5 ± 30*	120.4 ± 29.7*	125.6 ± 32.3	114.1 ± 33.5	123.8 ± 32.7
Selenium (µg)	66.7 ± 23.6	62.9 ± 17.3	63.6 ± 20.3	69.3 ± 23.2	66.8 ± 22.1	66.4 ± 24.4	67.3 ± 17.0	60.9 ± 20.4	63.0 ± 19.9*	75.8 ± 24.7*	65.0 ± 21.2
Zinc (mg)	12.4 ±2.5*	10.5 ± 3.2*	11.3 ± 3.1*	12.5 ± 2.4*	11.4 ± 3.0*	12.4 ± 2.0*	12.9 ± 3.0*	10.9 ± 3.1*	11.4 ± 3.0*	12.7 ± 2.6*	11.6 ± 3.0

Vitamin C (mg)	109.9 ± 27.5*	100.5 ± 27.3	104.3 ± 27.0	110.5 ± 29.6	106.8 ± 28.2	106.8 ± 29.6	109.0 ± 29.3	102.0 ± 25.8	103.4 ± 27.6	118.7 ± 25.0*	105.8 ± 27.7
Manganese (mg)	3.0 ± 1.8*	3.5 ± 1.8*	3.0 ± 1.8*	3.7 ± 1.9*	2.8 ± 1.8*	4.0 ± 2.1*	3.4 ± 1.9*	3.2 ±1.5*	3.1 ± 1.8	3.5 ± 2.1	3.2 ± 1.8
Copper (mg)	1.1 ± 0.5	1.1 ± 0.4	1.1 ± 0.5	1.1 ± 0.4	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.5	1.2 ± 0.4	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.5
Phosphorus (mg)	507.7 ± 132.0	503.7 ± 119.7	503.4 ±126.8	513.8 ± 126.8	502.5 ± 119.2	502.0 ± 135.7	511.9 ± 159.9	509.2 ± 115.4	503.6 ± 123.2	518.7 ± 144.8	506.0 ± 126.6
Niacine (B_3) (mg)	1.3 ± 0.5	1.24 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.1 ± 0.5	1.3 ± 0.4	1.3 ± 0.5	1.3 ± 0.5	1.2 ± 0.5	1.3 ± 0.5
Cobalamin (B_{12}) (µg)	4.2 ± 1.1	4.5 ± 1.2	4.4 ± 1.1	4.1 ± 1.2	4.4 ± 1.3	4.4 ± 0.9	4.1 ± 1.1	4.4 ± 1.0	4.4 ± 1.2	4.1 ± 1.0	4.3 ± 1.1
Thiamine (B_1) (mg)	0.4 ± 0.7	0.5 ± 0.6	0.5 ± 0.8	0.4 ± 0.4	0.34 ± 0.5*	0.4 ± 0.5*	0.38 ± 0.4*	0.7 ± 0.9*	0.5 ± 0.7*	0.3 ± 0.1*	0.5 ± 0.7
Pantothenate (B_5) (mg)	2.9 ± 1.3	2.8 ± 1.3	2.9 ± 1.3	2.9 ± 1.4	3.0 ± 1.4	2.5 ± 1.0	3.2 ± 1.2	2.7 ± 1.3	2.8 ± 1.3	3.2 ± 1.3	2.9 ± 1.3

Pyridoxal phosphate (B_6) (mg)	1.2 ± 0.4	1.3 ± 0.4	1.3 ± 0.4*	1.1 ± 0.4*	1.2 ± 0.4	1.3 ± 0.5	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.5	1.2 ± 0.4
Biotine (µg)	28.0 ± 7.1	28.4 ± 7.3	28.3 ± 7.4	27.8 ± 6.6	27.3 ± 7.0	30.6 ± 6.0	28.3 ± 7.0	28.3 ± 7.9	28.3 ± 7.2	27.3 ± 7.3	28.2 ± 7.2
Vitamin K (µg)	60.4 ± 15.6	61.6 ± 15.7	61.2 ± 15.6	60.1 ± 15.8	61.4 ± 16.0	60.4 ± 16.9	60.9 ± 14.2	60.4 ± 15.7	60.7 ± 15.6	62.0 ± 16.0	60.9 ± 15.6

Estimates in Table 5.4 are marked with " * " to indicate statistical significance ($p < 0.05$).

The findings in Table 5.5 indicate that men had higher dietary nutrient density with regards to vitamin A ($p< 0.0001$), iron ($p= 0.02$), calcium ($p= 0.04$), zinc ($p< 0.0001$) and vitamin C ($p= 0.01$) than women, while the dietary nutrient density of manganese for women was higher than for men ($p= 0.02$). In older participants (aged 30-39 years), the dietary micronutrient density of vitamin D ($p= 0.02$), zinc ($p= 0.01$) and manganese were higher than in younger participants (aged 18-29 years). Younger participants however had higher dietary nutrient density regarding vitamin B_6 (pyridoxal phosphate) ($p= 0.02$) and iodine ($p= 0.004$). Participants who were on a special diet had higher dietary nutrient density with respect to selenium ($p= 0.01$), zinc ($p=0.04$), and vitamins C ($p= 0.01$) and D ($p= 0.01$), but lower for vitamins B_{12} ($p= 0.03$) and B_1 ($p= 0.001$) compared to those who were not on a special diet. Participants' dietary nutrient density with regards to thiamine, manganese, zinc, potassium, vitamin K and magnesium differed significantly among ethnic categories ($p< 0.05$). When the difference was analysed between the ethnic groups using Bonferroni post-hoc t-test, white-British, Africans and those of mixed race had higher vitamin K, vitamin B_5 (pantothenic acid), potassium and magnesium compared to Asians ($p< 0.0001$). Asians, however, had a higher dietary nutrient density for manganese than Africans ($p= 0.004$). Mixed race participants also had a higher dietary nutrient density for zinc compared to their white-British ($p= 0.002$) and African ($p= 0.003$) counterparts. The diet of mixed-race participants was also more nutrient-dense with regards to potassium compared to that of white-British participants ($p< 0.0001$).

Table 5.5. Dietary micronutrient density (mean ± SD) based on the demographic characteristics of participants.

	Gender		**Age**		**Ethnicity**				**Special diet**		**All participants**
Micronutrient density (Nutrient unit per 1000 kcal)	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Vitamin A (µg)	265.1 ± 58.8*	224.5 ± 71.2*	244.7 ± 68.6	256.4 ± 63.3	242.4 ± 67.3	243.6 ± 54.8	260.0 ± 62.6	249.0 ± 75.1	247.4 ± 68.0	248.8 ± 64.6	247.6 ± 67.4
Riboflavin (B_2) (mg)	0.6 ± 0.1	0.6 ±0.1	0.6 ± 0.1	0.7 ±0.1	0.6 ±0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Folate (µg)	109.1 ± 18.0	107.1 ± 20.7	107.9 ± 20.2	109.2 ± 15.7	109.4 ±19.4	104.1 ± 18.4	113.7 ± 21.2	1.2 ± 17.2	107.8 ± 19.8	110.9 ± 15.1	108.2 ± 19.2
Vitamin D (µg)	3.1 ± 2.0	2.7 ±1.6	2.8 ± 1.8*	3.5 ± 2.1*	2.9 ± 2.0	2.4 ± 1.4	3.6 ± 2.1	2.9 ± 1.7	2.8 ± 1.8*	3.9 ± 2.1*	3.0 ± 1.9

Iron (mg)	4.25 ± 1.4*	3.8 ± 1.2*	4.0 ± 1.4	4.1 ± 1.2	4.1 ± 1.4	3.7 ± 1.1	4.3 ± 1.3	4.1 ± 1.5	4.0 ± 1.4	4.5 ± 1.2	4.1 ± 1.4
Calcium (mg)	396.7 ± 64.7*	376.7 ± 73.8*	384.7 ± 71.2	398.4 ± 62.5	383.1 ± 65.0	396.0 ± 68.7	410.4 ± 70.9	379.8 ± 73.5	385.8 ± 69.0	400.4 ± 70.3	388.1 ± 69. 3
Vitamin E (mg)	5.7 ± 1.6	5.9 ± 1.6	5.8 ± 1.7	5.6 ± 1.6	5.6 ± 1.5	5.7 ± 1.8	6.3 ± 1.9	5.8 ± 1.5	5.8 ± 1.6	5.8 ± 1.7	5.8 ± 1.6
Magnesium (mg)	109.8 ± 18.6	108.9 ± 18.2	109.4 ± 18.6	109.3 ± 17.9	109.8 ± 19.5*	109.5 ± 19.1*	109.1 ± 16.2*	108.7 ± 17.9*	110.8 ± 18.8	101.8 ± 14.2	109.4 ± 18.4
Potassium (mg)	1256 ± 218.7	1269.1 ± 212.0	1250.7 ± 217.8	1295.9 ± 206.4	1252.7 ± 212.8*	1305 ± 206.2*	1355.1 ± 233.9*	1201.4 ± 194.5*	1256.4 ± 212.8	1291.8 ± 230.2	1262.0 ± 215.4
Iodine (µg)	55.5 ± 13.0	56.5 ± 17.7	58.1 ± 14.5*	49.4 ± 15.2*	56.5 ± 15.3	55.8 ± 17.5	58.4 ± 15.4	54.2 ± 13.7	56.8 ± 15.0	51.2 ± 15.3	55.9 ± 15.2
Selenium (µg)	30.3 ± 11.6	28.4 ± 8.6	28.8 ± 10.4	31.4 ± 10.5	30.0 ± 10.7	30.2 ± 11.3	31.4 ± 9.0	27.5 ± 10.3	28.6 ± 10.0	34.2 ± 11.3*	29.5 ± 10.4
Zinc (mg)	5.6 ± 1.28*	4.8 ± 1.7*	5.1 ± 1.6*	5.7 ± 1.2*	5.1 ± 1.4*	5.6 ± 1.2*	6.0 ± 1.6*	4.9 ± 1.6*	5.2 ± 1.5*	5.7 ± 1.2*	5.3 ± 1.5

Vitamin C (mg)	49.9 ± 13.6*	45.3 ± 13.1	47.1 ± 12.8	50.6 ± 15.4	48.0 ± 13.7	48.5 ± 1.6	50.7 ± 14.3	46.0 ± 12.5	46.9 ± 13.3	53.7 ± 13.3*	47.9 ± 13.5
Manganese (mg)	1.3 ± 0.8*	1.6 ± 0.8*	1.4 ± 0.8*	1.7 ± 0.8*	1.3 ± 0.8*	1.8 ± 1.0*	1.6 ± 0.8*	1.4 ± 0.7*	14 ± 0.8	1.5 ± 0.9	1.4 ± 0.8
Copper (mg)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
Phosphorus (mg)	230.5 ± 66.5	227.8 ± 60.8	227.7 ± 63.0	234.3 ± 67.2	225.6 ± 60.0	229.8 ± 71.2	239.3 ± 82.2	228.6 ± 5.8	228.2 ± 61.2	234.6 ± 78.1	229.4 ± 64.0
Niacine (B_3) (mg)	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2
Cobalamin (B_{12}) (µg)	1.9 ± 0.5	2.0 ± 0.6	2.0 ± 0.6	1.8 ± 0.5	2.0 ± 0.6	2.0 ± 0.4	1.9 ± 0.6	2.0 ± 0.5	2.0 ± 0.6*	1.8 ± 0.4*	2.0 ± 0.6
Thiamine (B_1) (mg)	0.2 ± 0.3	0.2 ± 0.3	0.2 ± 0.4	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.4	0.2 ± 0.3*	0.1 ± 0.1*	0.2 ± 0.3
Pantothenate (B_5) (mg)	1.3 ± 0.6	1.3 ± 0.6	1.3 ± 0.6	1.3 ± 0.7	0.5 ± 0.2*	1.2 ± 0.5*	1.5 ± 0.6*	1.2 ± 0.6*	1.3 ± 0.6	1.5 ± 0.7	1.3 ± 0.6

Pyridoxal phosphate (B_6) (mg)	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2*	0.5 ± 0.2*	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.2
Biotine (µg)	12.7 ± 3.7	12.8 ± 3.5	12.8 ± 3.7	12.7 ± 3.5	12.2 ± 3.4	13.9 ± 3.3	13.2 ± 3.6	12. ± 4.0	12.9 ± 3.6	12.4 ± 3.7	12.8 ± 3.6
Folate (µg)	130.3 ± 23.7	133.9 ± 23.3	133.4 ± 23.0	127.2 ± 24.9	132.2 ± 21.0	13.7 ± 27.7	130.8 ± 22.1	130.7 ± 25.9	132.2 ± 23.6	129.9 ± 24.5	131.8 ± 23.6
Vitamin K (mg)	27.3 ± 7.1	27.8 ± 7.8	27.7 ± 7.6	27.1 ± 7.0	27.5 ± 7.5*	27.6 ± 8.9*	28.2 ± 6.8*	27.1 ± 7.1*	27.5 ± 7.5	27.8 ± 7.2	27.5 ± 7.4

Estimates in Table 5.5 are marked with " * " to indicate statistical significance ($p < 0.05$).

After adjusting for gender, age, being on a special diet, ethnicity, dietary phytate intake, appetite, alcohol intake, physical activity, hours of night-time sleep and smoking status, overall participants, there was a significant inverse relationship between the dietary nutrient density for vitamins A and K, iron, calcium and magnesium at baseline (Table 5.6) and for vitamins A, C, E and K, iron, zinc, calcium, selenium, folate, magnesium and phosphorus and body fat percentage ($p< 0.05$) at 6 months (Table 5.7). A unit increase in the dietary nutrient density for each micronutrient corresponded to a decrease in body fat percentage. At baseline, among males, a unit increase in the dietary nutrient density for vitamins A and K, iron, calcium and magnesium corresponded to an average body fat percentage decrease of 0.07%. While among vegetarians and participants of 18-29 years of age, a unit increase in the dietary nutrient density for magnesium and vitamin K corresponded to an average body fat percentage decrease of 0.006% and 0.141% respectively (Table 5.6).

Furthermore, at 6 months among men and women, a unit increase in the dietary nutrient density for vitamin A, folate, iron, calcium, magnesium, selenium, zinc, vitamin C, phosphorus and vitamin K corresponded to an average body fat percentage decrease of 0.06%, 0.03%, 0.33%, 0.01%, 0.05%, 0.03%, 0.19%, 0.04%, 0.01%, 0.04% respectively. Also, among participants aged 18-29 years and 30-39 years, a unit increase in the dietary nutrient density for vitamin A, folate, iron, calcium, magnesium, selenium, zinc, vitamin C, phosphorus and vitamin K corresponded to an average body fat percentage decrease of 0.04%, 0.02%, 0.26%, 0.01%, 0.11%, 0.05%, 0.06%, 0.41%, 0.05%, 0.03% and 0.05% respectively. Similarly, among vegetarians and non-vegetarians, a unit increase in the dietary nutrient density for vitamin A, folate, iron, calcium, magnesium, selenium, zinc, vitamin C, phosphorus and vitamin K corresponded to an average body fat percentage decrease of 0.02%, 0.18%, 0.62%, 0.05%, 0.70%, 0.12%, 0.03%, 0.38%, 0.01%, 0.06%, 0.10% (Table 6.7).

Table 5.6. The relationship between dietary nutrient density and body fat percentage at baseline (adjusted for dietary phytate and covariates).

	Gender		**Age (years)**		**Ethnicity**				**Special diet**		**All participants**
Micronutrient density (Nutrient per 1000 kcal)	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Vitamin A (µg)	-0.027 ±0.001 (0.01)*	0.020 ±0.01 (0.09)	-0.001 ±0.010 (0.08)	-0.004 ±0.012 (0.08)	-0.015 ±0.007 (0.04)*	-0.012 ±0.050 (0.10)	-0.005 ±0.011 (0.15)	0.014 ±0.060 (0.30)	0.007 ±0.007 (0.19)	1.861 ±22.412 (0.14)	-0.001 ±0.001 (0.02)*
Riboflavin (B_2) (mg)	-6.850 ±5.617 (0.19)	-7.330 ±8.480 (0.33)	2.518 ±5.479 (0.26)	0.977 ±7.024 (0.70)	-2.101 ±3.043 (0.29)	-2.540 ±1.738 (0.14)	-1.300 ±1.290 (0.30)	3.010 ±5.72 (0.41)	-0.514 ±5.141 (0.51)	-0.420 ±0.341 (0.38)	2.386 ±4.376 (0.09)
Folate (µg)	-0.011 ±0.042 (0.14)	0.020 ± 0.04 (0.11)	0.114 ±0.030 (0.09)	-0.043 ±0.046 (0.17)	-0.028 ±0.022 (0.08)	0.222 ±0.507 (0.10)	0.053 ±0.311 (0.41)	-0.024 ±0.031 (0.10)	-0.031 ±0.029 (0.09)	-1.743 ±1.172 (0.13)	0.013 ±0.025 (0.55)
Vitamin D (µg)	-0.594 ±0.293 (0.08)	-0.050 ±0.420 (0.07)	0.351 ±0.283 (0.10)	-0.047 ±0.382 (0.08)	0.050 ±0.211 (0.11)	1.607 ±1.935 (0.34)	-0.043 ±0.211 (0.33)	0.140 ±0.366 (0.53)	0.015 ±0.275 (0.16)	2.312 ±3.526 (0.40)	0.836 ±0.218 (0.72)

Iron (mg)	-0.254 ±0.435 (0.03)*	0.010 ±0.680 (0.08)	0.048 ±0.374 (0.11)	-0.429 ±0.674 (0.53)	-0.537 ±0.313 (0.31)	-0.636 ±0.515 (0.71)	-0.390 ±0.223 (0.70)	-0.154 ±0.707 (0.31)	0.390 ±0.369 (0.09)	0.089 ±0.088 (0.16)	-0.176 ±0.317 (0.04)*
Calcium (mg)	0.009 ±0.011 (0.04)*	0.010 ±0.01 (0.14)	-0.009 ±0.010 (0.09)	0.013 ±0.011 (0.18)	0.003 ±0.017 (0.42)	-0.023 ±0.134 (0.17)	-0.016 ±0.007 (0.20)	-0.024 ±0.033 (0.17)	-0.004 ±0.010 (0.07)	2.080 ±2.256 (0.18)	0.001 ±0.008 (0.04)*
Vitamin E (mg)	-0.278 ±0.338 (0.38)	0.120 ±0.51 (0.54)	-0.492 ±0.339 (0.28)	-0.260 ±0.408 (0.41)	-0.044 ±0.270 (0.14)	-1.200 ±0.201 (0.18)	-0.402 ±0.260 (0.19)	-0.180 ±0.450 (0.14)	0.077 ±0.320 (0.11)	0.284 ±0.242 (0.17)	0.549 ±0.265 (0.50)
Magnesium (mg)	-0.003 ±0.032 (0.02)*	0.080 ±0.05 (0.07)	0.018 ±0.033 (0.20)	-0.048 ±0.047 (0.47)	-0.012 ±0.068 (0.86)	-0.204 ±0.071 (0.40)	-0.034 ±0.028 (0.54)	-0.060 ±0.014 (0.50)	0.003 ±0.032 (0.82)	-0.006 ±0.016 (0.01)*	-0.035 ±0.026 (0.03)*
Potassium (mg)	0.005 ±0.003 (0.10)	-0.002 ±0.004 (0.18)	0.007 ±0.003 (0.24)	-0.008 ±0.005 (0.14)	-0.003 ±0.002 (0.11)	0.023 ±0.037 (0.52)	0.017 ±0.012 (0.33)	0.011 ±0.004 (0.17)	-0.001 ±0.002 (0.44)	-0.070 ±0.103 (0.11)	0.002 ±0.002 (0.32)
Iodine (µg)	-0.026 ±0.042 (0.18)	-0.004 ±0.05 (0.09)	0.008 ±0.038 (0.22)	0.002 ±0.036 (0.09)	0.005 ±0.042 (0.14)	0.020 ±0.271 (0.39)	0.060 ±0.030 (0.30)	0.016 ±0.023 (0.57)	0.040 ±0.034 (0.26)	-0.060 0.106 (0.14)	0.001 ±0.021 (0.29)
Selenium (µg)	0.042 ±0.056 (0.44)	-0.044 ±0.101 (0.14)	0.066 ±0.056 (0.51)	0.084 ±0.049 (0.18)	-0.032 ±0.077 (0.42)	0.005 ±0.142 (0.45)	0.008 ±0.047 (0.72)	0.063 ±0.470 (0.24)	0.007 ±0.054 (0.12)	-2.585 ±2.458 (0.09)	-0.054 ±0.043 (0.30)

Zinc (mg)	-0.042 ±0.511 (0.19)	1.333 ±0.45 (0.43)*	0.389 ±0.359 (0.33)	0.153 ±0.639 (0.60)	0.053 ±0.301 (0.16)	1.082 ±0.270 (0.08)	0.261 ±0.378 (0.14)	0.235 ±0.430 (0.23)	-0.654 ±0.345 (0.18)	-0.253 ±0.341 (0.02)*	-0.323 ±0.292 (0.22)
Vitamin C (mg)	0.043 ±0.050 (0.14)	0.130 ±0.060 (0.14)	-0.040 ±0.041 (0.07)	0.024 ±0.050 (0.42)	0.015 ±0.021 (0.11)	-0.033 ±0.101 (0.09)	-0.052 ±0.042 (0.16)	0.058 ±0.072 (0.19)	-0.047 ±0.038 (0.08)	0.873 ±1.506 (0.14)	0.016 ±0.032 (0.24)
Manganese (mg)	-0.440 ±0.732 (0.11)	0.700 ±0.955 (0.26)	-0.256 ±0.648 (0.31)	0.089 ±0.742 (0.58)	-0.134 ±0.520 (0.71)	-1.024 ±0.440 (0.19)	1.010 ±0.584 (0.34)	-0.298 ±0.603 (0.23)	1.694 ±0.619 (0.09)	2.372 ±12.956 (0.44)	-0.748 ±0.496 (0.71)
Copper (mg)	-2.250 ±2.797 (0.08)	-3.665 ±3.711 (0.17)	2.785 ±2.815 (0.14)	-0.100 ±2.937 (0.29)	-0.560 ±2.401 (0.21)	-1.700 ±1.413 (0.08)	-2.072 ±2.629 (0.27)	-3.505 ±2.104 (0.19)	0.690 ±2.452 0.07	0.008 ±2.324 (0.11)	-1.152 ±2.220 (0.13)
Phosphorus (mg)	0.006 ±0.010 (0.64)	-0.020 ±0.013 (0.40)	0.008 ±0.009 (0.51)	0.001 ±0.012 (0.02)*	-0.022 ±0.014 (0.24)	0.046 ±0.020 (0.40)	-0.011 ±0.029 (0.51)	-0.052 ±0.048 (0.77)	0.001 ±0.009 (0.38)	-1.919 ±0.014 (0.84)	-0.007 ±0.007 (0.66)
Niacin (mg)	-1.689 ±2.591 (0.89)	2.334 ±3.142 (0.81)	-0.286 ±2.589 (0.58)	-0.708 ±2.90 (0.44)	0.770 ±1.450 (0.41)	0.282 ±1.632 (0.76)	1.044 ±1.030 (0.64)	-1.081 ±2.522 (0.14)	-0.689 ±2.059 (0.12)	5.321 ±2.033 (0.72)	2.048 ±1.900 (0.52)
Cobalamin (B_{12}) (µg)	0.435 ±1.169 (0.21)	1.264 ±1.294 (0.42)	1.976 ±1.110 (0.27)	-0343 ±1.121 (0.80)	-0.067 ±0.263 (0.52)	-0.091 ±0.192 (0.73)	-0.229 ±0.692 (0.88)	2.282 ±1.175 (0.19)	0.826 ±0.927 (0.77)	0.871 ±0.937 (0.59)	-0.086 ±0.843 (0.58)
Thiamine (B_1) (mg)	-3.335 ±1.668 (0.12)	1.975 ±2.556 (0.29)	1.227 ±1.611 (0.47)	-0.792 ±3.611 (0.31)	-0.361 ±1.109 (0.12)	-0.900 ±1.092 (0.23)	-1.720 ±1.011 (0.67)	1.150 ±1.029 (014)	1.791 ±1.392 (0.22)	0.995 ±2.493 (0.09)	1.510 ±1.369 (0.07)

Pantothenate (B_5) (mg)	-0.833 ±0.976 (0.60)	1.740 ±1.236 (0.33)	0.139 ±0.910 (0.51)	-1.050 ±1.230 (0.79)	0.211 ±0.400 (0.19)	-1.221 ±1.310 (0.54)	1.420 ±0.101 (0.38)	0.033 ±1.070 (0.21)	-1.016 ±0.824 (0.37)	1.526 ±0.941 (0.59)	-0.007 ±0.756 (0.84)
Pyridoxal phosphate (B_6) (mg)	0.760 ±3.074 (0.92)	-0.200 ±3.305 (0.72)	-0.021 ±2.878 (0.80)	-0.488 ±3.420 (0.54)	0.632 ±2.110 (0.87)	-0.421 ±1.901 (0.23)	-0.291 ±2.440 (0.59)	-2.181 ±1.005 (0.91)	0.408 ±2.486 (0.98)	-0.151 ±2.606 (0.31)	-1.179 ±2.187 (0.47)
Biotin (µg)	0.240 ±0.209 (0.41)	0.418 ±0.194 (0.74)	0.081 ±0.167 (0.69)	0.139 ±0.230 (0.43)	-0.180 ±0.050 (0.29)	-0.120 ±0.101 (0.71)	-0.242 ±0.150 (0.77)	-0.184 ±2.54 (0.30)	0.197 ±0.148 (0.38)	-0.116 ±0.173 (0.70)	0.023 ±0.135 (0.60)
Vitamin K (mg)	-0.036 ±0.086 (0.01)*	0.121 ±0.100 (0.39)	-0.141 ±0.073 (0.02)*	-0.137 ±0.100 (0.09)	-0.039 ±0.033 (0.69)	0.170 ±0.270 (0.29)	-0.014 ±0.103 (0.43)	-0.030 ±0.041 (0.71)	0.059 ±0.067 (0.28)	0.063 ±0.086 (0.53)	-0.024 ±0.060 (0.04)*

Estimates represent the change in body fat percentage (mean ± SE (p-value)) per unit increase in nutrient density. Those marked with " * " to indicate statistical significance ($p < 0.05$). A negative value indicates a decrease, while a positive value indicates an increase in body fat per unit increase in nutrient density.

Table 5.7. The relationship between dietary nutrient density and body fat percentage at 6 months (adjusted for dietary phytate and covariates)

	Gender		**Age (years)**		**Ethnicity**				**Special diet**		**All participants**
Micronutrient density (Nutrient per 1000 kcal)	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Vitamin A (µg)	-0.033 ±0.010 (0.01)*	-0.080 ±0.012 (0.03)*	-0.070 ±0.006 (0.01)*	-0.004 ±0.010 (0.01)*	0.204 ±0.016 (0.07)	0.021 ±0.041 (0.09)	-0.072 ±0.023 (0.10)	-0.014 ±0.060 (0.06)	-0.006 ±0.007 (0.01)*	-0.014 ±0.062 (0.01)*	-0.006 ±0.006 (0.03)*
Riboflavin (B_2) (mg)	-2.992 ±4.359 (0.21)	4.31 ±7.82 (0.32)	1.392 ±4.464 (0.10)	-6.203 ±6.154 (0.09)	0.211 ±0.400 (0.13)	1.201 ±1.310 (0.31)	-1.410 ±0.101 (0.11)	-0.033 ±1.070 (0.09)	-2.091 ±4.493 (0.25)	-2.695 ±1.969 (0.14)	-3.793 ±3.875 (0.10)
Folate (µg)	-0.041 ±0.345 (0.02)*	-0.022 ±0.039 (0.01)*	-0.020 ±0.025 (0.02)*	-0.028 ±0.036 (0.01)*	0.049 ±0.034 (0.31)	0.418 ±0.310 (0.22)	-0.013 ±0.011 (0.42)	-0.024 ±0.031 (0.11)	-0.021 ±0.025 (0.01)*	-0.333 ±0.705 (0.03)*	-0.016 ±0.022 (0.04)*
Vitamin D (µg)	-0.030 ±0.232 (0.24)	0.160 ±0.378 (0.09)	0.184 ±0.238 (0.09)	-0.396 ±0.250 (0.10)	0.180 ±0.405 (0.07)	1.402 ±1.015 (0.15)	-0.070 ±0.101 (0.52)	-0.133 ±0.336 (0.09)	-0.049 ±0.231 (0.31)	-1.867 ±1.935 (0.27)	-0.154 ±0.193 (0.51)

Iron (mg)	-0.501 ±0.362 (0.01)*	-0.167 ±0.627 (0.03)*	-0.213 ±0.308 (0.01)*	-0.316 ±0.519 (0.03)*	0.790 ±0.281 (0.11)	0.650 ±0.515 (0.51)	-0.310 ±0179 (0.09)	-0.162 ±0.168 (0.33)	-0.597 ±0.313 (0.001)*	-0.636 ±0.355 (0.002)*	-0.450 ±0.280 (0.03)*
Calcium (mg)	-0.006 ±0.009 (0.02)*	-0.010 ±0.012 (0.01)*	0.012 ±0.008 (0.001)*	-0.009 ±0.009 (0.03)*	0.003 ±0.017 (0.05)	0.022 ±0.134 (0.22)	-0.016 ±0.007 (0.28)	-0.024 ±0.033 (0.42)	-0.004 ±0.008 (0.04)*	-0.093 ±0.144 (0.01)*	-0.001 ±0.007 (0.03)*
Vitamin E (mg)	-0.439 ±0.287 (0.002)*	-0.280 ±0.460 (0.01)*	-0.073 ±0.281 (0.03)*	-0.153 ±0.314 (0.01)*	0.044 ±0.270 (0.33)	1.200 ±0.201 (0.16)	-0.402 ±0.260 (0.31)	-0.180 ±0.450 (0.09)	-0.024 ±0.270 (0.01)*	-1.367 ±0.340 (0.31)*	-0.062 ±0.235 (0.03)*
Magnesium (mg)	-0.034 ±0.028 (0.006)*	-0.070 ±0.044 (0.01)*	-0.019 ±0.028 (0.02)*	-0.086 ±0.037 (0.01)*	-0.012 ±0.068 (0.08)	0.204 ±0.071 (0.11)	-0.034 ±0.028 (0.51)	-0.060 ±0.014 (0.24)	-0.001 ±0.027 (0.03)*	-0.214 ±0.393 (0.004)*	-0.004 ±0.023 (0.01)*
Potassium (mg)	-0.007 ±0.002 (0.19)	0.001 ±0.003 (0.22)	-0.003 ±0.002 (0.41)	-0.008 ±0.004 (0.58)	0.003 ±0.002 (0.17)	0.023 ±0.037 (0.27)	-0.017 ±0.012 (0.11)	-0.011 ±0.004 (0.46)	-0.003 ±0.002 (0.61)	-0.023 ±0.038 (0.09)	-0.001 ±0.002 (0.09)
Iodine (µg)	-0.041 ±0.031 (0.31)	0.016 ±0.023 (0.58)	0.010 ±0.031 (0.11)	-0.001 ±0.028 (0.71)	0.005 ±0.042 (0.36)	0.020 ±0.271 (0.09)	-0.060 ±0.030 (0.20)	-0.016 ±0.023 (0.91)	-0.005 ±0.028 (0.54)	-0.119 ±0.127 (0.16)	0.013 ±0.024 (0.35)
Selenium (µg)	-0.008 ±0.042 (0.002)*	-0.059 ±0.87 (0.04)*	-0.034 ±0.046 (0.01)*	-0.080 ±0.038 (0.04)*	0.032 ±0.077 (0.13)	-0.005 ±0.142 (0.46)	-0.008 ±0.047 (0.08)	-0.063 ±0.470 (0.25)	-0.026 ±0.047 (0.003)*	-0.023 ±0.142 (0.01)*	-0.017 ±0.038 (0.01)*

Zinc (mg)	-0.161 ±0.368 (<0.001)*	-0.218 ±0.404 (0.02)*	0.601 ±0.294 (0.002)*	-0.218 ±0.500 (0.01)*	-0.053 ±0.301 (0.14)	-1.082 ±0.270 (0.10)	-0.261 ±0.378 (0.07)	-0.235 ±0.430 (0.09)	-0.043 ±0.298 (0.02)*	-1.071 ±0.297 (0.03)*	-0.020 ±0.258 (0.01)*
Vitamin C (mg)	-0.027 ±0.035 (0.006)*	-0.048 ±0.058 (0.04)*	-0.051 ±0.043 (0.009)*	-0.041 ±0.040 (0.02)*	0.015 ±0.021 (0.58)	0.033 ±0.101 (0.26)	-0.052 ±0.042 (0.39)	-0.058 ±0.072 (0.06)	-0.024 ±0.032 (0.03)*	-0.003 ±0.162 (0.001)*	-0.018 ±0.029 (0.01)*
Manganese (mg)	-0.900 ±0.544 (0.23)	0.292 ±0.890 (0.05)	0.030 ±0.392 (0.13)	-0.052 ±0.570 (0.21)	0.134 ±0.520 (0.18)	1.024 ±0.440 (0.40)	-1.010 ±0.584 (0.29)	-0.298 ±0.603 (0.11)	-0.134 ±0.520 (0.08)	-2.314 ±0.340 (0.27)	-0.206 ±0.439 (0.09)
Copper (mg)	-2.339 ±2.086 (0.38)	4.120 ±3.104 (0.18)	2.431 ±1.78 (0.49)	-0.687 ±2.482 (0.09)	0.560 ±2.401 (0.51)	1.700 ±1.413 (0.07)	-2.072 ±2.629 (0.25)	-3.505 ±2.104 (0.11)	-0.436 ±2.047 (0.19)	-1.367 ±1.754 (0.31)	-0.899 ±1.784 (0.10)
Phosphorus (mg)	-0.001 ±0.007 (0.03)*	-0.014 ±0.010 (0.03)*	-0.003 ±0.008 (0.01)*	0.020 ±0.010 (<0.001)*	-0.022 ±0.014 (0.12)	0.046 ±0.020 (0.40)	-0.011 ±0.029 (0.08)	-0.052 ±0.048 (0.17)	-0.003 ±0.007 (0.01)*	0.013 ±0.006 (0.04)*	0.001 ±0.006 (0.02)*
Niacin (mg)	-1.012 ±1.930 (0.27)	2.301 ±2.483 (0.09)	3.900 ±1.95 (0.11)	-3.367 ±2.447 (0.08)	0.770 ±1.450 (0.21)	0.282 ±1.632 (0.1)	-1.044 ±1.030 (0.07)	-1.081 ±2.522 (0.16)	-0.826 ±1.725 (0.14)	-0.832 ±1.70 (0.12)	-0.785 ±1.526 (0.08)
Cobalamin (B_{12}) (µg)	-0.213 ±0.879 (0.10)	2.296 ±1.002 (0.22)	0.166 ±0.862 (0.58)	-0.226 ±1.020 (0.32)	0.067 ±0.263 (0.73)	0.091 ±0.192 (0.31)	-0.229 ±0.692 (0.13)	-2.282 ±1.175 (0.49)	-0.706 ±0.771 (0.77)	-0.058 ±0.950 (0.13)	-0.587 ±0.678 (0.20)
Thiamine (B_1) (mg)	-1.942 ±1.282 (0.34)	1.100 ±1.969 (0.56)	1.397 ±1.252 (0.09)	-1.748 ±3.052 (0.42)	0.361 ±1.109 (0.11)	0.900 ±1.092 (0.78)	-1.720 ±1.011 (0.23)	1.150 ±1.029 (0.29)	-0.533 ±1.167 (0.41)	0.644 ±1.280 (0.55)	-0.254 ±1.100 (0.32)

Pantothenate (B_5) (mg)	-1.495 ±0.703 (0.07)	0.038 ±1.000 (0.38)	0.263 ±0.734 (0.29)	-0.379 ±1.039 (0.80)	0.211 ±0.400 (0.10)	1.221 ±1.310 (0.34)	-1.420 ±0.101 (0.78)	-0.033 ±1.070 (0.52)	-0.418 ±0.690 (0.17)	-1.550 ±1.000 (0.08)	-0.065 ±0.607 (0.25)
Pyridoxal phosphate (B_6) (mg)	-0.495 ±2.203 (0.26)	6.959 ±2.548 (0.14)	1.353 ±2.232 (0.66)	-3.064 ±2.890 (0.32)	0.632 ±2.110 (0.49)	0.421 ±1.901 (0.13)	-0.291 ±2.440 (0.78)	-2.181 ±1.005 (0.67)	-0.795 ±2.051 (0.28)	-0.763 ±2.703 (0.17)	-0.001 ±1.757 (0.48)
Biotin (µg)	-0.104 ±0.135 (0.11)	-0.117 ±2.548 (0.46)	-0.068 ±0.125 (0.15)	-0.068 ±0.194 (0.32)	-0.180 ±0.050 (0.71)	-0.120 ±0.120 (0.53)	-0.242 ±0.160 (0.26)	-0.184 ±2.54 (0.44)	-0.026 ±0.124 (0.57)	-0.170 ±0.151 (0.28)	-0.044 ±0.109 (0.31)
Vitamin K (mg)	-0.054 ±0.062 (0.03)*	-0.030 ±0.079 (0.03)*	-0.053 ±0.060 (0.001)*	-0.054 ±0.084 (0.004)*	0.039 ±0.033 (0.06)	-0.170 ±0.270 (0.10)	-0.014 ±0.103 (0.32)	-0.030 ±0.041 (0.26)	-0.087 ±0.056 (0.01)*	-0.109 ±0.090 (0.04)*	-0.040 ±0.048 (0.01)*
Estimates represent the change in body fat percentage (mean ± SE (p-value)) per unit increase in nutrient density. Those marked with " * " to indicate statistical significance ($p < 0.05$). A negative value indicates a decrease, while a positive value indicates an increase in body fat per unit increase in nutrient density.											

In Table 5.8 below, no association between dietary nutrient density and body fat percentage was observed overall for participants, and within demographic groups ($p > 0.05$). However, after adjusting for gender, age, being on a special diet, ethnicity, dietary phytate intake, appetite, alcohol intake, physical activity, hours of night-time sleep and smoking status, overall participants, there was a significant inverse relationship between the dietary nutrient density for vitamins A, C, E and K, folate, iron, zinc, calcium, magnesium, selenium and phosphorus and body fat percentage ($p < 0.001$). A unit increase in the dietary nutrient density for each micronutrient corresponded to a decrease in body fat percentage (Table 5.9). Among males and females, a unit increase in the dietary nutrient density for vitamin A, folate, magnesium, vitamin C, phosphorus, iron, vitamin E, zinc, selenium, biotin and vitamin K corresponded to an average body fat percentage decrease of 0.27% ($p < 0.05$) and 2.21% ($p < 0.05$) respectively. Similarly, among participants aged 18-29 and 30-39 years, a unit increase in dietary nutrient density for vitamin A, folate, magnesium, vitamin C, phosphorus, iron, vitamin E, zinc, selenium and vitamin K corresponded to an average decrease of 0.50% ($p < 0.05$) and 0.1% ($p < 0.05$) respectively (Table 5.9).

Table 5.8. The relationship between dietary nutrient density and body fat percentage showing the change in body fat percentage (mean ± SE (p-value)) with a unit increase in dietary nutrient density based on participant characteristics.

	Gender		**Age (years)**		**Ethnicity**				**Special diet**		**All participants**
Micronutrient density (Nutrient per 1000 kcal)	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Vitamin A (µg)	-0.11 ± 0.76 (0.74)	-0.05 ± 0.79 (0.58)	-0.03 ± 0.60 (0.11)	-0.06 ± 0.02 (0.42)	0.01 ± 0.03 (0.62)	0.01 ± 0.04 (0.40)	-0.01 ±1.07 (0.17)	-0.01 ± 0.05 (0.33)	0.02 ± 0.03 (0.78)	0.14 ±0.06 (0.61)	-0.25 ± 0.01 (0.31)
Riboflavin (B_2) (mg)	-7.10 ± 12.02 (0.14)	17.87 ±13.45 (0.22)	12.57 ± 9.87 (0.48)	-4.54 ± 9.84 (0.10)	3.27 ± 16.22 (0.82)	2.08 ± 17.4 (0.16)	-1.84 ±21.20 (0.29)	-5.97 ± 21.82 (0.71)	9.36 ± 11.85 (0.12)	6.02 ± 16.84 (0.33)	-8.95 ± 7.93 (0.74)
Folate (µg)	-0.03 ± 1.18 (0.83)	0.12 ± 1.24 (0.24)	0.08 ± 0.95 (0.16)	-0.01 ± 0.06 (0.15)	0.02 ± 0.10 (0.18)	0.01 ± 0.11 (0.47)	-0.02 ± 1.68 (0.36)	-0.04 ± 0.13 (0.22)	0.06 ± 0.07 (0.14)	0.35 ±0.16 (0.53)	-0.05 ± 0.64 (0.18)

Vitamin D (μg)	-4.19 ± 7.53 (0.20)	11.48 ± 8.4 (0.09)	8.03 ± 6.17 (0.32)	-2.59 ± 6.14 (0.16)	2.07 ± 10.25 (0.29)	1.32 ± 11.00 (0.07)	-1.43 ± 13.19 (0.13)	-3.77 ± 13.79 (0.27)	5.91 ± 7.49 (0.20)	5.05 ± 10.07 (0.31)	-15.59 ± 2.43 (0.11)
Iron (mg)	-0.52 ± 3.91 (0.17)	-2.83 ± 5.01 (0.08)	-1.87 ± 3.39 (0.21)	-0.24 ± 3.37 (0.16)	-0.46 ± 2.30 (0.30)	-0.29 ± 2.46 (0.14)	-0.62 ± 7.02 (0.09)	-0.84 ± 3.09 (0.19)	-1.32 ± 1.68 (0.10)	-8.06 ± 3.80 (0.38)	-14.69 ± 0.54 (0.24)
Calcium (mg)	-0.01 ± 0.68 (0.39)	-0.37 ± 0.71 (0.12)	-0.02 ± 0.54 (0.18)	-0.004 ± 0.02 (0.09)	0.01 ± 0.03 (0.32)	0.01 ± 0.03 (0.17)	-0.01 ± 0.95 (0.24)	-0.01 ± 0.04 (0.09)	0.02 ± 0.02 (0.16)	0.11 ± 0.05 (0.08)	-0.20 ± 0.01 (0.13)
Vitamin E (mg)	-0.48 ± 4.1 (0.21)	2.52 ± 5.15 (0.10)	1.67 ± 3.54 (0.31)	-0.22 ± 3.52 (0.09)	-0.41 ± 2.05 (0.28)	-0.24 ± 4.96 (0.13)	-0.55 ± 7.35 (0.16)	-0.75 ± 2.76 (0.10)	-1.19 ± 1.50 (0.20)	-7.20 ± 3.39 (0.08)	-13.12 ± 0.48 (0.17)
Magnesium (mg)	-0.29 ± 1.21 (0.15)	-0.12 ± 1.27 (0.09)	-0.08 ± 0.97 (0.24)	-0.02 ± 0.07 (0.11)	0.02 ± 0.10 (0.08)	0.01 ± 0.10 (0.19)	-0.02 ± 1.72 (0.16)	-0.04 ± 0.14 (0.07)	0.06 ± 0.08 (0.28)	0.37 ± 0.17 (0.14)	-0.67 ± 0.02 (0.10)
Potassium (mg)	-0.03 ± 0.41 (0.18)	0.01 ± 0.42 (0.24)	-0.01 ± 0.32 (0.17)	-0.01 ± 0.01 (0.38)	0.01 ± 0.01 (0.15)	0.01 ± 0.01 (0.20)	-0.01 ± 0.57 (0.18)	-0.01 ± 0.01 (0.11)	0.01 ± 0.01 (0.40)	0.39 ± 0.02 (0.13)	-0.07 ± 0.003 (0.15)
Iodine (μg)	-0.04 ± 1.47 (0.24)	0.19 ± 1.55 (0.09)	0.13 ± 1.18 (0.36)	-0.02 ± 0.10 (0.11)	0.03 ± 0.16 (0.61)	0.02 ± 0.15 (0.15)	-0.04 ± 2.1 (0.21)	-0.06 ± 0.21 (0.10)	0.09 ± 0.11 (0.27)	0.55 ± 0.26 (0.33)	-1.01 ± 0.04 (0.22)

Selenium (µg)	-0.16 ± 2.53 (0.08)	-0.65 ± 2.67 (0.32)	-0.44 ± 2.04 (0.54)	-0.08 ± 0.34 (0.58)	0.11 ± 0.54 (0.29)	0.07 ± 6.17 (0.81)	-0.13 ± 3.87 (0.10)	-0.20 ± 0.73 (0.61)	0.31 ± 0.40 (0.13)	1.90 ± 0.90 (0.77)	-3.47 ± 0.13 (0.39)
Zinc (mg)	-0.61 ± 3.51 (0.72)	3.45 ± 4.60 (0.37)	2.27 ± 3.07 (0.12)	-0.26 ± 3.05 (0.20)	0.56 ± 2.8 (0.45)	0.31 ± 4.33 (0.17)	-0.77 ± 6.41 (0.33)	-1.03 ± 3.75 (0.28)	1.61 ± 2.04 (0.22)	9.78 ±4.61 (0.43)	-17.83 ± 0.65 (0.62)
Vitamin C (mg)	-0.09 ± 2.00 (0.25)	-0.37 ± 2.12 (0.33)	-0.25 ± 1.61 (0.11)	-0.05 ± 1.0 (0.12)	0.06 ± 0.31 (0.09)	0.04 ±011 (0.30)	-0.07 ± 2.89 (0.09)	-0.11 ± 0.42 (0.36)	0.18 ± 0.23 (0.18)	1.10 ± 0.52 (0.15)	-2.00 ± 0.07 (0.08)
Manganese (mg)	-3.34 ± 6.20 (0.44)	9.52 ± 6.89 (0.17)	6.64 ± 5.06 (0.32)	-2.04 ± 5.04 (0.08)	1.70 ± 8.44 (0.23)	1.42 ± 6.84 (0.41)	-1.26 ± 10.69 (0.19)	-3.11 ±11.40 (0.07)	4.87 ± 6.17 (0.33)	9.03 ± 8.55 (0.15)	-4.89 ± 1.98 (0.50)
Copper (mg)	-10.10 ±16.62 (0.38)	4.12 ±18.60 (0.23)	17.00 ± 13.67 (0.08)	-6.55 ± 13.63 (0.18)	4.45 ± 22.08 (0.52)	4.32 ± 18.69 (0.26)	-2.21 ± 29.28 (0.11)	-8.12 ± 29.71 (0.33)	10.22 ± 16.14 (0.58)	5.38 ± 16.51 (0.84)	-8.36 ± 8.80 (0.21)
Phosphorus (mg)	-0.01 ± 0.87 (0.07)	-0.06 ± 0.91 (0.14)	-0.04 ± 0.70 (0.26)	-0.01 ± 0.03 (0.07)	0.01 ± 0.05 (0.30)	0.01 ± 0.05 (0.15)	-0.01 ± 1.23 (0.16)	-0.02 ±0.07 (0.42)	0.03 ± 0.04 (0.19)	0.18 ± 0.09 (0.22)	-0.31 ± 0.01 (0.14)
Niacin (mg)	-8.57 ± 14.30 (0.09)	-10.33 ±15.98 (0.17)	-14.78 ± 11.73 (0.30)	-5.53 ± 11.71 (0.28)	-3.86 ± 19.14 (0.24)	-3.67 ± 16.04 (0.13)	-2.03 ± 15.20 (0.10)	-7.04 ± 15.75 (0.71)	-11.04 ± 13.98 (0.32)	-9.03 ± 11.07 (0.06)	-13.02 ± 2.95 (0.15)

Cobalamin (B_{12}) (µg)	-3.54 ± 4.64 (0.45)	7.59 ± 5.09 (0.12)	5.28 ± 3.77 (0.08)	-1.52 ± 3.74 (0.18)	1.34 ± 6.68 (0.23)	1.08 ± 5.05 (0.11)	-1.21 ± 8.12 (0.07)	-2.45 ± 8.98 (0.27)	3.85 ± 4.88 (0.09)	13.43 ± 11.05 (0.38)	-7.29 ± 1.31 (0.19)
Thiamine (B_1) (mg)	-7.4 ± 12.03 (0.22)	3.64 ± 6.49 (0.34)	4.51 ± 2.20 (0.09)	-7.84 ± 9.40 (0.65)	10.21 ± 19.30 (0.22)	1.37 ± 3.30 (0.42)	-5.14 ± 9.18 (0.49)	-11.01 ± 13.01 (0.31)	8.92 ± 12.43 (0.82)	3.75 ± 7.54 (0.51)	-8.14 ± 17.03 (0.45)
Pantothenate (B_5) (mg)	-2.00 ± 9.98 (0.06)	6.05 ±10.26 (0.09)	8.38 ± 14.78 (0.08)	-7.19 ± 14.75 (0.12)	4.82 ± 13.52 (0.15)	4.73 ± 12.25 (0.27)	-2.32 ± 11.80 (0.63)	-8.80 ± 12.19 (0.45)	4.80 ± 7.48 (0.09)	3.96 ±5.59 (0.21)	-4.67 ± 8.71 (0.07)
Pyridoxal phosphate (B_6) (mg)	-7.44 ± 12.54 (0.12)	18.59 ±14.02 (0.50)	13.08 ± 11.19 (0.31)	-4.77 ± 13.16 (0.19)	3.40 ± 16.89 (0.28)	3.18 ± 14.05 (0.40)	-1.88 ± 22.10 (0.16)	-6.21 ± 12.00 (0.13)	9.74 ± 12.34 (0.25)	9.72 ± 9.39 (0.15)	-1.90 ± 3.32 (0.19)
Biotin (µg)	-0.29 ± 3.08 (0.39)	-1.39 ± 5.91 (0.11)	-0.93 ± 2.02 (0.15)	-0.17 ± 0.73 (0.20)	0.23 ± 1.15 (0.14)	0.14 ± 5.64 (0.07)	-2.91 ± 5.74 (0.10)	-0.42 ± 1.54 (0.28)	0.66 ± 0.84 (0.18)	4.03 ± 1.90 (0.08)	-6.93 ±0.23 (0.27)
Vitamin K (mg)	-0.17 ± 2.28 (0.25)	-0.77 ± 3.05 (0.05)	-0.51 ± 2.19 (0.07)	-0.09 ± 0.41 (0.15)	0.13 ± 0.64 (0.22)	-0.24 ± 0.71 (0.13)	-0.16 ± 4.42 (0.08)	-0.04 ± 0.14 (0.19)	0.37 ± 0.47 (0.10)	0.29 ± 0.14 (0.06)	-3.85 ± 0.13 (0.09)
Estimates are marked with " * " to indicate statistical significance ($p< 0.05$). A negative value indicates a decrease, while a positive value indicates an increase.											

Table 5.9. The relationship between dietary nutrient density and body fat percentage showing the change in body fat percentage (mean ± SE (p-value)) with a unit increase in dietary nutrient density based on participant characteristics (adjusted for gender, age, appetite, ethnicity, special diet, phytate intake, physical activity, average hours of night-time sleep, smoking and alcohol intake).

	Gender		**Age (years)**		**Ethnicity**				**Special diet**		**All participants**
Micronutrient density (Nutrient per 1000kcal)	Male	Female	18-29	30-39	African	White British	Mixed	Asian	No special diet	Vegetarian	
Vitamin A (µg)	-0.01 ± 0.76* (0.02)	-0.05 ± 0.79* (0.01)	-0.03 ± 0.60* (0.04)	-0.01 ± 0.02* (0.01)	0.0001 ± 0.04 (0.08)	0.005 ± 0.04 (0.21)	-0.01 ± 1.07 (0.11)	-0.01 ± 0.05 (0.07)	-0.02 ± 0.03* (0.01)	-0.14 ± 0.06* (0.001)	-0.02 ± 0.41* (0.01)
Riboflavin (B_2) (mg)	-7.10 ± 12.02 (0.11)	17.87 ± 13.45 (0.06)	12.57 ± 9.86 (0.05)	-4.54 ± 9.84 (0.17)	0.43 ± 13.43 (0.13)	3.03 ±13.46 (0.09)	-1.84 ± 21.20 (0.20)	-5.97 ±21.82 (0.09)	-9.36 ± 11.85 (0.16)	-6.72 ± 16.84 (0.07)	-8.95 ± 7.93 (0.13)
Folate (µg)	-0.03 ± 1.18* (0.01)	-0.12 ± 1.24* (0.04)	-0.08 ± 0.95* (0.001)	-0.01 ± 0.06* (<0.001)	0.001 ± 0.11 (0.05)	0.01 ± 0.09 (0.08)	-0.02 ± 1.68 (0.05)	-0.04 ± 0.13 (0.19)	-0.06 ± 0.07* (0.02)	-0.35 ± 0.16* (0.01)	-0.05 ± 0.64* (0.04)
Vitamin D (µg)	-4.19 ± 7.53 (0.18)	11.48 ± 8.4 (0.24)	8.03 ± 6.17 (0.33)	-2.59 ± 6.14 (0.09)	0.08 ± 8.24 (0.07)	1.78 ± 8.37 (0.30)	-1.43 ± 13.19 (0.14)	-3.77 ± 13.79 (0.11)	-5.91 ± 7.49 (0.32)	-3.60 ± 6.97 (0.07)	-5.67 ± 4.95 (0.14)

Iron (mg)	-0.52 ± 3.91* (0.03)	-2.83 ± 5.01* (0.01)	-1.87 ± 3.38* (0.01)	-0.24 ± 3.37* (<0.001)	0.09 ± 2.67 (0.05)	0.26 ± 4.75 (0.09)	-0.62 ± 7.02 (0.10)	-0.84 ± 3.09 (0.16)	-1.32 ± 1.68* (0.09)	-8.06 ± 3.80* (0.06)	-1.24 ± 2.72* (0.09)
Calcium (mg)	-0.01 ± 0.68 (<0.001)*	-0.04 ± 0.71 (0.04)*	0.02 ± 0.54 (0.01)*	-0.004 ± 0.02 (0.03)*	0.001 ± 0.04 (0.08)	0.004 ± 0.28 (0.17)	-0.01 ± 0.95 (0.10)	-0.01 ± 0.04 (0.15)	-0.02 ± 0.02 (0.01)*	-0.11 ± 0.05 (0.03)*	-0.02 ± 0.36 (0.01)*
Vitamin E (mg)	-0.48 ± 4.10 (0.03)*	-2.52 ± 5.15 (0.01)*	-1.67 ± 3.54 (0.03)*	-0.22 ± 3.52 (0.004)*	0.03 ± 2.24 (0.07)	0.24 ± 4.96 (0.19)	-0.55 ± 7.35 (0.08)	-0.75 ± 2.76 (0.10)	-1.18 ± 1.50 (0.01)*	-7.20 ± 3.39 (0.002)*	-1.11 ± 2.84 (0.04)*
Magnesium (mg)	-0.03 ± 1.21 (0.006)*	-0.12 ± 1.27 (0.002)*	-0.08 ± 0.97 (0.01)*	-0.02 ± 0.07 (0.04)*	-0.001 ± 0.11 (0.08)	0.01 ± 0.10 (0.07)	-0.02 ± 1.72 (0.15)	-0.04 ± 0.14 (0.05)	-0.06 ± 0.08 (0.02)*	-0.37 ± 0.17 (0.005)*	-0.06 ± 0.65 (0.01)*
Potassium (mg)	-0.003 ± 0.41 (0.07)	0.01 ± 0.42 (0.09)	-0.01 ± 0.32 (0.05)	-0.002 ± 0.01 (0.05)	0.002 ± 0.01 (0.08)	0.001 ± 0.01 (0.06)	-0.003 ± 0.57 (0.11)	-0.004 ± 0.01 (0.05)	-0.01 ± 0.01 (0.09)	-0.04 ± 0.02 (0.07)	-0.01 ± 0.22 (0.07)
Iodine (µg)	-0.04 ± 1.47 (0.05)	0.19 ± 1.55 (0.09)	0.13 ± 1.18 (0.07)	-0.02 ± 0.10 (0.09)	0.001 ± 0.17 (0.05)	0.02 ± 0.14 (0.05)	-0.04 ± 2.10 (0.08)	-0.06 ± 0.21 (0.09)	-0.09 ± 0.11 (0.06)	-0.55 ± 0.26 (0.05)	0.086 ± 0.80 (0.08)
Selenium (µg)	-0.16 ± 2.53 (0.003)*	-0.65 ± 2.67 (0.01)*	-0.44 ± 2.04 (0.03)*	-0.08 ± 0.34 (0.02)*	0.03 ± 0.68 (0.09)	-0.07 ± 6.17 (0.07)	-0.13 ± 3.87 (0.07)	-0.20 ± 0.73 (0.06)	-0.31 ± 0.40 (0.01)*	-1.90 ± 0.90 (0.02)*	-0.30 ±1.18 (0.04)*

Zinc (mg)	-0.61 ± 3.51 (0.02)*	-3.45 ± 4.50 (0.01)*	2.27 ± 3.07 (0.02)*	-0.26 ± 3.1 (0.04)*	-0.01 ± 2.79 (0.08)	-0.31 ± 4.33 (0.06)	-0.77 ± 6.40 (0.07)	-1.03 ± 3.75 (0.09)	-1.61 ± 2.04 (0.03)*	-9.78 ± 4.61 (0.01)*	-1.50 ± 2.46 (0.04)*
Vitamin C (mg)	-0.09 ± 2.0 (0.03)*	-0.37 ± 2.12 (0.004)*	-0.25 ± 1.61 (0.02)*	-0.05 ± 0.20 (0.03)*	0.001 ± 0.34 (0.05)	0.04 ± 287 (0.10)	-0.07 ± 2.89 (0.13)	-0.11 ± 0.42 (0.06)	-0.18 ± 0.23 (0.004)*	-1.10 ± 0.52 (0.01)*	-0.17 ± 1.10 (0.04)*
Manganese (mg)	-3.34 ± 6.20 (0.07)	4.52 ±6.89 (0.05)	6.64 ± 5.06 (0.07)	-2.04 ± 5.04 (0.14)	0.16 ± 7.43 (0.09)	1.42 ± 6.84 (0.09)	-1.26 ± 10.69 (0.12)	-3.11 ± 11.4 (0.18)	-4.87 ± 6.17 (0.10)	-9.63 ± 13.97 (0.06)	-4.67 ± 4.06 (0.09)
Copper (mg)	-5.06 ± 16.61 (0.10)	4.09 ± 18.60 (0.17)	1.00 ± 13.67 (0.11)	-6.55 ± 13.63 (0.08)	0.81 ± 19.66 (0.10)	4.32 ±18.69 (0.07)	-2.21 ± 29.29 (0.09)	-8.12 ± 12.71 (0.07)	-2.74 ± 16.14 (0.08)	-4.50 ± 13.54 (0.08)	-12.16 ± 10.99 (0.07)
Phosphorus (mg)	-0.01 ± 0.87 (0.003)*	-0.06 ± 0.91 (0.001)*	-0.04 ± 0.70 (0.03)*	-0.01 ± 0.03 (0.01)*	-0.003 ± 0.06 (0.07)	0.01 ± 0.05 (0.09)	-0.01 ± 1.23 (0.10)	-0.19 ± 0.07 (0.07)	-0.03 ± 0.04 (0.04)*	-0.18 ± 0.09 (0.01)*	-0.03 ± 0.47 (0.02)*
Niacine (B_3) (mg)	-8.57 ± 14.30 (0.09)	2.97 ± 15.98 (0.11)	4.78 ± 11.73 (0.08)	-5.53 ± 11.71 (0.07)	0.61 ± 16.36 (0.09)	3.67 ±16.04 (0.05)	-2.03 ± 25.20 (0.10)	-7.04 ± 25.75 (0.06)	-11.04 ± 13.98 (0.06)	-7.07 ± 13.09 (0.09)	-10.55 ± 9.43 (0.07)
Cobalamin (B_{12}) (µg)	-2.54 ± 4.64 (0.12)	7.59 ± 5.09 (0.08)	5.28 ± 3.77 (0.17)	-1.52 ± 3.74 (0.10)	0.14 ± 6.33 (0.09)	1.08 ± 5.05 (0.06)	-1.21 ± 8.13 (0.10)	-2.46 ± 8.98 (0.05)	-3.85 ± 4.88 (0.07)	-23.43 ± 11.04 (0.42)	-3.69 ± 3.02 (0.16)

Thiamine (B_1) (mg)	-3.02 ± 12.20 (0.09)	4.82 ± 12.49 (0.09)	0.9 ± 14.33 (0.12)	-4.12 ± 10.82 (0.10)	3.03 ± 10.57 (0.14)	4.66 ±13.18 (0.16)	-5.69 ± 7.40 (0.08)	-2.79 ± 10.11 (0.15)	-2.81 ± 7.57 (0.09)	5.75 ± 6.03 (0.11)	-5.32 ± 8.84 (0.09)
Pantothenate (B_5) (mg)	-11.0 ± 17.98 (0.07)	6.04 ± 20.26 (0.09)	8.68 ± 14.78 (0.07)	-5.13 ± 14.74 (0.11)	0.94 ± 21.89 (0.23)	4.73 ±20.35 (0.19)	-2.32 ± 11.80 (0.06)	-8.60 ± 32.19 (0.12)	-1.80 ± 17.48 (0.010)	-3.26 ± 39.59 (0.14)	-13.17 ± 11.88 (0.15)
Pyridoxal phosphate (B_6) (mg)	-7.44 ± 12.54 (0.08)	18.58 ± 14.02 (0.05)	13.08 ± 10.29 (0.25)	-4.77 ± 10.26 (0.15)	0.47 ± 14.08 (0.05)	3.18 ±14.05 (0.12)	-1.88 ± 22.10 (0.10)	-6.21 ± 22.72 (0.09)	-9.74 ± 12.34 (0.07)	-9.37 ± 17.04 (0.17)	-9.32 ± 8.27 (0.08)
Biotin (µg)	-0.29 ± 3.08 (0.21)	-13.39 ± 5.91 (0.08)	-0.93 ± 2.01 (0.10)	-0.17 ± 0.73 (0.09)	-0.02 ± 1.17 (0.11)	-0.14 ± 5.64 (0.09)	-0.29 ± 5.74 (0.06)	-0.42 ± 1.54 (0.14)	-0.66 ± 0.84 (0.07)	-4.03 ± 1.90 (0.08)	-0.62 ± 2.22 (0.18)
Vitamin K (mg)	-0.17 ± 2.16 (0.04)*	-0.77 ± 3.05 (0.01)*	-0.51 ± 2.19 (0.07)*	-0.09 ± 0.41 (0.003)*	0.004 ± 0.70 (0.07)	-0.08 ± 6.08 (0.09)	-0.16 ± 4.42 (0.09)	-0.23 ± 0.86 (0.05)	-0.37 ± 0.47 (0.06)*	-2.24 ± 1.06 (0.004)*	-0.35 ± 1.45 (0.03)*

Values in Table 5.9 represent changes in body fat percentage ± standard error for a unit increase in the dietary nutrient density for specific nutrients. For instance, with 1mg per 1000 kcal increase in the dietary nutrient density of zinc, body fat percentage decreased by 1.50 ± 2.46%.

Estimates are marked with " * " to indicate statistical significance ($p< 0.05$). Where the relationship was analysed within gender, age, ethnicity or special diet categories, other covariates other than the category involved were considered.

When participants were categorised based on their level of physical activity; low, moderate and high (Table 5.10- below), it was observed that among those with a low level of physical activity, a unit increase in dietary nutrient density of vitamins A, C, E and K, folate, iron, calcium, potassium, iodine, selenium, zinc and phosphorus corresponded to an average body fat percentage decrease of 0.2%. Among those with a moderate level of physical activity, a unit increase in the dietary nutrient density of these nutrients corresponded to an average body fat percentage decrease of 0.56%. For those who were engaged in a high level of physical activity, there was no relationship between dietary nutrient density and body fat percentage.

Table 5.10. Change in body fat (mean ± SE (p-value)) with a unit increase in dietary nutrient density for participants with weekly low, moderate and high levels of physical activity per week.

Micronutrient density (Nutrients per 1000 kcal)	Low-level physical activity per week	Moderate physical activity per week	High physical activity per week
Vitamin A (µg)	-0.01 ± 0.02 (0.01)*	-0.03 ± 0.04 (0.03)*	0.02 ± 0.02 (0.08)
Riboflavin (B_2) (mg)	-4.34 ± 9.96 (0.06)	-13.6 ± 15.30 (0.19)	-8.06 ± 9.95 (0.10)
Folate (µg)	-0.03 ± 0.06 (0.04)*	-0.08 ± 0.09 (0.10)	-0.05 ± 0.06 (0.22)
Vitamin D (µg)	-2.74 ± 6.30 (0.08)	-8.57 ± 9.68 (0.09)	-5.10 ± 6.28 (0.07)
Iron (mg)	-0.61 ± 1.41 (0.01)*	-1.92 ± 2.17 (0.02)*	-1.14 ± 1.41 (0.15)
Calcium (mg)	-0.01 ± 0.02 (0.003)*	-0.03 ± 0.03 (0.01)*	-0.02 ± 0.01(0.31)
Vitamin E (mg)	-0.55 ± 1.20 (0.01)*	-1.78 ±1.96 (0.06)	-1.02 ± 1.26 (0.19)
Magnesium (mg)	-0.03 ± 0.06 (0.09)	-0.09 ± 0.10 (0.12)	-0.05 ± 0.06 (0.27)
Potassium (mg)	-0.01 ± 0.01 (0.02)*	-0.01 ± 0.01 (0.09)	-0.01 ± 0.01 (0.10)
Iodine (µg)	-0.04 ± 0.10 (0.001)*	-0.14 ± 0.15 (0.19)	-0.08 ± 0.10 (0.16)
Selenium (µg)	-0.14 ± 0.33* (0.02)*	-0.04 ± 0.5 (0.06)	-0.27 ± 0.33 (0.18)
Zinc (mg)	-0.75 ± 1.71* (0.01)*	-2.41 ± 2.66 (0.14)	-1.38 ± 1.71 (0.09)
Vitamin C (mg)	-0.08 ± 0.19* (0.04)*	-0.27 ± 0.30 (0.01)*	-0.55 ± 0.19 (0.13)
Manganese (mg)	-2.3 ± 5.18 (0.08)	-7.3 ± 8.07 (0.11)	-4.19 ± 5.18 (0.34)
Copper (mg)	-5.91 ± 13.56 (0.11)	-9.12 ± 21.10 (0.19)	-1.0 ± 13.54 (0.22)
Phosphorus (mg)	-0.01 ± 0.03* (0.02)*	-0.05 ± 0.05 (0.07)	-0.03 ± 0.03 (0.14)
Niacin (B_3) (mg)	-5.12 ± 11.75 (0.08)	-16.57 ±18.28 (0.10)	-9.51 ± 11.74 (0.53)
Cobalamin (B_{12}) (µg)	1.78 ± 4.01 (0.06)	-5.78 ± 6.38 (0.19)	-3.32 ± 4.09 (0.11)
Thiamine (B_1) (mg)	-2.10 ± 12.06 (0.08)	-4 ±12.17 (0.19)	-6.11 ± 10.49 (0.16)
Pantothenate (B_5) (mg)	-6.40 ± 14.68 (0.06)	-20.71 ± 22.86 (0.23)	-11.88 ± 14.67 (0.36)
Pyridoxal phosphate (B_6) (mg)	-4.51 ± 10.36 (0.13)	-14.62 ± 16.1 (0.17)	-8.39 ± 10.36 (0.20)
Biotin (µg)	-0.31 ± 0.71 (0.09)	1.0 ± 1.1 (0.14)	-0.57 ± 0.70 (0.15)
Vitamin K (mg)	-0.17 ± 0.39 (0.04)*	-0.55 ± 0.61 (0.02)*	-0.32 ± 0.39 (0.19)

The values in Table 5.10 are effect size estimates ± standard error. Estimates are marked with " * " to indicate statistical significance ($p < 0.05$). Each estimate represents the change in body fat percentage for a unit increase in dietary micronutrient density for specific micronutrients. Low, moderate and high levels of physical activity per week refer to the metabolic equivalent of task (MET)-minutes of activity < 600, $\leq 600 < 1500$, and ≥ 1500, respectively. The different levels of physical activity per week are classified according to the guideline for grading physical activity assessed by the Global Physical Activity Questionnaire.

After adjusting for all covariates assessed, and without adjusting for dietary phytate, there was a relationship between dietary nutrient density and body fat percentage; a unit increase in the dietary nutrient density of vitamins A, C, E and K, folate, magnesium, calcium, iodine, selenium and phosphorus corresponded to a decrease in body fat percentage (Table 5.11).

Table 5.11. The association between dietary nutrient density and body fat percentage after adjusting for the covariates assessed, and without adjusting for dietary phytate.

Dietary micronutrient density (Nutrients per 1000 kcal)	% Body fat change per unit increase in dietary micronutrient density	p-value
Vitamin A (µg)	-0.02 ±0.02	0.01*
Riboflavin (B_2) (mg)	-8.06 ±9.94	0.63
Folate (µg)	-0.05±0.06	0.04*
Vitamin D (µg)	-5.09±6.28	0.55
Iron (mg)	-1.14±1.41	0.43
Calcium (mg)	-0.02±0.02	0.01*
Vitamin E (mg)	-1.02±1.26	0.02*
Magnesium (mg)	-0.05±0.06	0.03*
Potassium (mg)	-0.01±0.01	0.41
Iodine (µg)	-0.08±0.10	0.68
Selenium (µg)	-0.27± 0.33	0.03*
Zinc (mg)	-1.38± 1.71	0.76
Vitamin C (mg)	-0.16± 0.19	0.01*
Manganese (mg)	-4.19± 5.18	0.52
Copper (mg)	-10.97± 13.54	0.39
Phosphorus (mg)	-0.03 ± 0.03	0.03*
Niacine (B_3) (mg)	-9.50 ± 11.74	0.63
Vit B_{12} (µg)	-3.32 ± 4.09	0.54
Thiamine (B_1) (mg)	-5.50 ± 8.01	0.12
Pantothenate (B_5) (mg)	-11.88 ± 14.67	0.69
Pyridoxal phosphate (B_6) (mg)	-8.39 ± 10.36	0.31
Biotin (µg)	-0.57 ± 0.70	0.74
Vitamin K (mg)	-0.32 ± 0.39	0.02*
The values in Table 6.9 are effect size estimates ±standard error. The estimates marked with " * " are statistically significant ($p< 0.05$). Each estimate represents the change in body fat percentage for a unit increase in dietary micronutrient density for specific micronutrients.		

5.3.2. Supplementary Findings

Further description of participants' nutrient intake is provided in the given tables below (Tables 5.12 and 5.13). In Table 5.12 below, the mean body fat percentage for participants whose body fat increased by 1% or more (28.5 ± 6.7%), and those whose body fat increased by less than 1% (29.7 ±7.4%) were significantly different ($p < 0.001$). However, there was no statistically significant difference between the average nutrient intakes between both participant groups.

Table 5.12. Average daily dietary nutrient intake of participants based on changes in body fat percentage.

Dietary nutrient	Participants whose body fat percentage increased by 1% or more	Participants whose body fat percentage increased by less than 1% or remained constant	p-value
Energy (kcal)	2338.3±199.1	2184 ± 265.4	0.63
Vitamin A (µg)	539.1 ± 184.0	540 ± 128.5	0.79
Riboflavin (B_2) (mg)	1.43 ± 0.2	1.4 ± 0.2	0.48
Folate (µg)	244.6 ± 41.6	231.6 ±39.7	0.79
Vitamin D (µg)	5.4 ±3.4	6.6 ± 4.1	0.78
Iron (mg)	8.5 ± 2.6	9.1 ± 2.9	0.79
Calcium (mg)	828.8 ± 142.8	822.1 ± 161.1	0.79
Vitamin E (mg)	12.1 ± 3.4	12.4 ± 3.6	0.79
Magnesium (mg)	246.8 ± 41.5	240.6 ± 37.6	0.79
Potassium (mg)	2774.6 ± 468.9	2762 ± 417.1	0.79
Iodine (µg)	139.9 ± 39.3	117.1 ± 28.5	0.75
Selenium (µg)	61.6 ± 19.5	64.1 ± 24.7	0.77
Zinc (mg)	11.4 ± 3.2	11.9 ± 2.9	0.85
Vitamin C (mg)	106.9 ± 23.5	105.9 ± 31.2	0.83
Manganese (mg)	3.5 ± 2.0	3.3 ± 1.9	0.78
Copper (mg)	1.1 ± 0.4	1.1 ± 0.5	0.81
Phosphorus (mg)	504.4 ± 84.9	497.2 ± 139.3	0.79
Niacine (mg)	1.1 ± 0.4	1.3 ± 0.5	0.78
Vit B12 (µg)	4.6 ± 1.2	4.2 ± 1.1	0.79
Thiamine (mg)	0.5 ± 0.7	0.4 ± 0.7	0.58
Pantothenate (mg)	2.9 ± 1.5	2.9 ± 1.3	0.80
Pyridoxal phosphate (mg)	1.3 ± 0.4	1.1 ± 0.5	0.79
Biotine (µg)	28.2 ± 6.5	28.4	0.79
Vitamin K (mg)	62.0 ±15.6	57 ± 15.9	0.76
The overall average increase in body fat for the participants was 1%. The mean body fat percentages for participants whose body fat increased by 1% or more, and those whose body fat increased by less than 1% were 28.5 ± 6.7% and 29.7 ±7.4% respectively. P-values marked with an asterisk (*) are statistically significant.			

In Table 5.13 below, there was no statistically significant difference between the mean body fat percentage for participants whose body fat increased (28.5 ±7.1%) and those whose body fat decreased or remained constant (33.8 ±9.0%) (p= 0.84). However, there was a statistically significant difference between the average nutrient intakes for some nutrients between the participants in both groups.

Table 5.13. Average daily dietary nutrient intakes of participants based on changes in percentage body fat.

Dietary nutrient	**Participants whose body fat percentage increased**	**Participants whose body fat percentage decreased or remained constant**	**p-value**
Energy (kcal)	2269.6 ± 231.6	2176.0 ±207.8	0.27
Vitamin A (µg)	537.3 ± 149.0	564.1 ±128.5	0.04*
Riboflavin (B_2) (mg)	1.4 ± 0.2	1.4 ± 0.2	0.06
Folate (µg)	238.0 ± 39.7	246.4 ± 32.5	0.04*
Vitamin D (µg)	6.1 ± 3.7	7.1 ± 4.3	0.04*
Iron (mg)	8.9 ± 2.9	9.0 ± 2.8	0.04*
Calcium (mg)	851.2 ± 116.1	835.0 ± 118.8	0.04*
Vitamin E (mg)	12.1 ± 3.3	13.7 ± 3.0	0.04*
Magnesium (mg)	247.4 ± 31.9	239.4 ± 35.9	0.04*
Potassium (mg)	2780.2 ± 420.1	2794.1 ± 394.9	0.04*
Iodine (µg)	128.9 ± 34.0	121.9 ± 32.1	0.04*
Selenium (µg)	65.2 ± 22.0	66.2 ± 21.0	0.04*
Zinc (mg)	11.3 ± 2.9	11.8 ± 3.0	0.06
Vitamin C (mg)	102.2 ± 29.4	106.7 ± 26.5	0.06

Manganese (mg)	3.2 ± 1.9	3.3 ± 1.8	0.06
Copper (mg)	1.0 ± 0.5	1.2 ± 0.5	0.05
Phosphorus (mg)	507.2 ± 112.6	501.8 ± 150.8	0.04*
Niacin (B_3) (mg)	1.3 ± 0.5	1.2 ± 0.5	0.04*
Cobalamin (B_{12}) (µg)	4.4 ± 1.1	4.3 ± 1.1	0.04*
Thiamine (B_1) (mg)	0.4 ± 0.7	0.5 ± 0.7	0.03*
Pantothenate (B_5) (mg)	2.7 ± 1.3	3.0 ± 1.4	0.05
Pyridoxal phosphate (B_6) (mg)	1.2 ± 0.5	1.2 ± 0.4	0.07
Biotin (µg)	28.4 ± 7.1	27.5 ± 7.6	0.04*
Vitamin K (mg)	60.0 ± 14.3	64.0 ± 14.9	0.04*
The overall average increase in body fat for the participants was 1%. The mean body fat percentages for participants whose body fat increased and those whose body fat decreased or remained constant were 28.5 ± 7.1% and 33.8 ± 9.0% respectively. P-values marked with an asterisk (*) are statistically significant.			

The analysis conducted to investigate the influences of sleep, cigarette smoking, appetite, and alcohol intake on the association between change in dietary nutrient density and change in body fat percentage showed that neither of these factors influenced the relationship (Table 5.14-below).

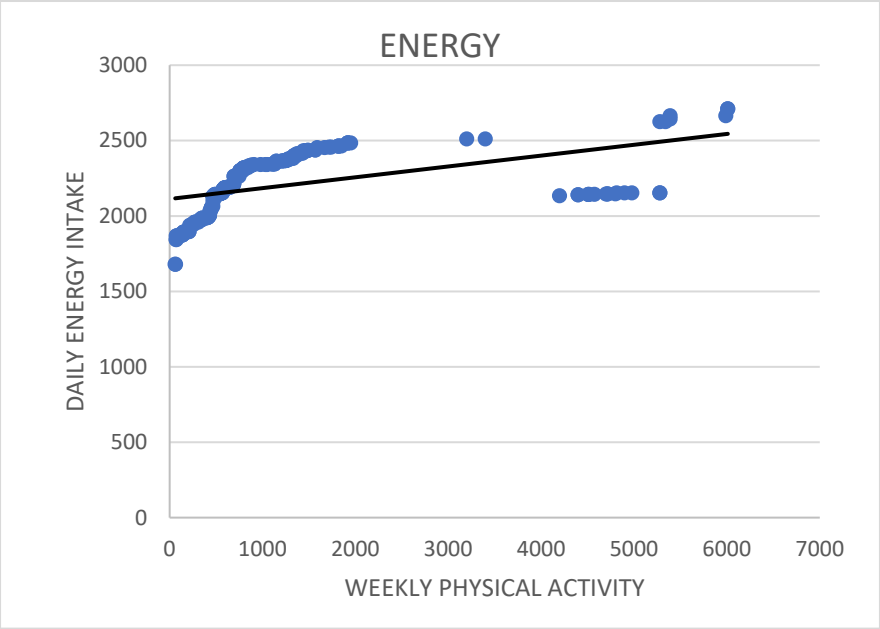
Table 5.14. The influence of sleep, cigarette smoking alcohol intake and appetite on the association between change in dietary nutrient density and change in body fat percentage.

Dietary Component	Sleep		Cigarette Smoking		Alcohol Intake		Appetite Score	
	<7 hours	≥7 hours	No	Yes	No	Yes	≤14	>14
Vitamin A (µg)	-0.01 ± 0.02 (0.03)*	-0.09 ± 0.05 (0.02)*	-0.03 ± 0.03 (0.04)*	-0.01 ± 0.04 (0.001)*	-0.04 ± 0.02 (0.04)*	-0.01 ± 0.03 (0.01)*	-0.02 ± 0.06 (0.04)*	-0.02 ± 0.02 (0.12)*
Riboflavin (mg)	-4.20 ± 10.22 (0.06)	-3.72 ± 19.72 (0.06)	-11.16 ± 11.68 (0.07)	-2.49 ± 17.01 (0.06)	-16.72 ± 9.91 (0.83)	-2.48 ± 14.07 (0.12)	-6.55 ± 20.64 (0.69)	-10.23 ± 10.33 (0.33)
Folate (µg)	-0.03 ± 0.06 (0.01)*	-0.23 ± 0.12 (0.04)*	-0.07 ± 0.07 (0.01)*	-0.02 ± 0.10 (0.02)*	-0.10 ± 0.06 (0.02)*	-0.02 ± 0.09 (0.04)*	-0.05 ± 0.15 (0.003)*	-0.06 ± 0.06 (0.001)*
Vitamin D (µg)	-2.66 ± 6.46 (0.06)	-23.52 ± 12.47 (0.06)	-7.06 ± 7.39 (0.06)	-1.57 ± 10.8 (0.04)	-10.57 ± 6.27 (0.73)	-1.57 ± 8.90 (0.001)	-4.10 ± 13.06 (0.30)	-6.46 ± 6.52 (0.42)
Iron (mg)	-0.60 ± 1.45 (0.001)*	-5.28 ± 2.79 (0.003)*	-1.58 ± 1.65 (0.01)*	-0.35 ± 2.41 (0.02)*	-2.37 ± 1.40 (0.002)*	-0.35 ± 1.99 (0.04)*	-0.93 ± 2.96 (0.004)*	-1.45 ± 1.46 (0.04)*
Calcium (mg)	-0.01 ± 0.19 (0.02)*	-0.07 ± 0.04 (0.02)*	-0.02 ± 0.02 (0.004)*	-0.01 ± 0.03 (0.003)*	-0.04 ± 0.03 (0.02)*	-0.01 ± 0.03 (0.02)*	-0.02 ± 0.05 (0.04)*	-0.02 ± 0.02 (0.01)*
Vitamin E (mg)	-0.53 ± 1.29 (0.03)*	-4.70 ± 2.49 (0.002)*	-1.48 ± 1.48 (0.03)*	-0.31 ± 2.15 (0.04)*	-2.11 ± 1.25 (0.02)*	-0.31 ± 1.78 (0.002)*	-1.20 ± 1.35 (0.001)*	-1.29 ± 1.31 (0.01)*
Magnesium (mg)	-0.03 ±0.07 (0.01)*	-0.24 ± 0.13 (0.002)*	-0.07 ± 0.07 (0.04)*	-0.02 ± 0.11 (0.12)*	-0.11 ± 0.06 (0.04)*	-0.02 ± 0.09 (0.003)*	-0.06 ± 0.16 (0.002)*	-0.07 ± 0.07 (0.03)*
Potassium (mg)	-0.01 ± 0.01	-0.03 ± 0.01	-0.01 ± 0.01	0.01 ± 0.01	-0.01 ± 0.01	-0.01 ± 0.01	0.01 ± 0.02	-0.01 ± 0.01

	(0.04)*	(0.06)*	(0.01)*	(0.002)*	(0.003)*	(0.004)*	(0.02)*	(0.001)*
Iodine (µg)	-0.04 ± 0.10 (0.09)	-0.36 ± 0.19 (0.06)	-0.11 ± 0.11 (0.34)	-0.02 ± 0.17 (0.22)	-0.16 ± 0.10 (0.62)	-0.02 ± 0.14 (0.35)	-0.09 ± 0.24 (0.76)	-0.10 ± 0.10 (0.45)
Selenium (µg)	-0.14 ± 0.34 (0.004)*	-1.24 ± 0.66 (0.04)*	-0.37 ± 0.39 (0.06)*	-0.08 ± 0.57 (0.04)*	-0.74 ± 0.46 (0.03)*	-0.08 ± 0.47 (0.01)*	-0.36 ± 0.86 (0.03)*	-0.34 ± 0.34 (0.02)*
Zinc (mg)	-0.72 ± 1.76 (0.002)*	-6.39 ± 3.39 (0.005)*	-1.92 ± 2.01 (0.03)*	-0.43 ± 2.92 (0.01)*	-2.87 ± 1.70 (0.004)*	-0.43 ± 2.42 (0.02)*	-1.63 ± 1.84 (0.001)*	-1.76 ± 1.77 (0.01)*
Vitamin C (mg)	-0.08 ± 0.20 (0.01)*	-0.71 ± 0.38 (0.02)*	-0.21 ± 0.22 (0.06)*	-0.05 ± 0.33 (0.004)*	-0.43 ± 0.26 (0.003)*	-0.05 ± 0.27 (0.12)*	-0.17 ± 0.47 (0.03)*	-0.20 ± 0.20 (0.004)*
Manganese (mg)	-2.19 ± 5.39 (0.08)	-19.37 ± 10.27 (0.72)	-5.81 ± 6.08 (0.16)	-1.29 ± 8.86 (0.41)	-11.54 ± 7.12 (0.36)	-1.29 ± 7.33 (0.42)	-4.12 ± 11.87 (0.68)	-5.32 ± 5.38 (0.87)
Copper (mg)	-5.72 ± 13.91 (0.12)	-5.07 ± 26.85 (0.09)	-15.20 ± 15.91 (0.12)	-3.38 ± 23.17 (0.83)	-3.20 ± 18.61 (0.49)	-3.37 ± 19.16 (0.11)	-9.71 ± 30.00 (0.42)	-13.92 ± 14.06 (0.12)
Phosphorus (mg)	-0.01 ± 0.03 (0.07)*	-0.12 ± 0.06 (0.002)*	-0.04 ± 0.04 (0.01)*	-0.01 ± 0.05 (0.04)*	-0.07 ± 0.04 (0.01)*	-0.01 ± 0.05 (0.02)*	-0.03 ± 0.08 (0.001)*	-0.03 ± 0.03 (0.03)*
Niacin (mg)	-4.96 ± 12.06 (0.17)	-13.2 ± 23.27 (0.62)	-13.17 ± 13.79 (0.79)	-2.93 ± 20.08 (0.22)	-16.16 ± 16.13 (0.77)	-2.92 ± 16.60 (0.69)	-8.06 ± 25.73 (0.42)	-12.07 ± 12.18 (0.55)
Vitamin B_{12} (µg)	-1.73 ± 4.21 (0.32)	-15.31 ± 8.12 (0.25)	-4.60 ± 4.81 (0.41)	-1.02 ± 7.00 (0.01)	-9.12 ± 5.63 (0.67)	-1.02 ± 5.79 (0.12)	-3.60 ± 10.05 (0.54)	-4.21 ± 4.25 (0.73)

Thiamine (mg)	-4.12 ± 9.42 (0.11)	-3.64 ± 8.54 (0.11)	-3.20 ± 10.42 (0.16)	-2.00 ± 15.58 (0.09)	1.68 ± 12.96 (0.35)	-4.03 ± 12.41 (0.72)	-5.01 ± 10.41 (0.12)	-9.50 ± 4.38 (0.39)
Pantothenate (mg)	-6.20 ±15.07 (0.16)	-4.09 ± 29.10 (0.06)	-16.48 ± 17.24 (0.60)	-3.67 ± 25.10 (0.09)	-32.70 ± 20.16 (0.12)	-3.66 ± 20.76 (0.86)	-10.07 ± 31.54 (0.09)	-15.08 ± 15.23 (0.43)
Pyridoxal phosphate (mg)	-4.38 ± 10.63 (0.33)	-8.22 ± 20.53 (0.42)	-11.62 ± 12.17 (0.06)	-2.59 ± 17.71 (0.47)	-13.08 ± 12.23 (0.07)	-2.58 ± 14.65 (0.45)	-9.87 ± 11.13 (0.28)	-10.65 ± 10.75 (0.42)
Biotin (µg)	-0.30 ± 0.72 (0.44)	-2.63 ± 1.40 (0.21)	-0.79 ± 0.83 (0.06)	-0.18 ± 1.20 (0.58)	-1.57 ± 0.97 (0.18)	-0.18 ± 1.00 (0.24)	-0.69 ± 1.71 (0.63)	-0.72 ± 0.73 (0.88)
Vitamin K (mg)	-0.17 ± 0.40 (0.02)*	-1.46 ± 0.78 (0.04)*	-0.44 ± 0.46 (0.01)*	-0.10 ± 0.66 (0.001)*	-0.87 ± 0.54 (0.02)*	-0.10 ± 0.55 (0.002)*	-0.42 ± 1.01 (0.04) *	-0.40 ± 0.41 (0.002)*
Estimates in Table 5.14 represent the change in percentage body fat ± standard error, for a unit increase in dietary nutrient density of specific micronutrients. Estimates with asterisk "*" are statistically significant.								

A positive correlation was observed between participants' physical activity and energy intake (Pearson correlation coefficient= 0.51, p< 0.001) (Figure 6.1). Physical activity did not show a corresponding relationship with micronutrient intake (Table 5.15; Figures 5.2 and 5.3).

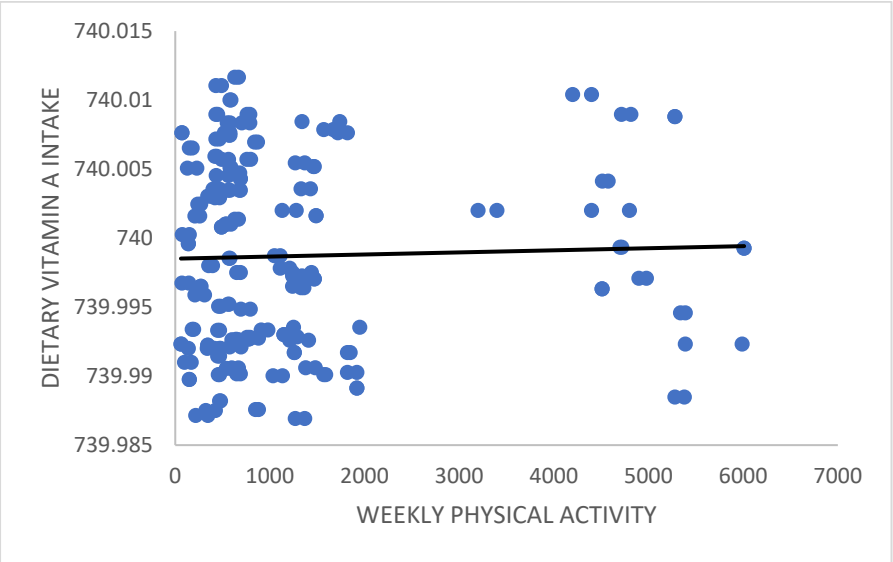


Daily energy intake is measured in kilocalories, and weekly physical activity is measured in Metabolic Equivalent of Task- minutes (MET-minutes). Pearson correlation coefficient = 0.51 (p< 0.001).

Figure 5.1. A line chart of average daily energy intake plotted against weekly physical activity

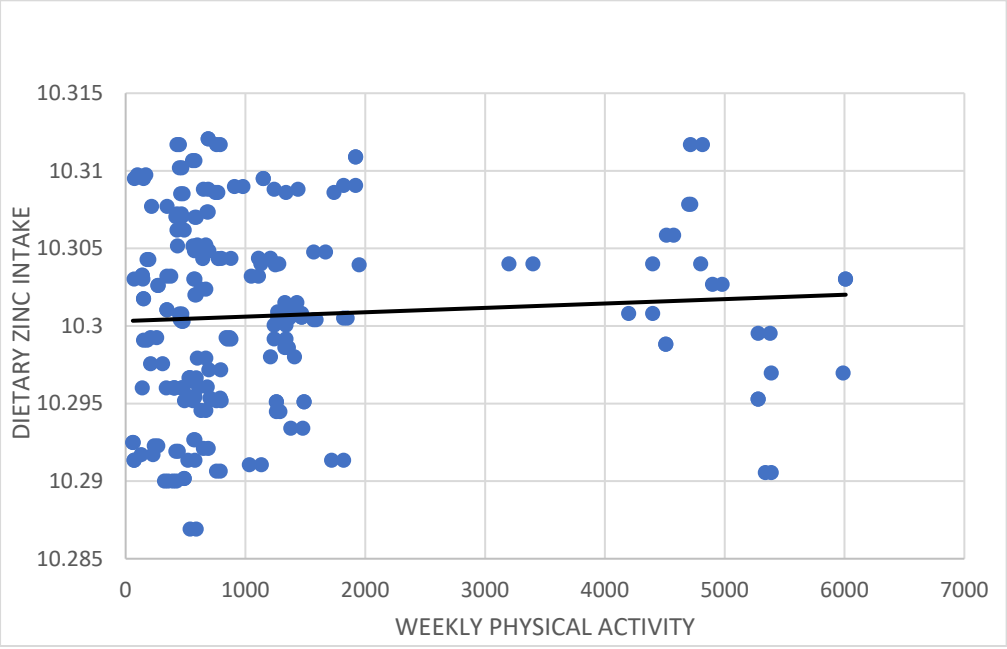
Table 5.15. The correlation between physical activity and the average daily dietary micronutrient intake

Daily Dietary Micronutrient Intake	Pearson correlation coefficient	p-value
Vitamin A (μg)	0.002	0.98
Riboflavin (mg)	0.014	0.84
Folate (μg)	0.001	0.98
Vitamin D (μg)	0.001	0.98
Iron (mg)	0.020	0.98
Calcium (mg)	0.002	0.98
Vitamin E (mg)	0.002	0.98
Magnesium (mg)	0.015	0.82
Potassium (mg)	0.020	0.82
Iodine (μg)	0.014	0.82
Selenium (μg)	0.015	0.82
Zinc (mg)	0.002	0.97
Vitamin C (mg)	0.002	0.09
Manganese (mg)	0.001	0.98
Copper (mg)	0.020	0.83
Phosphorus (mg)	0.011	0.98
Niacin (mg)	0.001	0.90
Vitamin B_{12} (μg)	0.061	0.19
Thiamine (mg)	0.044	0.52
Pantothenate (mg)	0.004	0.95
Pyridoxal phosphate (mg)	0.005	0.94
Biotin (μg)	0.002	0.97
Vitamin K (mg)	0.001	0.63



Average daily vitamin A intake is measured in micrograms and weekly physical activity is measured in Metabolic Equivalent of Task- minutes (MET-minutes). Pearson correlation coefficient = 0.002 (p= 0.98).

Figure 5.3. A line chart of the average daily dietary vitamin A intake plotted against weekly physical activity.



Average daily zinc intake is measured in milligrams and weekly physical activity is measured in Metabolic Equivalent of Task- minutes (MET-minutes). Pearson correlation coefficient = 0.001 (p= 0.97).

Figure 5.4. A line chart of the average daily dietary zinc intake plotted against weekly physical activity.

5.4. Discussion

The primary objective of this prospective cohort study was to investigate the association between change in dietary nutrient density and change in body fat percentage among adults in the United Kingdom. The results showed that a unit increase in the dietary nutrient density of vitamins A, K, C and E, folate, iron, calcium, magnesium, zinc, selenium and phosphorus corresponded to a significant decrease in body fat percentage ($p< 0.05$) after adjusting for age, gender, ethnicity, being on a special diet, dietary phytate intake, appetite, alcohol intake, physical activity, hours of night-time sleep and smoking status. Based on these findings, the null hypothesis was rejected. This is the first study to demonstrate the association between change in dietary nutrient density and change in body fat percentage among adults in the United Kingdom.

The discussion of this chapter describes the nutrient intake of the participants, the association between changes in the dietary nutrient density of specific micronutrients and body fat percentage in relation to other studies. It also discusses the influence of several variables on the association between change in dietary nutrient density and change in body fat percentage, and the implication of exclusively associating energy or micronutrient intake with body fat changes in comparison with dietary nutrient density.

5.4.1. Nutrient intake of participants

The findings of this study indicate that energy intake and the dietary intakes of all micronutrients studied, but thiamine, manganese and vitamin B_{12} were lower than the reference nutrient intakes (RNI) (RNI according to the European Food Safety Authority) for men and women. Similarly, the National Diet and Nutrition Survey (NDNS) (2012/13- 2013/14) (Public Health England, 2017) observed that the energy intake for men and women were below the reference intakes; 2107 kcal and 1595 kcal, respectively. However, in contrast to the present

study, some of the micronutrients (vitamin A, vitamin D, magnesium, potassium, iodine, zinc, calcium, iron and folate) assessed in the NDNS were close to or above the reference intake values. The findings of the present study are of public health significance, given the clinical importance of the affected micronutrients. For instance, vitamin A plays a crucial role in immune competence, tissue differentiation and the visual cycle (Somer and Vyas, 2012), while zinc is a known antioxidant associated with fertility in men (Colagar, Marzony and Chaichi, 2008) and plays regulatory roles which help to support immunity and avert age-related diseases (Chasapis, et al., 2012). Hence, a low intake of these micronutrients can compromise their physiological function.

The cohort study also found no significant variation between the dietary energy intakes of both sexes in contrast to other studies which have reported gender differences in energy intake. For instance, a cross-sectional population-based study constituting 210,106 individuals from the UK Biobank (mean difference of energy intake per day of 1358 kJ per day) indicated that the mean difference of energy intake between both sexes was 1358 kJ per day (Bennett, Peters and Woodward, 2018). The NDNS (2012/13- 2013/14) also showed that men had higher energy intake compared to women (Public Health England, 2017). Data from the National Health and Nutrition Examination Survey (NHANES III) similarly showed that when daily energy intake was compared in a cohort of participants, men consumed more energy than women (187 kJkg−1 versus 170 kJkg−1) (Chumlea, et al., 2002; Kant and Graubard, 2006). This has been explained to be due to the tendency of women to experience more food-related conflict and consume less energy to lose or maintain their body mass (Rolls, Federoff and Guthrie, 1991). Such detail regarding participants' food preference cannot be deduced from the present study as it was not assessed. However, considering that 5.4% more women than men claimed to be vegetarians (Table A11.1- Appendix 11), a higher energy intake among men than women

was expected. Research shows that vegetarians tend to have lower energy intake compared with meat-eaters (Clarys, et al., 2014; Alles, et al., 2017).

Furthermore, between both sexes in the present research, women had lower dietary intakes of 4 micronutrients (iron, calcium, zinc and vitamin C) compared to the men, but had a higher intake of manganese. A secondary analysis of data from 3,238 adults who participated in the NDNS (years 1-6) did likewise show that women in the UK had a significantly lower intake of iron and calcium compared to men (Derbyshire, 2018). Amongst adults (aged 19-64) in the United Kingdom, it has been shown that women are at a higher risk of iron deficiency compared to men (Bates, et al., 2014). Inadequate iron intake in the diet increases the risk of iron deficiency anaemia which can increase one's susceptibility to infection and impact cognitive development in children and foetal development in pregnancy (EFSA, 2009; Alwan, et al., 2015).

The intake of calcium among women in the UK has also been of concern, given that the NDNS data (2008/2009 – 2011/2012) indicated the increased likelihood of inadequate calcium intake in women (Public Health England, 2014). Inadequate calcium intake is known to increase the risk of osteoporosis in later life (Beto, 2015). Osteoporosis affects an estimated three million people in the UK, with more than a sixth receiving treatment for fragility fractures each year due to this condition (NHS, 2019). Although both men and women can be affected by osteoporosis, it is four times more common in women than men (Feldstein, et al., 2003; Alswat, 2017) due to hormonal changes especially occurring at menopause (NHS, 2019).

In the present study, it was observed that younger adults (aged 18-29) had a lower intake of vitamin D, zinc and manganese compared to older adults (aged 30-39). A secondary analysis of the NDNS (years 1–6) UK Diet and Nutrition Survey similarly found that young adults were more likely to have micronutrient shortfalls (Derbyshire, 2018). Based on the analysis, young

adults in their twenties had significantly lower intakes of 8 micronutrients ($p < 0.05$) (vitamin A, riboflavin, folic acid, calcium, magnesium, potassium, iodine, and copper) compared to adults in their thirties, forties and fifties (Derbyshire, 2018). Although the micronutrients affected in previous research (Derbyshire, 2018) varies from those of the present study, lower micronutrient intake in young adults (in their twenties) compared to older adults has been attributed the tendency of young adults to adopt emerging food trends and avoid consuming some food groups (Fayet-Moore, Petocz and Samman, 2014). A similar trend is observed in the present study, as 70.6% of those on special diets were young adults (aged 18-29) (Table A12.1- Appendix 12). Previous studies have associated the intake of special diets with low micronutrient status. For instance, vegetarians have been shown to have lower intake of vitamin B12 (Draper, et al., 1993; Karanudak, Kiziltan and Cigerim, 2008; Turner, Sinclair and Knez, 2014) and zinc (Turner, Sinclair and Knez, 2014) compared to omnivores, and veganism has been associated with low vitamin D, iodine, selenium, calcium and vitamin B12 intakes (Kristensen, et al., 2015; Schupbach, et al., 2017). Also, having diets low in red meat (less than 40g daily) has been associated with low vitamin D and zinc intakes (Derbyshire, 2018).

5.4.2 The Association between Change in Dietary Nutrient Density and Change in Body Fat

5.4.2.1. The dietary nutrient density of vitamin A and adiposity

In this research, a unit increase in the dietary nutrient density of vitamin A was associated with a 0.02% decrease in body fat percentage. There is a dearth of evidence regarding the relationship between the change in dietary nutrient density of vitamin A and change in body fat percentage in the literature, however previous studies have consistently indicated an inverse relationship between vitamin A and measures of adiposity. A case-control trial of 144 Thai adults found a negative correlation between serum vitamin A concentration and body mass, hip circumference and BMI in those who were overweight (Viroonudomphol, et al., 2003). Also,

after adjusting for energy intake, Zulet, et al. (2008) found that vitamin A intake was inversely associated with measures adiposity (BMI, waist circumference, and WHR) in healthy adults. Other studies have also reported corresponding findings (Moor, Wartanowicz and Ziemlanski, 1992; Vaughan, Benyshek and Martin, 1997; de Souza, et al., 2007; Johnston, et al., 2007; Villaca, et al., 2008; Aasheim, et al., 2008; Mills, Furr and Tanumihardjo, 2008; Suano, et al., 2008; Villaça, et al., 2008; Pereira, et al, 2012).

Studies have posited that vitamin A influences body fat through promoting fat cell apoptosis (Kim, et al., 2000) and reducing adiposity by inhibiting adipogenesis, especially during the early stages of adipocyte differentiation (Xue, et al., 1996).

5.4.2.2. The dietary nutrient density of vitamin K and adiposity

The present research found that a unit increase in the dietary nutrient density of vitamin K corresponded to 0.35% decrease in body fat percentage. Despite the lack of evidence associating the dietary nutrient density of vitamin K and body fat in the existing literature, several authors have shown an inverse relationship between vitamin K and body fat, and the role of vitamin K in energy metabolism. Knapen and colleagues in a recent 3-year randomised controlled intervention involving 214 participants tested the hypothesis that increased intake of vitamin K decreased body fat mass (Knapen, Jardon and Vermer, 2018). The researchers observed that vitamin K (menaquinone-7) intake resulted in a decrease in visceral body fat. Corresponding findings have been reported by Shea, et al. (2010) who found that vitamin K status in men and women was inversely related to body fat percentage. Considering that vitamin K accumulates in the human adipose tissue compared to other tissues (Thijssen and Drittij, 1996), it has been argued that the low vitamin K status in those with high body fat percentage is due to the sequestration of vitamin K in adipose tissue (Wortsman, et al., 2000; Harris, Dawson-Hughes, 2007). While this is plausible, the findings of Shea and colleagues support those of the present study.

Vitamin K influences glucose homeostasis and body fat through reducing the risk of insulin resistance (Sakamoto, et al., 2008; Yoshida, et al., 2008; Yoshida, Jacques and Meigs, 2008).

5.4.2.3 The dietary nutrient density of vitamin C and adiposity

This study found that a unit increase in the dietary nutrient density of vitamin C corresponded to 0.17% decrease in body fat percentage. Data from previous studies have not stated the relationship between the dietary nutrient density of vitamin C and body fat percentage. However, consistent evidence indicates an inverse relationship between plasma vitamin C and adiposity.

In an early study, Schectman, et al. (1989) reported a significant inverse association between plasma vitamin C and body mass index among 11,592 participants who took part in the NHANES (II). More recently, Johnston, et al. (2007) in a cross-sectional study of 118 non-smoking adults similarly concluded that plasma vitamin C concentration was inversely related to BMI independent of vitamin C supplement use, body mass and age. Plasma vitamin C concentration was also inversely related to abdominal obesity (estimated by hip circumference) among 19,000 participants of the European Prospective Investigation into Cancer and Nutrition Norfolk cohort study (Canoy, et al., 2005). Other researchers have also noted reduced blood concentration of vitamin C in obese individuals (Moor, et al., 1992; Galan, et al., 2005).

Vitamin C has been suggested to influence adiposity through various processes involving increased oxidation of body fat (Johnston, Corte and Swan, 2006). Vitamin C acts as a cofactor for the synthesis of carnitine, a metabolite needed for transporting long-chain fatty acids across the mitochondrial membrane for oxidation (Rebouche, 1991). Studies have shown that carnitine supplementation at 3g per day for 10 days increases fat oxidation by 20% (Douillet, et al., 1998), and carnitine deficiency is associated with reduced fat oxidation and lipid accumulation in the muscle (Kim, et al., 2000; Wutzke and Lorenz, 2004; Foster, 2005).

Furthermore, vitamin C increases adiponectin concentration which promotes fatty acid uptake in the skeletal muscle by activating sensors controlling energy metabolism; AMPK (5-adenosine monophosphate-activated kinase) and PPARα (Peroxisome proliferator-activated receptor) (Lafontan and Viguerie, 2006). Other mechanisms through which vitamin C reduces adiposity include modulating adipocyte lipolysis (Carcamo, et al., 2002; Garcia-Diaz, 2009) and inflammatory response (Carcamo, et al., 2002).

5.4.2.4 The dietary nutrient density of vitamin E and adiposity

This study found that a unit increase in the dietary nutrient density of vitamin E corresponded to 1.11% decrease in body fat percentage. Evidence linking the dietary nutrient density of vitamin E and body fat is limited. Nevertheless, some studies have found an association between vitamin E and excess body fat and emphasised the influence of vitamin E on glycaemic control.

Singh, et al. (1998) and Wallstrom, et al. (2001) in their studies, found an inverse relationship between the blood concentration of vitamin E and obesity. In a randomised controlled trial (Manning, et al., 2004), an increase in plasma vitamin E concentration was significantly correlated with a decrease in plasma insulin, fasting glucose concentration, oxidative stress, and an improvement in insulin resistance. Oxidative stress is known to be associated with the development of insulin resistance (Evans, et al., 2003), which is considered an important contributor to the development of excess body fat (Kahn and Flier, 2002). Although the present research focused on the dietary nutrient density of vitamin E, perhaps the same mechanisms can explain the results.

5.4.2.5 The dietary nutrient density of folate and adiposity

This study found that a unit increase in the dietary nutrient density of folate corresponded to 0.05% decrease in body fat percentage. Not much is known regarding the association between

the dietary nutrient density of folate and body fat percentage in previous studies. Nevertheless, the present finding is supported by the consistent inverse association between folate and measures of adiposity have been noted in some studies. Gunanti, et al. (2014) for instance, found that in 1,131 Mexican American children, serum folate concentration was inversely related to total body fat mass, BMI and trunk fat mass. The authors also found that folate intake was inversely associated with BMI. Other studies have reported corresponding findings (Gallistl, et al., 2000; Kimmons, et al., 2006; Tinker, et al., 2012), and emphasised the role of folate in lipid, lipoprotein and carbohydrate metabolism (Picciano, et al., 2010; Lim, Choi and Choue, 2008).

Various mechanisms underlie the relationship between folate and adiposity, such as the activation of beta-adrenergic receptors which are associated with increased tissue lipolysis and reduced obesity (de-Souza and Burkey, 2001). Furthermore, being a vital source of the one-carbon group for DNA methylation, folate may also influence adiposity through eliciting alterations in DNA methylation and insulin resistance which in turn influences fat deposition (Li, et al., 2017). In a cross-sectional study of 1530 non-diabetic adults who participated in the National Health and Nutrition Examination Survey (NHANES 2011-2012), a 25% increase in serum folate corresponded to a 3.06% decrease in insulin resistance (Li, et al., 2017).

5.4.2.6 The dietary nutrient density of iron and adiposity

In the present study, a unit increase in the dietary nutrient density of iron corresponded to 1.24% decrease in body fat percentage. There is inadequate evidence in the literature linking the dietary nutrient density of iron and body fat percentage. That notwithstanding, a substantial body of research has consistently reported an association between the body iron status and adiposity. Among the first documented association between serum iron concentration and adiposity was among adolescents in the 1960s (Wenzel, Stults and Mayer, 1962; Seltzer and Mayer, 1963). Later research has confirmed this relationship in adults and children (Pinhas-

Hamiel, et al., 2003; Nead, et al., 2004; Chambers, et al., 2006). For instance, a cross-sectional study of 321 children and adolescents found a higher prevalence of iron deficiency and low serum iron concentration in obese participants compared to their normal-weight counterparts (Pinhas-Hamiel, et al., 2003). Also, overweight children and those at risk of being overweight have been shown to be twice as likely to be iron-deficient compared to normal-weight individuals based on an analysis of the NHANES (III) (Nead, et al., 2004). More so in both studies, obese children and adolescents who were iron-deficient were reported to have inadequate iron intake (Pinhas-Hamiel, et al., 2003; Nead, et al., 2004). The mechanism underlying the link between iron and adiposity has not been elucidated. However, it is thought to be associated with changes in insulin resistance (Wlazlo and van Greevenbroek, 2012).

5.4.2.7 The dietary nutrient density of calcium and adiposity

A unit increase in the dietary nutrient density of calcium corresponded to a 0.02% decrease in body fat percentage in this study. Evidence linking the dietary nutrient density of calcium with body fat percentage is lacking in the existing literature. However, some studies have revealed the influence of the dietary intake of calcium on measures of adiposity. A cross-sectional survey of Chinese women found that after adjusting for potential confounding factors, there was an inverse relationship between dietary calcium intake and fat mass, BMI, waist circumference and WHR (Huang, et al., 2011). The researchers also observed that the risk of abdominal obesity significantly decreased for each quartile increase in dietary calcium. Other studies in Western populations have similarly found an inverse association between dietary calcium and measures of adiposity (body fat mass, and BMI) (Lin, et al., 2000; Zemel, et al., 2000).

Calcium is recognised as a critical regulator of fat metabolism, and different mechanisms through which calcium may influence adiposity have been proposed. Low dietary calcium intake can promote the increased concentration of 1,25, dihydro-vitamin D which favours

increased intracellular calcium levels that stimulate the expression of lipogenic enzymes and reduces lipolysis leading to fat accumulation in adipocytes (Zemel, et al., 2000). Some researchers have also posited that calcium promotes the formation of insoluble calcium fatty acid soap by binding to bile acids thereby resulting in the malabsorption of fat (Denke, Fox and Schulte, 1994; Shahkhalili, et al., 2001). Despite these proposed mechanisms, some studies have reported somewhat inconsistent findings. For instance, Jacqmain, et al. (2003) in the second phase of the cross-sectional Quebec Family Study found that after adjusting for protein intake, there was a negative relationship between daily calcium intake and adiposity in women, but not in men. In another study, Kamycheva and colleagues noted a positive association in women and no effect in men (Kamycheva, et al., 2003). The findings of both studies show that no consistent gender-specific patterns exist; however, do not refute the association between calcium intake and adiposity.

5.4.2.8 The dietary nutrient density of magnesium and adiposity

In the present research, a unit increase in the dietary nutrient density of magnesium corresponded to 0.06% decrease in body fat percentage. Previous literature lacks evidence showing this association, but there is abundant evidence highlighting the inverse relationship between magnesium and various measures of adiposity. Data from the Mexican National Health and Nutrition Survey (2012) demonstrated that the intake of magnesium was associated with low waist circumference and BMI (Castellanos-Gutierrez, et al., 2018). Other studies in different populations have also reported consistent findings (Song, et al., 2005; He, 2006).

Several studies have concluded on insulin resistance as the mechanism through which magnesium influences adiposity. For instance, after following 42,872 men and 85,060 women for 12 years and 18 years respectively, a prospective study found an inverse relationship between magnesium intake and the risk of developing type-2 diabetes mellitus (Lopez-Riduara, et al., 2003), which is known to result from insulin resistance (Banerji. et al., 1995; van Haeften,

et al., 1998). A later study on a Canadian population of normal weight, overweight and obese participants likewise showed that higher dietary intake of magnesium was significantly associated with reduced insulin resistance (Cahill, et al., 2013). A double-blind placebo-controlled randomised trial also demonstrated that an increase in dietary magnesium specifically improved insulin sensitivity (Guerrero, et al., 2004). More so, other studies have stated the effect of magnesium in reducing markers of insulin resistance (Villegas, et al., 2009; Kim, et al., 2010). Magnesium is posited to be functionally linked to the metabolism of glucose through interacting with the activity of tyrosine-kinase on the insulin receptor which plays a crucial role in insulin resistance (Kolterman, et al., 1982).

5.4.2.9 The dietary nutrient density of zinc and adiposity

This study found that a unit increase in the dietary nutrient density of zinc corresponded to a 1.5% decrease in body fat percentage. This finding is supported by epidemiological evidence indicating the association between zinc (intake and status) and body fat. In a cross-sectional study of 850 men in India, Singh, et al. (1998) identified zinc deficiency as a risk factor for central obesity. Similarly, a Guatemalan study observed that zinc-deficient children were fatter than those with adequate zinc status (Cavan, et al., 1993).

Zinc is considered to influence adiposity through eliciting changes in insulin resistance. Garcia, et al. (2013), in a study of Mexican children, showed that low zinc status was associated with higher insulin resistance. Ortega, et al. (2012) similarly found that the risk of insulin resistance increased with low zinc concentration in blood. The role of zinc in the synthesis, secretion and storage of insulin is known (Chausmer, 1998; Cruz, et al., 2018). Zinc also modulates the action of insulin by acting through a series of molecular pathways involving stimulating the phosphorylation of the β-subunit of the insulin receptor and promoting the activity of protein kinase-β and phosphatidylinositol-3 kinase which enhance the entry of glucose into the cell (Vardatsikos, Pandey and Srivastava, 2013; Ranasingh, et al., 2015).

5.4.2.10 The dietary nutrient density of selenium and adiposity

In the present study, a unit increase in the dietary nutrient density of selenium corresponded to 0.3% decrease participants' body fat percentage. Scientific evidence regarding the relationship between the dietary nutrient density of selenium and adiposity is lacking in previous research, but selenium is well known for its role adipogenesis and adipocyte hypertrophy. Studies posit that selenium may inhibit adipogenesis and fat accumulation (Kim, et al., 2012a; 2012b). Selenium intake has also been significantly negatively associated with body fat percentage, BMI and waist circumference (Wang, et al., 2016). Wang and colleagues further suggested that the dietary intake of selenium may account for up to 27% of the observed differences in body fat percentage.

Several other studies have observed a negative relationship between serum selenium and adiposity (Stranges, et al., 2010; Ortega, et al., 2012; Azab, et al., 2014). Despite these findings, intervention studies have revealed contrasting findings (Hawkes and Keim, 2003; Navas-Carretero, et al., 2011). In the former study involving 12 participants (Hawkes and Keim, 2003), body mass increased with increased selenium intake (297µg per day) in one group of participants and decreased with decreased selenium intake in another group (14µg per day). In the latter study, Navas-Carretero (2011) noted that among 24 participants, the consumption of selenium-enriched diet did not accrue to more weight loss compared to the consumption of selenium non-enriched diet. The authors of both studies have, however, recommended caution in interpreting their results due to the small sample sizes involved.

Elaborate details of the mechanism through which selenium influences body fat is unclear, however, the modulation of thyroid hormone concentration and metabolism leading to changes in energy metabolism and body weight have been documented (Hawkes and Keim, 2003). The mechanism of reducing insulin resistance has also been suggested (Wang, et al., 2017). Selenium intake can influence insulin sensitivity through various mechanisms, which include

inducing insulin-like action, reducing oxidative stress and decreasing pro-inflammatory cytokines. Studies demonstrate that selenium may mimic insulin and stimulate the uptake of glucose (Ezaki, 1990; Furnsinn, et al., 1996; Hei, et al., 1998). More so, selenium may increase insulin sensitivity by inhibiting the production and activity of proinflammatory cytokines. Selenium may also improve insulin resistance by acting as a potent antioxidant and reducing reactive oxygen species (Puchau, et al., 2010; Dhanya, Swathy and Indira, 2014). Insulin signalling is influenced by a balance of the activity of antioxidant defences and the production of reactive oxygen species (Truong and Carroll, 2013). Excessive production of reactive oxygen species can increase insulin resistance (Chen, Xu and Zhang, 2014; Farinha, et al., 2015; Razavi, et al., 2016).

5.4.2.11 The dietary nutrient density of phosphorus and adiposity

This research found that a unit increase in the dietary nutrient density of phosphorus corresponded to 0.03% decrease in body fat percentage. Currently, there is a scarcity of evidence associating the dietary nutrient density of phosphorus with body fat percentage. Nevertheless, extant literature posits that phosphorus intake may be associated with increased body mass. In a randomised clinical trial, Ayoub, et al. (2015) observed that a 12-week supplementation with phosphorus significantly decreased measures of adiposity including BMI, body mass and waist circumference. Other studies involving various populations have observed likewise (Haglin, 2001; Foley, et al., 2009; Park, et al., 2009; Obeid, 2013; Obeid, Hachem and Ayoub, 2014).

The suggested mechanisms by which phosphorus influences body fat include regulation of food intake and energy use (Obeid, 2013). The availability of phosphorus stimulates the production of Adenosine triphosphate (Morris, Nigon and Reed, 1978; Friedman, 2007), which transmits neural signals to the central nervous system and decreases food intake by influencing satiation (Friedman, 2007). Other studies have suggested that phosphorus influences energy expenditure

(Jaedig and Henningsen, 1991; Kaciuba-Uscilko, et al., 1993; Jaedig, Lindgarde and Arborelius, 1994; Nazar, et al., 1996). For instance, with the addition of phosphorus to orange juice, increased postprandial thermogenesis was observed in obese individuals (Jaedig and Henningsen, 1991; Jaedig, Lindgarde and Arborelius, 1994). Similarly, phosphorus supplementation was found to increase the resting metabolic rate in obese participants (Kaciuba-Uscilko, et al., 1993; Nazar, et al., 1996). These findings indicate that the influence of phosphorus on body fat can be significant especially as the resting energy expenditure is the largest component of energy expenditure (among others-Thermic Effect of Food and Activity Energy Expenditure) (Hall, et al., 2012).

This study is the first to report findings on the association between change in dietary nutrient density and change in body fat percentage in adults. The results are supported by evidence on the various roles played by micronutrients in energy balance and the inverse relationship between micronutrient intake and body fat in various populations.

5.4.2.12 The dietary nutrient density of vitamins D and B_{12}, copper, manganese, and adiposity

Existing research has associated low intakes and serum levels of vitamin D (Walsh, Bowles and Evans, 2017), vitamin B_{12} (Baltaci, et al., 2013), copper (Azab, et al., 2014; Gonzalez-Reimers., et al., 2014), and manganese (Aschner, et al., 2007) with various measures of adiposity. However, the present cohort study does not provide any supporting evidence. Perhaps no supporting evidence was found, since micronutrient and energy intakes (dietary nutrient density) were considered in the present study, rather than only micronutrient intake as in the previous studies. It has already been argued in this thesis that dietary energy and micronutrient intakes are both crucial in energy metabolism; therefore, should both be considered in relation to adiposity.

5.4.3. The Influence of Physical Activity on the Association between Change in Dietary Nutrient Density and Change in Body Fat Percentage

The findings of the cohort study show that the relationship between dietary nutrient density and body fat percentage tends to diminish with the increase in physical activity and does not hold for individuals with a high level of physical activity (MET- minutes of activity ≥ 1500) (Table 6.10 in section 6.1). This occurred because there was a positive correlation between participants' physical activity and energy intake (Pearson correlation coefficient= 0.51, $p<0.001$; Figure 6.1 in section 6.4.2) without a corresponding correlation with micronutrient intake (Table 6.15; Figures 6.2 and 6.3 in section 6.4.2). Previous studies have similarly reported the tendency for energy intake to increase with physical activity. A 2-year prospective study of 538 adolescent students in the Harvard School of Public Health found that although physical activity resulted in an energy deficit, for each extra hour of exercise engaged by the students, they consumed an extra 292 calories (Sonneville and Gotmaker, 2008). Similarly, a Canadian randomised cross-over study involving thirteen women concluded that high-intensity exercise increased energy intake (Pormerlaeu, et al., 2004). The researchers observed that participation in high-intensity physical activity resulted in increased energy intake of 127 kcal. Other researchers have also stated that increased physical activity is usually matched by increased energy intake to compensate for the energy expended in accordance with the biological principle of homeostasis (Chaput, et al., 2011; Jodhun, Pem and Jeewon, 2016).

5.4.4. The Influence of Phytate on the Association between Change in Dietary Nutrient Density and Change in Body Fat Percentage.

The influence of phytate on the association between change in dietary nutrient density and change in body fat percentage was investigated by adjusting for all covariates and without adjusting for dietary phytate. When this was done, dietary nutrient densities for zinc and iron

were not associated with body fat percentage. This result was expected given the conclusion of the systematic review (Chapter 3), although it is still unclear why magnesium was not affected (Table 5.11-section 5.4.1). It might be due to the variation in the affinity of phytates for the various minerals. In-vitro studies have shown that inositol phosphates (phosphate groups in phytic acid) have a higher affinity for iron and zinc compared to magnesium (Vohra et al., 1965; Persson et al., 1998; Bohn, Meyer and Rasmussen, 2008). While this may explain the result in the present cohort study, it is acknowledged that other factors might have had an influence such as the gastrointestinal integrity and the solubility of the mineral-phytate complexes (Bohn, Meyer and Rasmussen, 2008).

5.4.5. Body Fat Percentage, Average Daily Dietary Energy and Micronutrient Intakes

When the estimates of body fat percentage of those who experienced an increase in body fat percentage (28.5±7.1%), and those whose body fat percentage decreased or remained constant were compared (33.8±9.0%), no statistically significant difference was observed. Despite this finding, there were statistically significant differences in the average nutrient intakes between the participants in both groups for some nutrients (vitamins A, B_1, B_3, B_{12} D, E and K, folate, iron, calcium, magnesium, potassium, iodine, selenium, phosphorus and biotin).

More so, when the participants were grouped into those whose body fat increased by 1% or more and those whose body fat increased by less than 1% and the mean body fat percentage estimates of both groups were compared (28.5± 6.7% versus 29.7±7.4% respectively), there was a statistically significant difference ($p< 0.001$). However, there was no statistically significant difference between the average nutrient intakes between both groups. These findings put together show that differences in percentage body fat change between individuals might not necessarily reflect differences in their average daily energy or micronutrient intake. The findings also suggest the likelihood of bias when exclusively associating either nutrient or

energy intake with body fat percentage. The simultaneous consideration of energy and micronutrient intakes as represented by the dietary nutrient density is rather recommended.

In this thesis, it has been argued that since both micronutrients and energy intake play a crucial role in energy metabolism, exclusively associating either of them with body fat percentage is inadequate from a nutritional perspective. Hence the use of dietary nutrient density, an index which includes both variables. The selection of the 1% change category while analysing the results was not based on a standard from previous literature, but as a measure of change in this thesis to investigate if exclusively considering only energy intake or micronutrient intake in association with body fat percentage would have sufficed rather than the dietary nutrient density.

5.4.6. The Influence of Sleep, Cigarette Smoking, Appetite, being on a Special Diet and Alcohol Intake on the Association between change in Dietary Nutrient Density and change in Body Fat Percentage.

a) Sleep

In this study, sleeping for less or at least 7 hours at night-time did not influence the relationship between dietary nutrient density and body fat percentage (Table 5.14- section 5.4.2) after adjusting for other covariates. This finding was unexpected since previous studies have associated sleep deprivation (having less than 7 hours of sleep) (Cooper, et al., 2018) with increased body fat. For instance, an examination of the data from the National Health and Nutritional Examination Survey I, conducted between 1982 and 1992, revealed that subjects between the ages of 32 years and 49 years with self-reported sleep duration for less than 7 hours had higher average BMIs. Also, they were more likely to be obese than subjects with sleep durations of at least 7 hours (Gangwisch, 2005). Other studies similarly found an association between sleep deprivation and increased body fat (Nielsen, Danielsen

and Sørensen, 2011; Xi, et al., 2014). It has been suggested that experimental sleep restriction is associated with increased levels of ghrelin and decreased levels of leptin, which are correlated with increased hunger, especially for fat and carbohydrate-dense foods (Klok, Jakobsdottir, Drent, 2007). While the present study neither provides evidence to support nor refute the mechanism by which sleep duration influences body fat, it shows that although those who slept for less than 7 hours each night had a significantly higher energy intake (Table A12.1-Appendix 12) compared to those who slept for at least 7 hours, there was no significant difference in the body fat percentage of both groups.

b) Cigarette smoking

Although previous studies suggested that that cigarette smoking decreased body fat through increasing the metabolic rate (Chiolero, et al., 2008; Parsons, et al., 2009), this cohort study did not find any influence of cigarette smoking (Table 5.12- section 5.4.2) on the association between change in dietary nutrient density and change in body fat percentage. Perhaps the influence of this factor was not observed due to the categorisation of smokers used. In the present study, participants were categorised into smokers and non-smokers, which could have undermined the effect of the number of cigarettes smoked per day for smokers. Apparently, despite that smoking can lower body fat (Chiolero, et al., 2008), the number of cigarettes smoked per day can modify this influence. Previous research has positively associated heavy smoking with a greater risk of obesity (Rasky, Stronegger and Freidl, 1996; John, et al., 2005; Chiolero, 2007). For instance, in the Cancer Prevention Study I, heavy smokers (≥ 2 packs of cigarettes per day) were more likely to be overweight compared to light smokers (Rasky, Stronegger and Freidl, 1996). John, et al. (2005), in a national sample of 7123 adult German residents, likewise found that the number of cigarettes smoked per day was positively related to being overweight.

c) Appetite

Extant literature indicates that increased appetite can result in overeating and increase in body fat (Blundell and Finlayson, 2004; Dalton, et al., 2013). Research on appetite assessment also states that a lower appetite score (≤14 on the Simplified Nutritional Appetite Questionnaire) can predict weight loss (Wilson, et al., 2005). The present study, however, shows that one's appetite score does not influence the relationship between change in dietary nutrient density and change in body fat percentage (Table 5.14- section 5.4.2).

d) Special diet

Furthermore, this study finds that being on a special diet (vegetarian or omnivore) neither showed any significant difference with regards to body fat percentage (Table 5.3- Chapter 5) nor influenced the relationship between change in dietary nutrient density and change in body fat percentage (Table 5.9- Chapter 5). In contrast, previous studies have indicated the association between being on a special diet and body fat. For instance, a Korean study of 75 adults found that vegetarians had a significantly lower body fat percentage (21.6 ± 6.4%) compared to omnivores (25.4 ± 4.6%) (Kim, Cho and Park, 2012). Similarly, in the EPIC-Oxford Study involving 37,875 adults, vegetarians (23.28 kg/m^2) had a significantly lower BMI than omnivores (24.41 kg/m^2) (Spencer, et al., 2003).

The reason for the difference between the findings of these previous studies and that of the present study is not known. However, it might be related to differences in the duration of vegetarianism. In both studies (Spencer, et al., 2003; Kim, Cho and Park, 2012), the authors indicated that the vegetarians included had maintained the dietary pattern for at least 20 years. Although this detail was not investigated in this cohort study, it is unlikely that those on a special diet had adhered to vegetarianism for up to 20 years, since most participants were young

adults (18-29 years). Similar results should not be expected given the variation in the period of adherence to vegetarianism.

e) Alcohol Intake

Alcohol consumption did not influence the relationship between change in dietary nutrient density and change in body fat percentage in this study (Table 5.14- section 5.4.2). This finding was unexpected since alcohol contributes to the total energy intake, influences metabolic pathways able to alter fat metabolism (Traversy and Chaput, 2015) and has been positively associated with an increase in body fat (Arif and Roher, 2005; Fan, et al., 2008; Lee, 2008). Perhaps no influence of alcohol was observed in the present study as alcohol intake was categorised based on drinkers and non-drinkers without considering the amount and frequency of consumption. Traversy and Chaput (2015) have stated that some of the factors which can modify the influence of alcohol include gender, type and frequency of alcohol consumed, drinking patterns, history of alcohol use, predisposition to weight gain, physical activity and dietary behaviour. For instance, the relationship between alcohol intake and body fat is generally stronger in men than women (French, et al., 2010), particularly due to differences in the amount and type of alcohol consumed. Research shows that men consume three times the amount of alcohol consumed by women (Nielsen, et al., 2012) and are more likely to drink beer, which contains more energy than wine per standard drink (Yeomans, 2010).

5.5. Conclusion

This study provides evidence to suggest that the increase in the dietary nutrient densities of vitamins A, C, E and K, folate, iron, calcium, magnesium, zinc, selenium and phosphorus influence a decrease in body fat percentage in adults. It also indicates that moderate to high levels of physical activity and dietary phytate intake can influence the association between change in dietary nutrient density and change in body fat percentage. Furthermore, the findings

of this research show that one's dietary nutrient density is a better predictor of their body fat changes than either energy or micronutrient intake.

CHAPTER 6. THESIS DISCUSSION

The current recommendations for reducing the risk of obesity have highlighted the importance of consuming nutrient-dense foods (Troesech, et al., 2015; Smethers and Rolls, 2018). However, there remains a dearth of evidence on the association between dietary nutrient density and body fat. Hence, the overall aim of the thesis was to investigate the association between change in dietary nutrient density and change in body fat percentage. This aim was achieved through specific objectives:

i. To evaluate the influence of phytate on the bioavailability of micronutrients through a systematic review.
ii. To validate a food photography method for estimating dietary micronutrients against the weighed food record method as a reference.
iii. To assess the dietary nutrient density and body fat percentage of participants and examine the association between change in dietary nutrient density and change in body fat percentage using a prospective cohort study design over 6 months.

These objectives have been reported in chapters 3, 4, 5, respectively, and the related findings are discussed as follows.

Chapter 3 focused on a systematic review of the influence of phytate on the bioavailability of micronutrients in the diet. This objective was included considering that the association between change in dietary micronutrients and change in body fat percentage, which is hypothesised in the thesis is premised on the absorption of micronutrients in the diet. Therefore, it was necessary to know if phytate in the diet should be assessed as a moderator. The systematic review concluded that phytate in the diet reduced the bioavailability of zinc, magnesium, and iron.

The interaction between phytate and trace minerals has hitherto received much attention in both food processing and nutrition research. The increasing studies published on the alleged anti-

nutrient characteristics of phytates served as a rationale for the removal of phytate during food processing (dephytinisation) to improve the bioavailability of minerals, especially in populations mainly developing countries consuming unrefined cereals and, or pulses as a staple (Kumar, et al., 2009). This has however been contested by studies stating that phytate did not have antinutrient properties, but rather had beneficial health effects, such as protection against renal stones, cancer (Vucenik and Shamsuddin, 2003), diabetes mellitus (Larsson, et al., 1997; Barker and Berggren, 1999), coronary disease (Jariwalla, et al., 1990; Persson, et al., 1998), dental caries (Kaufman and Kleinberg, 1971), and human immunodeficiency virus (Otake, et al., 1989; 1999).

Due to the lack of unanimous consensus on the role of phytate, there was a need to identify and appraise available evidence and conclude on the influence of phytate on mineral bioavailability, to provide a rationale for its inclusion as a moderator of the association between changes in the dietary nutrient density and body fat percentage in the cohort study. Based on the findings of this systematic review, phytate can reduce the bioavailability of zinc, magnesium, and iron in the diet. Besides the relevance of the conclusion of the systematic review in the cohort study, it also has implications for the policy on dephytinisation. The conclusion provides supporting evidence for dephytinisation.

In chapter 4, the objective was to validate a food photography method for assessing micronutrients in the diet using weighed food record as a reference. This objective was included as a step towards achieving the overall aim of the thesis to ensure that the dietary assessment method for estimating micronutrients was valid and reliable. The process of validation involved assessing the diet by the test and reference method and comparing the results. Findings from the validation study showed that the test method (food photography method) was reasonably valid and reliable for assessing dietary micronutrients. Other food photography techniques have

similarly been demonstrated to be valid and reliable for dietary assessment in children (Martin et al., 2007; Nicklas et al., 2012) and adult populations (Williamson et al., 2002; Williamson et al., 2003; Williamson et al., 2004).

The finding that food photography is a reasonably valid and reliable method for the dietary assessment of micronutrients highlights the role that technology can play in assessing diet and some implications. First, using smartphones for dietary assessment makes it possible for the easy transport and storage of images. Secondly, unlike the more traditional methods of dietary assessment, the food photography method is less time consuming and reduces the participant burden associated with estimating food portion sizes (Olafsdottir, et al., 2016). Also, with the development of a data management software or website, where the photographs can be stored, raters can be trained and inter- and intra-rater reliability can be assessed. The stored food photographs can also provide an opportunity for monitoring of changes in participants' diet over time. Overall, the findings in this chapter indicate that the food photography method is a reasonably valid, reliable and convenient way to assess micronutrients in the diet.

Chapter 5 of this thesis focussed on the association between change in dietary nutrient density and change in body fat percentage and found that an increase in the dietary nutrient densities of vitamins A, C, E and K, folate, iron, calcium, magnesium, zinc, selenium and phosphorus corresponded to a decrease in body fat percentage. Investigating this association involved assessing dietary micronutrients using the food photography method, which was validated in chapter 4 and assessing dietary phytate as a moderator given the conclusion of the systematic review carried out in chapter 3. The association between change in dietary nutrient density and change in body fat percentage observed in chapter 5 is supported by the findings of previous studies suggesting the contribution of suboptimal micronutrient levels to the risk of obesity

(Manning, et al., 2004; Nead, et al., 2004; Johnston, et al., 2007; Gunanti, et al., 2014; Castellanos-Gutierrez, et al., 2018; Knapen, Jardon and Vermer, 2018).

The mechanisms by which the increased dietary nutrient densities of the selected micronutrients (vitamins A, C, E and K, folate, iron, calcium, magnesium, zinc, selenium and phosphorus) influence body fat percentage was not investigated in this thesis. However, previous studies have suggested some mechanisms such as promoting glucose homeostasis and body fat through reducing the risk of insulin resistance (Sakamoto, et al., 2008; Yoshida, et al., 2008; Yoshida, Jacques and Meigs, 2008; Puchau, et al., 2010; Wlazlo and van Greevenbroek, 2012; Dhanya, Swathy and Indira, 2014; Li, et al., 2017), modulating adipocyte lipolysis (Carcamo, et al., 2002; Garcia-Diaz, 2009) and inflammatory response (Carcamo, et al., 2002).

In addition, the data analysed in the cohort study showed that the association between changes in the dietary nutrient density and body fat percentage could be influenced by the level of physical activity and the intake of phytate in the diet. The association tended to diminish with the increase in physical activity and was not observed for individuals with a high level of physical activity per week (MET- minutes of activity per week ≥ 1500). More so, with the presence of phytate in the diet, no association was observed between the dietary nutrient densities of zinc and iron, and body fat percentage. It was also noted that phytate had no association with body fat percentage (Figures A13A and A13B- Appendix 13).

Finally, data from the cohort study clarified that the exclusive association of micronutrient or energy intake with changes in body fat could be erroneous. Rather, changes in dietary nutrient density are more predictive of body fat changes. Since both micronutrients and energy intake can influence energy balance, the use of dietary nutrient density would be an adequate consideration from a dietary perspective.

CHAPTER 7. THESIS CONCLUSION, IMPLICATIONS FOR PRACTICE, STRENGTHS, LIMITATIONS, AND RECOMMENDATIONS FOR FURTHER RESEARCH

7.1 Conclusion

This thesis provides evidence to suggest that the food photography method is reasonably valid and reliable for assessing dietary micronutrients and that the increase in the dietary nutrient densities of vitamins A, C, E and K, folate, iron, calcium, magnesium, zinc, selenium, and phosphorus influence a decrease in body fat percentage in adults. The findings also show that the association between dietary nutrient density and body fat percentage can be influenced by physical activity and dietary phytate intake. Furthermore, the findings indicate that changes in dietary nutrient density are a better predictor of body fat changes compared to only either energy or micronutrient intake with regard to the influence of nutrition on body fat percentage. Hence, the dietary nutrient densities of vitamins A, C, E and K, folate, iron, calcium, magnesium, zinc, selenium, and phosphorus should be considered as a critical aspect of diet planning in weight-loss interventions.

7.2 Public Health Implications of Findings

Micronutrient deficiencies remain a public health problem in various parts of the world, affecting both underdeveloped and developed countries. It has been suggested that the rates of obesity are increasing more rapidly in populations with more prevalent micronutrient deficiencies (Via, 2012). The finding of the present cohort study suggests that low dietary nutrient density (particularly for vitamins A, C, E, and K, folate, iron, calcium, magnesium, zinc, selenium, and phosphorus) and high dietary intake of phytate are potential contributors to this phenomenon. The findings also suggest that micronutrient intake together with energy

intake are necessary for tackling obesity from a nutritional perspective, especially as previous research shows that weight-loss interventions which focussed only on altering energy intake as a means of achieving sustained weight loss fail to record meaningful results (Hafekost, et al., 2013).

Furthermore, given the relationship observed in the present study, incorporating the nutrient density of specific micronutrients to the nutrient labelling scheme can be potentially useful to inform consumers on specific micronutrient to calorie intake ratio (nutrient density) of various food products and enable them to make informed decisions based on their body fat composition and health concerns.

Another important implication which can be derived from the present findings is that exclusively associating either energy intake or micronutrient intake with body fat percentage can be misleading. Unfortunately, this is the case for the predominant body of extant literature, but inadequate from a nutritional perspective since both energy and micronutrient intakes have the propensity to influence body fat.

The interpretation and application of the findings of this thesis derived from the implications discussed above should, however, be considered carefully, as some potential sources of error have been acknowledged. For instance, the estimates for body fat percentage were obtained using the BIA technique, which is known to be liable to error owing to the varying hydration status of the participants (Kyle, et al., 2004). Also, the use of convenient sampling might have led to the selection of participants of similar characteristics and resulted in limited generalisability of the findings. In addition, the cohort study controlled for phytate among several other dietary factors such as polyphenols, which may affect the bioavailability of micronutrients. It is also worth mentioning that the association observed is based on the assumption that the participants captured all the diet which they consumed during the research.

However, since a self-reported measure for dietary intake was applied, there remains the risk of a selective recording of dietary intake due to social acceptability and body image factors. These potential sources of error have also been highlighted in the study limitations below and possible measures for improvement indicated in the recommendations for future research.

7.3 Strengths and Limitations

The major strengths of this thesis include the dietary assessment technique used, the estimation of body fat percentage, and the effort made in controlling the factors which might affect the results. The method of dietary assessment used is a dietary record technique (food photography method), which was validated before use with the weighed food record as a standard. Body fat was estimated directly by measuring body fat percentage, rather than using surrogates. Scientific evidence has emphasised the limitations of using surrogates for estimating body fat (Cornier, et al., 2011; Li, et al., 2011; Gurunathan and Myles, 2016). In this study, various covariates were considered and controlled to limit bias while the data was collected and during the analysis of the results (see sections 5.3.9, 5.3.10 and 5.3.12).

This thesis is not without limitations. First, in the cohort study, the BIA technique provided a means for simple, low-cost, and non-invasive assessment of body fat percentage. While the device was indicated by the manufacturer to reasonably compare with the DXA, it is acknowledged that the BIA is not the gold-standard and is liable to bias owing to the assumption of a fixed hydration (Kyle, et al., 2004). Second, convenience sampling technique was applied in the cohort study due to its advantages such as being cheap, inexpensive and fast. Nevertheless, it is liable to selection bias and may have resulted in the recruitment of participants who were more health or nutrition-conscious, thereby limiting the generalisability of the conclusions drawn. Third, in the cohort study, as is the case with any study that relies on self-reported food intake, there is a likelihood of reporting bias. Reporting bias may be related

to an individual's body mass or other attributes. For instance, it is known that obese individuals tend to underreport energy intake (Orcholski, et al., 2015). However, it is unclear whether this occurrence is uniform across all dietary components (Suchanek, Poledne, Hubacek, 2011; Lafay, Mennen, Basdevant, 2015). Another limitation worth mentioning is that due to the challenge associated with estimating some dietary factors which could influence micronutrient bioavailability such as oxalates and polyphenols using available food nutrient databases, they were not considered. Hence, it is unclear how this might have influenced the results. Also, given the sample size, ethnic profile, and age categories of the cohort, the findings of the cohort study may not be representative of the UK adult population. For instance, considering that the participants' ages ranged from 18- 39 years, it might be erroneous to assume that similar results would be achieved if older adults were recruited. This is especially due to the likely variation in dietary behaviour and technology use. Furthermore, various mechanisms that associate micronutrient intake and body fat have been suggested in previous studies, as discussed in the thesis. However, the present study does not demonstrate or verify these mechanisms.

Also, with regard to the cohort study in this thesis, like in any cohort study, the loss of participants to follow up threatened the validity of results. Although the loss may be thought to be associated with little bias given that it did not exceed 20% (Sacket, Richardson, and Rossemberg, 1997), the actual bias introduced could be more if those lost were of unique characteristics. Additionally, the findings of the supplementary analysis (section 5.3.2) suggest that change in dietary nutrient density better reflects a change in body fat percentage compared to either nutrient intake or energy intake. While this supports the rationale for the use of dietary nutrient density, caution may be applied in interpreting this result, considering that the analysis was not planned prior to beginning the study, and the study was not powered for the analysis.

Other limitations of this thesis are with regard to the systematic review and the validation study reported in chapters 3 and 4, respectively. In the systematic review, only studies published in

English were included. Although this criterion was observed because English is the only language understood by the researcher, it introduced a language bias which might have influenced the results of the systematic review. Also, a narrative review rather than a meta-analysis was performed due to the heterogeneity of protocols and inconsistency of reporting of outcomes of the included studies. Notwithstanding that the choice was warranted, it is known that the decisions of a narrative review tend to be less transparent since it does not apply statistical analysis to yield an objective measure of the integrated evidence (Lee, 2019).

Regarding the validation study, the use of convenient sampling might have introduced selection bias favouring health-conscious participants or those interested in nutrition. Hence, not representative of adult university students. Also, notwithstanding the demonstration of the validity of the food photograph method for dietary assessment, the method may not be suitable for people who are not knowledgeable about computers and smartphones.

Due to all these limitations put together, caution should be applied in interpreting the results of this thesis.

7.4 Recommendations for Future Research

Due to the specific research question addressed in this thesis, and the time and resources available at the time of carrying out the project, some issues regarding the association between diet and body fat have not been addressed. Some of these issues would benefit from further research.

In this thesis, the association between change in dietary nutrient density and change in body fat percentage was investigated among 108 participants using a prospective cohort study design, which lasted for 6 months. Further research may investigate the association in a larger sample size and for a more extended period. It might also be necessary to assess the variables at

multiple intervals during the study period to evaluate the consistency of the relationship. More so, since the cohort study in this thesis focused on young adults (aged 18-39 years), it might be worth investigating the association in other populations, such as in children and older adults.

Furthermore, this thesis has clarified that dietary phytate and physical activity can influence the relationship between change in dietary nutrient density and change in body fat percentage. While those remain important factors, further studies may also consider the influence of gut microbiota on the relationship between dietary nutrient density and body fat percentage. Research evidence indicates that gut microbiota plays a crucial role in the harvest and utilisation of energy derived from the diet (Krajmalnik-Brown, et al., 2012).

Additionally, in the cohort study (chapter 5), body fat percentage was measured using BIA. Although this method has its strengths, which have been mentioned in chapters 2 and 5, and was used with the necessary precautions, the researcher acknowledges that it is not the gold standard. Future research may retest the hypothesis on the association between change in dietary nutrient density and change in body fat percentage using DXA or air-displacement plethysmography for assessing body fat percentage. Future studies may also retest the hypothesis using a valid and reliable objective method for dietary assessment to avoid the bias associated with using subjective methods.

In the systematic review on the influence of phytate on the bioavailability of micronutrients (chapter 3), only primary studies published in English were included. Future research may broaden the language criterion to achieve more generalisable findings.

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APPENDIX 1- General Methods

A1.1 Introduction

This chapter highlights the research paradigm, the study designs and rationale for the study designs applied in the studies reported in chapters 3, 4 and 5 of this thesis. Considering that some protocols and other procedural details used within each study are unique, such details are not included in this chapter but are outlined in the specific chapters.

Chapter 3 of this thesis reports a study aimed to conduct a systematic review on the influence of phytate on the bioavailability of micronutrients in the diet. Chapter 4 aimed to investigate the validity of a food photography method for dietary assessment using weighed food record method as a reference, while chapter 6 reports a study which investigates the association between change in dietary nutrient density and change in body fat percentage. Since similar study designs have been used in the studies reported in chapters 5 and 6, the research paradigm underpinning the study design will be discussed first, followed by the concept and scientific rationale for the systematic review reported in chapter 4.

A1.2. Research paradigm for chapters 4 and 5

A research paradigm refers to the researcher's philosophical positions regarding epistemology (how reality can be understood), ontology (the nature or essence of a phenomenon being investigated) and axiology (ethical issues involved), which shape the theory and practice of a methodological investigation (Saunders, Lewis and Thornhill, 2016). A myriad of research paradigms exists; however, they can be grouped into four main taxonomies namely, positivist paradigm, interpretivist paradigm, critical paradigm (Candy, 1989) and pragmatic paradigm (Tashakkori and Teddlie, 2003a; 2003b). The positivist paradigm postulates that knowledge and understanding of reality can be gained from experimentation, observation and reason gained from experience, while the interpretivist paradigm attempts to understand and interpret reality from the viewpoint of the subject being observed rather than the observer's perspective.

The critical paradigm, also known as the transformative paradigm, addresses social, economic and political issues which lead to conflict and oppression regarding a given situation to transform the political structures to improve social justice. The pragmatic paradigm asserts that reality cannot be understood and interpreted by a single paradigm but by a combination of paradigms necessary to capture the elements of reality being studied (Tashakkori and Teddlie, 2003a; 2003b). Based on assumptions undergirded by the various paradigms, the positivist paradigm was chosen for the studies reported in chapters 4 and 5 of this thesis. Further details on the positivist paradigm and the justification for choosing it are presented as follows.

A1.2.1. Positivist paradigm

First proposed by Auguste Comte (1798- 1857), this paradigm is grounded in scientific methodology (experimentation, observation and reason based on experience), is considered the basis for understanding human behaviour and hence a legitimate means of extending human understanding and knowledge. In this paradigm, experimentation serves as a means of exploring observations, answering questions and investigating the relationship between variables in nature. It also attempts to interpret observations based on facts or measurable quantities (Fadhel, 2002). Research underpinned by this paradigm relies on deductive reasoning, hypothesis formulation and testing, providing operational definitions and mathematical calculations and extrapolations to achieve conclusions. Its main objective is to provide explanations and make predictions based on quantifiable outcomes undergirded by four assumptions; determinism, empiricism, parsimony and generalisability (Cohen, Manion and Morrison, 2000). Determinism implies that events observed are influenced by other factors. Hence by understanding the relationship among factors, the potential impacts of explanatory factors on dependent factors can be predicted and controlled. Assuming empiricism implies that verifiable empirical data supporting the theoretical framework chosen, which enables a hypothesis to be tested is necessary for investigating a research problem. The assumption of

parsimony refers to the use of the most economical means possible to explain research phenomena, while the generalisability assumption implies that the findings obtained from research should be transferable or applicable in other situations by inductive conclusions. In other words, with the premise of generalisability, a positivist can predict what is obtainable in different regions or populations. Due to these assumptions, quantitative research methods are advocated by the positivist paradigm as fundamental for a meaningful description of parameters in data collection analysis and interpretation (Cohen, Manion and Morrison, 2000).

Based on the assumptions of the positivist paradigm, its epistemology is objectivist, its ontology is naïve realism, its methodology is experimental, and its axiology is beneficence. By the objectivist epistemology, it means that human understanding is gained through the application of reason (Fadhel, 2002). The naïve realist ontology means that material objects with perceptible characteristics exist in the world regardless of being perceived or not. It also assumes that the world can be perceived as it is through senses. By the assumption of an experimental methodology, the paradigm implies that research involves investigating a changing variable with reference to the changes occurring in another variable (Smith and Heshusius, 1986). The attribute of this methodology allows hypothesis testing by the researcher. Finally, the beneficence axiology implies that the research achieves good outcomes for the research participants and humanity in general.

The choice of a philosophical paradigm has methodological implication and relates to the research questions, participant selection, data collection instruments and procedures for data analysis (Guba and Lincoln, 1988; Saunders, Lewis and Thornhill, 2016). A positivist paradigm implies that the data being collected is quantitative, can be provided by the participants selected, is measurable by the instrument(s) used, and can be analysed using quantitative methods to achieve research outcomes which either validate or nullify a given hypothesis.

Given that the objectives of the studies in chapters 4 and 5 of this thesis are aligned with the epistemology, ontology and axiology of the positivist paradigm, a quantitative study design was applied to both of them. For instance, in chapter 5, a prospective cohort study, the researcher aims to investigate the association between change in dietary nutrient density and change in the body fat percentage among research participants by observation and quantitative measurement of these variables. Also, in the research, the changes in one variable (body fat percentage of the participants) are observed relative to changes in the other (dietary nutrient density) without any attempt to gain the participants' viewpoint on these variables. Furthermore, the research aims to test the hypothesis that an increase in dietary nutrient density is associated with a decrease in body fat percentage and to generate findings based on which nutritional and health recommendations will be made and published for the benefit of the research participants and humanity in general. Similarly, in chapter 4, the researcher aims to investigate the validity of a food photography method for assessing micronutrients in the diet while using weighed food record method as a standard. To achieve this aim, micronutrients will be estimated by both methods without any attempt to gain the participants' viewpoint on these variables. Also, the study aims to test the hypothesis that the food photography method is valid for the estimation of micronutrients in the diet and make publishable diet-related recommendations to benefit the research participants.

A1.2.2. The concept and scientific rationale for a systematic review

A systematic review is a scientific investigation which focusses on answering a clearly formulated question through a systematic process of analysing and summarising original primary studies. The process of summarising results usually applies some strategies to limit error, such as:

i. Conducting a systematic search for relevant primary studies based on the subject area of concern.

ii. Defining and applying a clear and reproducible method for selecting primary studies for inclusion in the review.

iii. Describing the design and protocol of the primary studies.

iv. Synthesising the data arising from the original studies and interpreting the findings.

In a systematic review, data from the primary studies may be synthesised with or without statistical methods. Where data is synthesised statistically, the review can be known as a meta-analysis. Otherwise, it is called a systematic review with a narrative or tabular synthesis. Regardless of the method applied in synthesising the data, the primary objective of a systematic review is to provide a comprehensive summary of evidence regarding a research question. In keeping with this objective, the systematic review reported in chapter 4 of this thesis aims to collect data from primary studies and provide a complete summary of evidence on the influence of phytate on the bioavailability of micronutrients in the diet. Also, the data arising from the primary studies included in the review will be qualitatively synthesised and the findings interpreted.

APPENDIX 2- Tables for the screening of 7671 search record based on the inclusion and exclusion criteria, and the quality assessment of included papers for systematic review using the Critical Appraisal Skills Programme (CASP).

Table A2.1. Screening of 7671 search record based on the inclusion and exclusion criteria

Screening criteria	**Number of papers included**
Before full application of the inclusion and exclusion criteria	7671
Language	7625
Publication type	458
Participants and study design	51
Intervention, comparison and outcome	33

Tables A2.2a and A2.2b- Quality Assessment of included papers for systematic review (n=33) using the Critical Appraisal Skills Programme (CASP).

Tables A2.2a. Quality Assessment of cohort studies

	CASP CHECKLIST FOR COHORT STUDIES										
STUDY	**1**	**2**	**3**	**4**	**5 (a, b)**	**6(a,b)**	**7**	**8**	**9**	**10**	**11**
1. Kennedy, Hambidge and Manary, 2010	+	+	-	-	+, +	+, +	+	±	+	-	-

1. Did the study address a clearly focused issue?
2. Was the cohort recruited in an acceptable way?
3. Was the exposure accurately measured to minimise bias?
4. Was the outcome accurately measured to minimise bias?
5. (a) Have the authors identified all important confounding factors? (b) Have they taken account of the confounding factors in the design and/or analysis?
6. (a) Was the follow up of subjects complete enough? (b) Was the follow up of subjects long enough?
7. What are the results of this study?
8. How precise are the results?
9. Do you believe the results?
10. Can the results be applied to the local population?
11. Do the results of this study fit with other available evidence?
12. What are the implications of this study for practice?

+ = Yes; - = No; ± = Cannot tell; NS= Not stated

Tables A2.2b. Quality Assessment of RCTs and Cross-over studies

	CASP CHECKLIST FOR RCTs AND CROSS-OVER TRIALS										
STUDY	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Bohn, et al. (2004)	+	+	+	+	+	+	19.5%	NS	+	+	+
Brnic, et al. (2014)	+	+	+	+	+	+	80%	95%	+	+	+
Brune, Rossander and Hallberg, (1989)	+	-	+	-	+	+	92%	NS	+	+	+
Couzy, et al. (1993)	+	+	+	+	+	+	40%	NS	+	+	+
Couzy, et al., 1998 (a&b)	+	-	+	±	-	+	57%, 55%	NS	+	+	+
DellaValle, et al. (2015)	+	+	+	+	+	+	90%	NS	-	+	+
Egli, et al. (2003)	+	+	+	-	+	+	11.8%	NS	-	-	-
Fredlund, et al. (2006)	+	-	+	-	+	+	8%	95%	+	+	+
Gillooly, et al. (1984)	+	-	+	-	+	+	20%	NS	-	+	+
Hall, et al., 1989	+	+	+	+	+	+	68.1%	NS	-	+	+
Hallberg, Brune and Rossander (1989)	+	+	+	+	+	+	18%	NS	+	+	+
Hallberg, Rossander and Skanberg (1987)	+	-	+	-	+	+	54.8%	NS	+	+	+
Hambidge, et al. (2004).	+	-	+	-	+	+	17%	NS	+	+	+
Hanson, et al. (2006)	+	+	+	+	+	+	53%	NS	-	+	+
Hurrel, et al., 1992 (a, b, c, d)	+	-	+	-	+	+	52.3%, 51.8%, 98.3%, 78.8%	NS	+	+	+
Hurrell, et al. (2003).	+	-	+	-	+	+	67.6%	NS	+	+	+
Jaramillo, et al. (2015).	+	-	+	-	+	+	24.4	NS	-	+	+
Kim, et al. (2007)	+	-	+	+	+	+	21%	NS	-	+	+
Lonnerdal. (1984)	+	+	+	+	+	+	48.4%	NS	-	+	+
Manary, et al. (2000) (a&b)	+	+	+	+	+	+	41%	NS	-	+	+
Mazariegos, et al. (2006)	+	+	+	+	+	+	0	NS	-	+	+
McCance and Widdowson (1942) (1)	+	-	+	-	+	+	38.95%, 33.2%	NS	+	+	+
McCance and Widdowson (1942) (2)	+	-	+	-	+	+	9.5%, 20.8%	NS	+	+	+
Mendoza, et al. (1998)	+	-	+	-	+	+	49%	NS	-	+	+
Navert, Sandstrom, Cederblad (1985)	+	+	+	+	+	+	9.6%	NS	+	+	+
Petry, et al. (2014)	+	+	+	+	+	+	37%	95%	-	+	+
Sandberg, et al. (1999)	+	-	+	-	+	+	39%	NS	+	+	+
Sandberg, Hasselblad and Hasselblad (1982)	+	-	+	-	+	+	40%	NS	-	+	+
Sandstrom and Sandberg (1992)	+	-	+	-	+	+	67.4%	NS	-	+	+
Sandstrom, et al. (2000)	+	-	+	-	+	+	0	NS	+	+	+

Sandstrom, et al. (1987)	+	-	+	-	+	+	68.7%	NS	+	+	+
Thacher, et al. (2009)	+	-	+	+	+	+	26.9%	95%	-	+	+

1. Did the trial address a clearly focused issue?
2. Was the assignment of patients to treatments randomised?
3. Were all of the patients who entered the trial properly accounted for at its conclusion?
4. Were patients, health workers and study personnel 'blind' to treatment?
5. Were the groups similar at the start of the trial
6. Aside from the experimental intervention, were the groups treated equally?
7. How large was the treatment effect?
8. How precise was the estimate of the treatment effect?
9. Can the results be applied to the local population, or in your context?
10. Were all clinically important outcomes considered?
11. Are the benefits worth the harms and costs?

+ = Yes; - = No; ± = Cannot tell; NS= Not stated

APPENDIX 3- Research Ethics Approval for Validation Study

Anglia Ruskin University

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Ref: NS/jc/FMSFREP/15/16 082
Enquiries: Joanne Corney
Direct Line: 01245 684779
Date: 24th May 2016

Osinachi Ekeagwu

Dear Charmaine

Re: Application for Ethical Approval

Principal Investigator:	Osinachi Ekeagwu
FREP number:	15/16 082
Project Title:	Validation of a food diary

Thank you for your application for ethical approval from the Faculty of Medical Science Faculty Research Ethics Panel (FREP).

Following discussion of your proposal, the Panel decided to offer a conditional offer to your project application; the condition being upon the following:

- It is not specified anywhere where or how long the data will be kept for (we advise applicants that the data will be destroyed within three years upon completion of the project). Please can you add both to the Participant Information Sheet and Participant Consent Form?
- Section A of your Participant Information Sheet informs participants that they will be required to photograph their food and drink but there is no mention of it in Section B. Please can you amend this so it is consistent and fully inform the participants of exactly how long they need in order to devote to the study (presumably 8 days) so they can make an informed choice of taking part.
- Your Participant Information Sheet and Participant Consent Form need to include a statement to inform participants that no nutritional advice will be given on their food diary and intake.
- It would also be beneficial to add a statement to Appendix 3 inform Participants that a benefit would be that they are helping to develop a new dietary tool.
- The statement regarding the benefits of the project in Appendix 3 is incomplete. Please complete this sentence.
- The Participant Information Sheet also needs to inform participants of who to go to if they wish to make a complaint. Please visit: http://web.anglia.ac.uk/anet/staff/sec_clerk/feedback.phtml for details.

APPENDIX 4- Participant Information Sheet for Validation Study

Anglia Ruskin University

Participant Information Sheet

Title of the project: Validation of a Food Photograph Diary

Main investigator and contact details: Osinachi Ekeagwu
Osinachi.ekeagwu@student.anglia.ac.uk

Supervisor: Dr Marie-Ann Ha
Marie-Ann.Ha@anglia.ac.uk

Section A: The Research Project

Title of project: Validation of a Food Photograph Diary

Purpose and value of study:
To validate an electronic food diary as a useful measure for dietary data collection with a potential to reduce the burden of collecting data.

.

Invitation to participate:
You are being invited to take part in a research study. Before you make your decision, it is important for you to understand why the research is being done and what it will involve. Please take some time to read the following information. If there is anything you are not clear about or would like more information, please feel free to contact me:

There are a limited number methods for measuring dietary intake. The gold standard is seen as a 4-day written food diary (Gibney, et al., 2009). However this is quite work intensive for the subject.

The proposed research aims to validate an electronic food diary in order to be able to apply it as a useful measure of dietary data thereby reducing the burden of data collection on the participants. You will be asked to record everything you eat and drink for four days, one being on a weekend, on two separate occasions.

On one occasion you will be asked to write everything down on paper and estimate the amounts you have eaten and drunk using standard measure such as plates and cups or weighing. This will be a four-day record.

On another occasion, you will be asked to photograph your meals and drinks and fill in some information electronically about your meals also for four days.

Both sets of dietary data will be analysed using food tables for nutrient content. The nutrient contents will then be compared to see if they are similar and if the electronic diary is as accurate as the written format. Any data that is published will be anonymous.

Who is organising the research:

Osinachi Ekeagwu is the only researcher in this study and is carrying out this research as part of his PhD in Public Health with Anglia Ruskin University. This research is being supervised by Dr Marie-Ann Ha.

What will happen to the results of the study?

The results will be submitted to the Department of Allied and Public health, of the Faculty of Medical Sciences at Anglia Ruskin University. In addition, I will discuss the findings of the study with all the participants. I may also publish these results in peer reviewed scientific literature. At no stage will any participant be able to be identified.

Contact for further information:

Osinachi Ekeagwu at osinachi.ekeagwu@student.anglia.ac.uk

Dr Marie-Ann Ha at Marie-Ann.Ha@anglia.ac.uk

Section B: Your Participation in the Research Project

Why you have been invited to take part:
You have been invited to participate in this study to provide measures of dietary intake as part of the process required for the validation of a food diary to be used in a larger research for my doctoral thesis. Your participation is considered valuable as it will lead to the development of a new food diary tool with a potential benefit of reducing the burden of recording dietary details.
.

Whether you can refuse to take part:
Your participation in the study is entirely voluntary. You are under no pressure to take part.

Whether you can withdraw at any time, and how
You may withdraw from the study at any time without having to explain why. This you can do by signing the slip at the end of the consent form

What will happen if you agree to take part (brief description of procedures/tests)
If you agree to take part in the study, first, you will be given the log-in details of an electronic food diary requiring you to fill in details of your dietary intake for four days. Subsequently, you will be required to fill out an estimated food diary for an equivalent length of time.

Whether there are any risks involved (e.g. side effects from taking part) and if so what will be done to ensure your wellbeing/safety:
Taking part in the study will not in any way affect your well-being or safety. Also, no information regarding nutritional advice or intake recommendation will be indicated on the food diary.

Agreement to participate in this research should not compromise your legal rights should something go wrong:
No legal rights will be compromised by taking part in this study should something go wrong.

Whether there are any special precautions you must take before, during or after taking part in the study:
As there are no risks foreseen with participating in this study, there are no special precautions on your part to be taken before, during or after taking part in the study. However if you have any complaints, you may send your concerns to complaints@anglia.ac.uk.

What will happen to any information/data/samples that are collected from you

1. All information collected from participants will be kept safe and confidential all through the study. All information provided will be safeguarded, locked in a drawer within the university and destroyed upon three years of completion of the project

Whether there are any benefits from taking part:

Taking part in this study will not have any benefits to the individual participants. However, by participating, you will be contributing towards developing a new tool for keeping dietary record.

How your participation in the project will be kept confidential:

Your anonymity will be guaranteed by allocating you a subject number. Only the researcher will have knowledge of the information provided in the questionnaire and identifiable data if any, will be destroyed upon three years of completion of the study.

Ethical Approval

Approval for this study has been agreed by ARU Faculty of Medical Sciences Research Ethics' Panel.

YOU WILL BE GIVEN A COPY OF THIS TO KEEP
TOGETHER WITH A COPY OF YOUR CONSENT FORM

APPENDIX 5- Bland Altman Plot for Food Photograph and Weighed Food Record Estimates of Micronutrients in Participants' Diet in the Validation Study.

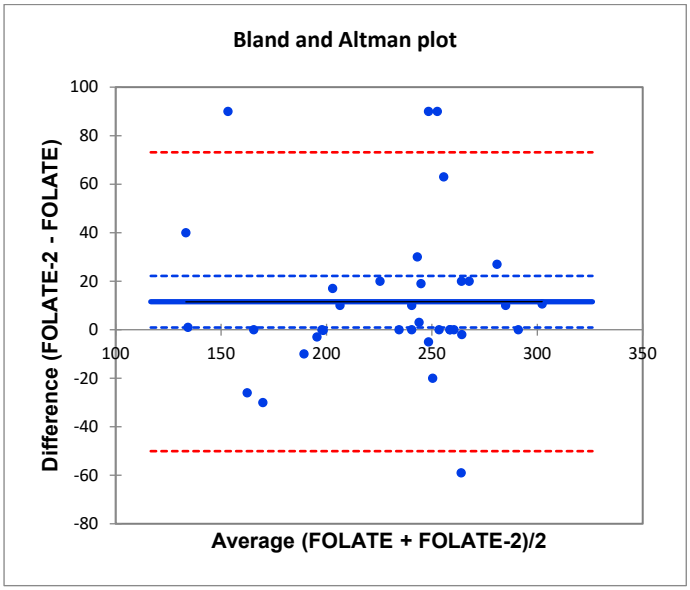


Figure A3.1. Bland Altman plot for food photograph and weighed estimates of folate in participants' diet.

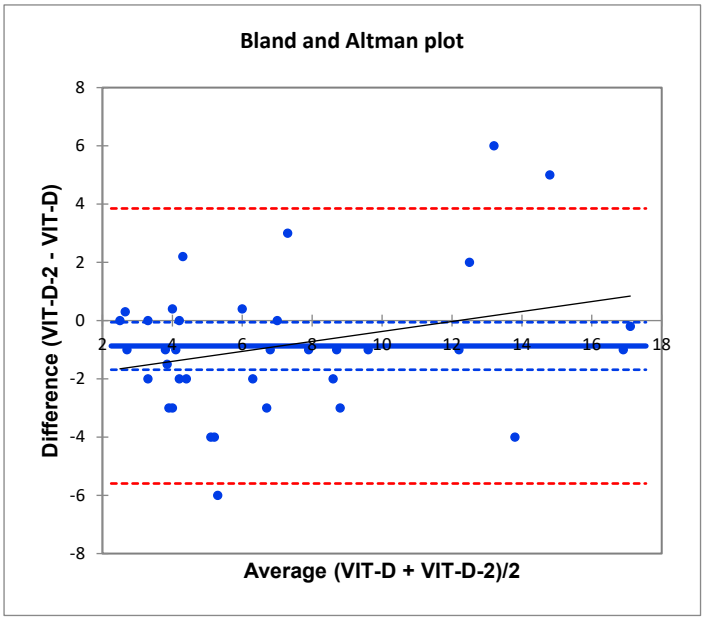


Figure A3.2. Bland Altman plot for food photograph and weighed estimates of vitamin D in participants' diet.

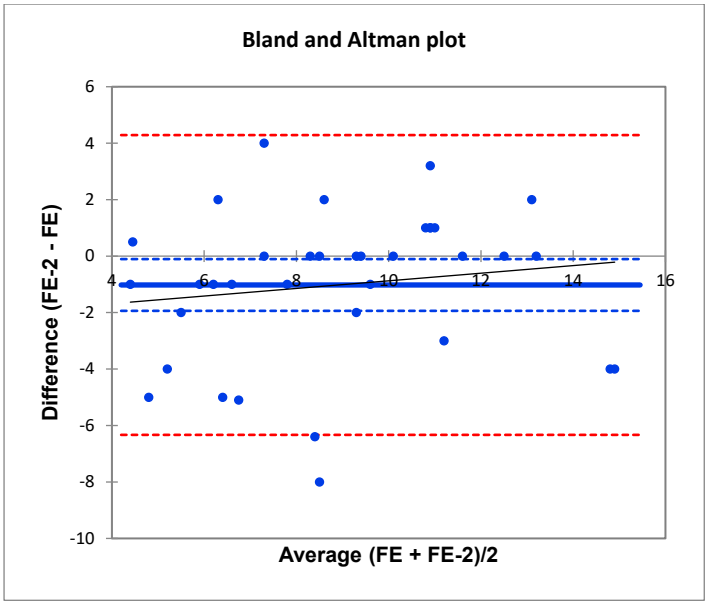


Figure A3.3. Bland Altman plot for food photograph and weighed estimates of iron in participants' diet.

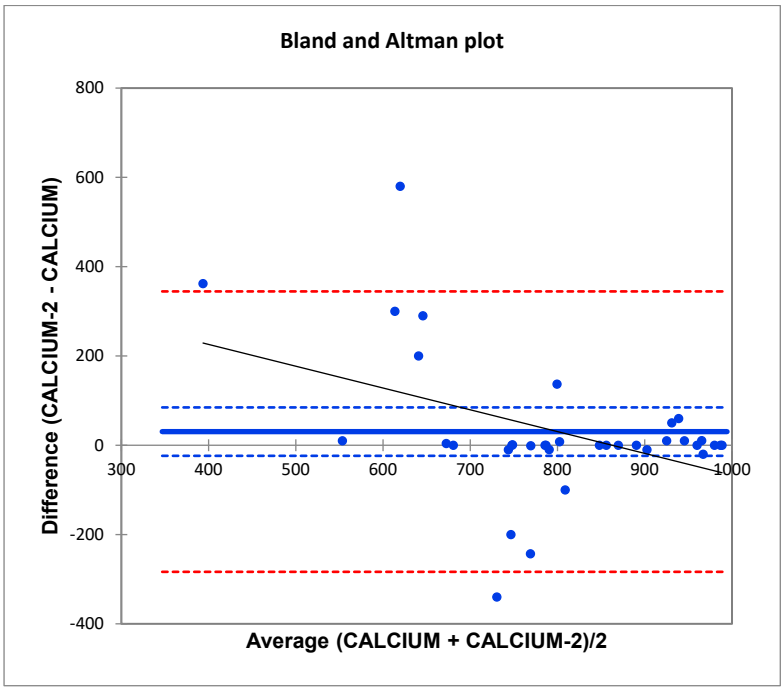


Figure A3.4. Bland Altman plot for food photograph and weighed estimates of calcium in participants' diet.

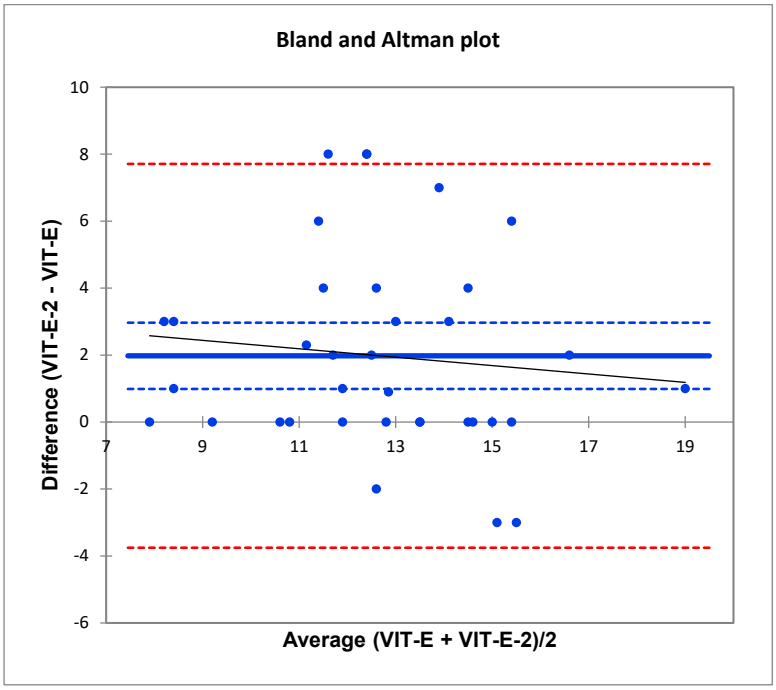


Figure A3.5. Bland Altman plot for food photograph and weighed estimates of vitamin E in participants' diet.

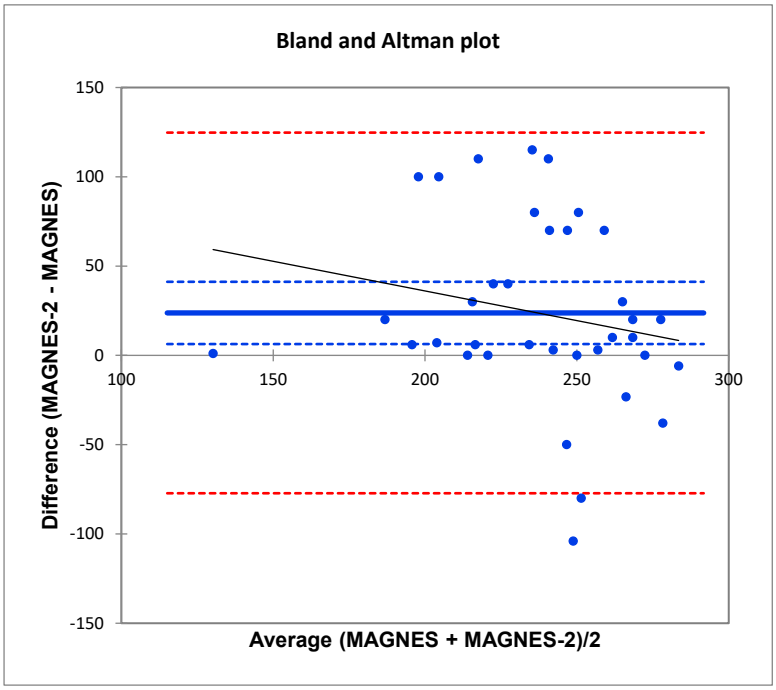


Figure A3.6. Bland Altman plot for food photograph and weighed estimates of magnesium in participants' diet.

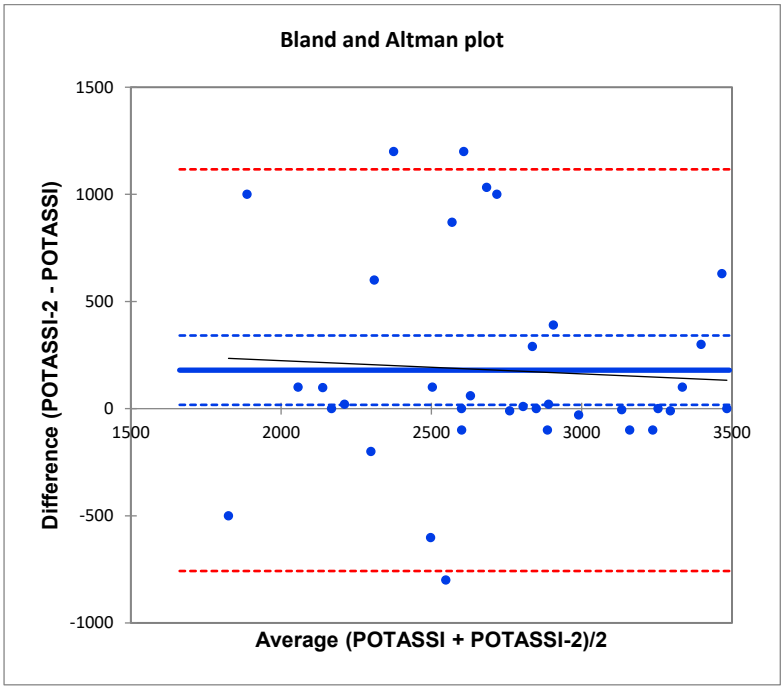


Figure A3.7. Bland Altman plot for food photograph and weighed estimates of potassium in participants' diet.

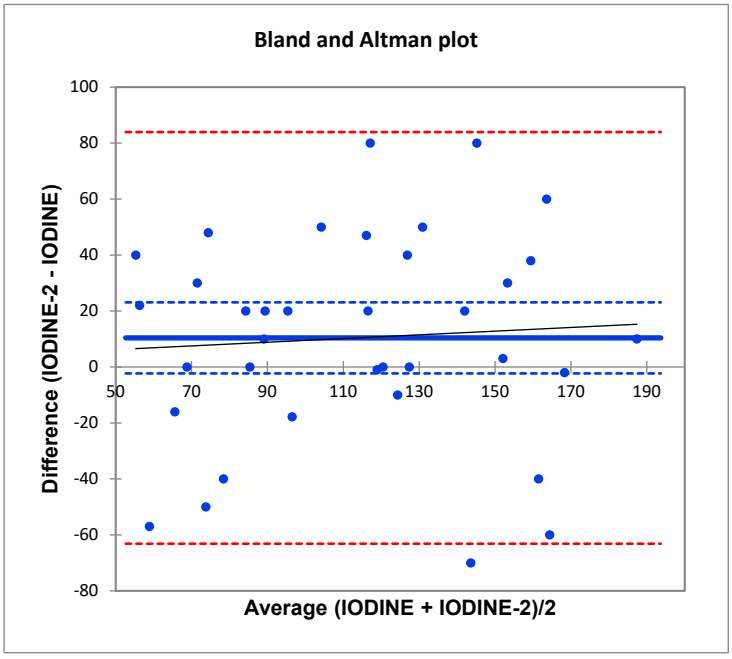


Figure A3.8. Bland Altman plot for food photograph and weighed estimates of iodine in participants' diet.

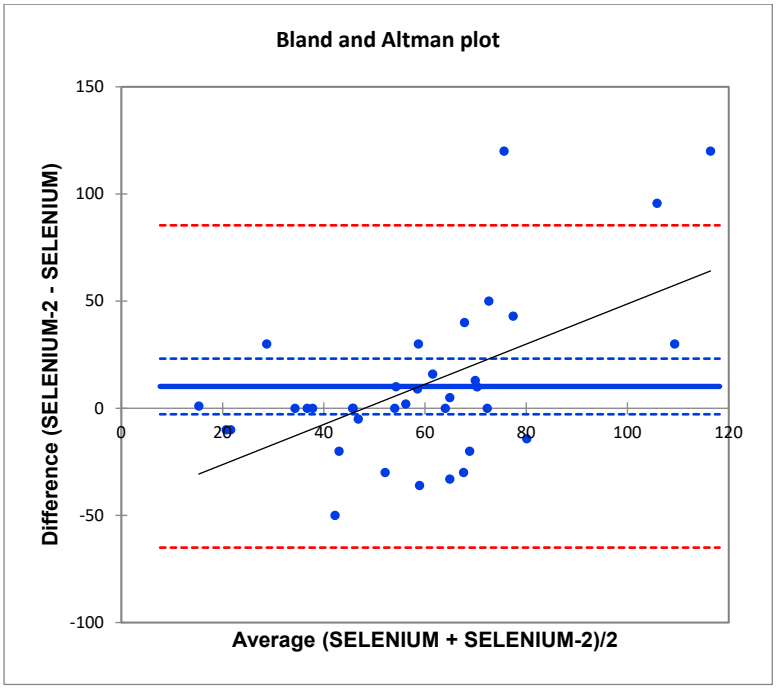


Figure A3.9. Bland Altman plot for food photograph and weighed estimates of selenium in participants' diet.

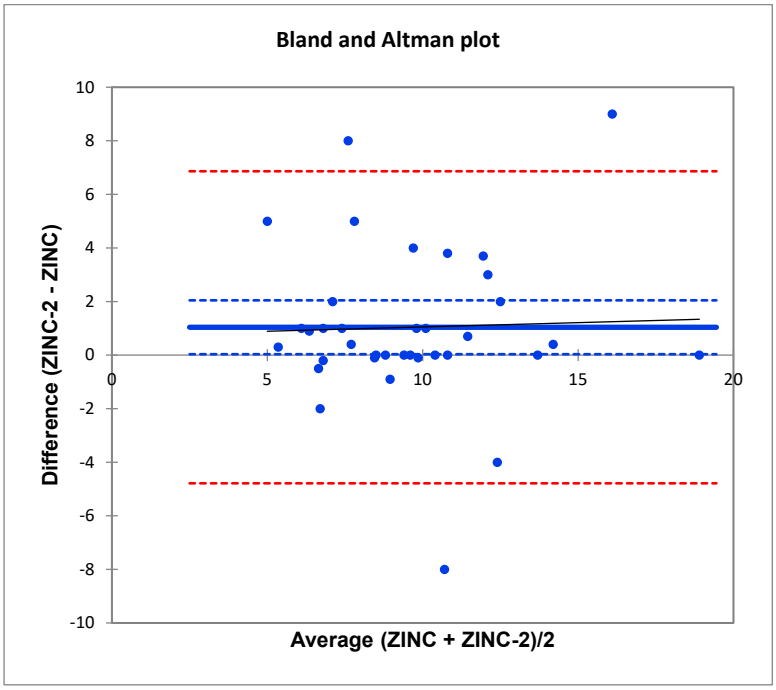


Figure A3.10. Bland Altman plot for food photograph and weighed estimates of zinc in participants' diet.

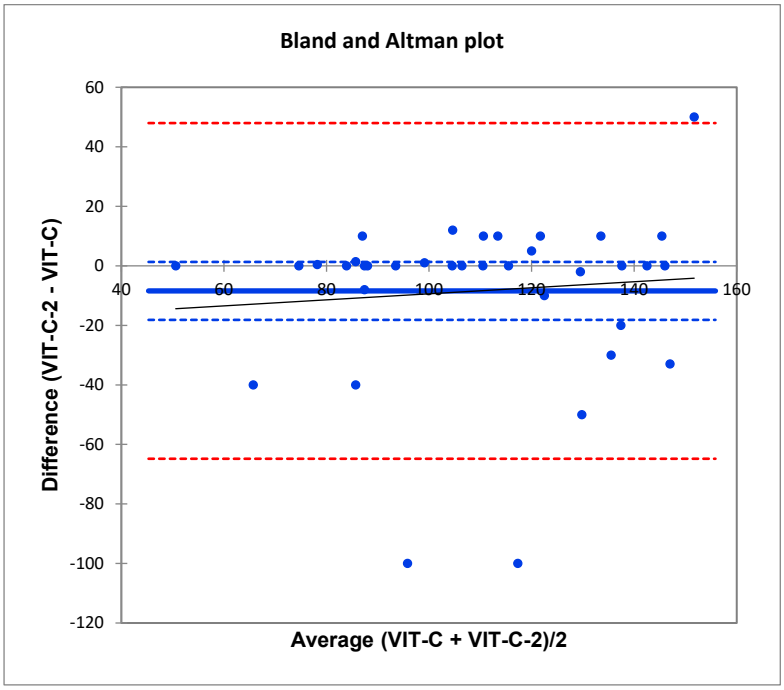


Figure A3.11. Bland Altman plot for food photograph and weighed estimates of vitamin C in participants' diet.

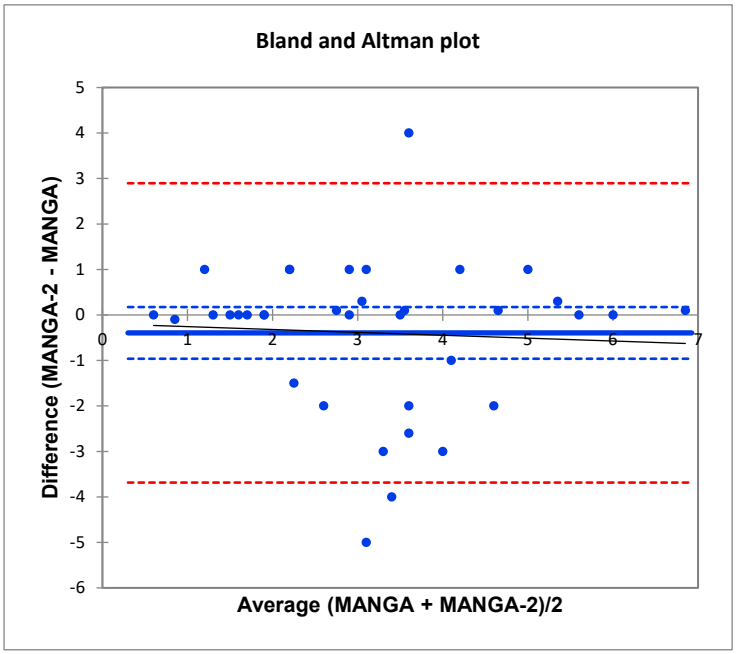


Figure A3.12. Bland Altman plot for food photograph and weighed estimates of manganese in participants' diet.

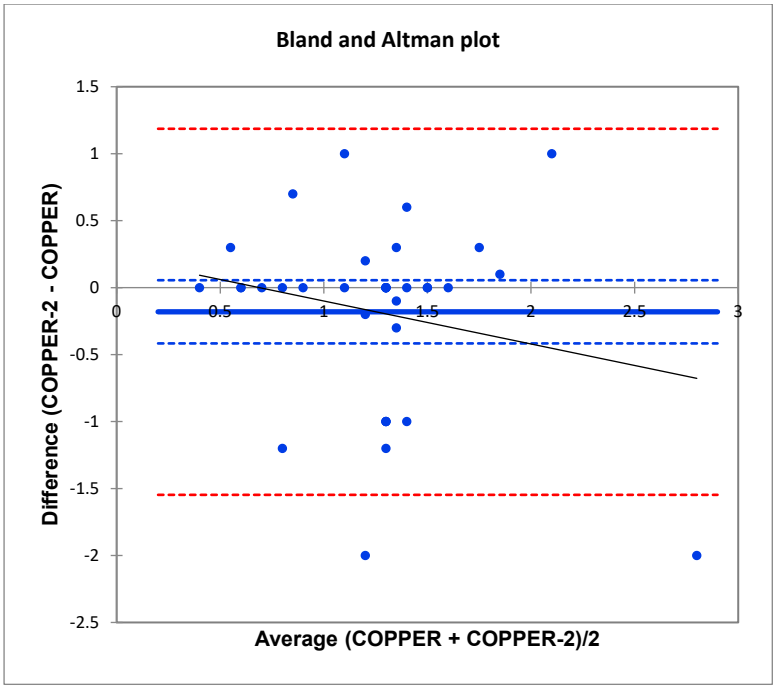


Figure A3.13. Bland Altman plot for food photograph and weighed estimates of copper in participants' diet.

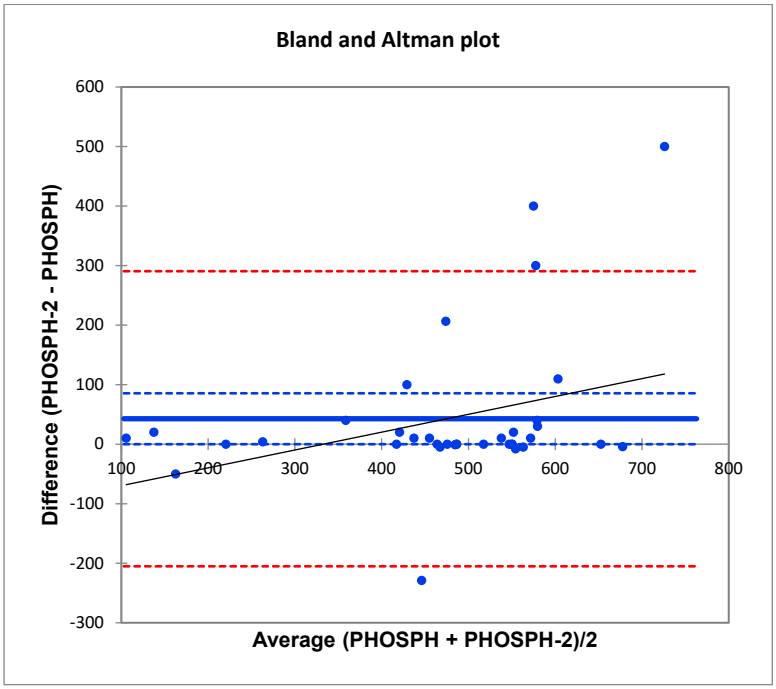


Figure A3.14. Bland Altman plot for food photograph and weighed estimates of phosphorus in participants' diet.

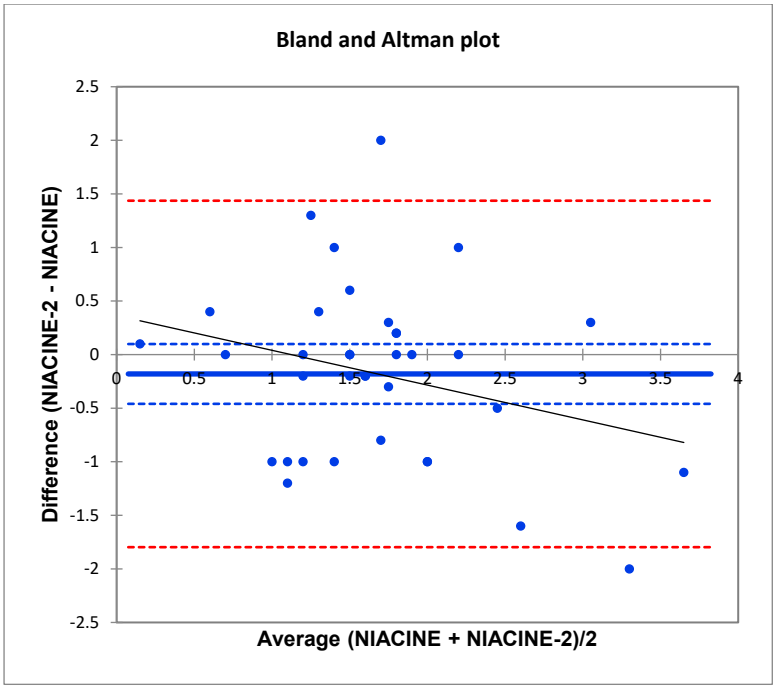


Figure A3.15. Bland Altman plot for food photograph and weighed estimates of niacin in participants' diet.

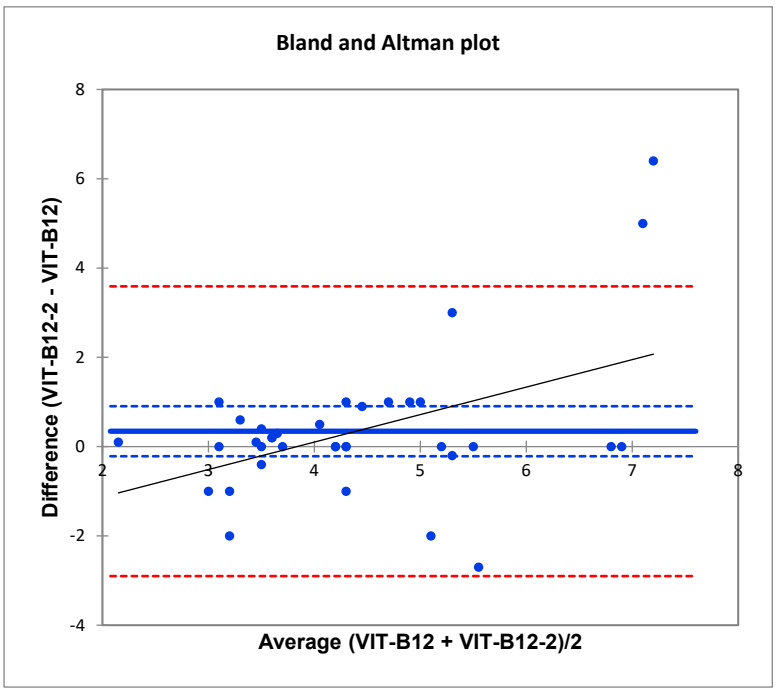


Figure A3.16. Bland Altman plot for food photograph and weighed estimates of vitamin B12 in participants' diet.

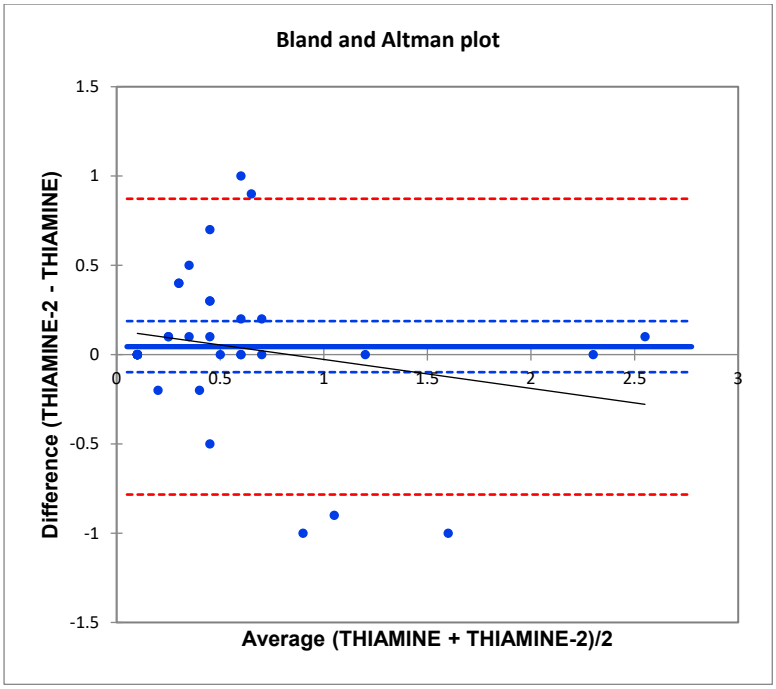


Figure A3.17. Bland Altman plot for food photograph and weighed estimates of thiamine in participants' diet.

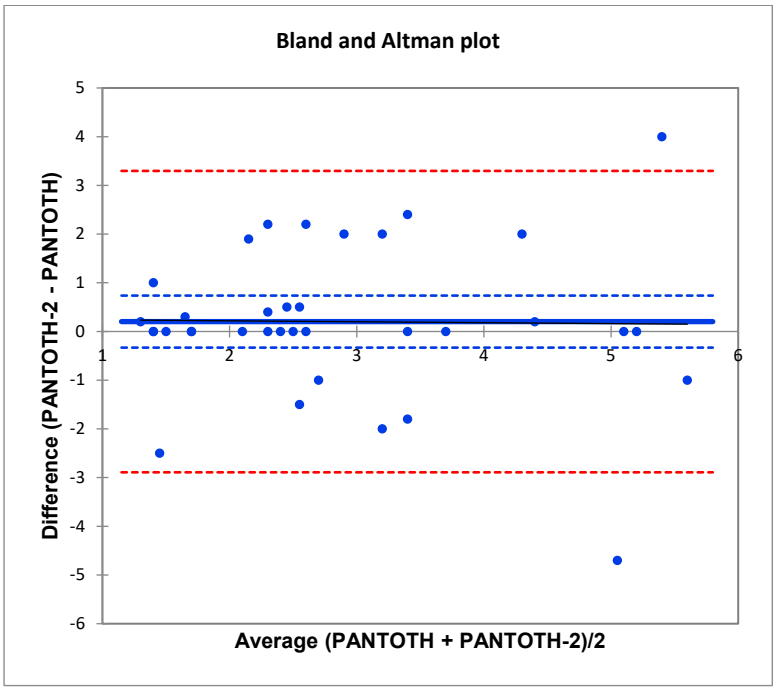


Figure A3.18. Bland Altman plot for food photograph and weighed estimates of vitamin B5 in participants' diet.

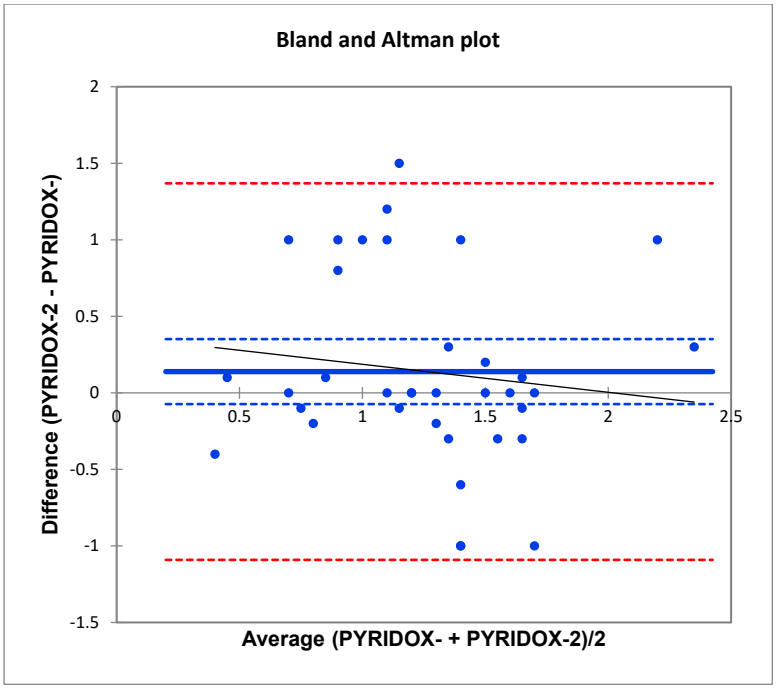


Figure A3.19. Bland Altman plot for food photograph and weighed estimates of pyridoxal phosphate in participants' diet.

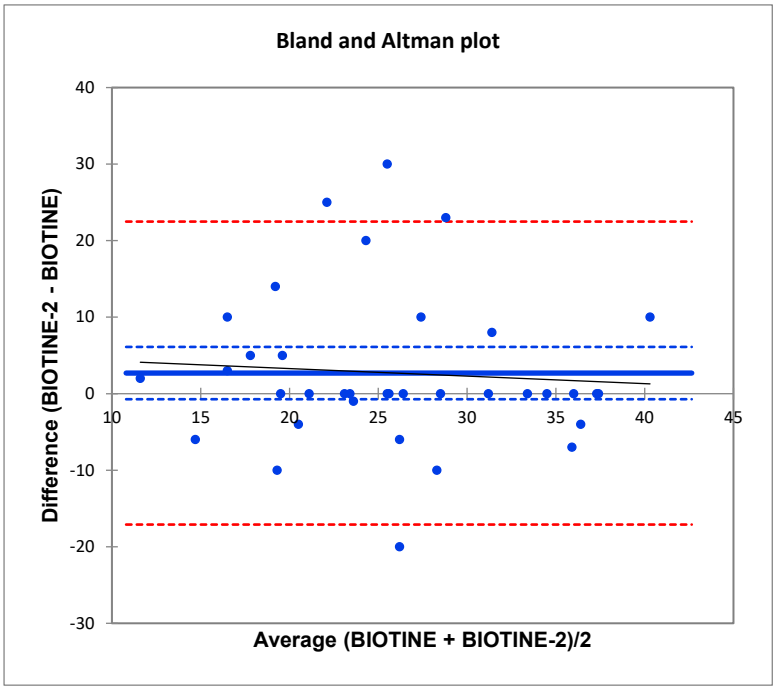


Figure A3.20. Bland Altman plot for food photograph and weighed estimates of biotin in participants' diet.

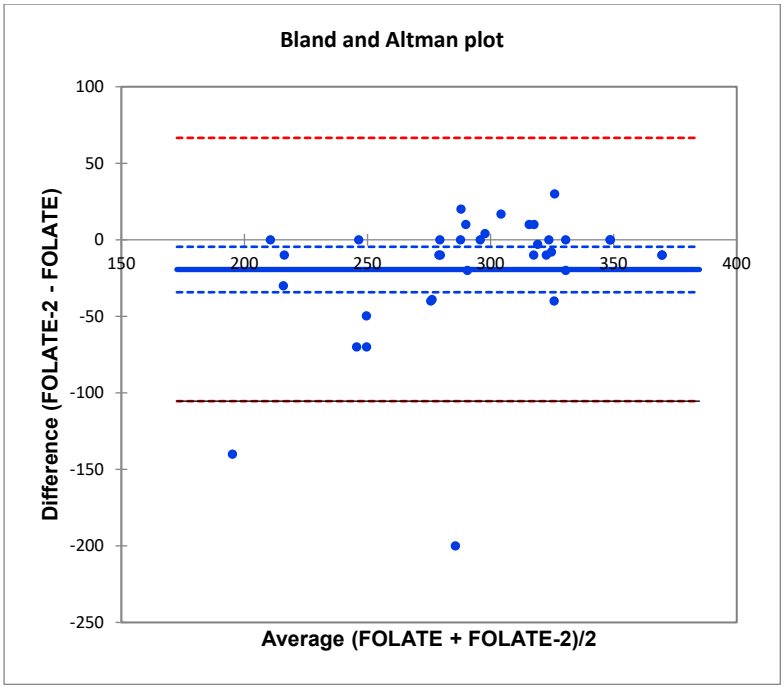


Figure A3.21. Bland Altman plot for food photograph and weighed estimates of folate in participants' diet

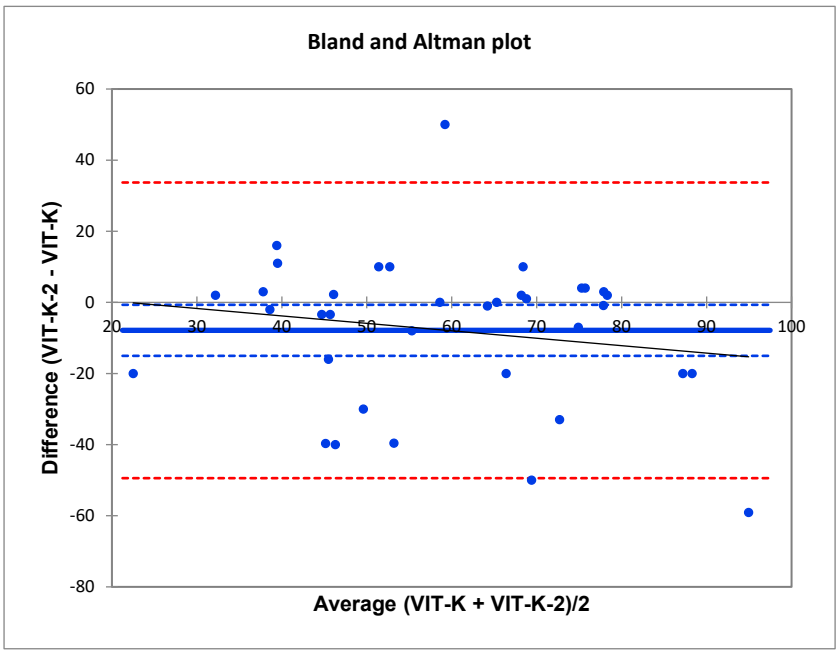


Figure A3.22. Bland Altman plot for food photograph and weighed estimates of vitamin K in participants' diet

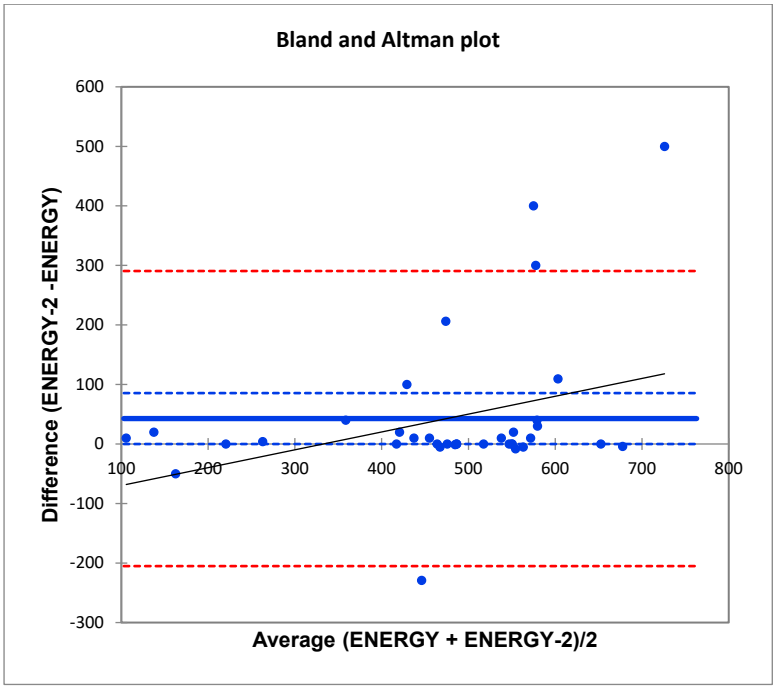


Figure A3.23. Bland Altman plot for food photograph and weighed estimates of energy intake in participants' diet

LEGEND FOR ALL THE FIGURES IN APPENDIX 3

Each Bland-Altman plot for food photography and weighed estimates of micronutrients in participants' diet showing the bias and limits of agreement. The graphs also indicate a trend line to show the tendency of the test method to overestimate or underestimate (———).

---------- = Limits of agreement; a range within which 95% of the differences between both measurements are included.

——— = Bias; the difference between the estimates obtained by both methods.

APPENDIX 6- The Simplified Nutritional Appetite Questionnaire

Simplified Nutritional Appetite Questionnaire (SNAQ)

Participant Number: _____

Are you male or female? Male ☐ Female ☐

What is your ethnic origin?

White	Mixed	Asian/Asian British	British/Black British	Other
British ☐	White/Black Caribbean ☐	Indian ☐	Caribbean ☐	Chinese ☐
Irish ☐	White/Black African ☐	Pakistani ☐	African ☐	
Other ☐	White & Asian ☐	Bangladeshi ☐	Other ☐	
	Other ☐	Other ☐		

Age: _____ **Weight:** _____ **Height:** _____

Date: _____

Please, complete the following questions by circling the correct answers.

1. My appetite is

a. Very poor
b. Poor
c. Average
d. Good
e. Very good

2. When I eat

a. I feel full after eating only a few mouthful
b. I feel full after eating about a third of a meal
c. I feel full after eating over half a meal
d. I feel full after eating most of the meal
e. I hardly ever feel full

3. Food tastes

a. Very bad
b. Bad
c. Average
d. Good
e. Very good

4. Normally I eat

a. Less than one meal a day
b. One meal a day
c. Two meals a day
d. Three meals a day
e. More than three meals a day

APPENDIX 7- The Global Physical Activity Questionnaire

GPAQ

Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. *[Insert other examples if needed].* In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

	Questions	Response	Code
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *[carrying or lifting heavy loads, digging or construction work]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 4*	P1
2	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days ⌊__⌋	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *[or carrying light loads]* for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 7*	P4
5	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days ⌊__⌋	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P6 (a-b)
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship. [insert other examples if needed]			
7	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days ⌊__⌋	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P9 (a-b)

Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure), [insert relevant terms].			
10	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like [*running or football,*] for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P 13*	P10
11	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P12 (a-b)

Continued on next page

GPAQ, Continued

Physical Activity (recreational activities) contd.			
Questions		**Response**	**Code**
13	Do you do any moderate-intensity sports, fitness or recreational (*leisure*) activities that causes a small increase in breathing or heart rate such as brisk walking,(*cycling, swimming, volleyball*)for at least 10 minutes continuously? *[INSERT EXAMPLES] (USE SHOWCARD)*	Yes 1 No 2 *If No, go to P16*	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational (*leisure*) activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P15 (a-b)
Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping. *[INSERT EXAMPLES] (USE SHOWCARD)*			
16	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs min s	P16 (a-b)

APPENDIX 8- The Perceived Stress Scale

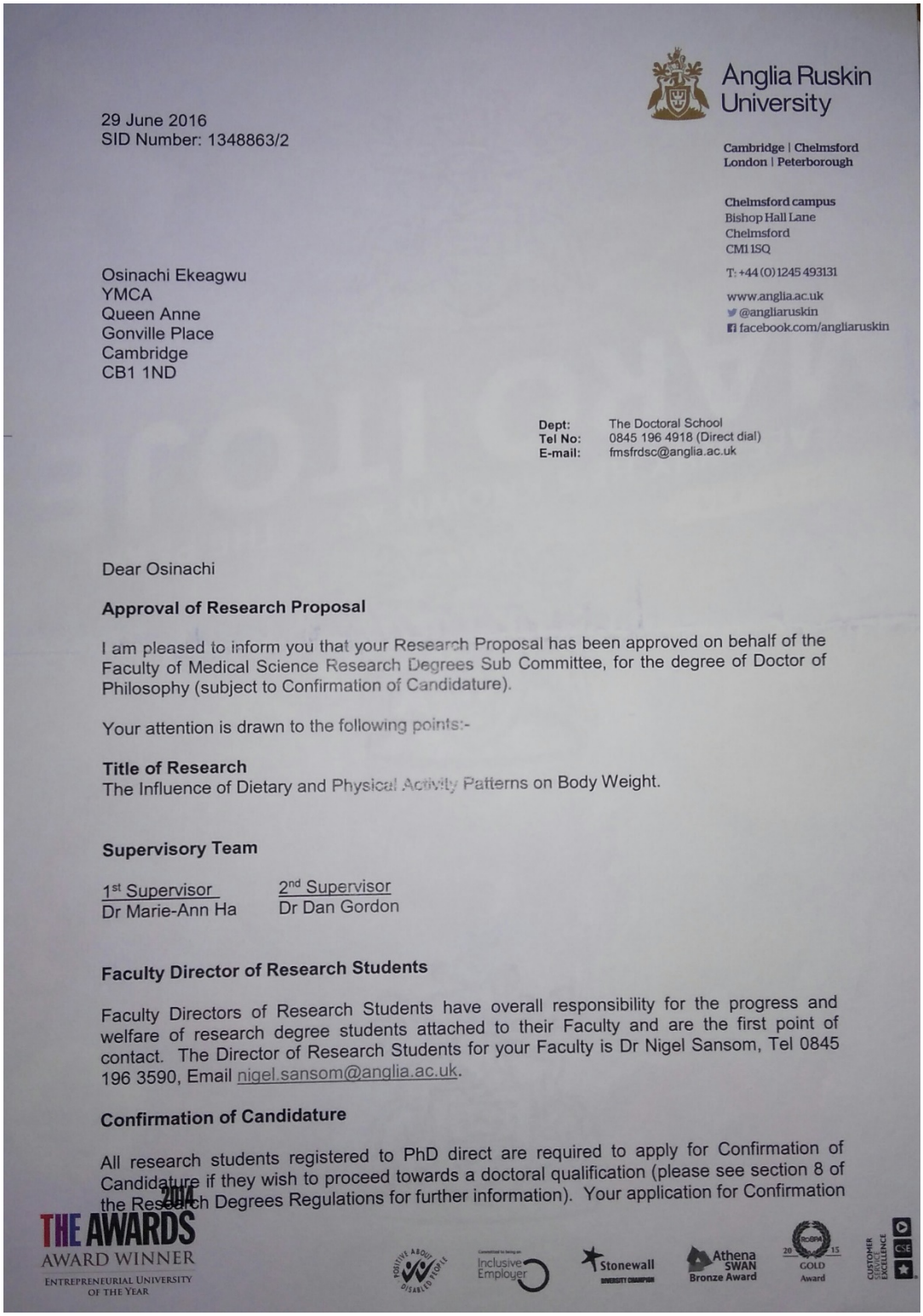
PERCEIVED STRESS SCALE

The questions below ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1.	In the last month, how have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2.	In the last month, how often have you felt that you were unable to control important things in your life?	0	1	2	3	4
3.	In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4.	In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5.	In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6.	In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7.	In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8.	In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9.	In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10.	In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

APPENDIX 9- Research Ethics Approval for Cohort Study



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29 June 2016
SID Number: 1348863/2

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Queen Anne
Gonville Place
Cambridge
CB1 1ND

Dept: The Doctoral School
Tel No: 0845 196 4918 (Direct dial)
E-mail: fmsfrdsc@anglia.ac.uk

Dear Osinachi

Approval of Research Proposal

I am pleased to inform you that your Research Proposal has been approved on behalf of the Faculty of Medical Science Research Degrees Sub Committee, for the degree of Doctor of Philosophy (subject to Confirmation of Candidature).

Your attention is drawn to the following points:-

Title of Research
The Influence of Dietary and Physical Activity Patterns on Body Weight.

Supervisory Team

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Faculty Director of Research Students

Faculty Directors of Research Students have overall responsibility for the progress and welfare of research degree students attached to their Faculty and are the first point of contact. The Director of Research Students for your Faculty is Dr Nigel Sansom, Tel 0845 196 3590, Email nigel.sansom@anglia.ac.uk.

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APPENDIX 10- Histogram, Normal Q-Q Plot and Box Plot for Body Fat Percentage Data Distribution.

Figure A10-1. Histogram

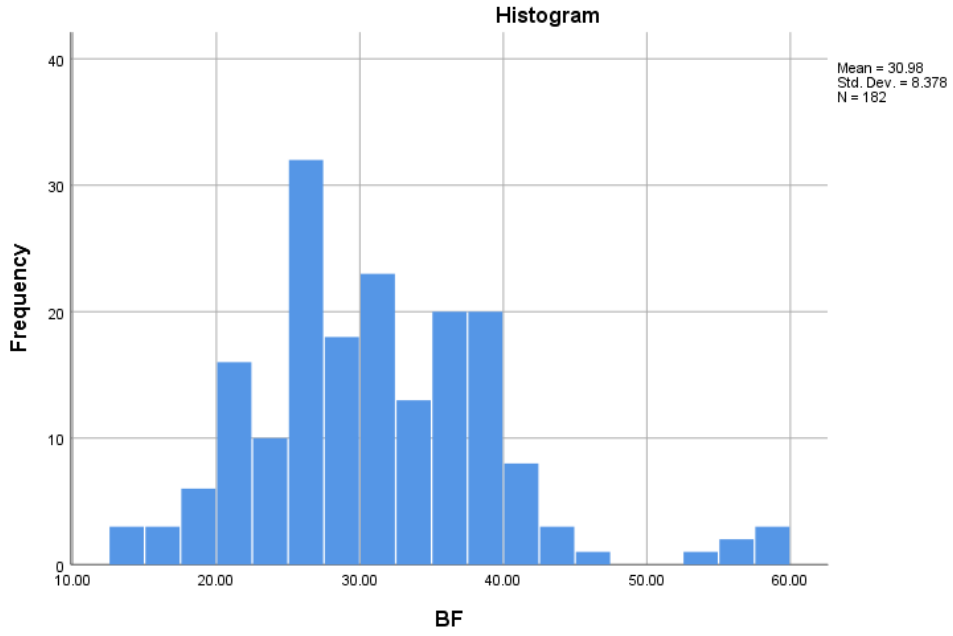


Figure A10-2- Normal Q-Q plot

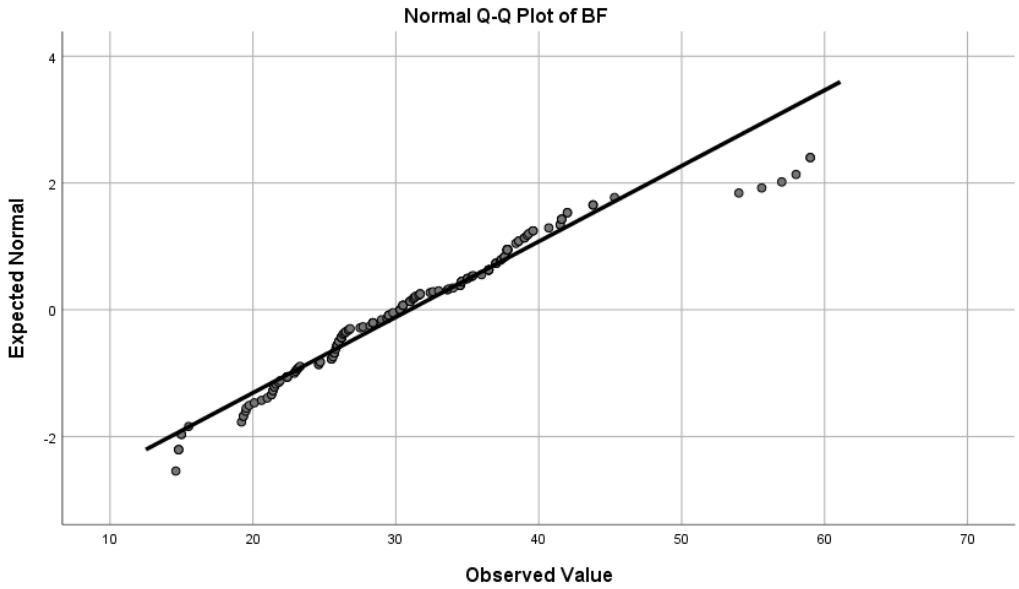
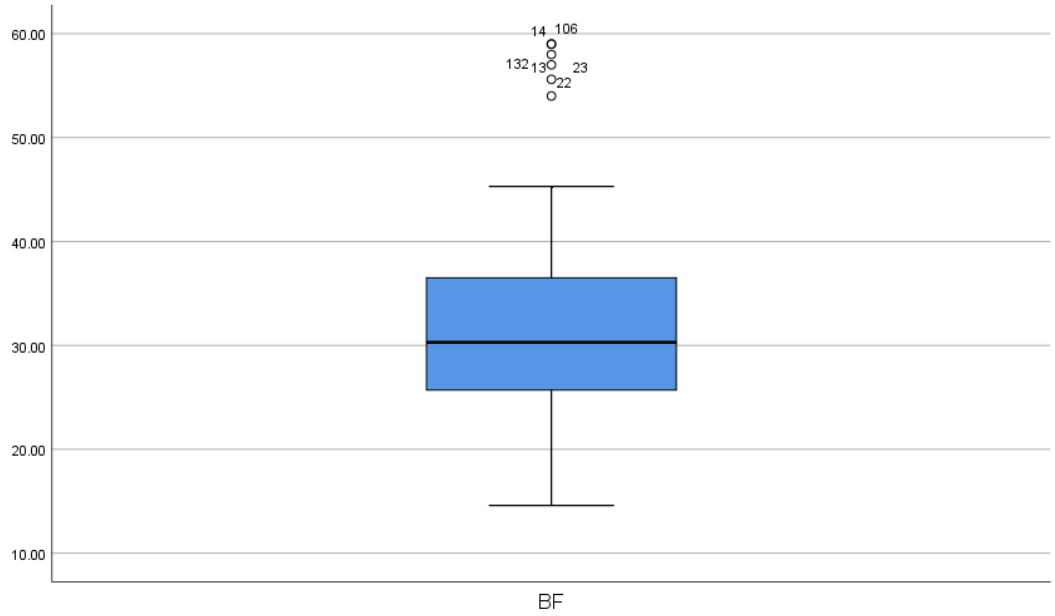


Figure A10-3. Box plot



APPENDIX 11- Tables for the proportion of cohort study participants on a special diet based on age and gender, and the covariates assessed in the cohort study.

Table A13.1. The proportion of cohort study participants on a special diet based on age and gender.

	Special Diet	
	Vegetarian n (%)	**Non-vegetarian n (%)**
Gender		
Male	8 (13.3)	52 (86.7)
Female	9 (18.7)	39 (81.3)
Age		
18- 29	12 (14.8)	69 (85.2)
30-39	5 (18.5)	22 (81.5)
Total	17 (15.7)	91 (84.3)

APPENDIX 12- Table for the energy intake among participants categorised based on the average hours of night-time sleep, cigarette smoking status, alcohol intake and appetite (Table A12.1) In Table A12.1 shows that the estimates for those who slept for less and at least 7 hours each night were statistically significantly different ($p<0.03$).

Table A12.1. Energy intake among participants categorised based on the average hours of night-time sleep, cigarette smoking status, alcohol intake, and appetite.

Dietary Component	Sleep		Cigarette Smoking		Alcohol Intake		Appetite	
	<7 hours	≥7 hours	No	Yes	No	Yes	≤14	>14
Energy (kcal)	2232.9 ± 262.2*	2208.1 ± 241.5	2176 ± 213.6	2172 ± 211.9	2242.9 ± 257.3	2212 ± 234.6	2232.4 ± 267.4	2193.1 ± 224.4
Estimates with an asterisk (*) are statistically significant.								

APPENDIX 13- Charts of the linear correlation between body fat percentage and dietary phytate intake at baseline and at 6 months showing the correlation coefficient and corresponding p-value.

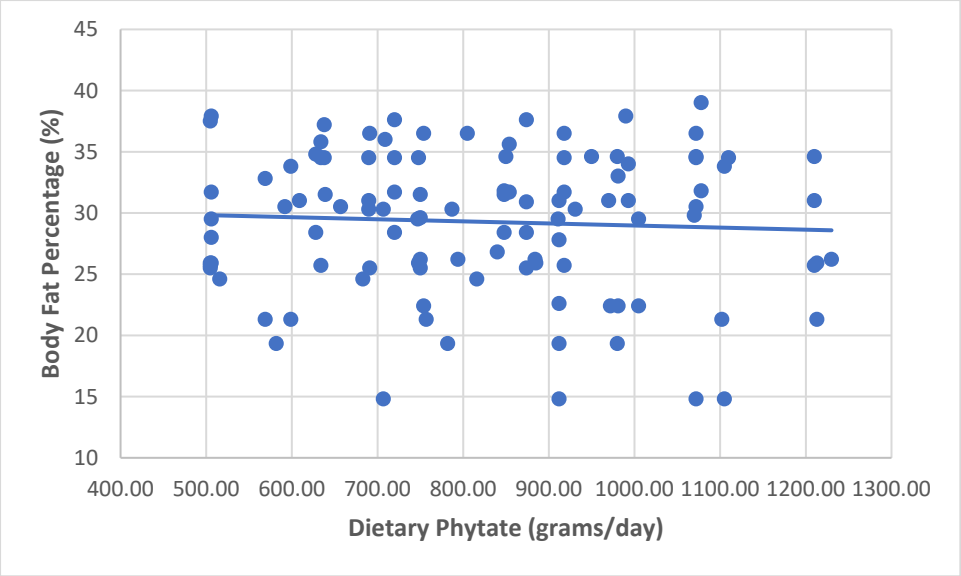


Figure A13a: A chart showing the linear relationship between dietary phytate and body fat percentage at baseline (r = - 0.06; p=0.61).

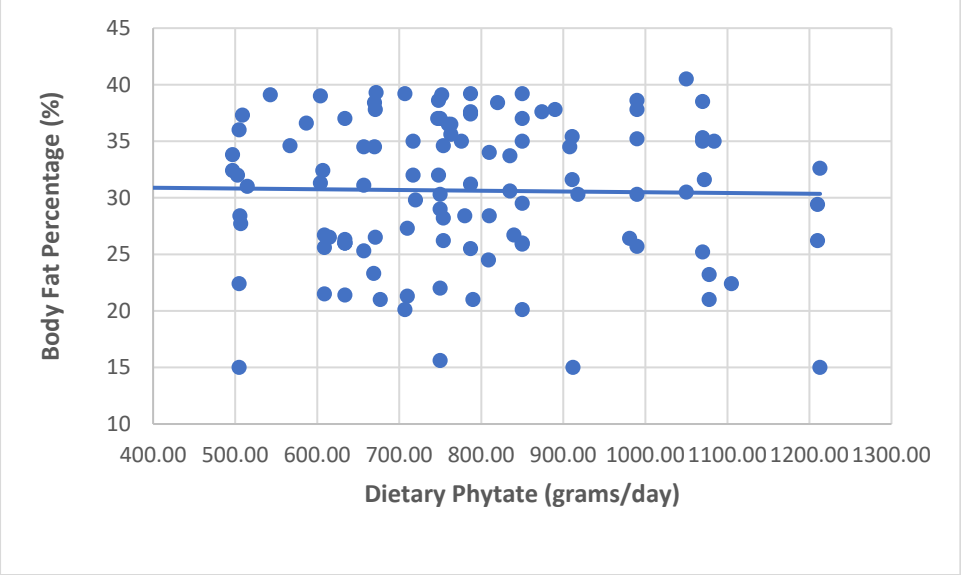


Figure A13b: A chart showing the linear relationship between dietary phytate and body fat percentage at 6 months (r = -0.02; p= 0.85).