Dietary diversity and micronutrient intake in women in North West UK and North West Pakistan: the impact of phytate

by

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Abstract

Micronutrient malnutrition continues to be the major public health problem globally, especially in South Asian (SA) countries including India, Pakistan, Bangladesh (Akhtar *et al*.,2013, Tidemann-Andersen *et al*., 2011). Currently, global statistics indicate a widespread increase in iron, zinc, vitamin A deficiencies among vulnerable populations(Sharma, 2003) Also, it is important to consider the bioavailability of micronutrients from the diet (Frontela et al., 2011) as cereal based staple diets consumed in SA countries contain high amount of phytates, which are known for inhibiting micronutrient absorption. Considering this, the purpose of the present study was to assess dietary diversity among SA women in northwest UK (NW UK) and northwest Pakistan (NW PK), along with investigating the amount of micronutrients (iron, zinc) and inhibitory phytates present in the flour used to make dietary staples from both the study locations. In addition, the micronutrient status (iron and zinc) of women in NW PK was assessed.

Dietary data from 40 female participants (18-30 years) from NW PK and 15 participants (18-30years) was obtained using three 24 hour dietary recalls. These were used to calculate the dietary diversity score for the women (WDDS). Wheat flour samples obtained from both the geographical regions and these were analysed for their iron and zinc content by atomic absorption spectrophotometry and for total phytate content by a spectrophotometric method. Finally to explore micronutrient status of women, blood samples were obtained for the participants in NW PK and analysed for haemoglobin and plasma zinc concentration.

The results revealed that the WDDS for participants across two study regions were significantly different ($p<0.05$), with those in the UK having a significantly higher WDDS (range: 4.33-5.06 in the UK vs 2.55-3.02 in PK). Analysis of flour samples revealed that the

phytate content of the flour used in the PK community ranged from 230 – 565 mg/100g. This is comparable in terms of phytate content to flour obtained from SA shops in NW UK, which ranged from 273 mg/100g for white flour to 584 mg/100g for wholemeal flour. The average iron and zinc content of the PK flour was also similar to that of wholemeal flour purchased in the UK. Biochemical measures of zinc and iron status of the NW PK women revealed they are likely to be deficient in both of these trace minerals.

In conclusion, the study highlighted the paucity of the diet consumed by SA women living in NW PK compared with that of SA women living in NW UK. The low dietary diversity, particularly the infrequent consumption of meat or fish, is likely to have contributed to the poor micronutrient status of the PK women. Strategies to improve this could include demonstration kitchens to provide basic nutritional information on how to make best use of available, affordable foods in order to improve dietary diversity in this community.

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Abbreviations

AAS – Atomic Absorption Spectroscopy

AF PK – Abaseen Foundation Pakistan

AF UK – Abaseen Foundation United Kingdom

HC – Health Centre

DDS – Dietary Diversity Score

DoS – Director of studies

EAR – Estimated Average Requirement

FAO - Food and Agriculture Organization of the UN

FATA - Federally Administered Tribal Area

FVS – Food Variety Score

GR – Guaranteed Reagent

HAZ – Height for Age Z scores

HDDS – Household dietary diversity score

iINSAFSs - International Institute of Nutritional Sciences and Food Safety Studies

KP – Khyber Pakhtunkhwa

LRNI – Lower Reference Nutrient Intake

NHANES - National Health and Nutrition Examination Survey

NW – North West

PK – Pakistan

RNI – Reference Nutrient Intake

SA – South Asia

STEM - Science, Technology, Engineering and Medicine

UCLan – University of Central Lancashire

UK – United Kingdom

UNICEF - United Nations International Children's Emergency Fund

WDDS – Women dietary diversity score

WFP – World Food Program

WHO – World Health Organisation

1 Chapter One: Introduction

1.1 Significance of micronutrients for human health

Micronutrients such as iron, zinc, calcium, iodine, and selenium although required by the body in small amounts, are necessary for maintaining optimal health. Their deficiencies can have long-lasting impact on an individual’s overall functionality. There are two classes of micronutrients, vitamins and minerals. Each vitamin and mineral performs specific structural and/or functional roles. In its structural roles, zinc helps to fold proteins into functional shapes and also maintains integrity of some proteins (Insel et al., 2013, Saunders et al., 2012). Functional roles include acting as co-enzymes required for metabolism. For example, zinc is the cofactor for over 300 enzymes, and selenium is required in the form of seleno-cysteine within the enzyme glutathione peroxidise (Shekin 2006).

Different foods contain different levels of vitamins and minerals, so it's important that a wide variety of foods are consumed from the different food groups and a variety within each food group, to ensure an adequate supply of all the micro-nutrients to meet body`s needs.

1.2 Significance of Iron for Human Nutrition

Iron is a part of haemoglobin molecule which accounts for two-thirds of the body's iron content (Barasi, 2002). The majority of body iron (60-70%) is utilized within haemoglobin in circulating red blood cells (Andrews, 1999). Also 20-30% of body iron is stored in hepatocytes and in reticulo-endothelial macrophages. To a large extent this is within ferritin and its degradation product hemosiderin. Rest of the body iron is present in myoglobin cytochromes and iron containing enzymes.

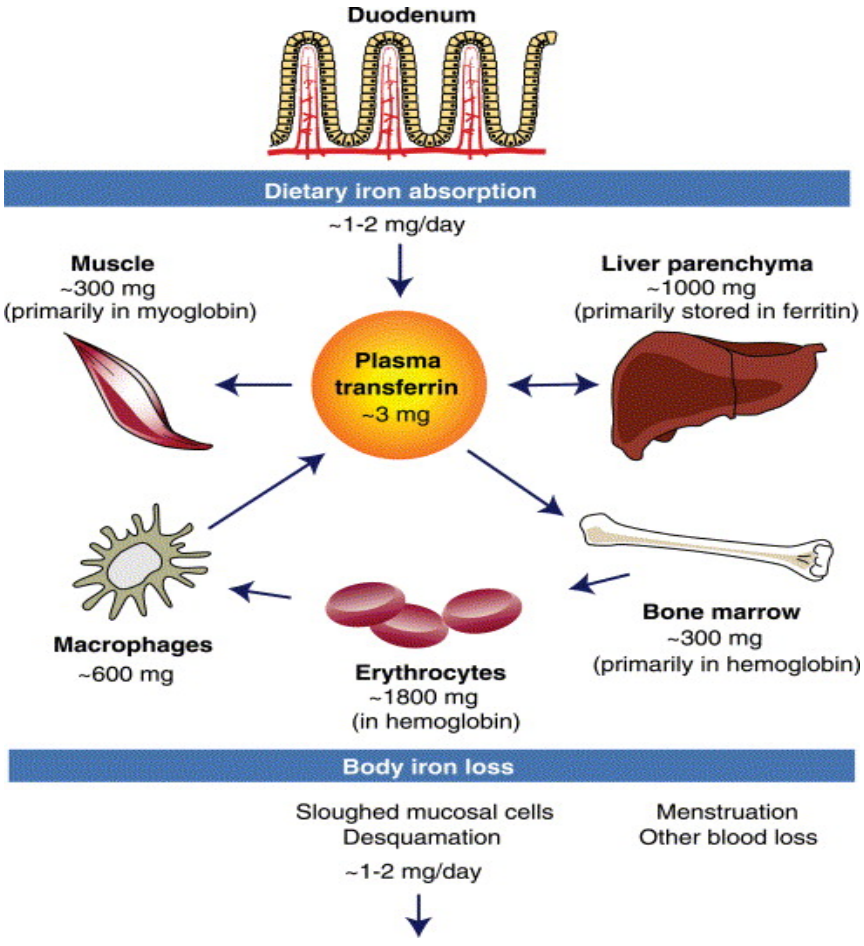


Figure 1-1Distribution of Iron in the body(Papanikolaou, et al., 2005)

As seen in the figure 1-1 healthy individual usually has been found to absorb 1-2mg of iron from the daily diet. However body loses its iron due to various processes like sloughed mucosal cell desquamation (1-2mg/day), losses during menstruation and also other blood

losses. Erythropoeisis requires approximately 30mg/day of iron and this is provided by recycling of iron via reticulo-endothelial macrophages. In blood the pool of transferrin bound iron (~ 3mg) is very dyanamic and undergoes changes several times. It is in this pool that the macrophages ingest red blood cells and release iron to circulating transferrin (Papanikolaou et al., 2005).

There is an inverse relationship between body iron stores and amount of iron absorbed by the body. In a study conducted by Hunt et al., (2003), it was reported that non- heme iron absorption is very limited in people with high iron stores, while this absorption is increased drastically in people with low iron stores.

Table 1-1Dietary reference values for iron (mg/day) for UK

Age group (both girls and boys)	LRNI[a] mg/day	EAR[b] mg/day	RNI[c] mg/day
15-50 years (females)	8.0	11.4	14.8
Pregnancy	No increase	No increase	No increase
Lactation	No increase	No increase	No increase

a – lower reference nutrient intake

b – Estimated Average Requirement

c – Reference Nutrient Intake

Source: Geissler and Powers, 2011

1.3 Dietary sources of iron

Various foods in each food group contain varying amounts of iron. While classifying foods as good sources of dietary iron, it is important to consider the bioavailability of iron from these food items in addition to the total amount of iron present. In terms of both, quantity and bioavailability, beef, red meat and liver are excellent dietary sources of iron. Other good sources of iron include oysters, lentils, shrimps, spinach, lima beans and whole grain cereals. Apart from these, certain cereals and millets typically consumed in South Asian countries like India, Pakistan contain good amount of iron. Cereals like wheat flour (whole), pearl millet, rice flakes and vegetables like cauliflower greens and amaranth leaves are moderate sources of iron.

1.4 Causes of Iron Deficiency Anaemia

Iron deficiency anaemia is multi-factorial. However, various studies have established certain common causes of anaemia which can be stated as follows:

1. **Low dietary intake** – Low dietary intake is the commonest cause of iron deficiency anaemia. In south Asian countries like Pakistan, Bangladesh, India, the diets are usually plant-based diets. These cereals does not contain good amount of iron with few exceptions. Moreover these diets are quite monotonous and contain very minimal amount of animal sources like meat, beef, liver, which are high sources of good quality iron.
2. **Impaired absorption** – This is concerned with the physiological state of the individuals. Diseases related to or those affecting gastrointestinal tract like helicobactor pyroli, inflammatory bowel disease infections can inhibit iron absorption. Another aspect that needs to be understood that is the regulation of dietary iron absorption by hepcidin. Hepcidin is a hormone, produced by the liver, which

decreases iron absorption when its levels are increased due to any kind of gastrointestinal inflammatory disorder (Nemeth, 2004). If the level of hepcidin is increased then the inorganic iron from the diet is not absorbed optimally, instead it gets trapped in intestinal epithelial cells and then removed out of the body via the stools (Anderson et al., 2009, Miller, 2013). This can subsequently lead to iron deficiency anaemia. Since this is caused due to inflammation, it is also referred as anaemia of inflammation.

3. **Increased blood losses** – Blood losses can contribute significantly to iron deficiency. Some of the reasons for blood loss include cow's milk enteropathy, menstruation and blood losses during delivery.
4. **Increased requirements of iron –** Iron requirements tend to increase in certain stages in life-cycle in order to cope up with the dynamic changes taking place in these stages.
    a. <u>In Pregnancy</u> - Iron is one of the essential micronutrients that play vital role during pregnancy and post-partum. The physiological requirement of iron in pregnancy is 3 times higher as compared to the requirements of non-pregnant women. According to Tapiero et al.,(2001) by the end of the pregnancy period , the women must have acquired approximately 1200mg of iron, either from the body iron stores or from diet. This is important to meet the increased requirements of iron for expansion of circulating red blood cells in mother as well for meeting the requirements of a developing foetus. However these increased requirements are mostly not met and thus leading to iron deficiency anaemia observed commonly in pregnant women. Denic.S and Agarwal.M.M (2007) have highlighted the statistics of anaemia in developing countries in order to emphasize the need to meet the increased requirements. According to them 56% of pregnant

women and 41% of non-pregnant women are anaemic. More specifically the statistics in South Asian region indicate that as high as 62% of pregnant women have iron deficiency anaemia. The reasons for this are complex, however the monotonous cereal based vegetarian diet is one of the major factors contributing to anaemia. Furthermore the researchers have also suggested that even though these women may enter into pregnancy state with sufficient iron stores, these stores decline considerably as pregnancy proceeds from the first trimester onwards. The dietary intakes are not sufficient to meet the increased requirements during second and third trimesters. Maintaining adequate haemoglobin levels during pregnancy is crucial. The significance of adequate haemoglobin levels has been elucidated in a study by Scanlon et al., 2000 where low haemoglobin levels during first and second trimester were associated with risk of preterm birth, while high haemoglobin levels resulted in small for gestational age babies. So this implies that both high and low haemoglobin levels can lead to unpleasant postnatal consequences and due to this, it is essential to adhere to optimal haemoglobin levels during pregnancy.

b. Adolescence - Adolescence is a life-stage with rapid growth and development. Early adolescence (15-18 years) is period of dynamic hormonal changes and also attainment of sexual maturity. Researchers (Oner et al., 2005, Chiplonkar and Tupe, 2010) have highlighted the influence of eating habits on the micronutrient status of adolescents. This inference was drawn from a study conducted by Oner et al (2005) with an aim to obtain data on nutritional intake of adolescent girls in Turkey. The data obtained using a 3 day self-reported food records indicated that the macronutrient intakes were adequate, however their micronutrient intake was

found to be much lower than the recommended values. The possible explanation for this observation was given later in 2007 by Rao and colleagues from their study in 164 adolescent girls from different schools in Hyderabad, India. Their results revealed that adolescents tend to have increased consumption of unhealthy fast foods, carbonated beverages, foods with high fat and sugar content and this predisposes them to deficiencies of vital minerals like iron and zinc. Growth spurt and onset of menstrual cycle significantly increases the requirement of iron in adolescent girls. Other studies in the context of iron status of adolescents have attributed the increased requirement of iron to rapid growth and menarche, low intake of iron-rich foods, inappropriate dietary choices, intestinal parasitic infestation and frequent consumption of beverages like tea and coffee (Bagchi, 2004, AI-Sharbatti et al., 2003, Mousa et al., 2003, Joharah and Al-Quaiz., 2001, EI-Sahn et al., 2000). Moreover, in developing countries like Pakistan, this age group also marks the beginning of child bearing years due to early marriageable age, typically 14 years in rural areas. So, by this stage, these girls may not be able to achieve sufficient iron stores to meet the increased requirements during pregnancy. Emphasizing the importance of iron in this adolescent age group, Tapiero et al (2001) points out that low iron stores in these young women can have serious health impact on pregnancy and its outcomes.

1.5 Concept of Bioavailability

Bioavailability of minerals can be defined as the proportion of total minerals in the food, meal or diet that is absorbed and utilized for normal body functions. Lestienne et al., (2005)

defined bioavailability as ‘the presence of the nutrient in the form that is available for absorption by enterocytes’. Gibson et al., (2006) defined bioavailability as ‘the proportion of an ingested trace element in the food that is absorbed and utilized for normal metabolic and physiological functions or storage’

Bioavailability of any mineral involves various stages and each stage is affected by various dietary and physiological factors.

The stages of bioavailability and the factors that affect them are as follows:-

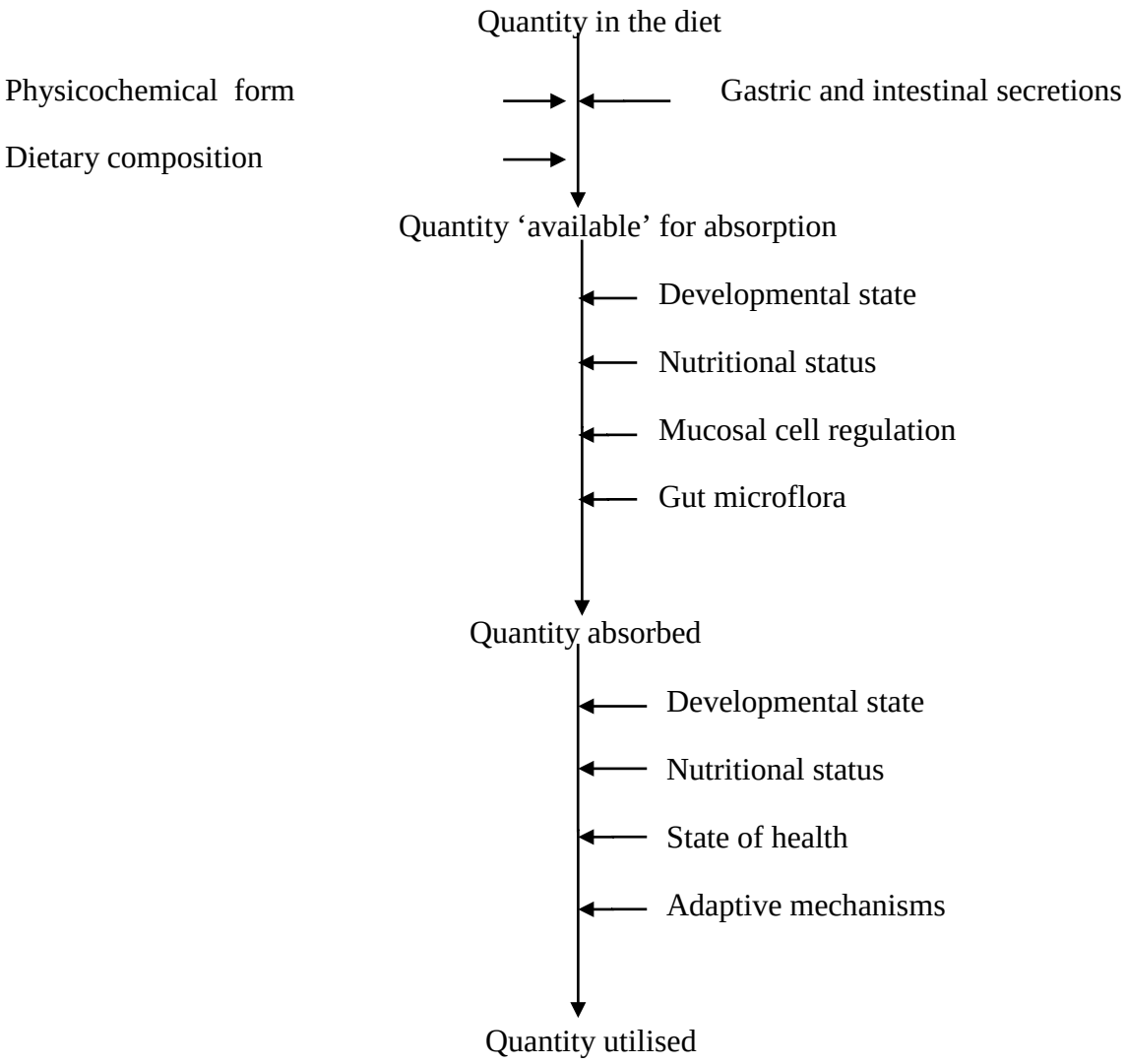


Figure 1-2Stages of bioavailability

Source: Fairweather- Trait, (1992)

As seen in the (Fig 1-2) there are different stages wherein the bioavailability of a mineral is affected. Also at each stage the bioavailability depends on various factors that influence the final utilization of that mineral in the body.

With respect to mineral availability many studies have established that there are innumerable factors which reduce intestinal absorption and thus resulting in high rates of iron and zinc deficiency especially in infants, children and women of child bearing age. In a study

conducted by Lestienne et al,. (2005) it was reported that the mineral absorption is influenced by level of mineral contents and by factors that enhance their absorption in the diet.

1.6 Factors Affecting Iron Absorption

According to another study conducted by Gibson et al., (2006) it was reported that factors affecting bioavailability include both dietary and host related factors.

Diet related factors include the chemical form of nutrient in the food, nature of food matrix, interactions occurring between nutrients and other organic components within the plant food and the pre-treatment of food during processing and/or preparation. Among these, chemical form of iron (haem or non-haem form) and food matrix significantly affects iron absorption in the body. Literature exists which suggests that haem iron (form of iron present in meat and meat products) accounts for approximately 10% of the total iron consumed. However even the total amount of haem iron in the diet is low, the percent absorption into the body is as high as 50% (Bohn et al., 2008).Data on iron absorption rates suggest that the percent of haem iron absorption is approximately 15-40% while that for non-haem it is 1-15% (Hunt, 2003, Roughead and Hunt., 2000, Hallberg et al., 1997) More work to gain clarity about higher absorption rates of haem iron has led to identification of specific receptors on the microvilli of the enterocytes and due to these the iron is easily absorbed and split from the complex by haem oxygenase (Bohn et al., 2008, Krishnamurthy et al., 2007, Worthington et al., 2001, Raffin et al., 1974).

Another important factor that affects iron absorption is the food matrix. This impact was observed in a study where the in-vitro non-haem iron solubility from typical Indian composite diets in Andhra Pradesh, West Bengal, Madhya Pradesh and Gujarat was estimated. It was found that iron solubility and phytate content were inversely proportional. In vitro solubility of iron from these meals decreased from 7.9 to 1.52% as phytate content

increased from 0.3 to 1.3g/day. Further rice based meals containing low iron and low phytate had better availability as compared to bajra based meals containing high iron and high phytate (ICMR,2000; Nair & Iyengar, 2009).

Host related factors include physiological status of the subjects, such as age, disease or stores of the mineral. From these, iron stores of an individual have shown to have considerable impact on iron absorption. Decreased body iron stores are associated with increased intestinal absorption of iron and vice-versa. Further, iron absorption may be increased in some pathological conditions like anaemia and haemorrhage, and in the physiological conditions such as menstruation, pregnancy and lactation. Similarly, with increasing iron stores, the absorption of iron is reduced to the level needed to cover the daily requirements.

Apart from this, disease states or any kind of functional abnormality seems to affect iron absorption through an indirect impact on iron bioavailability. Earlier studies suggest that after partial gastrectomy, there is a decrease in the bioavailability of non-haem dietary iron. The magnitude of the decrease depends on the type of gastric operation performed (Hallberg., 1981, Magnusson et al., 1979, Magnusson et al., 1976).

1.7 Assessment of iron status

There are various techniques used to assess iron status in individuals across the globe. However there is a lack of 'gold standard' which can be used to confirm the exact iron status, as each of the techniques used have some limitations. So it has been suggested that various laboratory techniques should be used in combination to get better understanding of physiological iron levels.

However for the present study Haemoglobin levels have been used as an indicator for assessing the prevalence of iron deficiency anaemia because of following reasons:-

1. Simplicity of measurement considering different geographical settings(urban and rural)
2. It has been regarded as the long term marker of individual's iron status
3. It is a stable biomarker and can be suitably used to assess iron status irrespective of age and gender
4. The apparatus required for assessment is minimal an so is extremely feasible for field/population studies

Source: Gibney et al., 2009

1.8 Significance of Zinc for Human Nutrition

There is no dearth of literature which has highlighted various vital functions of zinc over the years. Kristensen et al., (2006) reported that zinc is a part of substantial number of enzymes which have significant biological functions. More elaborative data on importance of zinc was stated by Gibson, (2006), which suggests that zinc is required for the activity of more than 100 enzymes that are a part of major metabolic pathways, thus indirectly affecting the entire functioning of the body. More precisely, Prasad (1995) stated that zinc is required by over 300 enzymes for their activities. This explains the role of zinc in nucleic acid metabolism, DNA synthesis, cell division, and protein synthesis. In addition to this, zinc is also a part of structure and functions of proteins which means that it is present as an important element in the various transcription factors, hormonal receptor sites and biologic membranes. Because of its ability to participate in strong but readily exchangeable ligand binding, it interacts with wide range of organic ligands and subsequently gets incorporated into various biological systems. The structural role of zinc has been established long back in eighties where it was reported that zinc is a part of certain intracellular proteins such as alcohol dehydrogenase, superoxide dismutase, keratin(hair)among others (Williams 1984) and plays an important role

in maintaining the structural and functional integrity of cellular membranes (Bettger and Odell 1981). More recently, the role of zinc as a part of zinc fingers has been highlighted (Klug 2010). The term 'zinc fingers' refers to various types of protein motifs. These fingers play pivotal role in correct positioning and binding of transcription factors to DNA (Thuren and Hay, 2006). This binding initiates the transcription process (Niragu, 2007).

Apart from these vital roles at molecular level, zinc is important from the point of view of social concern. It is the ubiquitous nature of zinc in biological systems that makes it important to explore the widespread consequences of zinc deficiency observed in vulnerable populations. Zinc deficiency on a broader spectrum affects pregnancy and pregnancy outcomes that is there are increased chances of pre-term deliveries, still births, spontaneous abortions and congenital malformations. If this deficiency is prevalent in large section of the population, then it will affect the productivity of the nation atlarge (Brown *et al*., 2001).

1.9 Zinc requirements of the vulnerable population

To convert physiological requirements of an individual into dietary requirement, the bioavailability of the zinc should be considered (Bel-Serrat *et al*., 2014, Sandstrom, 1998). According to recent literature on factors affecting zinc bioavailability (Bel-Serrat *et al*., 2014, Fairweather-Tait., 1998) 3 basic stages must be considered while assessing zinc intake and its absorption in the body. These include absorbability, mucosal transfer into systemic circulation and utilization of zinc within the body.

Table 1-2Dietary reference values for zinc (mg/day) for UK

Age group (both girls and boys)	LRNI[a]	EAR[b]	RNI[c]
15-50+ years (females)	4.0	5.5	7.0
Pregnancy	4.0	5.5	7.0
Lactation 0-4 months			+6.0
4 + months			+2.5

a – lower reference nutrient intake

b – Estimated Average Requirement

c – Reference Nutrient Intake

Source: Geissler and Powers, 2011

1.10 Dietary sources of zinc

Zinc is usually found in abundance in foods which are good sources of protein such as dark red meat, chicken, and seafood such as oysters (Lowe *et al*., 2009, Hunt, 2003). Other good sources of zinc include wheat germ, lobsters, and ham. Moreover various whole grains and cereals like pearl millet, whole wheat contain fairly high amount of zinc, but this zinc is poorly absorbed due to the presence of phytates that bind zinc in the gut lumen. Therefore, consumption of exclusive plant based diets excluding meat and fish can significantly contribute to zinc deficiency.

1.11 Factors affecting zinc absorption

1. Zinc intake :-

   The amount of zinc in the meal is one of the major factors affecting zinc absorption. Also with just increasing the amount of zinc in the diet, there is decrease in the fractional zinc absorption (Lonnerdal, 2000). Along with zinc intake, zinc status of an individual also significantly determines the extent of zinc absorption. In a study conducted by August et al.,(1989) it was found that young adult subjects absorbed 64% of zinc from the diet which contained 2.8-5mg/d but this zinc absorption was reduced to 39% when the diet contained 12.8-15mg/d. This study supports the aspect that with increasing zinc content, there is decrease in percent absorption, although the total amount absorbed does not change.

2. Source and amount of Protein :-

   The type of protein has been shown to affect zinc bioavailability. Animal protein (beef, eggs, meat, cheese) when incorporated in the diet has shown to counteract the inhibitory effect of phytate from the diets and thus enhance zinc absorption

(Lonnerdal, 2000). In contrast, studies have shown that when soy protein isolates were added to meals, zinc absorption was considerably reduced and this was attributed to the fact that these isolates normally contain considerable amounts of phytate.

Casein in the milk has been shown to have inhibitory effect on zinc absorption. In a study two milk formulas containing two different whey to casein ratios were studied. In one formula the whey: casein ratio was 60:40, while in the other ratio was 20:80. It was found that zinc absorption in adults was significantly higher (32%) when predominant formula was consumed as compared to one containing more of casein. In this case the percent of absorption was 21% (Lonnerdal, 2000). The mechanism suggested was that the phosphorylated serine and threonine residues on partially undigested casein subunits may be binding the zinc that is present and thus reducing its bioavailability.

1.12 Assessment of zinc status

There is lack of ideal biochemical indicator for assessing zinc status. Assessing zinc status also has been a challenge for long because the effective homeostatic mechanism of zinc regulation in the body buffers the functional response in an event of dietary zinc deficiency or zinc excess (Lowe *et al.,* 2009). Plasma zinc concentration is currently the most widely used biomarker, however it has well documented limitations (Hambidge, 2003). Furthermore Hess et al., (2007) explored the use of serum zinc concentration as a biomarker for assessing zinc status in population studies. Their results from depletion/repletion studies indicated that serum zinc concentrations respond appreciably to severe dietary zinc restriction, despite some individual variations in these responses. These authors also explained that while compiling

data from various intervention trials, they have found that both individual and population mean serum zinc concentrations increase consistently during zinc supplementation, regardless of the initial level of serum zinc concentration. So from these observations, it could be concluded that serum zinc concentration can be considered a useful biomarker of a population's risk of zinc deficiency. Apart from the above discussed biomarkers of zinc status, Lowe *et al*., (2009) in a systematic review have pointed out other indicators namely urinary zinc excretion, erythrocyte zinc concentration, mononuclear cell zinc concentration, polymorphonuclear cell zinc concentration, platelet zinc concentration, hair zinc concentration and plasma alkaline phosphatase activity. At the end of this vast systemic analysis, the authors revealed that only plasma zinc concentration and urinary zinc excretion (with moderate zinc status) were useful biomarkers of zinc status.

1.13 Significance of vitamin A for human nutrition

There are three forms of Vitamin A that are associated with the overall metabolism of Vitamin A in the human body. These include retinol, retinal and retinoic acid and altogether these are referred to as 'retinoids'. Retinyl ester is the storage form of Vitamin A. Vitamin A is required throughout life as it plays pivotal role in numerous cellular activities including reproduction, embryonic development, vision, growth, cellular differentiation and proliferation, tissue maintenance and lipid metabolism.

To add to the significance of vitamin A in the body, retinol and retinoic acid has been shown to be important for embryonic development and these are also important for the expression of gene that is required for growth regulation (Bennasir et al., 2010, Semba, 2001). It has been reported that during foetal development, retinoic acid is required for formation of limb, heart, eyes and ears (Solomons, 2001). In context of vitamin A required for normal growth, it has

been reported that vitamin A regulates bone remodelling that encompasses resorption and deposition of bone, and this remodelling is required for linear growth (Barasi, 2003).

From the above discussion, it becomes clear that vitamin A and its metabolites are required at various phases of lifecycle and this makes it important to explore vitamin A, its absorption and related factors in detail to understand the reasons for prevalence of vitamin A deficiencies at global level.

1.14 Requirements of Vitamin A for vulnerable population

Table 1-3Dietary reference values for vitamin A (µg retinol equivalent/day) for UK (Geissler and Powers, 2011)

Age group (both girls and boys)	LRNI[a]	EAR[b]	RNI[c]
11-50+ years (females)	250	400	600
Pregnancy	250	400	700
Lactation	250	400	950

a – lower reference nutrient intake

b – Estimated Average Requirement

c – Reference Nutrient Intake

1.15 Dietary sources of Vitamin A

Liver and fish liver oils (example - cod liver oil) are excellent dietary sources of Vitamin A. Other good sources of Vitamin A include milk fat as present in whole milk, butter and other dairy products, eggs and foods fortified with vitamin A. Margarines contain vitamin A as adding vitamin A to domestic size packs is the legal requirement in the UK. Plant sources provide carotenoids (yellow, red pigments present in many fruits and vegetables). In the UK, most of the provitamin A can be obtained in form of β carotene. The best sources of provitamin A carotenoids include carrots, spinach, broccoli, squash, sweet potatoes and some orange coloured fruits like cantaloupes, peaches, apricots and mangoes.

1.16 Consequences of Vitamin A Deficiency

An early sign of vitamin A deficiency is impaired dark adaptation (Geisslers and Powers, 2011, Barasi, 2003), mild anaemia, abnormalities of taste and smell and follicular hyperkeratosis. Night blindness is the most common sign of vitamin A deficiency, which can be corrected with early treatment. If this stage is left untreated, then it can progress to further damaging conditions like conjunctival xerosis with bitot spots, corneal xerosis, ulceration and finally irreversible blindness in form of corneal necrosis (Ahmed and Darnton –Hill, 2004). Maternal vitamin A deficiency can lead to visual impairment and other related health consequences. Global data on prevalence of night blindness in pregnant women indicates that 7-8% of pregnant women (9.7 million) have been suffering with night blindness and another 15.3% have deficient serum retinol concentrations (Bhutta *et al*., 2013). According to some researchers (Tielsch *et al*., 2008, Christian *et al*., 2001, Christian *et al*., 1998) maternal night blindness can result in increased chances of low birth weight babies and infant mortality. However this type of blindness can be reduced by vitamin A supplementation during pregnancy. Apart from these ocular consequences of vitamin A deficiency, there are other

physiological effects like decreased immunity, increased infections, diarrheal episodes in children, morbidity and other related micronutrient deficiencies.

1.17 Need to explore micronutrient deficiencies in vulnerable population

Since micronutrients play pivotal role in maintaining optimal health, it becomes crucial to explore their deficiencies especially in young women and women of reproductive age.Moreover research has highlighted that consequences of such nutritional deficits are not limited to a certain age group or gender, but can have serious devastating impact across the entire life cycle as seen in figure 1-3. This also implies that if these deficiencies are detected and controlled at an early stage in the life-cycle (adolescence, or young age) then the adverse consequences in later life can be reduced significantly.

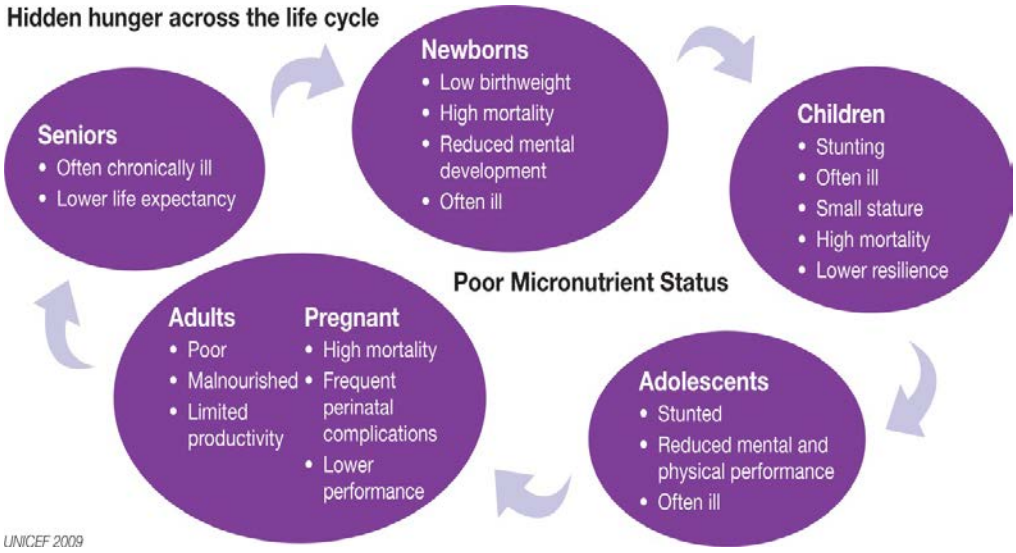


Figure 1-3Impact of micronutrient deficiencies across the entire life-cycle (Biesalski H.K., et al., (2013))

These long term effects of micronutrient deficiencies have been also highlighted in Barker's hypothesis (also called as foetal programming hypothesis) (Navaneetham and Jose, 2005). According to this hypothesis, it was proposed that the environment of the foetus and infant – determined by the mother's nutrition and the baby's exposure to infection after birth – determines the pathologies of later life. This again emphasises the above discussed point of

view that the micronutrient deficiencies should be overcome by good nutrition practices to avoid health problems later in life cycle.

1.18 Prevalence of micronutrient deficiencies in South Asian (SA) countries

A substantial amount of literature suggests that prevalence of micronutrient deficiencies (iron, zinc) are increasing especially in South Asian countries such as India, Pakistan, Bangladesh (Akhtar*et al*., 2013). According to a joint statement issued by WHO, World Food Program and UNICEF, deficiencies of micronutrients are a major public health problem. More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iron and zinc (WHO, WFP and UNICEFstatement, 2007). The WHO estimates that 35-75% of pregnant women in developing countries are anaemic(Jufar and Zewde, 2014, WHO 1993-2005).For women, the consequences of anaemia are very serious and includes reduced work capacity, poor pregnancy outcome (including premature births, low birth weight babies), increased risk of death during delivery and postpartum. It has been estimated that anaemia may be associated with 50% of maternal deaths world-wide (Galloway *et al*.,2002). Statistics also suggest that even though iron deficiency is present in most of the countries of the world, the numbers are greater in South Asia (57%) (WHO 2001,Tidemann-Andersen *et al*.,2011). In a review by Seshadri (2001) on the prevalence of iron, zinc and folic acid deficiencies in South Asia, it was concluded that iron deficiency and anaemia affects 50 % or more of pregnant women.

Prevalence of zinc deficiency is higher in developing countries as compared to developed countries. According to statistics from a study, about 71.2% of population in South Asiais at an increased risk of low dietary zinc intake (Ramakrishnan, 2002)**.** Also according to study conducted in Delhi (India) 49.4% of adolescents girls suffered from zinc deficiency (Akhtar 2013, U.Kapil *et al*., 2011).

Vitamin A deficiency at any age can have adverse effects on an individual's health and well-being. It has been reported by that nearly 50% of vitamin A deficiency cases are found in South-Asia, including India (35.5 million), Indonesia (12.6 million) and China (11.4 million) (Ejaz and Latif.,2010, Diaz et al., 2003). Severe form of vitamin A deficiency can lead to xerophthalmia, however mild and moderate forms of deficiency can also have considerable consequences due to the significance of vitamin A in range of biochemical functions in the body.

1.19 Prevalence of Iron deficiency in Pakistan

Substantialbody of literature has highlighted that micronutrient deficiencies relating to iron, zinc and vitamin A are a major public health problem in Pakistan and needs immediate attention to prevent long term consequences in the young population. More recently in 2011 a National Nutrition Survey was conducted in Pakistan and the results obtained indicated an increase in percentage of anaemia among women (15-49 years age range) in different regions of Pakistan (Table 1-4)

Table 1-4Iron Deficiency Anaemia among Non-pregnant women (15-49 yrs) across different regions in Pakistan (2011)

Total (Overall Pakistan)	Rural	Urban	Regions in Pakistan	% with anaemia
50.4%	50.9%	49.3%	Sindh	62.0%
			Balochistan	48.9%
			Punjab	48.6%
			AJK	41.0%
			KP	**35.6%**
			GilgitBaltistan	23.3%

Source: National Nutrition Survey, Pakistan 2011

The map of the region given in the National Nutrition Survey report helps in better understanding of the statistics of zinc deficiency among the target population (Figure 1-4)

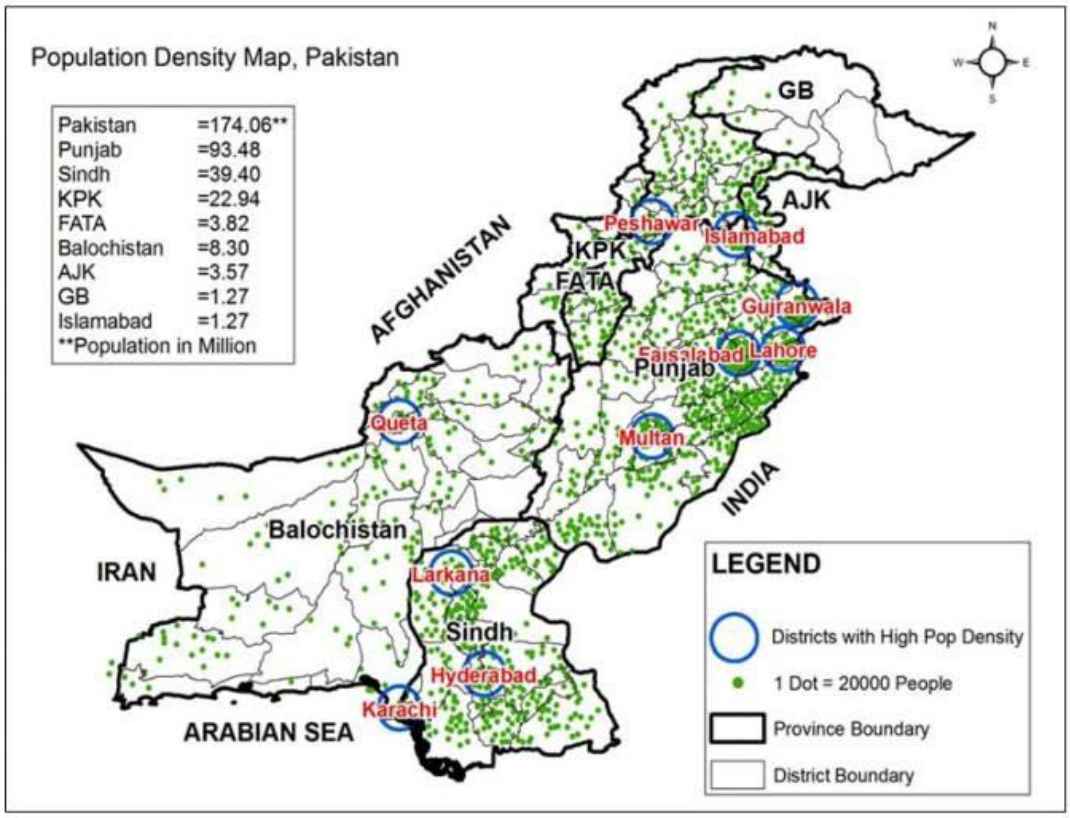


Figure 1-4Map of Pakistan highlighting the regions covered in National Nutrition Survey

1.20 Prevalence of zinc deficiency in Pakistan

Recently in a study Akhtar (2013) presented the statistics of zinc deficiency in Pakistan (Table 1-5).

Table 1-5Zinc deficiency in pregnant and non-pregnant women (15-49 years) in Pakistan (2011)

Area	Non - pregnant			Pregnant		
	Deficient (<60µg/dl)	Non-deficient (≥60µg/dl	N Number of participants	Deficient (<60µg/dl)	Non-deficient (≥60µg/dl	N Number of participants
Total	41.6	58.4	5953	48.3	51.7	791
Urban	38.2	61.8	2395	47.2	52.8	285
Rural	43.2	56.8	3558	48.7	51.3	506

Source – Akthar, 2013, National Nutrition Survey Report 2011

Statistics according to specific regions indicate suggest that 54% of pregnant women Sindh province of Pakistan suffered from zinc deficiency (Akhtar, 2013). More recently Akhtar et al (2014) conducted a cross-sectional study in rural area (Palosi, District Peshawar) in Khyber Pakhtunkhwa (KP) Provinceof Peshawar, Pakistan to estimate the prevalence of zinc deficiency among women in child bearing age (15-45yr) and also investigated if factors like marital status, pregnancy status has any influence on the zinc status. Blood samples were obtained from a total of 353 women and these were analysed for their zinc levels. It was found that 27.8% of women were zinc deficient, while 8.8% suffered from severe zinc deficiency. The statistics were radically higher for pregnant women. It was observed that women in the second and third trimester were 3.4 and 3.7 times respectively more likely to be deficient as compared to the control groups. This suggests that there is an urgent need to

planpreventiveand curative measures for this vulnerable age group because if these women continue to be deficient during nutritionally demanding periods such as pregnancy then it can have adverse pre and postnatal consequences. This scenario again sheds light on Barkar's hypothesis which states that low birth weight is the reflection of poor maternal nutrition. It also emphasizes on the importance of nutrition not only during pregnancy, but all throughout childhood and adolescence (Navaneetham and Jose, 2005).

1.21 Prevalence of vitamin A deficiency in Pakistan

According to a review, Vitamin A deficiency is defined as a public health problem if more than 2% of the population has serum retinol levels below 20 g/dL and the degree of severity is classified as follows: mild, 2–10%; moderate, 10–20%; and severe, ≥20%. (Ramkrishnan 2002, WHO,1995).TheNational Nutrition survey conducted in Pakistan in 2011highlights the prevalence of Vitamin A deficiency among the pregnant and non-pregnant women (15-49 years) in Pakistan (Table 1-6).

Table 1-6Vitamin A deficiency among pregnant and non-pregnant women (15-49 years) in Pakistan (2011) (National Nutrition Survey Report 2011)

Area	Pregnant		Non-pregnant	
	Severe deficiency (<0.35µmol/L)	Moderate deficiency (0.35-0.70µmol/L)	Severe deficiency (<0.35µmol/L)	Moderate deficiency (0.35-70µmol/L)
Pakistan	18.7%	27.3%	17.0%	25.1%
Urban	16.1%	25.4%	10.9%	24.0%
Rural	19.7%	28.1%	19.6%	25.5%
Punjab	19.6%	24.1%		
Sindh	15.1%	31.6%		
KP	**35.5%**	**40.7%**		
Balochistan	26.1%	34.6%		
AJK	3.3%	28.9%		
GB	20.0%	24.1%		

1.22 Prevalence of micronutrient deficiencies in UK

Growing body of work in this area of nutrition has shown that iron deficiency is also common across industrialised countries, even though the statistics are much lower as compared to developing countries. It has been found that in the UK , 21% of female teenagers between 11

and 18 years and 18% of women between 16 and 64 years are iron deficient (Heath and Faiweather-Tait., 2002, Zimmermann and Hurrell., 2007).

1.23 Collaboration with Abaseen Foundation

Abaseen Foundation United Kingdom (AFUK) is a Lancashire based charity which was set up in 2002 to provide technical and financial support to its sister organisation, Abaseen Foundation Pakistan (AFPK) for health, education, relief and research projects in North West Pakistan. The AFUK/AFPK partnership operates 2 primary schools and 2 health facilities (including the Health Centre (HC) at Baghbanan, also called BHU or Baghbanan Health Unit) in Khyber Pakhtunkhwa (KP) and in the Federally Administered Tribal Area (FATA) in North West Pakistan (NW PK). AFUK has a Board of Trustees and AFPK a Board of Governors who oversee the work of their respective organisations. Over the last 10 years, AFUK has supported a number of successful health service development projects. AFPK (with technical support from AFUK) has undertaken a number of activities, including a survey of 1,043 households in Baghbanan during July 2012, and a rapid needs assessment in November 2012 which involved 1 to 1 interviews with 8 key informants to identify the problems of girls, boys, women, men, ethnic minorities and the disabled on a range of factors affecting their health and wellbeing. In addition, Jirga meetings (Jirga is a Pukhtun term for a decision making assembly of male elders) have been held (as part of an ongoing ethnographic research study) to identify the most important health needs of vulnerable groups within the community (Figure 1-5).

Figure 1-5Jirga meetings conducted by staff at Abaseen Foundation PK in Baghbanan region

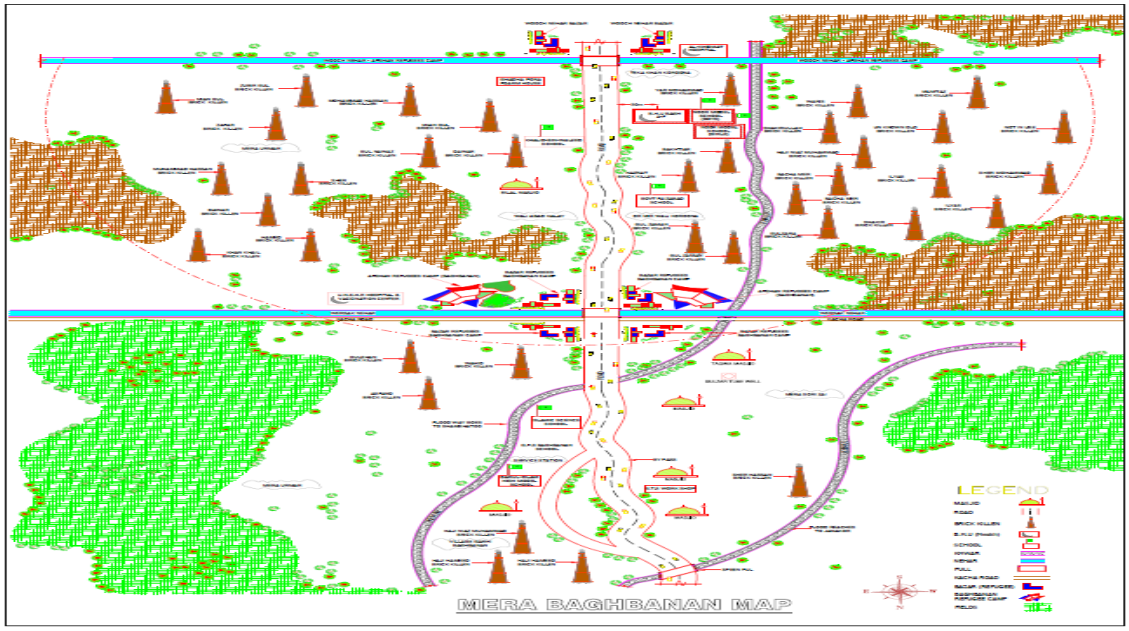
The Abaseen Foundation (AFUK and AFPK) has a close working relationship with the International Institute of Nutritional Sciences and Food Safety Studies (iINSAFSs) at the University of Central Lancashire (UCLan).

In continuation with this working relationship, the present study was also acollaborative research project developed as part of MRes degree at UCLan. The main focus of this study was to understand the dietary pattern and dietary diversity in Baghbanan region of North West Pakistan and compare it with the dietary pattern and diversity observed in the Pakistani community based in North West United Kingdom (NW UK)

1.24 Details about the geographical setting of the present study

The present study was undertaken in two different geographical settings, one rural setting in Bagbhanan (NW PK) and other in Preston (NW UK).The geographical maps can be representing the region are as follows

a. Map showing the study region – Baghbanan region in NW PK



b. Map showing the study region – Preston in NW UK

1.25 Rural setting in Baghnanan (North West Pakistan)

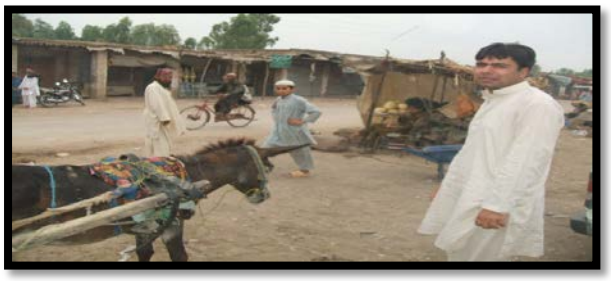


Figure 1-6Research assistant Mr. Akhtar Munir working in the Brick-kiln community

Figure 1-7Typical cooking area in the community

Figure 1-8Drying bricks in the sun

Figure 1-9Brick kiln

1.26 About Baghbanan region and Brick kiln community

Baghbanan region is located 20 kilometres South East of Peshawar. Baghbanan is a brick kiln community of 5,000 households of Afghan refugees, internally displaced people, and host population. They live in chronic rural poverty, with many households subsisting on an income of less than 1 US dollar per day. Children are born into bonded labour and start work on the brick kilns from an early age, and have limited life trajectories. Girls typically marry in their early teens and multiple pregnancies with short birth spacing are usual. The adult female literacy rate is less than 3% so intergenerational transfer of poverty is inevitable.

In 2009, a comprehensive survey of 200 households in Baghbanan by AF PK, highlighted high levels of acute malnutrition (26.6%) and chronic malnutrition (43.1%), poor uptake of ante-natal care (14.3%), low levels of infant and child immunisation (20%) and financial problems faced at delivery (89%).

1.27 Urban setting in Preston in North West UK

Figure 1-10Study setting in North West UK

A comparative view of the both the study setting can clearly indicate the differences in accessibility, availability of foods, socioeconomic status and these differences can significantly affect micronutrient intake in both the study populations.

1.28 Rationale for the present study

Considering the above discussed scenario it became evident that the community inBaghbhanan, especially women and young children were at high risk of nutritional deficits.

The reasons for these micronutrient deficiencies are complex and numerous. Poverty, food security, education and culture are all factors that play a role. So in order to explore these reasons, the research team within iINSAFSs at UClan undertook a research based at the Baghbanan health centre (HC) that serves a marginalised, poor community in Baghbanan (NW PK). To date, a detailed evaluation of the dietary patterns and nutrient intakes in this community has not been conducted. In addition, it is important to consider the bioavailability of micronutrients from the diet (Frontela *et al*., 2011). This is because cereals, which form the integral part of diets consumed in South Asian countries contain high amount of anti-nutritional factors including phytates, which inhibit the absorption of the micronutrients from such foods.

Another aspect about micronutrient deficiencies is that there are very rare chances of an individual having deficiency of a single micronutrient. This is because there are many interrelated functions of different micronutrients. So collecting data about staple diets and analysing them for all 3 micronutrients will help in obtaining a clear picture of prevalence of these deficiencies. Moreover a database of the micronutrient content of staple diet consumed by people in Bagbhanan region of NW PK is not currently available and this can be a hurdle in planning intervention strategies to overcome the highly prevalent micronutrient deficiencies. Therefore, the present study was developed where dietary pattern of 2nd generation females in age group 18-30yr was analysed to understand dietary diversity and this was also compared with the dietary data obtained from the urban counterpart of the study in Preston (NW UK).

1.29 Diet Assessment tools used in the present study

(ia) Dietary Diversity Score

Dietary diversity score (DDS) technique is a relatively new way of assessing dietary information in population studies. This technique is not very commonly used in studies, but research work relating dietary diversity scores and various parameters such as energy intake, adequate micronutrient intake, mortality among children at public level has always been going on since many years. One of the earliest studies using DDS was conducted by Kant etal.,1993, where the relationship between DDS and all-cause mortality was examined for the data that was obtained from the first National Health and Nutrition Examination Survey I (NHANES I). This study considered 5 food groups including meat, dairy, grains, fruits and vegetables and so the maximum possible score was 5. An inverse relationship between risk of mortality and DDS was observed. Furthermore, Arimond and Ruel 2004, examined the relationship between dietary diversity and height for age Z scores (HAZ) in 6-23 months infants using data from 11 Demographic and Health surveys. Significant associations were observed in 7 countries out of 11 countries studied between DDS and HAZ, considering factors like breast feeding, child age, urban or rural location. However the authors did point out that diversity scores depend largely upon socioeconomic status and that it should be considered while interpreting conclusions. As work progressed in this area of nutrition more food groups were included in measuring DDS. Moreover this technique was also validated against other methods of measuring food intake in order to ensure its usefulness as an indicator at population level. Torheim etal, 2004 conducted a detailed examination of relationship between nutrient adequacy and dietary diversity in adult female population (15-45years) in Mali, Africa. While examining this relationship, authors developed two different indexes of dietary diversity, namely food variety score (FVS) and dietary diversity score (DDS). FVS implied simply counting the number of food items while DDS involved

counting different food groups. Other information on demographic, socioeconomic and food production status was obtained at household and individual status using a pre-coded questionnaire. Mean adequacy ratio and mean recommended intake levels were used to assess nutritional adequacy. DDS was calculated as the numberof food groups consumed during the diet-recording period and it was based on food groups including cereals, legumes,oil/sugar, fruit, vegetables, meat, milk, fish, eggs and green leaves. Further dietary diversity was assessed in relation to age, gender, socioeconomic status and place of residence. This was an advanced modification of method described by Kant et al, 1991and similar one by Torheim et al., 2003. At the end of this comparison it could be concluded that dietary diversity score could be useful indicator of nutrient adequacy.

Continuing their work on similar lines, more recently FAO (Kennedy *et al*., 2011) developed the latest extremely modified version of DDS as a part of their work on developing guidelines for measuring household and individual dietary diversity. The authors emphasize the usefulness of this dietary diversity questionnaire as it is rapid, user friendly and low cost, requiring minimal dietary assessment tools. It is the qualitative measure of food consumption pattern and reflects individual and household access to a variety of foods.

This dietary diversity score has been categorised into two types:-

Household dietary diversity score (HDDS) for assessing diversity at household level and Womendietary diversity score (WDDS) for assessing diversity at individual level.

For the present study WDDS has been used with score range 0-9 (0- indicating lowest diversity and 9 indicating highest diversity).

(ib)Rationale for using Women Dietary Diversity Score

The WDDS technique has been adopted for the present study due to various reasons:-

Firstly the dietary data was obtained from two completely different geographical regions (NW UK and NW PK) and the participants from these two regions had distinct food

consumption pattern governed by cultural diversity. According to the researchers (Kennedy et al., 2011) this dietary diversity score questionnaire tends to be extremely flexible and can suit any cultural and local environment. Secondly, as stated earlier, there was great diversity in the diets consumed between both the study locations and so it was thought that it would be useful to obtain the diversity score instead of obtaining nutritional values of individual diets. Moreover, in depth dietary data (24 hour dietary recall format) could not be obtained from participants in NW PK. One of the prominent reasons for this was that the women participants were illiterate and could not explain accurately the portion size consumed. So the nutritionist recorded the intakes as 'one plate' 'one glass' which were not so useful for understanding the actual amount of food consumed. Later, the nutritionist was also asked to click pictures of the foods consumed. But again due to lack of any standard measurement, the amount could not be judged. So these pictures were useful for gaining idea about the variety of foods consumed over a period of week, but the data could not be entered in the food composition software such as WinDiets to understand entire micronutrient profile.However, it was important to analyse these diets in a way in which the quality of the information gathered would not have an impact on the results. Considering all these parameters, it was found that obtaining DDS score for the diets in both the study locations would be useful in interpreting the results appropriately.

(ii) Dietary data using 24 hour recall format

Gathering appropriate, reliable dietary information from participants of different cultural origin, age, gender, educational and economic backgrounds has been one of the crucial aspects in the context of studies aimed at assessing nutritional status of people in large population studies. From various techniques that are generally used, 24 hour dietary recall format is the most commonly used technique. Gibson and Ferguson (2008), in their

exhaustive work surrounding the use of 24 hour dietary recall as a tool for assessing dietary intakes have highlighted the advantages of using 24 hour recall format. Some of the important advantages of 24 hour recall format include:

- This type of interview format allows sufficient interaction between the interviewer and the participant. In addition to this these recalls can be administered face to face at participant's house or in any informal relaxed environment. This helps in getting good quality data, not only about the food consumed, but also some more quality characteristics like the cultural preferences of food, and ethnographic data.
- The time required for a single dietary recall maximum 20-30 minutes depending on the time required by the participant to recall the food items consumed.
- This format also requires minimum tools and tends to be low cost exercise and this makes it suitable for large scale surveys. (Pan et al., 1999)

In this context researchers have also suggested that the data obtained using this method becomes more reliable when there are 3-7 dietary recalls from a single participant, however 2 recalls have been found to be satisfactory (Bernard, 2006, Cupples et al., 1992). Moreover it has also been reported that use of photographs of portion sizes of different foods can be very useful to minimise the various biases encountered in this method (memory lapses, under-reporting of 'bad foods').

Considering all these parameters, 24 hour recall format (appendix 2h and 2i) was used in the present study. There were 3 dietary recalls for a single participant including 2 weekdays and one weekend. These recalls could also be used to calculate the DDS.

(iii) WinDiets

In the past, different food tables were used to analyse diet diaries, data obtained using 24 hour dietary recalls. One of the most common databases that have been used in UK is McCance and Widdowson (2002) food composition table. However with advancement in this area of

nutrition analysis, now we have electronic databases to facilitate this process, and there are many on the market, including WinDiets, CompEat, Dietplan6.

WinDiets (version 2010) is a software system, produced by the Robert Gordon University, for nutritional analysis of food and contains as many as ten food groups containing between 500-1000 core food items. It includes the complete UK dataset published in 2008.The US food tables are also available. These are not subdivided so you can only search the complete database. Local foods can be searched as well.WinDiets allows the user to calculate the nutritional status of a person by entering their daily diet from breakfast to late evening snacks. The available options like 'edit person data' allows user to enter all the person specific details like weight , height, age, gender and place of residence.

1.30 Traditional diet of people in NW Pakistan (Baghbanan)

Apart from various physiological factors and nutrient interactions, it is the plant (cereal) based traditional staple diet of people in Pakistan that contributes significantly to the prevalence of micronutrient deficiencies. As seen in the figure no.1-11, the daily diet of the people is highly monotonous with either rice or any other cereal preparation and with either one pulse or a vegetable. Lack of animal products in daily diet is clearly evident and this contributes to iron and zinc deficiency that is prevalent in this population. Limited published literature exists that highlights the diets to be monotonus as majority of the information that available is from the communication of lady health workers with community members. However a recent study by Dykes et al., (2011), points out some of the crucial aspects about diets consumed by a community in Nahaqi in KP in NW PK. This study also establishes that dietary pattern is highly influenced by economic status of people and that women tend to have cheapest foods like potatoes, chickpeas even during nutritionally demanding physiological states such as pregnancy. Dark meat (Lamb mutton or goat) or chicken balls

consumed with oven-baked bread called nan-kebab is a popular dish consumed in the north-west region of Pakistan. However the frequency of consumption of meat also depends on the economic status. In the area (Baghbanan Brick kiln) where the present study has been conducted, the frequency of consumption of meat and meat products is less as compared to the overall northwest region due to low affordability. The most common vegetables consumed include potatoes, onion, brinjal (aubergine), lady's finger, and green peas. These vegetables are not high sources of vitamin A. So overall a cereal based diet with low iron and zinc bioavailability and less variety in terms of vegetables consumed makes this population in Baghbanan susceptible to iron, zinc and vitamin A deficiencies.

Figure 1-11Different products (from cereals like wheat, rice, semolina) consumed in North West Pakistan as a part of daily diet

1.31 Wheat flour consumption pattern in Pakistan

Pakistan is the 8th largest wheat producer country, contributing about 3.17 % of the world Wheat production from 3.72% of wheat growing area (Nadeem et al., 2010, GOP, 2008).Wheat alone accounts for 14 percent of value added in agriculture. It is grown by about 80 percent of farmers on about 9 million ha, which is close to 40 percent of the country's total cultivated land, according to official sources in Pakistan. The crop also accounts for an estimated 37 percent of both food energy and protein intakes (FAO, 2013)

Most wheat produced in Pakistan is consumed domestically. Depending on domestic production, the country may import wheat in a deficit year or export the surplus in a year of high production.Pakistan was a net wheat importer until 2000, but became a net exporter of wheat in 2010. Pakistan exported up to 2 to 3 million tonnes of wheat in 2010/11 according to grain traders interviewed in Karachi in May 2011. (FAO, 2013).

Wheat is the staple diet of people in Pakistan and contributes significantly to the calorie and protein profile as far as the nutritional status of people in Pakistan is concerned. Wheat flour is obtained by grinding the wheat grain eliminating the bran and the germ. The medium refined flours are ground endosperm, while whole wheat flour is achieved by adding germ and bran to the flour (Febles et al., 2002).

According to researchers (Anjum et al., 2002), spring wheat is the most commonly grown variety of wheat in Pakistan. In Pakistan, wheat is consumed generally in form of products like unleavened flat bread (roti or chapati), wheat semolina sweet dessert (suji ka halwa), refined wheat flour (maida), wheat biscuits and other bakery products made from refined wheat flour, whole wheat grain and corn flour to make rotis (makai ki roti) and brown rice. There are regional variations in the wheat products that are generally consumed by the locals.

Punjab and Sindh provinces tend to have unleavened chappatis or rotis while in Baluchistan and Khyber pakhtun provinces fermented rotis are prepared (Nadeem et al., 2010).

1.32 Wheat flour consumption pattern in UK

There is lack of literature which can be useful in estimating the exact percentage of consumption of wheat flour and related products. But in the present study, some amount of good quality data have been obtained regarding the most commonly used brands of wheat flour as well as wheat flour products consumed by SA population (18-30 Years) in the UK. These included breads made from different flour like white flour bread, brown bread, wholemeal bread as well as traditional items like chapattis, rotis, parathas, naan. Also in this context it is important to mention that the some wheat flour varieties that are available in UK shops are enriched with essential nutrients like calcium, iron, B12, folic acid and fibre. However the wheat flour brands that are commonly available in SA shops include East End Premium Gold Chakki Atta, East End Medium Chapatti flour, Supreme Medium Chapatti flour, East End White flour, Elephant Atta with varieties like Wholemeal flour, medium chapatti flour, fine white flour. Apart from these various supermarkets have their own branded flour varieties like Asda Plain Flour 10.3%, Asda Strong White Bread Flour 11.5%McDougalls Plain Flour 10.4%, Sainsbury's Strong White Bread Flour 13.4%, Waitrose Strong White Bread Flour 12.9%.

Figure 1-12Types of wheat flour commonly consumed by South Asian population in Preston (NW UK)

1.33 Details about the origin of these flour samples

All the flour samples have been labelled as 'produced in UK', however if the origin of the grains is traced then it was found that all the flour samples produced under brand name East End were milled by East End Foods in the UK at their State of the art Flour Mill using hard wheat from Uttar Pradesh in India. These details on the brand Supreme could not be obtained

1.34 Nutritional profile of wheat flour

Considering the morphology of wheat grain kernel, it is important to note that each part of this grain has a specific nutritional profile. It is an important dietary source of carbohydrate

and proteins. It also forms a fairly good source of iron for vegans for whom cereals form the integral part of their diets (USDA Nutrient Database).The various nutrients present in different parts of the grain can be seen in the diagram below

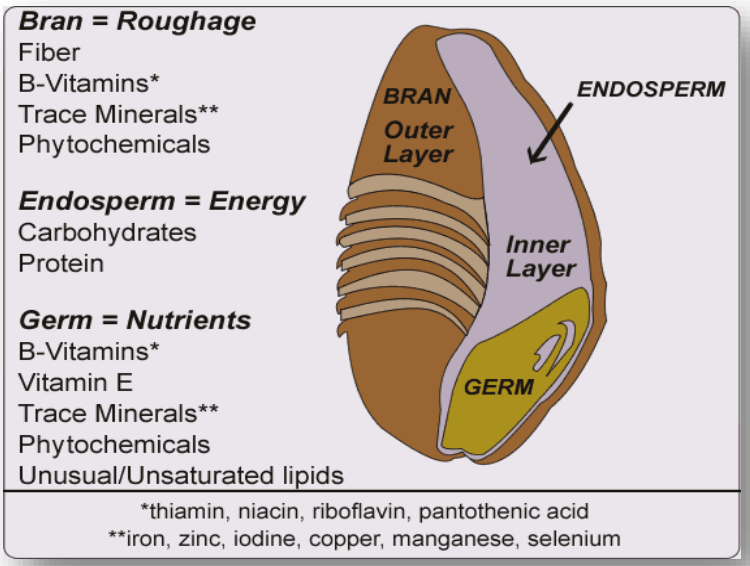


Figure 1-13Nutrients present in wheat kernel

Source - http://heathershearth.ca/?page_id=70

Despite of these nutritional values the main concern with consumption of wheat flour as major dietary staple is the presence of high levels of phytate.

1.35 Phytate

In addition to the individual factors that affect iron and zinc absorption respectively, one major factor which has greatest inhibitory effect on the bioavailability of both iron and zinc is phytate and its complexes.

Phytic acid (known as inositol hexaphosphate (IP6), or phytate) is the principal storage form of phosphorus in many plant tissues, especially in the grass family (wheat, rice, rye, barley

etc) and beans. Of the total amount of phosphorus in plants, approximately 60-90% is found as phytate (Bosscher et al., 2001). In wheat, rice, this phytic acid is largely present in the external that is the pericarp and the aleurone layer. Phosphorus in this form is generally not bioavailable to humans because humans lack the digestive enzyme, phytase, required to separate phosphorus from the phytate molecule.

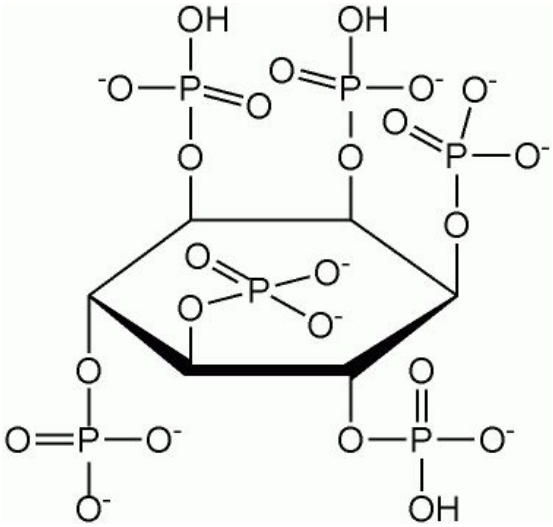


Figure 1-14Structure of Phytic acid Raboy et al., (2000)

Over the years numerous studies have established the inhibitory role of phytate on iron and zinc absorption in humans. The presence of high amount of phytate in cereal based diets predisposes individuals to micronutrient deficiencies especially in countries like India and Pakistan, where cereals form major part of their daily diet.

1.36 Phytate content of cereals in UK

Varieties of cereals either locally produced or imported are consumed by people in UK. During the 24 hour recall interviews it was found that wheat flour and wheat flour products like parathas, chapattis and different types of bread were consumed as a part of daily diet

Phytate content of various cereals and cereal products is not available in UK food composition tables (McCance and Widdowson, 2002), however in a study conducted by Amirabdollahian and Ash (2010), data from various published and unpublished sources has been compiled together in order to estimate the daily intake of phytate by different age-groups in UK. The findings of the study indicated that breakfast cereals and breads contributed significantly to the phytate intake for all age groups studied. This was primarily due to high frequency of consumption of these products. Moreover these cereals also tend to be high in phytate content (example. 780mg/100g for high fibre and whole grain cereals). The authors have also brought out potatoes and savoury snacks form the second most important source of phytate intake among young population (4-18 years age). It was observed that potato chips were consumed by 88% of girls in the study. This implies that if frequent consumption of these phytate rich products is not controlled then it can have adverse impact on mineral absorption from the diets and thereby lead to micronutrient deficiencies among UK population.

There are number of studies which have shown the inhibitory effect of phytate on iron and zinc availability.

1.37 Inhibitory effect of Phytate on Iron Absorption

With respect to the effect of phytate on iron absorption a study was conducted by Sandberg et al., (1999) to understand the effects of different forms of phytate that is inositol tri, tetra and

pentaphosphates (IP_3 , IP_4 , IP_5) on iron absorption in humans. Here iron absorption was measured in 5 experiments from single meals using the method of extrinsic labeling with ^{55}Fe and ^{59}Fe and then whole body retention and erythrocyte uptake of isotopes was determined. In first 3 experiments meals provided to the subjects contained white wheat rolls to which 10mg of phytate in the form of IP_5, IP_4, IP_3 respectively were added. In experiment 4, the effect of mixture of phytate isomers was studied wherein 10mg of phytate in form of IP_3 + IP_4 and 2mg of phytate in form of IP_5 + IP_4, were added. In experiment 5, 20mg of mixture IP_3 + IP_4 and 3mg of IP_5 + IP_4, were used. Each experiment had 8-11 subjects. It was found that in experiment 1, iron absorption was reduced by 39% with no effect on iron absorption in experiments 2 and 3. Higher inhibitory effect on iron absorption was observed in experiments 4 and 5 having reduction upto 54% and 64% respectively suggesting that IP_3 and IP_4 isomers were responsible for inhibition of iron absorption. Therefore one can conclude that isomer form of phytate present in the food must be known before predicting the inhibitory action.

On similar lines another study by Davidsson, (2003) showed strong inhibitory effect of phytic acid on iron bioavailability when soy formulaes were evaluated before and after dephytinization. It was found that when 83% of phytic acid was degraded, the bioavailability of iron increased from 5.5% to 6.8%. However, pronounced effect was observed when 100% degradation of phytic acid was carried out. It was seen that the geometric mean of bioavailability increased significantly from 3.9% to 8.7%. Further work was carried out by Hurrell et al., (2003) to understand the influence of phytic acid degradation on iron absorption from cereal porridges. An exogenous phytase was used to fully degrade phytic acid during manufacture of 9 roller-dried complementary foods based on rice, wheat, maize, oat, sorghum and wheat-soy blend. Iron absorption was measured by extrinsic label radio iron technique from phytate free as well as phytate containing porridges. Results revealed that dephytinization increased iron absorption in rice porridge from 1.73% to 5.34%, in oats from

0.33% to 2.79%, while in maize from 1.80% to 8.92%. In case of wheat porridge increase was much significant that is from 0.99% to 11.54% and for wheat-soy blend it increased from 1.15% to 3.75%. Later, when ascorbic acid was added to these mixtures iron absorption substantially increased from 2.40% to 8.46%. This study again establishes that phytate has inhibitory effect on iron absorption and that when this phytate content is reduced there is considerable increase in iron availability.

1.38 Inhibitory effect of phytate on zinc absorption

With respect to effect of phytate on zinc availability, it has been reported that zinc is one of the most essential mineral which is adversely affected by phytate. The binding of zinc by phytate is dependent on several factors such as the amount of phytate, pH and the presence of other metal ions. At raised pH values as found in infants, zinc complexes with phytate and it enters the duodenum in insoluble form. Further if infants are given supplements containing iron and calcium, zinc interacts with these minerals thereby reducing its bioavailability (Bosscher et al., 2001). In addition to this, zinc bioavailability is also affected by the composition of diet, regarding both the concentration of zinc as well as the presence of inhibitors especially phytate (Kristensen et al., 2006). Even though phytate inhibits absorption of zinc, it is dose dependent and the phytate:zinc molar ratio has been suggested as an effective tool to estimate the proportion of absorbable zinc (Brown et al., 2004). The relationship between zinc intake, the phytate:zinc molar ratio of the diet, and zinc absorption has been described by WHO, (1996) which defined three categories of diets with high (phytate:zinc molar ratio less than 5), medium (phytate:zinc molar ratio between 5-15) or low zinc availability (phytate:zinc molar ratio greater than 15) based on the proportion of energy from animal sources, the types of processing of cereals, the amounts of inorganic calcium salts, and the phytate:zinc molar ratio. WHO,(1996) estimated that about 45% to 55% of the

element is absorbed from a high-bioavailability diet, 30% to 35% from a medium-bioavailability diet, and 10% to 15% from a low-bioavailability diet depending on the zinc content of the meal.

1.39 Inhibitory mechanisms of phytate on mineral absorption

From the above discussion, it can be very well understood that phytate significantly hinders the absorption of iron and zinc in the body as well as from foods. However it is equally important to explore the underlying mechanisms for the observed inhibitory effects. Some of the postulated interactions between phytate and minerals has been discussed below

The phytate molecule tends to form stable complexes with mineral divalent and trivalent cations like Zn^{+2}, Cu^{+2}, Fe^{+2}, Fe^{+3}, Ni^{2+}. Moreover if two cations are present simultaneously, then the proportion of IP6 – mineral complex increases and this significantly reduces the absorption of minerals especially zinc absorption in the body (Sanstead, 2011). However this binding of phytate with zinc is affected by several factors like the amount of phytate, pH and the presence of other metal ions.Moreover, phytic acid in most plant foods is present as phytate salt or as a complex with proteins and this formation of salt complexes has been attributed to acid groups in the phytic acid molecule (Febles et al., 2002). This chelates with certain metal ions like calcium, zinc, copper and iron to form insoluble protein-mineral-phytate complexes (Duhan et al., 2002).

Phytic acid also forms strong ionic complexes not only in foods but also in the intestine (Bosscher et al., 2001). These complexes fail to break easily and thus inhibit absorption of essential minerals thereby leading to their deficiencies. The inhibitory mechanism of phytate on iron absorption can be seen in fig 1-15

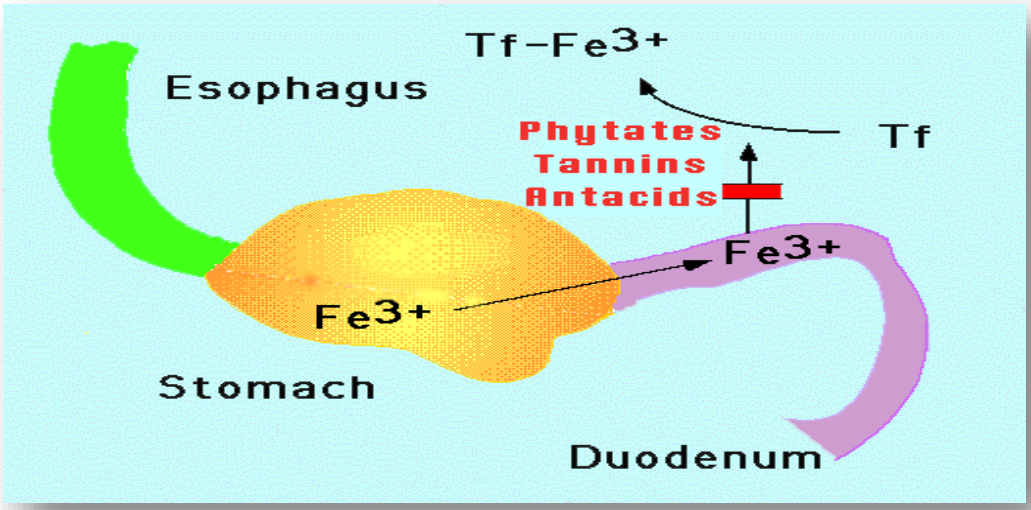


Figure 1-15Iron absorption in presence of inhibitors (phytate)

Source: http://sickle.bwh.harvard.edu/iron_absorption.html

As seen in figure 1.15, the consumed iron enters the stomach through oesophagus, where it is oxidised to its ferric form (Fe^{+3}). In the stomach, the gastric acidity and other solubilizing agents prevent its precipitation and thus iron is absorbed by the intestinal mucosal cells of the duodenum and jejunum. Further, in circulation this iron has to be coupled with transferrin (iron transport protein) for delivery to the cells where it is required. However when high amount of phytate is present in the diets, it blocks the coupling of iron to transferrin and thus iron is not fully absorbed by the required cells leading to iron deficiency.

A dose dependent inhibition of iron, zinc and calcium has been demonstrated in humans (Sandberg, 2002).

From these studies it appears that if these inhibitors could be reduced then there can be improvement in the availability of both iron and zinc. The effect of these inhibitors to some extent can be reduced by addition of factors that can enhance the availability.

1.40 Enhancers of mineral absorption

The inhibitory effect of phytates from the diets can be reduced by addition of enhancers of mineral absorption.

Enhancers are those substances which either are naturally present in the system or are added which aids in increasing bioavailability of zinc and iron from the complex diet. This role of enhancers is very vital in making the less bioavailable non-haem iron more available and thus increasing iron absorption. Non-haem iron is reduced within the lumen by dietary or endogenous factors such as ascorbic acid and glutathione, or by ferrireductase at brush border surface prior to translocation of membrane of Fe^{+2}(Camara et al., 2005).

Studies have shown that there are number of enhancers of non-haem iron absorption, however ascorbic acid has been regarded as the most effective enhancer of iron absorption

Ascorbic acid is the active form of vitamin C. It is the best known and most potent enhancer of non-haem iron absorption. There is enormous amount of work which has highlighted the beneficial role of ascorbic acid, both in its natural form in fruits and vegetables and also when added as a free compound. A study was conducted wherein phytase was added to whole wheat flour during bread preparation. This helped to increase the percentage of dialyzable iron. Authors also reported that the action of phytase could be maximized by addition of citric acid (6.25g/kg) to whole wheat flour. It was found that as compared to untreated bread, citric acid alone and the combination of phytase and citric acid enhanced the total iron dialyzability by 12 and 15 fold respectively (Porres et al., 2001). Emphasizing the beneficial role of ascorbic acid a Teucher et al., (2004) reported that ascorbic acid with its reducing and chelating properties is the most efficient enhancer of non-haem iron absorption, when its stability in the food is ensured. The mechanism by which ascorbic acid increases non-haem

iron absorption is that ascorbic acid has the ability to reduce ferric form of iron to ferrous form. Furthermore a study was conducted by Cook & Reddy, (2001) to estimate the effect of vitamin C on non-haem iron absorption from complete diet. Here iron absorption from complete diet was measured by performing 4 iron absorption tests. For the first test the subjects were asked to consume standard hamburger meal, so that results can be compared to previous studies, while in the remaining 3 tests subjects consumed radio-labelled bread rolls with each of 3 meals for 5 days. During 2^{nd} and 3^{rd} study periods subjects were required to either increase or decrease vitamin C intake. It was found that the beneficial effect of vitamin C in complete diet was not that significant when compared to the effect from single meals.

Inclusion of animal protein sources like meat, chicken, fish has beneficial effect on iron absorption as these foods contain haem form of iron which is more absorbed as compared to the non-haem form. In this context a study was conducted to examine effect of addition of small amounts of pork meat to a phytate rich meal on the extent of non-haem iron absorption. In this study 45 healthy women in age group of 24±3yrs were divided into 3 groups and each group was served 2 meals. Meal A was the basic meal and contained 2.3mg non- haem iron, 7.4 mg vitamin C and 220mg phytate. In contrast, meal B contained either 25g, 50g or 75g of pork meat. Since meals were extrinsically labelled with iron isotopes the determination of iron absorption was carried out from the measurements of ^{59}Fe whole body retention. Results indicated that 25 g of pork meat did not have significant effect on iron absorption, however when 50g and 75g of pork meat was added to the basic meal the non-haem iron absorption significantly increased to 44% and 57% respectively. This concludes that addition of small amounts of meat ($\geq$ 50g) can significantly improve non-haem iron absorption. It has been suggested that this beneficial effect of meat could be due to the potential ability of sulfhydryl-containing amino acids in the meat fractions will chelate non-haem iron and thereby facilitate intestinal absorption (Bach et al., 2003).

Apart from ascorbic acid, vitamin A and β carotene have also shown to improve non-haem iron absorption. In a study 100 human adults were fed 3 cereal based diets (rice, wheat, corn) labelled with radioisotope ^{59}Fe. Each diet contained different concentrations of vitamin A or β carotene. The presence of vitamin A increased iron absorption 0.2 fold for rice, 0.8 fold for wheat and 1.4 fold for corn. There was also considerable increase in iron absorption in presence of β carotene. It was more than 3 fold for rice and about 1.8 fold for both wheat and corn, indicating that vitamin A and β carotene prevented the inhibitory effect of phytate present in these cereals diets and also at the simultaneously increased iron absorption (Garcia-Casal et al., 1998).

Not only iron, enhancers do have beneficial effect on zinc absorption. Animal protein is a good source of zinc has been found to increase zinc absorption significantly (Kristensen et al., 2006).

1.41 Aims of the study

The primary aim of this study is to assess dietarydiversity and micronutrient intake in South Asian women in NW UK and NW PK.

1.42 Secondary aims

1. To investigate the quantity of dietary phytate, a major inhibitor of mineral absorption, present in the flour used to make chapatti/roti in the staple diets consumed by both communities.
2. To examine the relationship between dietary diversity and biochemical indices of micronutrient status for Fe and Zn

1.43 Objectives

1. To obtain data on diets consumed by young women, belonging to South Asian community in the age group of 18-30 years in North West Pakistan and North West UK using a 24 hour recall method.
2. Determine dietary diversity score of the diets consumed in both the study regions
3. Obtain wheat flour from markets in Baghbanan region of North West Pakistan and from traditional South Asian markets in North West UK and determine the total iron, total zinc and phytate content of these flours by laboratory methods.
4. Measure the micronutrient status of women in Pakistan using biochemical indices for Fe and Zn.

2 Chapter Two :Methods

The present study was designed in the quest to understand the micronutrient status of young women in the Baghbanan region of NW PK. The population survey previously conducted in the Baghbanan region (Brick kiln community) by Abaseen Foundation revealed that the typical diet consumed by the people tends to be extremely monotonous and also the availability of a variety of foods essential for good nutrition is limited. Moreover, previous work by our group (AF PK) and personal communication with women in the community provides evidence the diet consumed by women in reproductive age is limited in NW PK (Dykes et al, 2011). However, to date, a detailed evaluation of the dietary patterns and nutrient intakes in this community had not been conducted, and so it was likely that micronutrient deficiencies were prevalent.

So with this view the present study was proposed to assess dietary diversity and the micronutrient status of South Asian communities in two different settings namely a rural setting in Pakistan, the other in an urban setting in the UK. In addition, the presence of inhibitors of micronutrient absorption in the dietary staples (wheat flour) was also investigated in both settings.

2.1 Overview of the chapter

The entire methodology of the study has been divided into different phases

2.2Ethical Approval from the UK and Pakistan committees and data protection

2.3Recruitment of participants

2.4Collection and analysis of dietary data

2.5Collection and Laboratory analysis of wheat flour samples

2.6Collection of blood samples and biochemical analyses

2.7Statistical analysis

2.2 Ethical approval from the UK and Pakistan committees and data protection

In the UK the ethical approval was granted by the STEMH (Science, Technology, Engineering and Medicine) ethics committee at University of Central Lancashire (appendix 1e). In Pakistan, ethical clearance was obtained from Khyber Medical University, Peshawar (appendix 1b).

(i) Confidentiality of all the data – All the participants were allocated an ID number and this number was used at all the times. In the UK and in PK, a record linking names and contact details with the ID have been stored in a locked filing cabinet in the researcher's office (UK) or the Baghbanan Health Centre (PK) respectively until the end of the study. Thus adhering to the UCLan data protection policy, the data collection sheets (24 hour dietary recall sheets) only had the ID number on them.

(ii) Data Storage in both the study locations

UK Data:

The computers used for the storing the study data were UCLan Networked and password protected. No data has been taken off site. It was assured that only the researcher and the supervisory team (Professor. Nicola Lowe, Dr. Stephanie Dillon and Dr. Carol Wallace) had access to the data. Hard copies have been stored in a locked filing cabinet in the researchers office (Darwin Building) and these will be either destroyed or moved to the Director of studies (DoS) office filing cabinet for longer term storage (up to 5 years) if necessary.

PK Data:

Hard copies of data in PK have been stored in a locked filing cabinet at the HC for a maximum of 5 years. The computers used in PK were free standing and so the data has been

stored on an encrypted pen drive. Any information transmitted to the UK by email was anonymised. The data files were encrypted before emailing.

2.3 Recruitment of participants

As per the requirement of the study, female participants aged 18-30 years were recruited in both the study regions.

(i) **In the UK**- A total of 15 second generation, UK born females (18-30yr) were recruited through opportunistic sampling of students from University of Central Lancashire who readily volunteered to be the part of the study, following convenient sampling technique. The awareness of the present study was developed by word of mouth as well as with help of an advertisement flyer (appendix 1a)

(ii) **In Pakistan**- A total of 40 females (18-30yr) were recruited through our on-going collaboration with the AF PK through opportunistic sampling of women who attended the health centre following convenient sampling technique and this was conducted by the nutritionist based at the Baghbanan Health Centre (HC).

2.3.1 Procedure for recruiting participants in both the study regions

Every eligible participant was given a copy of the participant information sheet (appendix 1b and 1c) and the details of the study were clearly explained to the participant. If the participant was satisfied with the information provided and was willing to participate in the study they then signed an informed consent form (appendix 1d). This form was also signed by the researcher. Once this form was signed, the participant was fully enrolled in the study. It was made clear to all participants that they were free to withdraw from the study at any time up

until the end of their own data collection session without any consequence. A statement explaining this was included on both the consent form and participant information sheets.

In Pakistan (Baghbanan region), the female participants were illiterate and so all the information about giving voluntary consent and the details of the participant information sheet were explained to them verbally by the nutritionist in their local language.

2.4 Collection and analysis of dietary data

2.4.1 Collection of dietary data

Dietary data collection was conducted in both the study regions that is in Preston (NW UK) and in Bagbhanan HC (NW PK).

The dietary data was collected using a 24 hour dietary recall method. According to this method, the details of the food consumed by the participant in the last 24 hours were recorded on an approved format (appendix 2h and 2i). This data was collected from the participant on 3 different days between May 2014 and September 2014. Therefore there were 3 dietary recalls per participant. Also all these dietary recalls were interviewer administered and so only thing that the participant had to do was to answer all the questions asked by the interviewer in appropriate depth. The duration of each interview varied in the range of 10-15 minutes depending on the time taken by each participant to recall her intake. The time for the recalls should was mutually agreed by the participant and the interviewer.

In UK - the dietary data of the 15 recruited females (18-30yr) was collected by the main researcher (SP). Here there was flexibility of interviews to be taken on phone, skype or in person and this was totally based on the convenience of the participant. The researcher (SP) asked the participants to discuss about their dietary intake in detail. While doing so the participants were enquired about the brands of the foods consumed, portion sizes, detailed

recipe of the food item consumed. In order to get clear understanding of the portion sizes or amount consumed, the photos of the different portion sizes of different products were shown to the participants so that they can choose which is the closest to the amount of the food consumed (appendix 2g) In addition to this (wherever possible), if participants were not able to quantify the amount of liquids consumed then that amount of liquid (juice, water) was asked to be sampled in researcher's bottle and then this liquid was accurately measured in the laboratory at UCLan. All the participants in UK were well-educated and willing to participate actively in the study. They were also curious to know how the data will be analysed and what will be the outcome. Due to this rapport and positive co-operation, reasonably good quality data could be obtained. Since good quality information was obtained, it was analysed using nutrient database 'WinDiets as well as dietary diversity score technique DDS.

In Pakistan–In Bagbhanan, the recruited participants (18-30yr) were females that were attending the HC for their regular checkup following convenient sampling technique. The HC has a team of doctors and nutritionists who work for AF PK.All the 3 dietary recalls wereconductedface to face by the nutritionistat HC using the same 24 hour recall format as used by researcher (SP) in the UK.Also since the women participants in this region were illiterate,all these interviews were taken by the nutritionist in their local language. These data (24 hour dietary recall sheets) was then transported to UK for analysis.

During analysis it was found that portion size was not appropriate as all the foods consumed were reported in household measurements and these could not be converted into justified amounts. Although the dietary data did give a fair picture of the most commonly consumed products in the study region, the overall quality of the data was not good enough to allow detailed analysis for the micronutrient content using a database.

Therefore, the dietary data obtained from Pakistan could not be analysed using software such as WinDiets, where it is important to have accurate portion sizes in order to calculate micronutrient intake of individual participants.Considering all these confounding factors it was decided that the DDS should be calculated for both the study locations.

2.4.2 Analysis of dietary data

The raw dietary data obtained from the dietary recalls was analysed using DDS method developed by Food and Agricultural Organisation of United Nations (FAO) (Kennedy et al., 2011) for measuring household and individual dietary diversity.

In this method a DDS sheet was used (fig 2.1). According to the guidelines for this method, DDS can be used for assessing diversity scores at household level (HDDS) with score range of 0-12 and individual level (WDDS) with score range 0-9. Since the present study involved collection of dietary data at individual level, the WDDS format was used (Table 2-1)

Questionnumber	Foodgroup	Examples	YES=1NO=0
1	CEREALS	corn/maize,rice,wheat,sorghum,milletoranyothergrainsorfoodsmadefromthese(e.g.bread,noodles,porridgeorothergrainproducts)+*insertlocalfoodse.g.ugali,nshima,porridgeorpaste*	
2	WHITEROOTSANDTUBERS	whitepotatoes,whiteyam,whitecassava,orotherfoodsmadefromroots	
3	VITAMINARICHVEGETABLESANDTUBERS	pumpkin,carrot,squash,orsweetpotatothatareorangeinside+*otherlocallyavailablevitaminArichvegetables(e.g.redsweetpepper)*	
4	DARKGREENLEAFYVEGETABLES	darkgreenleafyvegetables,includingwildforms+*locallyavailablevitaminArichleavessuchasamaranth,cassavaleaves,kale,spinach*	
5	OTHERVEGETABLES	othervegetables(e.g.tomato,onion,eggplant)+*otherlocallyavailablevegetables*	
6	VITAMINARICHFRUITS	ripemango,cantaloupe,apricot(freshordried),ripepapaya,driedpeach,and100%fruitjucemadefromthese+*otherlocallyavailablevitaminArichfruits*	
7	OTHERFRUITS	otherfruits,includingwildfruitsand100%fruitjuicemadefromthese	
8	ORGANMEAT	liver,kidney,heartorotherorganmeatsorblood-basedfoods	
9	FLESHMEATS	beef,pork,lamb,goat,rabbit,game,chicken,duck,otherbirds,insects	
10	EGGS	eggsfromchicken,duck,guineafowlorany otheregg	
11	FISHANDSEAFOOD	freshordriedfishorshellfish	
12	LEGUMES,NUTSANDSEEDS	driedbeans,driedpeas,lentils,nuts,seedsorfoodsmadefromthese(eg.hummus,peanutbutter)	
13	MILKANDMILKPRODUCTS	milk,cheese, yogurt or other milkproducts	
14	OILSANDFATS	oil,fatsorbutteraddedtofoodorusedforcooking	
15	SWEETS	sugar,honey,sweetenedsodaorsweetenedjucedrinks,sugaryfoodssuchaschocolates,candies,cookiesandcakes	
16	SPICES,CONDIMENTS,BEVERAGES	spices(blackpepper,salt),condiments(soysauce,hotsauce),coffee,tea,alcoholic beverages	
Household levelonly	Didyouoranyoneinyourhouseholdeatanything(mealorsnack)OUTSIDEthehomeyesterday?		
Individuallevel	Didyoueatanything(mealorsnack)OUTSIDEthehomeyesterday?		

Table 2-1Dietary Diversity Score Sheet

Source: Kennedy et al., (2011)

This sheet has four columns namely ‘Question number’, ‘Food group’, ‘Examples of food items in each food group’ and ‘Scoring column with Yes=1 and NO=0’. In this context, there were total 16 questions and 16 food groups. The diets of the participants were sorted in this score sheet pattern, for example if participant ‘a’ had meat or meat products only on the first day of the recall out of the 3 dietary recall sessions, then it was noted as 1, 0, 0. This pattern was followed for analysis of all the dietary recalls. In addition to this, to arrive at the WDDS, it was suggested that some of the food groups should be combined. According to this the food group in question 1 and 2 (cereals and white roots and tubers) were combined into one as ‘Starchy staples’. This pattern was followed for other combinations as given in table 2-2.

Questionnumber(s)	Foodgroup
1,2	Starchystaples[1]
4	Darkgreenleafyvegetables
3,6andredpalmoilifapplicable	OthervitaminArichfruitsandvegetables[2]
5,7	Otherfruitsandvegetables[3]
8	Organmeat
9,11	Meatandfish[4]
10	Eggs
12	Legumes,nutsandseeds
13	Milkandmilkproducts

Table 2-2Combination pattern for different food groups as per guidelines for DDS

1 ThestarchystaplesfoodgroupisacombinationofCerealsandWhiterootsandtubers.
2TheothervitaminArichfruitandvegetablegroupisacombinationofvitaminArichvegetablesandtubersandvitaminArichfruit.
3Theotherfruitandvegetablegroupisacombinationofotherfruitandothervegetables.
4 Themeatgroupisacombinationofmeatandfish.
Source: Kennedy et al., (2011)

An SPSS 21 data sheet was prepared including all food groups, combinations of the questions (example – for combining questions 1 and 2 for day one it was entered as ‘D1Q1Q2’ and so on) and numbers of all the participants from both the study regions. Subsequently all the scores (either 1 or 0 were entered under individual headings and then a WDDS was computed

by adding all these combinations as per the guidelines. These scores enabled us to get clear understanding of the diversity in the diets consumed by participants in two different geographical areas. Higher scores indicated higher diversity and vice-versa.

The dietary data that was obtained from NW UK participants was in depth and appropriate portion sizes were obtained for the food items consumed by the participants, so along with DDS, this data was also analysed using WinDiets to obtain detailed nutritional intake of the participants in NWUK.

Data entry in WinDiets – A new workspace was created for each individual. Once the required workspace wasopened, the name food item or the food code (if known) wasentered in the left side box on the window. On entering the name of food, a list of relevant food items appeared, and then on selecting the required one, the corresponding code appeared in the box. After this the accurate weight of the food consumed was entered in the adjacent box and this enabled the entry of the food item in the worksheet. There was also an option to move between meals, which was used to move from breakfast to morning snacks and so on.

It is important to note that in order to obtain optimal nutritional profile, entering accurate weight of the food items consumed was crucial. The option , 'visualise portion size' helped in entering the correct portion sizes of various food items.

Also, if a certain recipe was not available in the list of local data then a new recipe was created and then this recipe was entered with the actual portion consumed.

Data Analysis in WinDiets– Once the data was entered completely the option 'Analysis' allowed the user to calculate the total nutritional content of the meal by day, or by DRV or up to a week.

2.5 Collection and Laboratory analysis of wheat flour samples

2.5.1 Collection of wheat flour samples

Wheat flour samples (staple diet of people in Pakistan) were collected from both the study settings, which is from NW UK (4 samples) and NW Pakistan (10 samples).

Collection from North West UK: - During 24 hour dietary recalls, the participants were asked about the brands of the food items consumed. Along with other food items the participants were also enquired about the brands of the wheat flour that were consumed by them. After collation of the data it was found that there were 4 most common types of wheat flour that were used by participants in NW UK . So these flour brands were purchased from traditional South Asian shops in NW UK (Preston) and analysed for iron, zinc and phytate content.

Collection from North West PK: - The nutrition team based at the HCcollected wheat flour samples from ten different households in Baghbanan. These samples were transported to the UK as per the advice from the high commission in Islamabad. A warden of the High Commission, who was familiar with our work, brought the flour in a diplomatic bag with prior clearance from customs.

2.5.2 Laboratory analysis of wheat flour samples

All wheat flour samples were analysed for their total iron, total zinc and phytate content using wet laboratory techniques.

All glassware used were acid washed in a 10% nitric acid solution (made up from a 70% concentrated stock) overnight and rinsed with deionized water followed by drying in a drying chamber. The acid used was of GR (Guaranteed Reagent) grade. Deionized water was used

for preparation of aqueous solutions. Samples were extracted for individual parameters in duplicates and were analysed in triplicates.

(i) Determination of total iron and total zinc content

The total iron and zinc content of the flour was determined using Atomic Absorption Spectroscopy (AAS) (Thermoscientific -ICE 3000 Series) adapted from method by Kim and Song (2014).

Principle:-

AAS technique was used to identify the presence and concentration of substances by analysing the spectrum produced when a substance is vaporised and absorbs certain frequencies of light. AAS has been used particularly for detecting the concentrations of metal ions in solutions.

Reagents:-

1. Nitric Acid (70% ACS reagent)
2. Iron standard solution (1000ppm Fisher scientific chemicals)
3. Zinc standard solution (1000ppm Fisher scientific chemicals)

Procedure:-

Wheat flour samples obtained from different sources were accurately weighed (1g). These samples were then digested overnight (o/n) at room temperature by adding 5ml of nitric acid (70% ACS reagent) in a closed 50-mL graduated polypropylene tube. Following the o/n incubation, samples were incubated in an oven at 60-70°C for 1 hour. At the end of the incubation period samples were cooled to room temperature and then the final volume was made up to 25 ml with deionised water. This solution was then analysed for total iron and total zinc content using AAS.

Prior to analysis of sample solutions for the individual element, a standard curve was plotted using standard solutions separately for iron and zinc.

Preparation of standard curve for iron - Iron standard solution was used to prepare dilutions for plotting standard curve. From this solution, five standard dilutions were made with concentrations from 1ppm to 5ppm. These standards were analysed using AAS and then this was followed by sample solution analysis for iron. The standard curve for iron is presented in fig 2-1

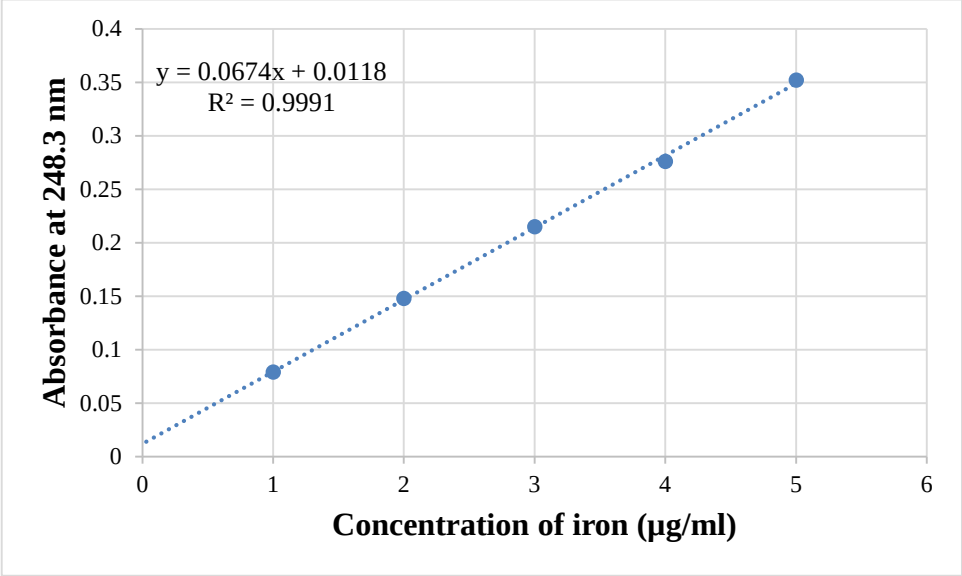


Figure 2-1Standard curve for iron

Preparation of standard curve for zinc - Zinc standard solution was used to prepare dilutions for plotting standard curve. From this solution, five standard dilutions were made with concentrations from 0.2ppm to 0.6ppm. These standards were analysed using AAS and then this was followed by sample solution analysis for zinc

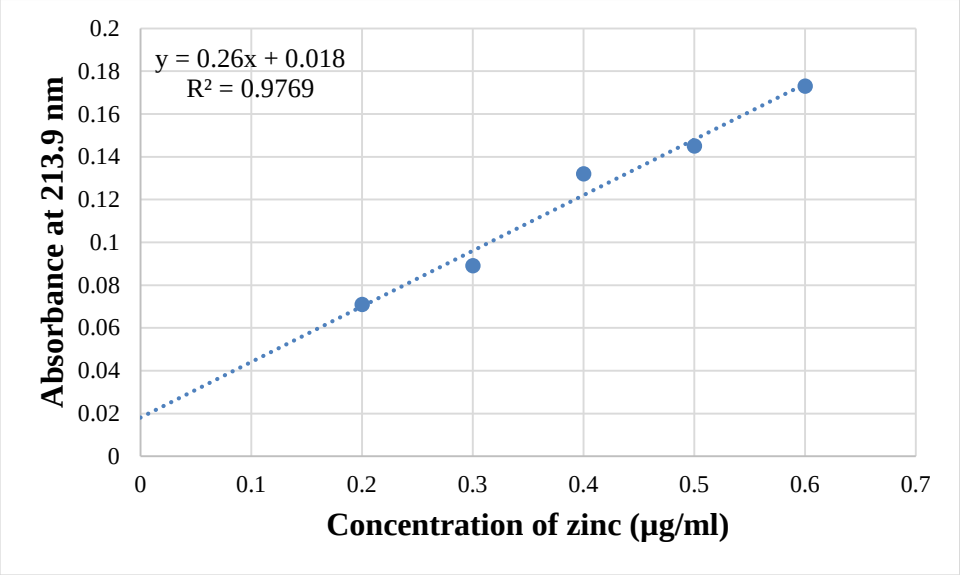


Figure 2-2Standard curve for zinc analysis

Validation of the method with reference material

In order to ensure that the results obtained by the method used for iron and zinc analysis were valid, a certified reference material (BCR – 191, Brown bread, Sample identification no – 0670) with known iron and zinc content (4.07mg/100g, 1.95mg/100g respectively) was run simultaneously and it was treated in the same way as the other samples.

(ii) Determination of phytate content

Phytate content was estimated by method described by Haug and Lantzsch (1983)

Principle:-

Phytate content was measured indirectly. A known amount of Fe+3 solution was added which binds with the phytate and the quantity of unbound ferric solution was estimated spectrophotometrically. Unbound Fe+3 ions complexed with with 2-2' bipyridyl solution and gave coloured complex (pink). The colour change was measured spectrophotometrically at wavelength 519 nm against a sample blank.

Reagents:-

1. Phytate reference solution - Sodium salt of phytic acid (Sigma, Aldrich 100969894)
2. Ferric solution (Ammonium iron (III) sulphate dodecahydrate $NH_4Fe(SO_4)_2.12H_2O$) (Sigma Aldrich 221260)
3. 2-2' bipyridyl solution (2,2'- Bipyridinium-N-N'-dipropylsulphonate Sigma Aldrich 7565)

Preparation of reagents is given in appendix 2j

Preparation of Standard Curve

Procedure

Dilutions from 1ml to 8ml were prepared using phytate reference solution. For example – for 1ml dilution – 1ml of phytate reference solution (was pipetted out in 100ml volumetric flask and then the volume was made up to 100ml with 0.2 N HCL. Similar procedure was followed for all 8 dilutions. Subsequently 0.5ml of each of these solutions (1,2,3,4,5,6,7,8ml) were pipetted out in 10ml test tubes with glass stoppers. To this 0.5 ml of ferric solution was added. These tubes were covered and kept in boiling water bath for 30 minutes. These tubes were then cooled in ice water for 15 minutes and then kept at room temperature for 15 minutes. To these tubes 2ml of 2-2'bipyridyl solution was added and absorbance was measured in spectrophotometer (JENWAY 7315 spectrophotometer) at 519 nm after a specified time period of 1 minute.

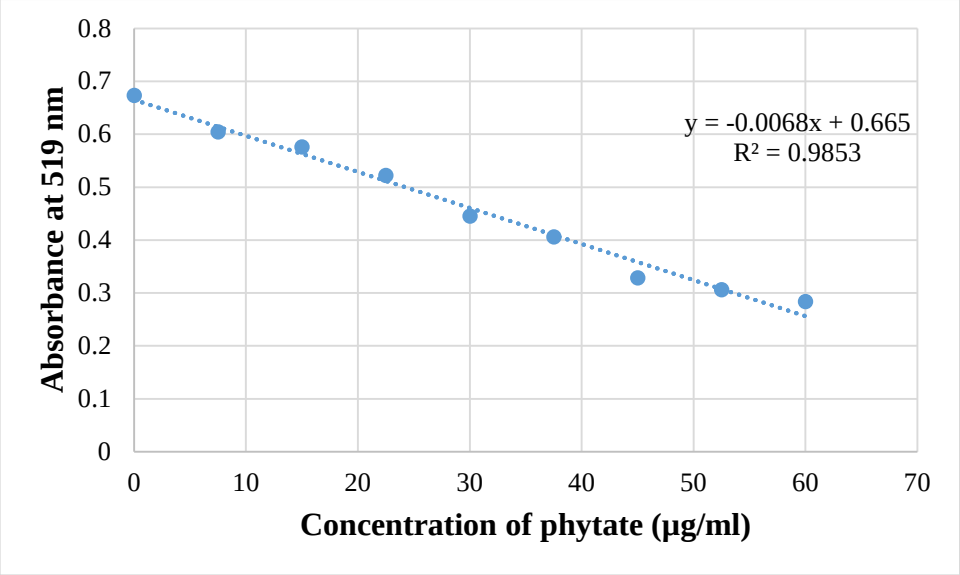


Figure 2-3Standard curve for phytate analysis

Sample Extraction

0.1g of dry sample was weighed in 100ml glass bottles and then mixed with 20ml of 0.2N HCL. These bottles with sample mixtures were incubated in an unstirred water bath (Clifton) for 3hrs at 37°C. At the end of incubation period this extract was filtered through ashless whatmann filter paper (Sigma Aldrich, Product code Z241180 - 110 mm diameter). 0.5 ml of this filtrate was added to 0.5ml of ferric solution and this was transferred to 1.5 ml eppendorf tubes. Tubes were then centrifuged at 13000 rpm for 15 minutes using a spectrafuge (Spectrafuge 24 D – JENCONS-PLS). The supernatant was used to estimate phytate content by the procedure used for standard curve.

Validation of the method

In order to ensure that the results obtained by the method used for phytate analysis were valid, a phytate recovery was carried out. A sample whose phytate content was analysed was

spiked with a fixed amount of phytate reference solution (2ml) and was subsequently treated in the same way as other samples. On obtaining the absorbance values for this spiked sample, the percent recovery was calculated using formula as given below:

$$\% \text{ recovery} = \frac{\text{Experimental value1 (original value of flour in µg/ml)}*100}{\text{Experimental value 2 (value obtained after spiking the flour in µg/ml)}}$$

Moreover one specific sample was used as internal standard. This means that this sample was run with every batch of samples, in order to assess between batch variations.

2.6 Collection of blood samples and its analysis for biochemical parameters

Blood samples were only taken from the PK based population for pragmatic reasons and ethical reasons. It was anticipated micronutrient deficiencies are present in the PK population (due to poverty and poor diet) and not in the UK population. There was full support through the HC (from where the Nutrition team operate) in PK to enable blood samples to be taken safely and stored appropriately. A single blood sample was taken. The haemoglobin levels of these blood samples were measured using an automated hematology ananlyzer (model: poch-100i, Sysmex corporation kobe japan, S.no B0697) at Baghbanan HC. The plasma zinc levels of these blood samples were analysed by AAS at UCLan.

2.6.1 Determination of plasma zinc levels

The plasma zinc content of the samples obtained from participants in NW PK was determined using AASby a method adapted from Lowe et al., (1998).

Reagents

1. Deionised water (Purity -18.2 micrograms)
2. 0.125 M HCL (Fisher scientific chemicals)
3. Seronorm™ Trace Elements Serum L-1 (Kit size 6*3ml, SERO AS, Norway)
4. Zinc standard solution (1000ppm Fisher scientific chemicals)

Procedure

Zinc standard solution was used to prepare dilutions for plotting standard curve. From this solution, six standard dilutions were made with concentrations from 0.05ppm to 0.5ppm. These standards were analysed using AAS and then this was followed by sample solution analysis for zinc.

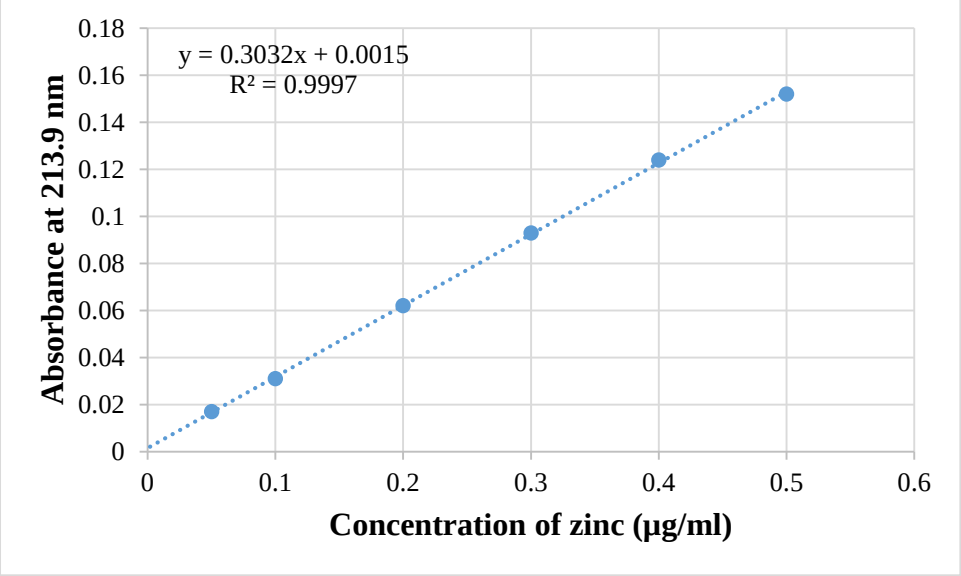


Figure 2-4Standard curve of zinc for plasma zinc analysis

Sample zinc analysis

The samples were diluted in the ratio of 1 in 5 before analysis for zinc content. For this purpose 0.2 ml of plasma sample was mixed with 0.8ml of 0.125 M HCL in an eppendorf tube (1.5ml). Subsequently these samples were vortex for 0.5-1minute, so that plasma mixes properly with HCL and a clear solution can be obtained for analysis. Following this the samples were analysed using AAS for their zinc content at 213.9nm.

Validation of the method

In order to ensure that the results obtained by the method used for zinc analysis were valid, an internal standard namely Seronorm Trace elements serum was used. This Seronorm was diluted prepared by adding 3ml of deionised water to the standard power sample (6*3ml). From this solution, it was further diluted in the ration of 1 in 5, similar to dilution as used for the sample. This Seronorm was run after fixed number of samples. This was to assess the validity of the results obtained for the samples.

2.7 Statistical Analysis

2.7.1 Comparison between dietary diversity scores of the diets consumed in NW UK and NW PK

The DDS obtained for 3 days (3 dietary recalls per participant) for all the participants in NW UK and NW PK were statistically analysed using statistical analysis package SPSS Version 22 for windows (IBM SPSS 22). Firstly the data was assessed for normality using Kolmogorov Smirnov test and the Sharpiro-Wilk test. The statistical significance was set at $p < 0.05$. Normally distributed data were comparing using t test and not-normally distributed data were compared using the Wilcoxon Signed Ranks test. Moreover mean and standard deviation values for all 3 days were calculated for all the participants.

2.7.2 Comparison between dietary diversity scores for consumption of specific food groups (starchy staples, vitamin A rich fruits/vegetables, other fruits/vegetables and meat/fish) across NW UK and NW PK

The DDS obtained for consumption of all the specific food groups (starchy staples, vitamin A rich fruits/vegetables, other fruits/vegetables and meat/fish) on 3 days (3 dietary recalls per participant) by all the participants in NW UK and NW PK were statistically analysed using statistical analysis package SPSS Version 22 for windows (IBM SPSS 22). The data was assessed for normality using Kolmogorov Smirnov test and the Sharpiro-Wilk test. The statistical significance was set at $p < 0.05$. Mean and standard deviation values were calculated for the scores of all 3 days for all the specified food groups. The data for starchy staples was normally distributed and so, an independent t-test was also performed using the scores of all the all the four specified food groups for all the participants in NW UK and NW PK. This test helped to gain clarity on the significant differences in between the scores for both the study regions. Since the data for other food groups was not normally distributed Wilcoxon Signed Ranks test was conducted to compare differences among the two study regions.

2.7.3 Comparison between iron, zinc and phytate content of the wheat flour samples obtained from NW UK and NW PK

Wheat flour samples from NW UK (4 samples from Preston, Lancashire) and NW PK (10 samples from Baghbanan community) were analysed using wet laboratory techniques at the UCLan. The values obtained for iron, zinc and phytate content of these samples were statistically analysed using statistical analysis package SPSS Version 22 for windows (IBM SPSS 22). The data was initially assess for normality using Kolmogorov Smirnov test and the Sharpiro-Wilk test. The statistical significance was set at $p < 0.05$. Mean and standard deviation values were calculated for all three parameters. The data for iron and phytate

content of samples was normally distributed and so an independent t-test was also conducted for all the samples. However the values for zinc content showed data which was very close being not normally distributed, so Wilcoxon Signed Ranks test was conducted.

In addition, a Chi-square test was used to test for an associate between community location (UK or PK) and frequency of food group consumption.

3 Chapter Three: Results

3.1 Overview of the chapter

In this chapter, the study results are presented in three sections.

In the first section, DDS for the diets consumed by participants in both the study regions (NW UK and NW PK) are presented. Along with this participant characteristics are also detailed in tabular format. Moreover, the frequency of consumption of specific food groups (for example, Vitamin A rich fruits and vegetables, meat and fish) by the participants istabulated.

In the second section, the results obtained from the laboratory analysis of wheat flour samples obtained from both the study locations (NW UK and NW PK) are presented. All the wheat flour samples were analysed for their iron, zinc and phytate content using wet laboratory techniques.

In the third section, data regarding the micronutrient status of women in Baghbanan region (NW PK) are presented. This was assessed using biochemical indices for iron status (haemoglobin levels) and zinc status (plasma zinc concentration).

3.2 Dietary diversity scores for the diets consumed in NW UK and NW PK

In order to estimate WDDS, dietary data was collected from the participants in both the study regions using 24 hour dietary recall format as described in section 2.4.1 . Also it can be noted that D1, D2, D3 in all the data related to diversity scores represents day 1, day 2 and day 3 respectively. The participant characteristics for NW UK and NW PK are presented in the table 3-1

Table 3-1Participant characteristics for NW UK and NW PK

Location	Mean age ± SD	% Married	% Pregnant	% Lactating	% taking nutritional supplements
NW UK	25.26 ± 6.83	40	6.66	0	6.66
NW PK	24.7± 3.64	100	50	50	12.5

The mean and standard deviation values were also calculated for all 3 for both the study regions. These values have been presented in Table 3-2

Table 3-2Mean and standard deviation values for dietary scores in UK and PK

Country	D1WDDS (Mean±SD)	D2WDDS (Mean±SD)	D3WDDS (Mean±SD)
UK(n=15)	5.06±1.33	4.86±1.84	4.33±1.49
Pakistan(n=40)	3.02±0.97*	2.85±0.69	2.55±0.78*

The symbol * indicates $p \leq 0.01$ for comparison between UK and PK on that day

As seen in table 3-2 the mean DDS for UK participants ranged from 4.33-5.06 whereas that for PK participants these varied from 2.55-3.02. So it can be concluded that the mean values of the scores for all 3 days were significantly lower for PK participants as compared to UK participants.

3.2.1 Frequency of consumption of specific food groups consumed by participants in NW UK

Tables 3-3 below shows the frequency of consumption of specific food groups, namely starchy staples, vitamin A rich foods, other fruit and vegetables and meat and fish for both the study locations over the 3 day monitoring period.

Table 3-3Consumption of specific food groups by participants in NW UK and NW PK

Food group	Location	Day 1 (%)	Day 2 (%)	Day 3 (%)
Starchy staples	NW UK	100	93.33	93.33
	NW PK	100	100	100
Vitamin A rich fruits and vegetables	NW UK	33.33	46.66	20
	NW PK	27.5	12.5	0
Other fruits and vegetables	NW UK	100	92.85	92.85
	NW PK	37.5	30	47.5
Meat and fish	NW UK	60	71.42	85.71
	NW PK	17.5	20	20

For each food group, a Chi- square test was used explore the association between study group and frequency of food group consumption on each day. Chi -square test revealed a significant association between community (PK or UK) and frequency of consumption of vitamin A rich foods ($p=0.003$), meat and fish ($p<0.005$) and fruit and vegetables ($p<0.005$), but not starchy foods.

3.2.2 Comparison between nutrient intake (iron, vitamin A) obtained from WinDiets and dietary diversity scores

The average iron and vitamin A intake obtained from WinDiets analysis were plotted in a graph against DDS scores in order to observe any impact of iron and vitamin A intake on the scores. However due to small sample size in NW UK (n = 15), no significant correlation was obtained.

3.3 Laboratory analysis of wheat flour samples from markets in NW UK and NW PK

3.3.1 Iron content of wheat flour samples

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their total iron content using AAS by a method adapted from Kim and Song (2014).

The bar diagram (Fig 4a.) shows the iron content of different flour samples. The first four bars indicate the iron levels in flour samples obtained from NW UK. The fifth bar reveals a range of values for 10 different flour samples obtained from 10 homes from the Baghbanan community in NW Pakistan.

The iron content of samples obtained from traditional south Asian markets in NW UK ranged from 3.68mg/100g for wholemeal flour to 1.35mg/100g for white flour. A gradual decrease was observed in the iron content of the flour samples with increase in the level of processing. This implies that the least processed whole meal flour had highest amount of iron and the iron levels decreased gradually in medium chapatti flour and then were found to be lowest for highly refined white flour. The iron content of the wheat flour samples obtained from 10 different households in Baghbanan varied widely from as low as 2.51mg/100g to as high as 7.00mg/100g. The iron content of various wheat flour samples has been depicted in figure 3-1

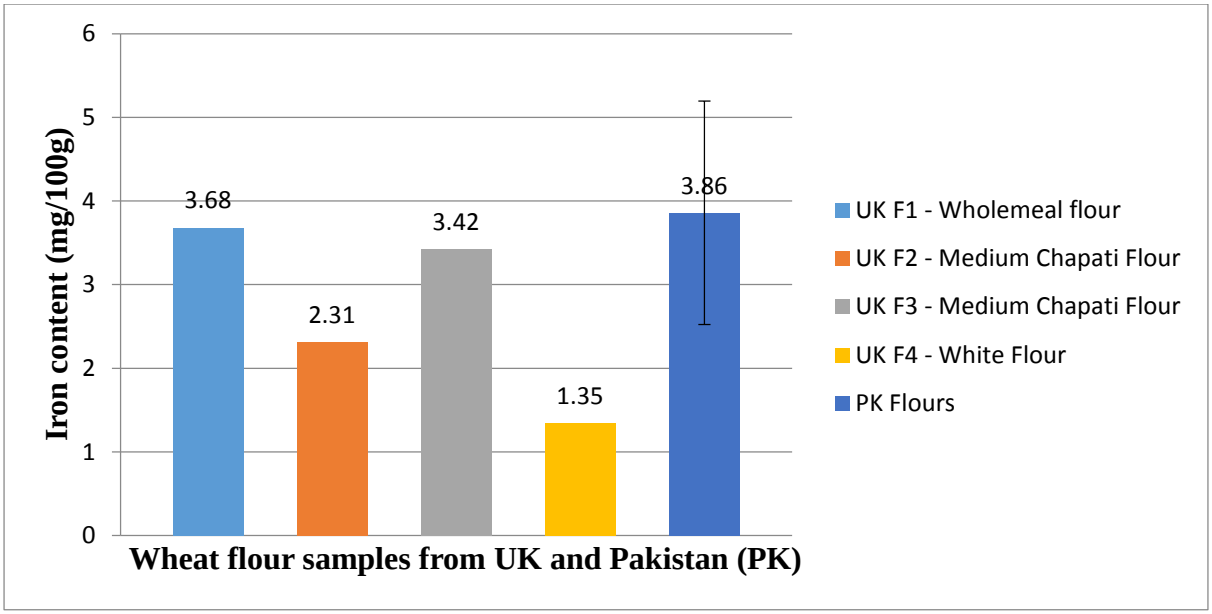


Figure 3-1Bar chart showing the iron content of different wheat flour samples from UK and PK

Further in order to ensure that the results obtained in the present study are valid, a certified reference material (BCR – 191, Brown bread, Sample identification no – 0670) was run simultaneously with other samples and was treated in the same manner as other samples. The known iron content of this reference material was 4.07mg/100g and the iron values obtained for the experimental sample were 4.29mg/100g. Since the experimental value was very close (within 5.5%) to the known value of certified material, it can be stated that the method adapted for determination of iron content in the present study was appropriate giving valid results.

3.3.2 Zinc content of wheat flour samples

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their total zinc content using AAS by a method adapted from Kim and Song (2014).

The results showed that the zinc content varied from 1.98mg/100g for UK wholemeal flour to 1.00mg/100g for UK white flour. Slight variations in the zinc values were also observed for

two medium chapatti flour samples 1.34mg/100g and 1.43mg/100g. These variations could be attributed to differences in brands of the two flours (East End and Supreme) respectively.

Wide variability was observed for zinc content of wheat flour samples obtained from NW PK ranging from as low as 1.32mg/100g to as high as 8.25mg/100g. Overall results revealed a pattern similar to the one observed for iron values of different flours. The zinc content was found to be highest for wholemeal flour and lowest for highly refined white flour. The zinc content of various wheat flour samples has been shown in fig.3-2

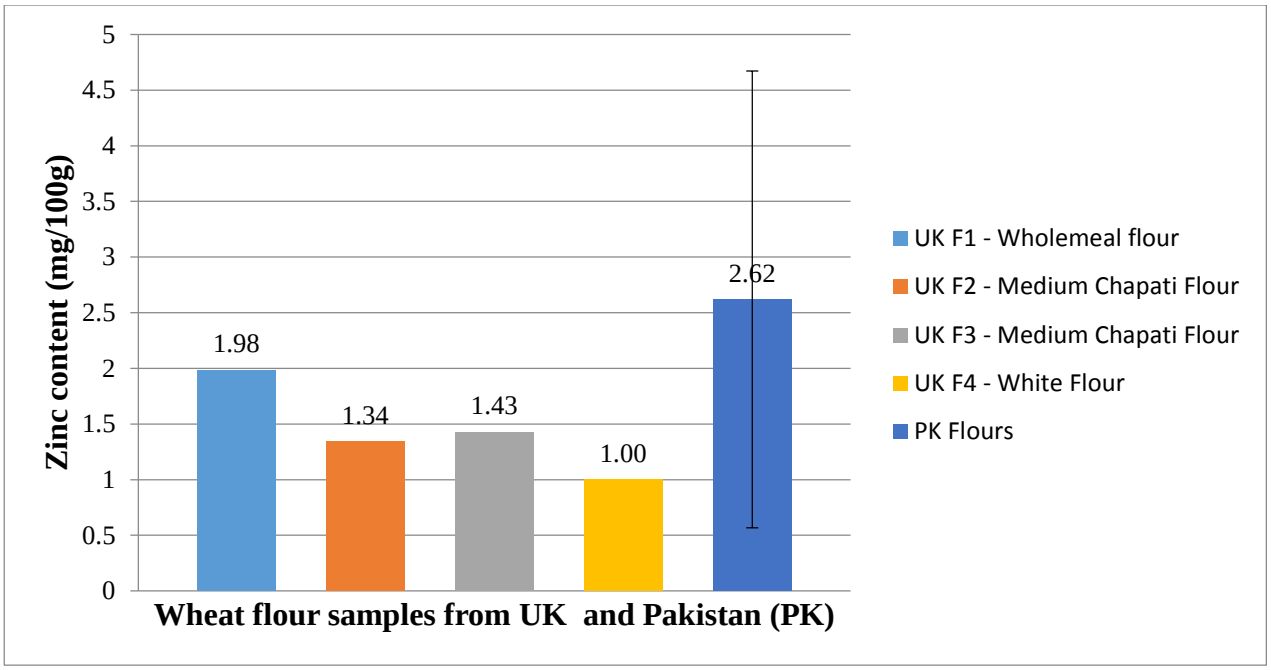


Figure 3-2Bar chart showing the zinc content of different wheat flour samples from UK and PK

Validity of the results for zinc content was ensured using a certified reference material (BCR – 191, Brown bread, Sample identification no – 0670). The known zinc content for this reference material was 1.95mg/100g. The experimental value obtained after analysing this material for zinc content was 1.93mg/100g. Since the values were extremely close to each other (within 2 %), it could be concluded that the method and the results were appropriate for determination of zinc content in the present study.

3.3.2 Phytate content of wheat flour samples

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their phytate content using spectrophotometric method adapted from Haug and Lantzch (1983).

The phytate content was found to be highest for UK wholemeal flour (583.82mg/100g) and lowest (273.52mg/100g) for UK white flour. This pattern was comparable to one observed in context of iron and zinc values for the same set of flour samples. A wide range of values were obtained for phytate content of wheat flour samples consumed by people in Baghbanan (NWPK) (230.39 - 565.44mg/100g). Fig.3-3 depicts phytate contents of various wheat flour samples analysed in the present study.

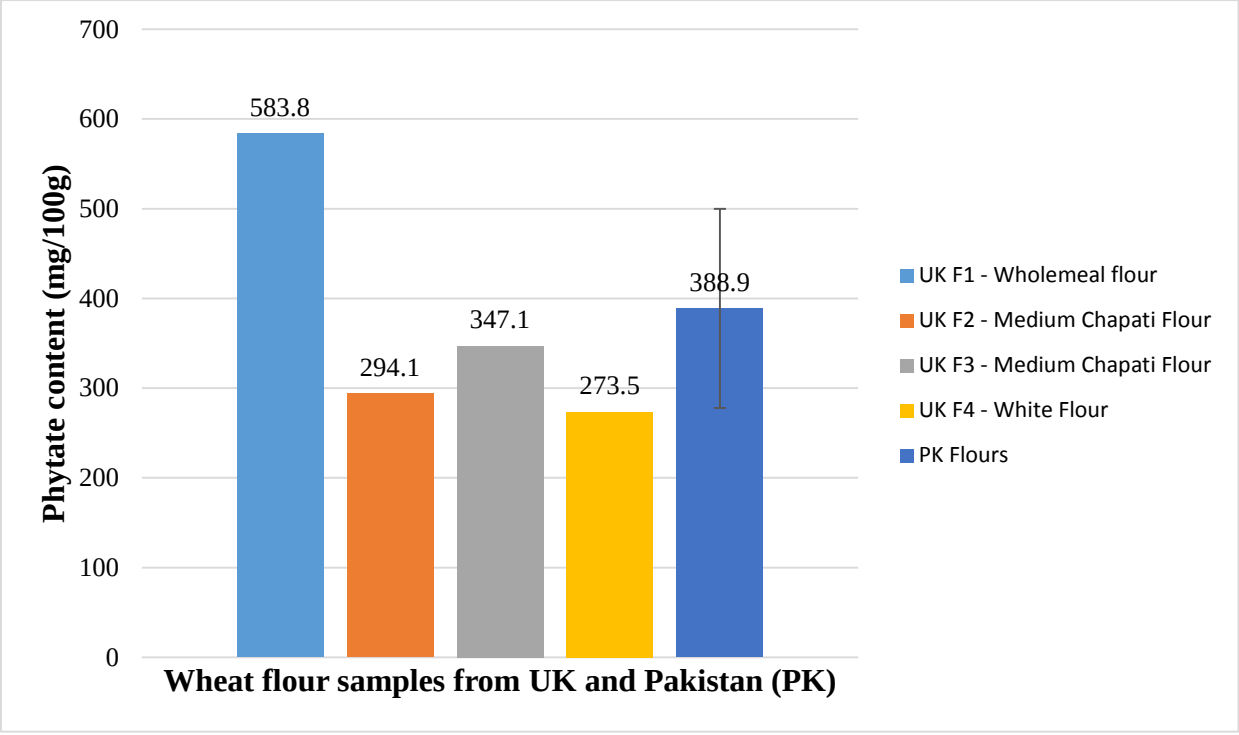


Figure 3-3Bar chart showing the phytate content of different wheat flour samples from UK and PK

In case of phytate content, the validity of the method and the results was determined using a phytate recovery method. The results obtained showed 92.19% phytate recovery. This confirmed that the method used for analysis was appropriate giving valid results.

In addition to these the coefficient of variation (CV) was calculated for all the three methods. Accordingly the average CV for phytate, iron and zinc was found to be 0.8, 3.7 and 4.9 respectively.

3.3.3 Determination of phytate:zinc molar ratios

The phytate:zinc molar ratio has been suggested as an effective tool to estimate the proportion of absorbable zinc (Brown et al., 2004, WHO,1996). WHO (1996) has defined three categories of diets with high (phytate-zinc molar ratio less than 5), medium (phytate-zinc molar ratio from 5-15) or low zinc availability (phytate-zinc molar ratio greater than 15). Phytate:zinc molar ratios has been calculated in the present study which involved analysis of wheat flour samples from NW UK and NW PK. Gargari et al., (2007) has given a formula for calculating phytate:zinc molar ratios in food samples. The formula is presented as follows:-

$$\text{Phytate:zinc molar ratios} = \frac{\text{Phytic acid (mg/100g)}/660}{\text{Zinc (mg/100g)}/65.4}$$

According to this formula, the phytate:zinc molar ratios has been calculated for 4 samples from NW UK and 10 samples from NW PK, These ratios have been presented in table 3-4

Table 3-4Phytate:zinc molar ratios of different wheat flour samples

Name of the flour	Phytate content (mg/100g)	Zinc content (mg/100g)	Phyate:zinc molar ratio
UK Wholemeal wheat flour	583.82	1.98	29.46
UK Medium chapatti flour (East End)	294.11	1.34	22.25
UK Medium chapatti flour (Supreme)	347.05	1.42	26.25
UK White flour	273.52	1.00	41.4
PK 1	493.38	2.65	18.67
PK 2	565.44	3.03	21.4
PK 3	416.91	2.10	21.03
PK 4	230.39	2.12	11.63
PK 5	286.27	1.82	21.65
PK 6	326.47	1.32	24.7
PK 7	302.20	8.25	3.80
PK 8	530.14	1.84	40.15
PK 9	368.38	1.30	55.8
PK 10	369.36	1.71	27.95

3.4 Determination of biochemical indices of micronutrient status in participants in Baghbanan

3.4.1 Haemoglobin levels

The iron status of the women in Baghbanan was determined by estimating haemoglobin levels at Baghbanan HC. The haemoglobin values for 40 participants has been given in table 3-5

Table 3-5Haemoglobin levels of participants in NW PK (Baghbanan)

Participant number	Physiological status	Haemoglobin levels (g/dl)	Participant number	Physiological status	Haemoglobin levels (g/dl)
1	Pregnant	10.2	21	Pregnant	10.0
2	Pregnant	10.6	22	Lactating	9.9
3	Lactating	10.6	23	Pregnant	9.4
4	Pregnant	10.5	24	Pregnant	11.7
5	Pregnant	9.9	25	Lactating	11.8
6	Lactating	9.4	26	Lactating	12.7
7	Pregnant	8.8	27	Pregnant	11.2
8	Lactating	9.3	28	Pregnant	11.9
9	Lactating	10.0	29	Lactating	12.9
10	Lactating	11.0	30	Lactating	11.0
11	Lactating	9.8	31	Lactating	11.5
12	Pregnant	11.0	32	Pregnant	10.9
13	Pregnant	13.0	33	Lactating	12.4
14	Pregnant	11.4	34	Pregnant	10.0
15	Pregnant	12.0	35	Pregnant	10.7
16	Lactating	12.9	36	Lactating	13.3
17	Lactating	13.4	37	Lactating	11.7
18	Lactating	12.0	38	Lactating	12.2
19	Lactating	10.3	39	Pregnant	11.6
20	Pregnant	10.0	40	Pregnant	12.4

The haemoglobin values in the PK participants ranged from 8.8-13.4g/dl. Gibson et al, (2005) reported that the normal haemoglobin value for non-pregnant women was 12g/dl and that for pregnant women was 11g/dl. According to these cutoff values 18 women had haemoglobin values lower than 11g/dl. However among 40 participants 7 had extremely low values ranging from 8.8-9.9g/dl.

The zinc status of women in Baghbanan region was determined by estimating plasma zinc values at University of Central Lancashire. A total of 40 plasma samples were obtained from Pakistan. However the zinc values of only 15 samples could be stated. All of the plasma samples were analysed, however due to unexpected technical fault with AAS, the values obtained for the remaining samples does not seem reliable. So in the present study zinc values of 15 samples have been presented. The plasma zinc values have been presented in table 3-6.

Table 3-6Plasma zinc values of participants in Baghbanan region

Participant number	Zinc levels (mg/L)	Physiological status
1	0.637	Pregnant
2	0.617	Pregnant
3	0.534	Lactating
4	0.518	Pregnant
5	0.486	Pregnant
6	0.590	Lactating
7	0.611	Pregnant
8	0.574	Lactating
9	0.464	Lactating
10	0.533	Lactating
11	1.350	Lactating
12	0.543	Pregnant
13	0.643	Pregnant
14	0.541	Pregnant
15	0.467	Pregnant

The zinc values obtained for the 15 participants range from 0.46-1.30 mg/L. The normal zinc range is from 0.7-1.2mg/L (Davis, 1993). So according to this range 14 participants out of 15 have low zinc levels.

4 Chapter Four:Discussion

4.1 Overview of the chapter

Thischapter will begin with briefing about significance of using DDS technique and then will proceed with explaining WDDS for the overall diets consumed by female participants (18-30yr) in NW UK and NW PK. The frequency of consumption of specific food groups (starchy staples, vitamin A rich fruits/vegetables, other local fruits/vegetables and meat/ fish) will also be highlighted. Furthermore any significant differences observed in the consumption pattern in relation to these specific groups across the two different geographical areas will also be brought out accompanying with reasons for the same. The discussion after this will move on to the second aim of the present study which involved laboratory analysis of wheat flour samples obtained from 10 different households in NW PK (Baghbanan community) and 4 samples from traditional south Asian shops in NW UK (Preston, Lancashire) for iron, zinc and phytate content. Each parameter will be highlighted in discussion and the results will be correlated with other published literature dealing with similar type of analysis. Finally adhering to the third and final aim of the study micronutrient status of women in NW PK will be explored based on the data obtained for haemoglobin levels (indicating iron status) and plasma zinc levels as analysed at UCLan using AAS. These levels will be discussed with the established normal ranges for these parameters.

4.2 WDDS of south Asian women in NW UK and NW PK

In the present study, the results revealed that there were significant differences ($p < 0.05$) in the WDDS for participants in the two distinct study settings. The range of scores for UK participants (n=15) for the 3 days were 0-6 with mean values ranging from 4.33-5.06. In contrast the scores for the PK participants (n=40) were from 1-5, with mean values much lower (2.55-3.02) than UK counterparts. The findings of the study in terms of the dietary scores points out the crucial nutrition situation in NW PK. The scores relate well with the observations of the population survey conducted in this Baghbanan community which stated that the diets are extremely monotonous over a long period of time. This could be attributed to the geographical area where this community is based. Baghbanan region is rural area which falls under marginalised section with poverty experienced all over this area. Other factors that could be contributing to this dietary pattern might include low awareness about importance of consuming variety of foods, low availability of foods due to low accessibility to main cities, affordability issues, political instability and poor educational background. In the present study, for gathering dietary data, the nutritionist had to explain the study in their local language as the women as majority of the women were illiterate. This raises another concern that if the women, who play pivotal role in preparation of day-to-day meals and bringing up children were illiterate and were not aware about the kind of foods that must be included in the daily diet, this can have adverse repercussions in the long run. The data gathered from these women also suggested that some of them were pregnant or had been pregnant recently. This becomes important as if the pregnant women does not get sufficient micronutrients (iron , zinc and vitamin A) then it can lead to this women giving birth to malnourished babies. If this situation is not interrupted with promotion of good nutrition practices, it can have adverse impact on the overall productivity of the region as a whole. In

contrast the economic status of the Pakistani community was much better in NW UK and this was reflected in the higher DDS as compared to participants in NW PK. Moreover participants in NW UK also had easy access to variety of foods, which was not possible in case of participants in NW PK. So difference in economic status and geographical settings did have an impact on dietary diversity in both the study locations.

4.3 Consumption of specific food groups (starchy staples, vitamin a rich fruits and vegetables by south Asian women in NW UK and NW PK

The WDDS sheet has been found to be useful not only to calculate the diversity score of the complete diet, but also for assessing the diversity in the consumption of specific food groups like starchy staples, vitamin A rich fruits and vegetables or meat and fish. In the present study these scores for individual food groups enabled us to obtain greater clarity on the dietary sources of micronutrients consumed by the participants. As far as the consumption pattern for starchy staples was concerned, there were no significant differences among the two study regions. Here it important to mention that under the category of starchy staples wheat in form of traditional products (Chapati, roti, paratha) and potatoes (in form of vegetable curry, or stuffed in paratha) were consumed almost daily by participants in both the study regions. Bread (as sandwiches or toasts with tea) and pasta were more commonly consumed in NW UK as compared to NW PK. There were significant differences between the two population with respect to consumption of Vitamin A rich fruits and vegetables. It is important to note that this significant difference was observed in the diets consumed during second and third dietary recall, but not in the first dietary recall. This could be attributed to increased awareness about the format in which diet must be reported, increased understanding of remembering all the foods consumed with reduction in inhibitions about reporting dietary intake to some extent, or some kind of personal bias. For example – the belief that since the diet is being recorded, more food should be eaten, more variety should be eaten so that more

data can be given to the interviewer. This was observed even during the data collection in NW UK. In context of consumption pattern of other fruits and vegetables across NW UK and NW PK, significant differences ($p < 0.05$ sig. 2 tailed) were observed for all 3 days. Critical analysis of the dietary recall sheets revealed that onions and tomatoes were most commonly consumed vegetables under this category in both the study regions. Similarly, fish and meat were consumed more frequently in the UK participants, compared with PK. A pattern similar to other fruits and vegetables has been observed for diversity score of consumption of fish and meat, with this the frequency of consuming this group significantly higher in the UK group. Fish consumption was not reported at all in PK, however chicken and lamb meat were consumed in both the study locations.

4.4 Laboratory analysis of wheat flour samples from NW UK and NW PK

4.4.1 Iron content

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their total iron content using AAS by a method adapted from Kim and Song (2014).

The iron content of samples obtained from traditional south Asian markets in NW UK ranged from 3.68mg/100g for wholemeal flour to 1.35mg/100g for white flour. This suggested that the iron content decreased with increase in the amount of processing, the highly processed refined white flour having the lowest iron content. As far as the iron content of wheat flour samples obtained from 10 different household in Baghbanan community in NW PK is concerned, a wide range of values have been observed, from 7.00 mg/100g to as low as 2.51mg/100g. Similar to these findings Anjum et al., (2003)in their study in Pakistan have also reported a wide range of values for iron content of different mill streams of flour of different wheat cultivars (Auqab 2000, Iqbal 2000 and Chennab 2000) from 9.79mg/100g to

12.45mg/100g. Further, in this study the authors also concluded that the highest iron content was found in wheat bran and bran shorts (ranging from 13.79 mg/100g to 15.02 mg/100g), followed by whole wheat flour having iron content in range of 12.56mg /100g to 13.72 mg/100g. This was because bran and surrounding portions of the grain are relatively rich in minerals and this content is reduced when processed to convert into whole wheat flour. These finding are in agreement with the pattern observed in the present study that is iron content reduces with an increase in the amount of processing. Ma et al., (2005) reported iron content of 18 commonly consumed wheat products in China and the values ranged from 0.41mg/100g for wheat flour with 50% extraction range to 5.41mg/100g for wheat gluten fried. From these 18 products the values obtained for only 3 products namely wheat flake (3.22mg/100g), wheat gluten baked (3.02mg/100g) and wheat gluten fried (5.41mg/100g) agreed with the results in the current study.

4.4.2 Zinc content

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their total zinc content using AAS by a method adapted from Kim and Song (2014).

The zinc content varied from 1.98mg/100g for UK wholemeal flour to 1.00mg/100g for UK white flour. The zinc content was found to be highest for wholemeal flour and lowest for highly refined white flour. The results obtained for the zinc content of the wheat flour samples obtained from Pakistan showed a wide range of values and so mean and standard deviation values were calculated 2.62±2.05 mg/100 (mean ± SD). Other group of researchers have also explored the zinc content of various food products across the globe. In context of the present study, it becomes important to assess zinc content of cereal and cereal products as these cereals form the staple diet of the people in developing countries and contribute towards dietary zinc intake. In this context in an exhaustive review Brown et al., (2001) pooled data from various sources and tabulated phytate and zinc content of various food groups.

Accordingly, the zinc values for whole grain cereals ranged from 0.5-3.2mg/100g which concur with the present study. Another such study was conducted by Ma et al., (2005) where zinc content of 18 wheat products consumed in China was determined and it was found that the zinc content ranged from 0.47 mg/100 g for unleavened wheat pancake and 2.75 mg/100 g for fresh wheat gluten. The values for zinc observed for whole wheat bread baked (1.25mg/100g), wheat gluten fried (1.98 mg/100g) and wheat gluten fresh (2.75mg/100g) are within range of the values in the present study. Apart from the wheat products, the zinc content of three types of wheat flour depending on the extraction rate was also determined and it was found to be 0.78mg/100g, 0.57mg/100g and 0.52mg/100g for wheat flour with extraction rate of 85%, 75% and 50% respectively. However, these values for wheat flour were much lower than in the present study. Anjum et al., (2003) in a study in Pakistan, reported zinc values for wheat flour from different wheat cultivars using different mill streams and these values varied from 3.05-9.49mg/100g. These values were within range of the results obtained for the flour samples from Pakistan in the present study. The zinc values of the present study have been found to agree with those reported by Gargari et al., (2007). These researchers reported zinc values for 7 wheat flour samples and 70 bread samples from various bakeries in Iran. For wheat flour values varied from 1.18-1.62 mg/100g, while breads ranged from 1.03-1.23mg/100g

4.4.3 Phytate content

A total of 14 wheat flour samples (4-NW UK and 10-NW PK) were analysed for their phytate content by method described by Haug and Lantzsch (1983). The phytate content for UK wheat flour samples varied from 583.82mg/100g to 273.52mg/100g for wholemeal flour and white flour respectively. Also the phytate content of medium chapatti flour was found to be

294.11mg/100g (East End) and 347.05mg/100g (Supreme). These differences seemed logical due to differences in the brands for these flour samples. Moreover the results for wheat flour samples from Baghbanan community revealed a wide range of values beginning with as high as 565.44mg/100 to as low as 230.39mg/100g. However it is important to note that despite this wide range, the highest value for phytate in PK flour was lower than the highest value found in UK flour. These values were analysed statistically for normality and it was found that the data was normally distributed. So an independent t-test was performed to compare differences between the values for UK and PK, however, there were no significant differences between the flours from the two geographical regions. Considering the varied range for PK samples, mean and standard deviation values (388.89±110.90) were determined. From these values it could be postulated that significant differences could not be detected between UK and PK flours may due to significant standard deviation value obtained for PK flour and this resulted in the overlapping of the values suggesting absence of notable differences. There is substantial amount of published literature dealing with phytate content of various cereals and cereal products or even staple diets of certain study regions. To begin with Ma et al., (2005) in their study investigated the phytate, calcium , zinc and iron content of commonly consumed products in China. So since wheat formed integral part of the diets consumed by Chinese population, phytate content of 18 wheat products was determined and the results showed that the phytate content ranged from 3mg/100g for wheat noodles to 420mg/100g for standard wheat flour. Their findings concur well with results from the present study.This also correlates with the observation in a study that 85-95% of anaemia in China is caused due to iron deficiency and that phytate was the major cause for low iron bioavailability leading to iron deficiency(Ma etal., 2006, He et al., 1994, Wang et al., 1990, Cai and Yan., 1990, Zhang Q., 1987). A study was conducted by Gargari et al., (2007) where different wheat flour samples and breads consumed commonly in Iran were analysed for their

phytic acid content. They reported that the values for phytic acid content in the flour samples ranged from 210.38–315.11 mg/100, while that for breads, it was found to be 7.76–10.06 mg/100g. The values for the flour samples are in good agreement with the values obtained for the flour samples in the present study. Moreover, even though in the present study only flour samples were analysed, it can be concluded from the results that with increase in the level of processing, there is significant decrease in phytic acid content, which justifies least processed UK wholemeal flour having highest phytate content and highly refined white flour having lower phytate content. So the findings of Gargia *et al*,(2007) stating products made from the wheat flour (breads) having significantly lower phytate levels are in absolute agreement with the pattern observed in the present study.

Other researchers have also reported wide range of values for phytic acid content in wheat flour samples and related products. Garcia-Estepa *et al*., (1999) have reported values for soft wheat flours ranging between 300 mg/100g to 400mg/100g, while that for hard wheat and whole wheat flours between 900mg/100g and 2220 mg/100g respectively. While the values observed for cereal flours were in range of the values obtained for flour samples in the present study, the values for whole wheat flour were much higher than in the present study. Furthermore, Febles et al., (2002) also in their study reported values for phytate content of different types of wheat flour. They found that the phytate content for refined flours was in range of 200-400 mg/100g, while whole flours were in range of 600-1000 mg/100g. Also an overview of the means from the study brought out that values for refined hand - made, commercial factory made and whole flour samples were 377mg/100g, 296mg/100g and 850mg/100g respectively. These values are within suitable range of values obtained in the present study. More recently a study was conducted by Peng et al., (2010) in Tai an, China which aimed at investigating the phytic acid contents of different streams from the flour milling process in 8 different varieties of Chinese hard white winter wheat. Here it is

important to note that this study used the same method was used for the present study. Findings from the study suggested that the coarse bran samples had the highest amount of phytic acid content (5385 mg/100g, while break flours (Qi and Feng, 2004) had lower phytate content (480mg/100g). This phytate content further reduced significantly for two types of reduction flours (Qi and Feng, 2004) and it was found to be 275mg/100g and 203 mg/100g respectively. The phytic acid values for different flour samples obtained in this study concurred with the values in the present study. In addition to this, the authors of this study also studied the phytate content of different bran samples with different bran sizes. They justified studying brans samples by stating that since fibre has been established as one of the beneficial ingredient for human health, there is increased consumption of bran and other fibre rich products in the daily diets. However it is important to understand that bran or fibre rich products are associated with high amount of phytic acid content and thereby leading to higher consumption of phytate under the pretext of consumption of fibre rich foods.

4.4.4 Phytate:zinc molar ratios

Phytate:zinc molar ratio has been recommended as one of the most effective indicators for assessing zinc bioavailability from food products (WHO, 1996 , International Zinc Nutrition Consultative Group (IZiNCG), 2004). Considering this in the present study, phytate:zinc molar were calculated by the formula given by Gargari et al., (2007). Accordingly the phytate:zinc ratios for UK wheat flour samples ranged from 22.25 for medium chapatti flour to 41.4 for white flour. The ratios for PK flour samples depicted an enormous range from 3.80-55.8. As defined by WHO (1996) depending on the scores high (phytate-zinc molar ratio less than 5), medium (phytate-zinc molar ratio from 5-15) or low zinc availability (phytate-zinc molar ratio greater than 15) can be assessed. In relation to these scores WHO (1996) has identified percent availability corresponding to the scores. If the score is < 5 then zinc

availability is high (50-55%), 5-15, it is moderate (30-35%) and scores more than 15, the availability is low (10-15%). The scores obtained in the present study indicate that only one flour sample out of the 14 samples analysed has high zinc availability. This implies that all the wheat flour samples (except one from PK) had low zinc availability (10-15%). This could be one of the major reasons for the high prevalence of zinc deficiency across the globe, especially in developing countries like India, Pakistan, and Bangladesh. This method of determining phytate:zinc molar ratios has been used across various studies by number of research groups (Gibson et al., 2003, Brown et al., 2001, Bosscher et al., 2001, Fitzgerald et al., 1993). Ma et al (2005) calculated phytate:zinc molar ratios for 60 food samples commonly consumed in China. More specifically in context of the present study it must be noted that the phytate:zinc molar ratios for 18 wheat products ranged from 0.58 for wheat noodle, fresh to 80.23 for wheat flour with 85% extraction rate. There is wide variability of scores for various products and this similar pattern has been observed for the present study. Following comparison between wheat flour samples in both the studies, it can be observed that the score for wheat flour with 85% extraction rate is significantly higher, but the scores for wheat flour with 75% and 50% extraction rate are much lower than those obtained in the present study. Further, the results of the current study strongly agrees with the scores reported by Gargari et al., (2007) for 7 wheat flour samples which ranged from 14.93–23.79.

4.5 Biochemical indices of micronutrient status of women (Baghbanan)

In accordance with the final aim of the study, biochemical indices for iron (haemoglobin) and zinc (plasma zinc) were measured in women (n=40 in 18-30 year age group) in Bagbanan (NW UK). The results revealed that the haemoglobin levels of women ranged from 9.3-13.0 (g/dL). When individual values were studied, it was observed that half of the participants (18 participants precisely) have low haemoglobin levels than normal(11mg/dl for pregnant

women and 12mg/dl for non-pregnant). This scenario is of a major public concern as most of the women were either pregnant at the time of the blood analysis or had been pregnant recently. Here it is important to note that the iron requirements during pregnancy are increased in order to support foetal development, for expansion of maternal blood supply and also to consider the potential blood losses at the time of delivery (Tapiero et al., 2001). However if these women are deficient as observed in the present study then it can have serious health consequences for the mother as well as for the baby postnatally. The results also bring out one of the major reasons for the high prevalence of maternal and infant deaths in developing countries especially in rural areas similar to the present study region.

In addition to haemoglobin levels for assessing iron status, zinc status of these women was also assessed using plasma zinc as an indicator. Plasma samples were obtained for all the 40 participants, however, due to technical problems with the instruments data has been discussed for only 15 samples. Even though the sample size is low as compared to haemoglobin levels, it can be considered as a representative sample and can be useful for giving an overview of the zinc status of the women in this Bagbanan community. The results revealed that all the 15 participants had low plasma zinc levels as compared to normal acceptable range (0.7-1.2mg/L). This reveals that the females in this community are seriously deficient in two most essential micronutrients. This can have long lasting irreversible consequences on the health of the women. In case this deficiency is found in pregnant women as observed in the present study, it can lead pregnancy related complications and thus adversely affect the outcome.

4.6 Conclusions

From all the results and discussions it can be concluded that the frequency of consumption of vitamin A rich fruits and vegetables, other local fruits and vegetables as well meat and fish products as a part of their daily diet is significantly lower in women in NW PK, while dietary

staple(wheat flour) is the integral part of their daily diet. However, these flours have high amount of phytate as revealed in the present study. So low consumption of good sources of micronutrients and higher intake of inhibitors of micronutrient absorption, make these women susceptible to micronutrient deficiencies. A level of micronutrient deficiency has been observed in the in present study as haemoglobin and plasma zinc levels were much lower than the normal healthy range.

4.7 Strengths and Limitations of the study

4.7.1 Strengths of the study

The present study contributed to the data on the phytate content of wheat flour samples in Pakistan. This is important because due to lack of concrete data on the phytate content of flour samples consumed by population in Baghbanan community, appropriate measures could not be taken in order to reduce that phytate content.

Moreover, in the present study a relatively new method of assessing dietary diversity (WDDS) has been used. Dietary diversity scores for diets consumed by people in Baghbanan (Brick kiln community) were not calculated in any of the previous studies. Diversity scores for consumption of specific food groups like vitamin A rich products, meat and fish were also added to the existing body of literature due to the present study.

4.7.2 Limitations of the study

The sample size of the participants recruited in both the study regions is not comparable as it was extremely challenging to recruit participants belonging to Pushtun community originally from NW region of Pakistan based in NW UK. This was because a very small cluster of this community is based in NW UK and among those, majority of them were reluctant to be part of the study. Due to this only 15 participants could be recruited in NW UK as opposed to 40 participants in NW PK.

In the present study haemoglobin levels were used as an indicator for iron status of women in Bagbhanan region. However it is important to note that this indicator is not very specific for indicating iron status or low dietary intake, but these levels can be affected by other physiological problems like worm infections. So it is generally recommended to use 2-3 indicators together to reach concrete conclusions.

Apart from this, communicating with PK based team about the details of the methodology of the study, data collection and data interpretation was difficult due to various factors like different time zones, political instability in NW PK and other accessibility issues due to the geographical location of the region.

4.8 Recommendations for future work

Firstly, the present study revealed that the variety of wheat flour samples that form an integral part of the daily diets of the people in Baghbanan region (NW PK) have high phytate content. So even though, these flour samples have sufficient amount of iron and zinc, these essential micronutrients are not fully bioavailable due to the high phytate content. In this context, studies have established that, this phytate content can be reduced by several household processing techniques like soaking of wheat grains for longer durations, fermentation, and germination. So as a part of future work, it is important to assess the feasibility of these methods in this community and then promote these techniques for improving the micronutrient status of people in this area.

Secondly, since the dietary pattern of this Brick kiln community is extremely monotonous, adequate awareness needs to be created among the community about incorporating foods belonging to different food groups in their daily diet. This awareness can be raised by using various techniques like demonstration kitchens, teaching in schools, teaching women in community settings.

Moreover economic considerations were not made for the sample population in NW UK as affordability of foods was not an issue for deciding the foods consumed, however this aspect about economic standing of NW UK population can be explored as a part of future work.

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6 Chapter Five. Appendices

5.1 Appendix 1

5.2 Appendix 2

5.3 Appendix 3

5.1 Appendix 1

a. Advertisement flyer for the study
b. Participant Information sheet for UK participants
c. Participant Information sheet for PK participants
d. Participant consent form
e. Ethics approval –UK
f. Ethics approval – Pakistan

Female Participants Needed!

Belonging to South Asian Community…….

Micronutrients – the small wonder

Required in small amounts……..but vital for good health

How well do you know them? Do you eat enough of them?

Don't know?……**Please Come along** and be a part of an interesting research project at UCLan and get your questions answered

You can be part of this if …….

- ✓ **You are a South Asian female in age group of 18-30years**
- ✓ **Your parents were born in Pakistan**

If you decide to be the part of this research then……..

- ➢ You will need to answer some questions about what you ate in the last 24 hours. Please note that this will be interviewer administered and so **only thing that you need to do is to answer all the questions asked by the interviewer in appropriate depth.** All the information collected will be kept anonymous, so if you decide to take part in the study, please answer all the questions as honestly as possible.
- ➢ There will be in total **only 3 dietary recalls**over a period of 3 months taken at appropriate intervals (between may and June)and at a time mutually agreed by both the interviewer and the participant. It will take **only 15 minutes for each interview**

If you areinterested in participating, or want to know more about it please feel free to contact:

Miss SuruchiPradhan – Research student

Contact No. - 01772 893751 / DB-329 Email – spradhan@uclan.ac.uk

'Thank you for considering taking part in this study and taking the time to read this sheet'.

PARTICIPANT INFORMATION SHEET for UK Participants

Project Title: Comparative study of micronutrients in traditional South Asian diets in North West UK and North West Pakistan

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Please feel free to contact us if there is anything that is not clear or if you would like more information. The contact information is given at the end of the form.

Take time to decide whether or not you wish to take part.

Purpose of the Study

The research team within International Institute of Nutritional Sciences and Food Safety Studies (iINSAFSs) at UClan are currently undertaking research based at the Basic Health Unit that serves a marginalised, poor community in north-west Pakistan.

The current research will help to gain an understanding of the amount of micronutrients (essential nutrients required by the body for normal growth and maintenance, such as iron, zinc and vitamin A) present in South Asian diets consumed in UK and that in Pakistan. This will also help understand the changes in diet that occur when families move from PK to the UK.

Why have I been invited to participate?

It is very important that young women of child bearing age have an adequate intake of micronutrients. Therefore we are looking for young women in 18-30 year age group who are willing to share information with us about their typical diet. So you are being invited on voluntary basis as you fulfil the following conditions necessary for participating in the study

1. Young women from South Asian Community
2. Your parents were born in Pakistan
3. Age group – 18-30 Years

Do I have to take part?

It is up to you to decide whether or not to take part in this study. Taking part in the study is completely on voluntary basis and if you decide to take part you are still free to withdraw at any time without giving a reason, up to the time when the final dietary recall is conducted. Simply inform the researcher (Suruchi) or her supervisor (Prof Nicola Lowe) by phone or email. Contact details are given at the end of the sheet.

What will happen to me if I take part?

If you decide to take part in the study, then you will have only 3interviews on 3 different days at a time that is convenient to you, between May and June. Each interview will take around 15 minutes. However, in this interview you will have to answer the questions about the food consumed by you for filling up a 24 hour diet recall form. This is the form where the foods consumed by you 24 hours prior to the day of interview are noted down on a piece of paper. In this you will have to mention all the details about the amount consumed by you, at what time, where, ingredients used in the recipe, method of preparation of the recipe and so on. Also no foods are good foods or bad foods, so do not hesitate to answer the questions in detail.

In addition in any circumstances, if face to face interview is not possible, it can be taken telephonically as well as on Skype. So there is no need for the participant to come to the University for their dietary recall.

Moreover all the information collected will be kept anonymous. You will be given an identification (ID) number and this will be used, rather than your name, during the data recording and analysis. Only the main investigators will have access to the link between your name and ID number. So, if you decide to take part in the study, please answer all the questions as honestly as possible.

What are the possible benefits of taking part?

There are no benefits to be gained by taking part in this study but it is hoped that the information gained may be of benefit to people in the future.Some general information about your diet and micronutrient intake will be given to you on request.

What are the possible risks of taking part?

To the best of our knowledge there are no possible risks to the participants for taking part in the study

Confidentiality and Data Handling

All information collected about you during the course of the study will be kept strictly confidential. All subjects will be assigned an identification number. This number will be used when discussing the information collected during the study and in any future publication of results. Only the researcher (Suruchi Pradhan) and her supervisory team (Prof Lowe, Dr Wallace, Dr Dillon) will be able to link your ID number to your name.

What should I do if I want to take part?

Every eligible participant will be given the required participant information sheet and the details of the study will be clearly explained to the participant. If the participant is satisfied with the information provided and is willing to participate in the study, then this participant will be given an informed consent form. The participant has to give her final agreement to be part of the study by signing this form. This form will also be signed by the researcher. Once this form is signed the participant is fully enrolled in the study.

What will happen to the results of the research study?

The information obtained about the diets from 24 hour dietary recall will be analysed and the micronutrient status of the participants will be determined. This data will form the part of dissertation which will be submitted as a part of the work to be conducted for gaining aMRes degree at University of Central Lancashire. Furthermore this data may also be published in relevant research journals. If this is the case, then we will ensure that you receive a copy of the published research if you wish.

Who is organising and funding the research?

My name is Miss Suruchi Pradhan. I am a student studying nutritionat International Institute of Nutritional Sciences and Food Safety Studies (University of Central Lancashire) and this present research is being conducted by me for gaining MRes degree in Nutrition under supervision of Professor Nicola Lowe (Professor of Nutritional Sciences at UCLAN).

Who has reviewed the study?

Ethical approval for this study has been granted by the University of Central Lancashire research ethics committee.

Further Information

Researchers contact details

Miss Suruchi Pradhan : 01772 893751email: spradhan@uclan.ac.uk

Professor Nicola Lowe: 01772 893599 email: nmlowe@uclan.ac.uk

Dean of School: Dr John Minten: 01772 895716

'Thank you for considering taking part in this study and taking the time to read this sheet'.

PARTICIPANT INFORMATION SHEET for PK Participants

Project Title: Comparative study of micronutrients in traditional South Asian diets in North West UK and North West Pakistan

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please listen carefully as I explain this to you.

Please feel free to ask questions if there is anything that is not clear or if you would like more information.

Take time to decide whether or not you wish to take part.

Purpose of the Study

The Abaseen Foundation is currently undertaking a survey in Baghabanto gain an understanding of the amount of micronutrients (essential nutrients required by the body for normal growth and maintenance, such as iron, zinc and vitamin A) present in typical diets of younger women living in this community. This information will help us develop the nutrition support services at the Basic Health Unit (BHU).

Why have I been invited to participate?

It is very important that young women of child bearing age have an adequate intake of micronutrients. Therefore we are looking for young women in 18-30 yearage group who are willing to share information with us about their typical diet. So you are being invited on voluntary basis as you fulfil the following conditions necessary for participating in the study

Do I have to take part?

It is up to you to decide whether or not to take part in this study. Taking part in the study is completely on voluntary basis and if you decide to take part you are still free to withdraw at

any time without giving a reason, up to the time when the final dietary recall is conducted.Simply inform the Nutritionist at the Basic Health Unit.

What will happen to me if I take part?

If you decide to take part in the study, then you will have only 3interviews on 3 different days at a time that is convenient to youin May and June. Each interview will take around 15 minutes.However, in this interview you will have to answer the questions about the food consumed by you. The nutritionist (I) will fill in the questionnaire based on the information you give me about the foods consumed by you 24 hours prior to the day of interview. In this you will have to mention all the details about the amount consumed by you, at what time, where, ingredients used in the recipe, method of preparation of the recipe and so on. Also no foods are good foods or bad foods, so do not hesitate to answer the questions in detail.

We will also take a single blood sample during your first interview. This will be used to measure the amount of important nutrients in your blood.

All the information collected will be kept anonymous. You will be given an identification (ID) number and this will be used, rather than your name, during the data recording and analysis. Only the main investigator will have access to the link between your name and ID number.

What are the possible benefits of taking part?

There are no direct benefits to you for taking part in this study but it is hoped that the information gained may be of benefit to the community through the development of nutrition support services at the BHU.Some general information about your diet and micronutrient intake will be given to you on request.

What are the possible risks of taking part?

To the best of our knowledge there are no possible risks to the participants for taking part in the study

Confidentiality and Data Handling

All information collected about you during the course of the study will be kept strictly confidential. All participants will be assigned an identification number. This number will be used when discussing the information collected during the study and in any future publication of results.

What should I do if I want to take part?

If you are satisfied with the information provided and are willing to participate in the study, then please give your verbal consent and I will record this on a form. Once I have done this, you are fully enrolled in the study.

What will happen to the results of the research study?

The information obtained about the diets from 24 hour dietary recall will be analysed and your micronutrient intakewill be determined. This information will help us understand the local diet and how best to improve our services. We may wish to publish a summary of this information to make it available to scientists and health care professionals worldwide. Rest assured that the location and participants will not be identified.

Who is organising and funding this work.

This is organised by Abaseen Foundation PK and our research support team at UCLan.

Who has reviewed the study?

Ethical approval for this study has been granted by the Khyber Medical Universityresearch ethics committee.

'Thank you for considering taking part in this study '

CONSENT FORM

Comparative study of micronutrients in South Asian diets in North West UK and North West Pakistan

Name : Suruchi Pradhan

Position :Research student at University of Central Lancashire

Researchers contact details

Miss Suruchi Pradhan : 01772 893751 Professor Nicola Lowe: 01772 893599

Please read the following statements and initial the boxes to indicate your agreement

Please initial box

I confirm that I have read and understand the information sheet, dated …….. for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason.	☐
I agree to take part in the above study.	☐
I agree that my data gathered in this study may be stored (after it has been anonymised) at UCLan and may be used for future research.	☐
I understand that it will not be possible to withdraw my data from the study after final analysis has been undertaken	☐
I agree to the interview being audio recorded	☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature

9th May 2014

Nicola Lowe and Suruchi Pradhan

School of Sport Tourism & the Outdoors

University of Central Lancashire

Dear Nicola & Suruchi

Re: STEMH Ethics Committee Application
Unique Reference Number: **STEMH 176**

The STEMH ethics committee has granted approval of your proposal application 'Comparative study of micronutrients (iron, zinc and vitamin A) in traditional South Asian diets in North West UK and North West Pakistan'. This approval is for the phase/work being undertaken in UK, with subsequent ethics submission required for the Pakistan element of this project. Approval is granted up to the end of project date* or for 5 years from the date of this letter, whichever is the longer.
It is your responsibility to ensure that

- the project is carried out in line with the information provided in the forms you have submitted
- you regularly re-consider the ethical issues that may be raised in generating and analysing your data
- any proposed amendments/changes to the project are raised with, and approved, by Committee
- you notify roffice@uclan.ac.uk if the end date changes or the project does not start
- serious adverse events that occur from the project are reported to Committee
- a closure report is submitted to complete the ethics governance procedures (Existing paperwork can be used for this purposes e.g. funder's end of grant report; abstract for student award or NRES final report. If none of these are available use e-Ethics Closure Report Proforma).

Yours sincerely
Paola Dey
Deputy Vice Chair
STEMH Ethics Committee

* for research degree students this will be the final lapse date

NB - Ethical approval is contingent on any health and safety checklists, as well as the MOU having been completed, and necessary approvals as a result of gained, together with any necessary home office requirements regarding importation of flour have been addressed

KHYBER MEDICAL UNIVERSITY
KMU-ETHICS BOARD
BLOCK –IV, PDA BUILDING, PHASE-V, HAYATABAD,
KHYBER PAKHTUNKHWA, PESHAWAR, PAKISTAN
☎091-9217258, 091-9217703 🖷091-9217704

No. DIR/KMU-EB/CS/ Dated: 16-06-2014

TO WHOM IT MAY CONCERN

Certified that Ethical Approval has been granted to the project title ***"Comparative study of micronutrients in traditional South Asian diets in North West UK and North West Pakistan.*** Submitted by Ms. Lailla (Nutrition Officer) from Abaseen Foundation.

After conducting a desk review, the proposal was found to be ethically sound.

Prof. Dr. Mukhtiar Zaman,
Secretary,
KMU Ethics Board

Prof. Dr. Shad Muhammad
Chairman,
KMU-Ethics Board.

5.2 Appendix 2

a. Study protocol

b. (i) Particulars of participants in NW UK

b. (ii) Particulars of participants in NW PK

c. CosHH Form

d. Risk assessment form

e. Photographs of the wheat flour samples from NW UK and NW PK

f. Photographs of the experiment (stepwise mineral analysis)

g. Photographs of portion sizes used during 24 hour dietary recalls from winDiets

h. 24 hour recall format – for UK participants

i. 24 hour recall format – for PK participants

j. Preparation of reagents for phytate estimation

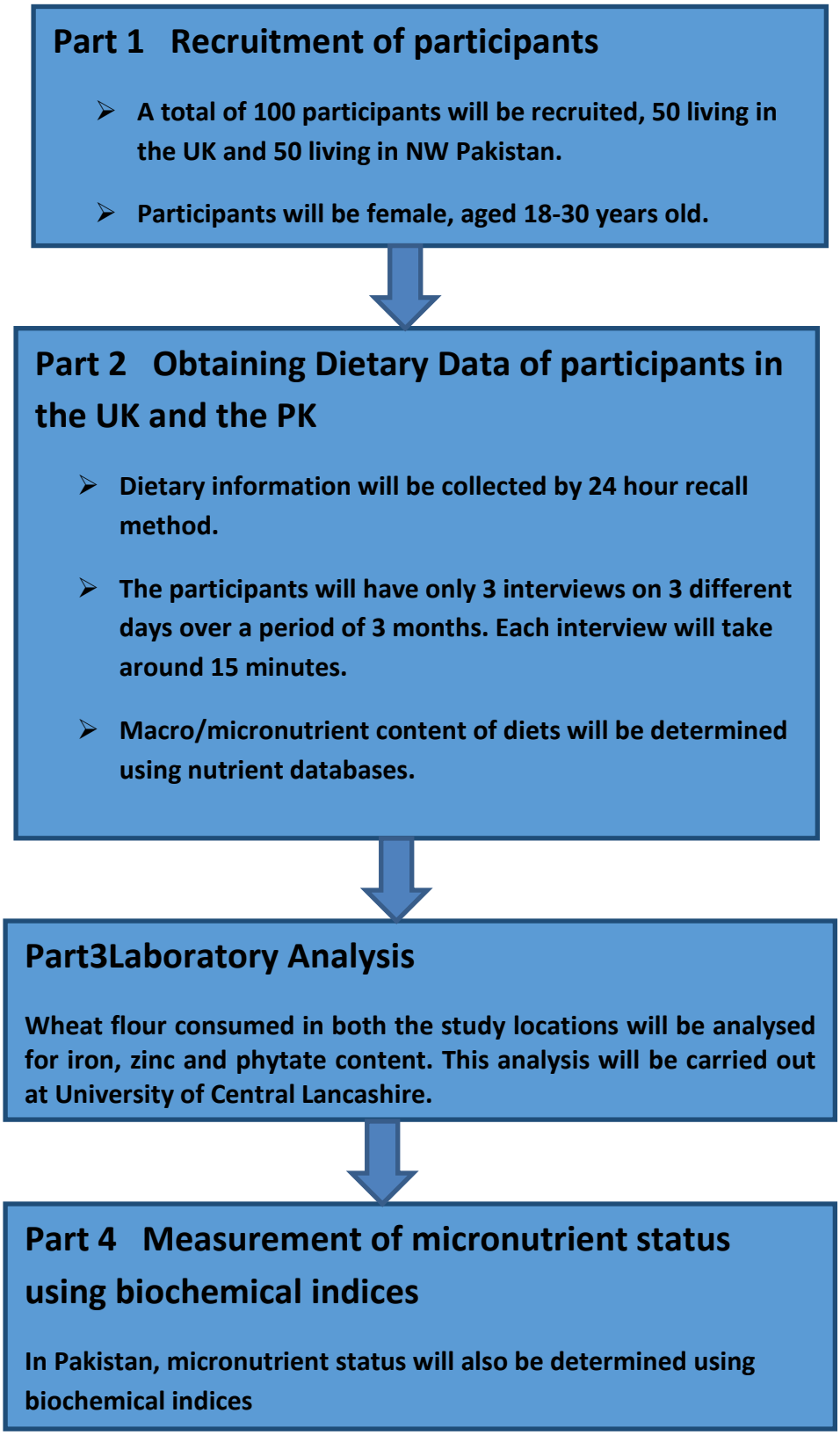
k. Raw data of DDS for NW UK participants

l. Raw data of DDS for NW PK participants

m. Raw data of frequency of consumption of specific food groups for NW UK and NW PK

a. Protocol of the study

The entire study is divided into 4 parts. These are as follows :-



b (i) Particulars of the participants recruited in NW UK

Participant Number	Age	Marital status	Physiological status	Nutritional supplement taken
1	18	Single	Not Pregnant	No
2	18	Single	Not Pregnant	No
3	18	Single	Not Pregnant	No
4	18	Single	Not Pregnant	No
5	30	Married	Not Pregnant	No
6	30	Single	Not Pregnant	No
7	20	Single	Not Pregnant	No
8	18	Single	Not Pregnant	No
9	22	Single	Not Pregnant	No
10	26	Married	Not Pregnant	No
11	35	Married	Not Pregnant	No
12	30	Married	Not Pregnant	No
13	26	Single	Not Pregnant	No
14	35	Married	Pregnant	Folic acid tablets - Preconceive
15	35	Married	Not Pregnant	No

b (ii) Particulars of the participants recruited in NW PK

Participant Number	Age	Marital status	Physiological status	Nutritional supplement taken
1	20	Married	Pregnant	
2	28	Married	Pregnant	
3	24	Married	Lactating	
4	30	Married	Pregnant	
5	26	Married	Pregnant	
6	22	Married	Lactating	
7	24	Married	Pregnant	
8	25	Married	Lactating	
9	22	Married	Lactating	
10	27	Married	Lactating	
11	18	Married	Lactating	
12	25	Married	Pregnant	Folic acid
13	28	Married	Pregnant	Folic acid
14	27	Married	Pregnant	Folic acid
15	23	Married	Pregnant	Folic acid
16	22	Married	Lactating	
17	29	Married	Lactating	
18	25	Married	Lactating	
19	23	Married	Lactating	
20	21	Married	Pregnant	
21	19	Married	Pregnant	
22	28	Married	Lactating	Micronutrient
23	20	Married	Pregnant	
24	23	Married	Pregnant	
25	29	Married	Lactating	
26	26	Married	Lactating	
27	30	Married	Pregnant	

28	24	Married	Pregnant	
29	29	Married	Lactating	
30	26	Married	Lactating	
31	27	Married	Lactating	
32	19	Married	Pregnant	
33	29	Married	Lactating	
34	19	Married	Pregnant	
35	22	Married	Pregnant	
36	26	Married	Lactating	
37	30	Married	Lactating	
38	18	Married	Lactating	
39	29	Married	Pregnant	
40	26	Married	Pregnant	

School/Service	Assessors Name(s)	Job Title/Position	☎
SSTO	Suruchi Pradhan	Student	01772 893751

Briefly describe the task/process. (description, use, users)

Phytate analysis of wheat flour using mass spectrophotometry

Substances (used or produced as by-products or wastes)	**Quantity**	**Hazard Class**	**WEL**	**Exposure Route(s)**	**Frequency and Duration of Exposure**	**Known Health Effects:**
Sodium salt of phytic acid	0.15g per experiment	Irritant	-	Inhale/Ingest/ Absorb	5 days/week for 2 months	Irritant to eye, skin and respiratory system
0.2 N Hydrochloric acid	3L per experiment	Corrosive/Irritant	2mg/m^3	Inhale/Ingest/ Absorb	5 days/week for 2 month	Burns and Irritant to respiratory system
Ammonium iron Fe^{+3} sulphate.$12H_2O$	0.2g per experiment	Irritant	2mg/m^3	Inhale/Ingest/ Absorb	5 days/week for 2 month	Irritant to skin and eyes
2,2' bipyridine	1g per experiment	Acute toxicity, oral and dermal	Contains no substances with occupational exposure limits	Inhale/Ingest/ Absorb	5 days/week for 2 months	Burns and Irritant to skin, eyes and respiratory tract. Toxic if swallowed.
Thioglycolic acid	1ml per experiment	Corrosive/Irritant	3.8mg/m^3	Inhale/Ingest/ Absorb	5 days/week for 2 months	Burns and toxic if inhaled, swallowed, corrosive to

						skin
Results of Relevant Health Surveillance				Results of Exposure Monitoring		

Control Measures				
☐ Elimination	☐ Substitution	☐ Reduction	☐ Isolation	☐Eng. Control
Details	*Details*	*Details*	*Details(glovebox)*	*Details(LEV, fumehood)*
Further Details (if required) Well ventilated lab space				
Personal Protective Equipment				
☑ Gloves	☑Eye protection	☑Coverall/lab coat	☐ Foot protection	☑Respiratory protection
Details Nitrile gloves	*Details* Safety goggles	*Details* Howie-style	*Details*	*Details:* Face mask
☐ Health Surveillance required		☐ Exposure monitoring required		

Emergency Arrangements

First Aid:	
Eyes	Irrigate thoroughly with water for at least 10 minutes. If discomfort persists, obtain medical attention.
Skin	Wash off thoroughly with soap and water. Remove contaminated clothing and wash before re-use. In severe cases, OBTAIN MEDICAL ATTENTION.
Ingestion	Wash out mouth thoroughly with water and give plenty of water to drink. OBTAIN MEDICAL ATTENTION.
Inhalation	Remove from exposure, rest and keep warm. In severe cases obtain medical attention.

Fire: Extinguisher Type			
☐Water	☑Foam	☑Powder	☐CO_2

Spillage/release:

Wear appropriate protective clothing.

Inform others to keep at a safe distance.

Spread soda ash/sand liberally over the spillage. Mop up cautiously with plenty of water and run to waste, diluting greatly with running water.

Otherwise transfer to container and arrange disposal via special waste route.

Wash site of spillage thoroughly with water.

For large spillages liquids should be contained with sand or earth and both liquids and solids transferred to salvage containers.

Any residues should be treated as for small spillages.

Waste Disposal procedure

Diluted acids will be flushed down drain with copious amounts of water. Undiluted acids will be collected and disposed off via specialist disposal routes

Persons likely to be exposed

☑Staff	☑ Student	☐ Visitor	☐ Contractor
☐Public	☐ Other (specify)		

Additional risks: for example circumstances where work will involve exposure to more than one substance hazardous to health, consider the risk presented by exposure to such substances in combination. Also, non-routine maintenance may present additional risk of exposure.
Use of concentrated (undiluted acids) will only be conducted in a fume hood under supervision. Use of diluted acids can be conducted on an open bench but special care will be taken with warmedHCl and a face mask will be used to protect against fumes.

Authorised by (sign):	N M Howe	Review date due:	14/02/15
Date:	14/02/14		

Notes:

Hierarchyofcontrol

Changethetaskorprocesssothatthehazardoussubstanceisnotrequiredorgenerated.
Replacethesubstanceswithasaferalternative.
Totallyisolateorenclosetheprocess.
Partiallyenclosetheprocessanduselocalexhaustventilation.
Ensuregoodgeneralventilation.
Useasystemofworkthatminimisesthechanceanddegreeofexposure.
Providepersonalprotectiveequipment(PPE).
Trainandinformstaffinthesafesystemofworkandrisks.
Additionalsupervision.
Examination,testingandmaintenanceofengineeringcontrolsand/orPPE.
Monitoringofexposure.
HealthSurveillance.
Other(specify).

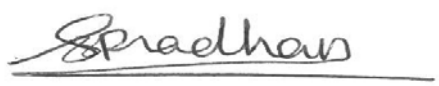
RISK ASSESSMENT FORM
(Medium & High Risk)

Use this form to risk-assess:

- ***Off-campus staff activities (research, fieldwork, educational visits etc) in medium/high risk environments such asfactories, farms, prisons, remote areas or participants' homes.***
- ***All staff activities involving medium/high riskprocedures or use of specialist equipment.***

For low risk locations and activities, use the appropriate low risk form.

This form should be completed by the staff member concerned, in conjunction with a qualified or otherwise competent person (normally a technician or Faculty HSE officer). Completed forms must be countersigned by the Head of School or the Chair of the School Health & Safety Committee.

Assessment Undertaken By: **(Student)**	**Assessment Verified By:** **(Technician or other competent person)**
Name: Suruchi Pradhan	Name: Nicola Lowe
Signed: 	Signed: 
Date: 13/02/14	Date*: 14/02/14
Note: Risk Assessment is valid for **one year from the date given above. Risk Assessments for activities lasting longer than one year should be reviewed annually.*	
Countersigned by Head of School or Chair of H&S Committee:	
Date:	

Risk Assessment For:
Activity: Preparation of Samples for ICP-MS
Location of Activity: MB308 and JB Firth Building Ground Floor

List significant hazards here:	List groups of people who are at risk:	List existing controls, or refer to safety procedures etc:	For risks which are not adequately controlled, list the action needed:	Remaining level of risk (high, medium or low):
Use of Hot Plate to warm acid ($HNO_3 + H_2O_2$) (Heat and Chemical)	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed.		Low
1% HNO_3 (Chemical)	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles).		Low
Trace Mineral Standards (2-5% HNO_3) (Chemical)	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles).		Low
Hydrogen peroxide solution (Chemical)	Students/Staff	Place samples in plastic tray and use lift rather		Low

Movement of Samples from MB308 to ICP-MS (JB Firth Building) ICP-MS Machine (Mechanical)	Students/Staff Students/Staff	than stairs. Use under supervision by ICM-MS technician		Low Low

Continue on another sheet if necessary.

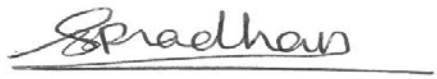
RISK ASSESSMENT FORM
(Medium & High Risk)

Use this form to risk-assess:

- ***Off-campus staff activities (research, fieldwork, educational visits etc) in medium/high risk environments such asfactories, farms, prisons, remote areas or participants' homes.***
- ***All staff activities involving medium/high riskprocedures or use of specialist equipment.***

For low risk locations and activities, use the appropriate low risk form.

This form should be completed by the staff member concerned, in conjunction with a qualified or otherwise competent person (normally a technician or Faculty HSE officer). Completed forms must be countersigned by the Head of School or the Chair of the School Health & Safety Committee.

Assessment Undertaken By: **(Student)**	**Assessment Verified By:** **(Technician or other competent person)**
Name: Suruchi Pradhan	Name:
Signed: 	Signed: 
Date: 13/02/14	Date*: 14/02/14
Note: Risk Assessment is valid for **one year from the date given above. Risk Assessments for activities lasting longer than one year should be reviewed annually.*	
Countersigned by Head of School or Chair of H&S Committee:	
Date:	

Risk Assessment For:
Activity: Preparation of Samples for Mass Spectrophotometry
Location of Activity: MB308 and JB Firth Building Ground Floor

List significant hazards here:	List groups of people who are at risk:	List existing controls, or refer to safety procedures etc:	For risks which are not adequately controlled, list the action needed:	Remaining level of risk (high, medium or low):
Use of shaker water bath	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles)		Low
Use of boiling water bath	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed.		Low
0.2 N Hydrochloric acid	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed.		Low
Sodium salt of phytic acid	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed. . Standard PPE (Gloves, Howie		

		Style Lab Coat, Goggles) plus use of face mask if needed.		Low
Ammonium iron Fe+3 sulphate.12H_2O	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed.		Low
Thioglycolic acid	Students/Staff	Standard PPE (Gloves, Howie Style Lab Coat, Goggles) plus use of face mask if needed.		Low
2,2’ bipyridine	Students/Staff	Place samples in plastic tray and use lift rather than stairs. Use under supervision by		Low

Movement of Samples from MB308 to Mass Spectrophotometer (JB Firth Building) Mass Spectrophotometer Machine (Mechanical)	Students/Staff Students/Staff	Mass Spectrophotometer technician		Low Low

Continue on another sheet if necessary.

Photographs of wheat flour samples purchased from SA shops in NW UK, Preston

East End – Premium gold chapatti atta

East End – Medium chapatti flour

Supreme - Medium chapatti flour

East End – White chapatti flour

Details of the origin of the flour

All the flour samples have been labelled as 'produced in UK', however if the origin of the grains is traced then it was found that all the flour samples produced under brand name East End were milled by East End Foods in the UK at their State of the art Flour Mill using hard wheat from Uttar Pradesh in India. These details on the brand Supreme could not be obtained

Photographs of wheat flour samples obtained from 10 different households in Brick-kiln community (Baghbanan region in NW PK).

Details of the samples – For obtaining these flour samples people in this Brick-kiln community purchase raw wheat grains and then these grains are milled using household level grinding machine. The names of these flour samples were not known as they did not belong to any particular brand. So in the present study these 10 flour samples were labeled PK1 to PK10

PK- 1 **PK- 2**

PK – 3 **PK - 4**

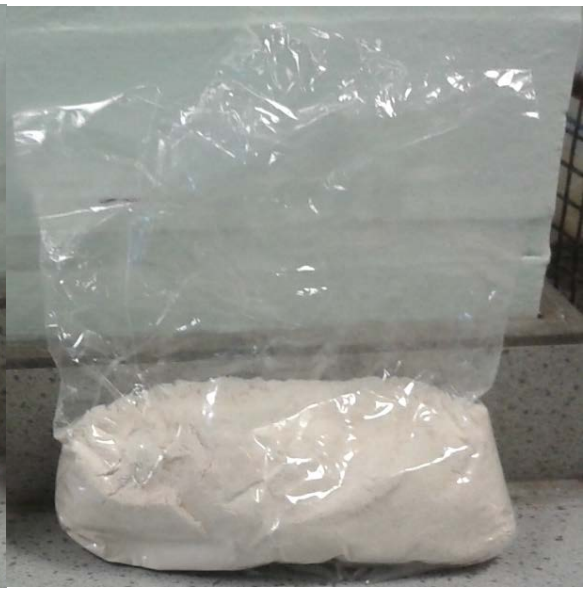
Photographs of wheat flour samples obtained from 10 different households in Brick-kiln community (Baghbanan region in NW PK).

PK - 5 **PK - 6**

PK - 7 **PK - 8**

Photographs of wheat flour samples obtained from 10 different households in Brick-kiln community (Baghbanan region in NW PK).

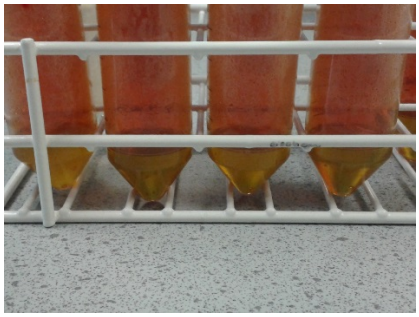
PK - 9



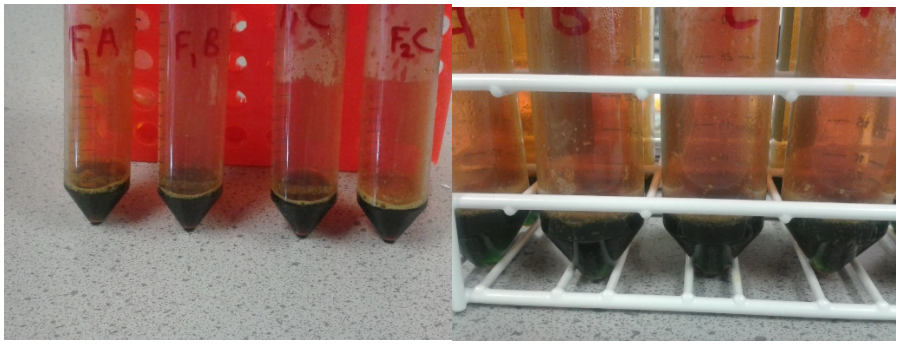
PK - 10

Photographs of the experiment (stepwise mineral analysis)

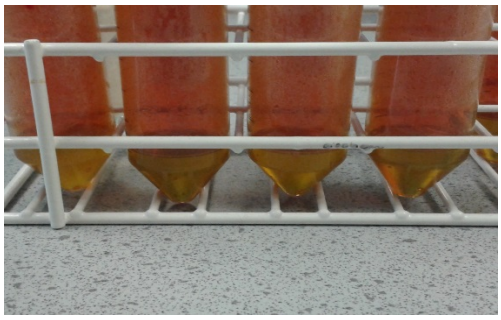
Step 1 - 1g of wheat flour + 5 ml of nitric acid (70%)



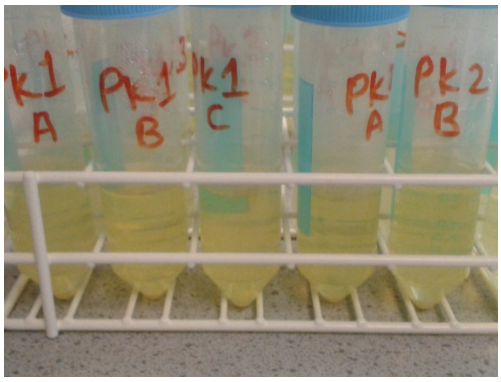
Step 2 - mixture of flour and acid after keeping overnight at room temperature



Step 3 – change in the colour of mixture after keeping in hot air oven at 60-70°C for 1 hour

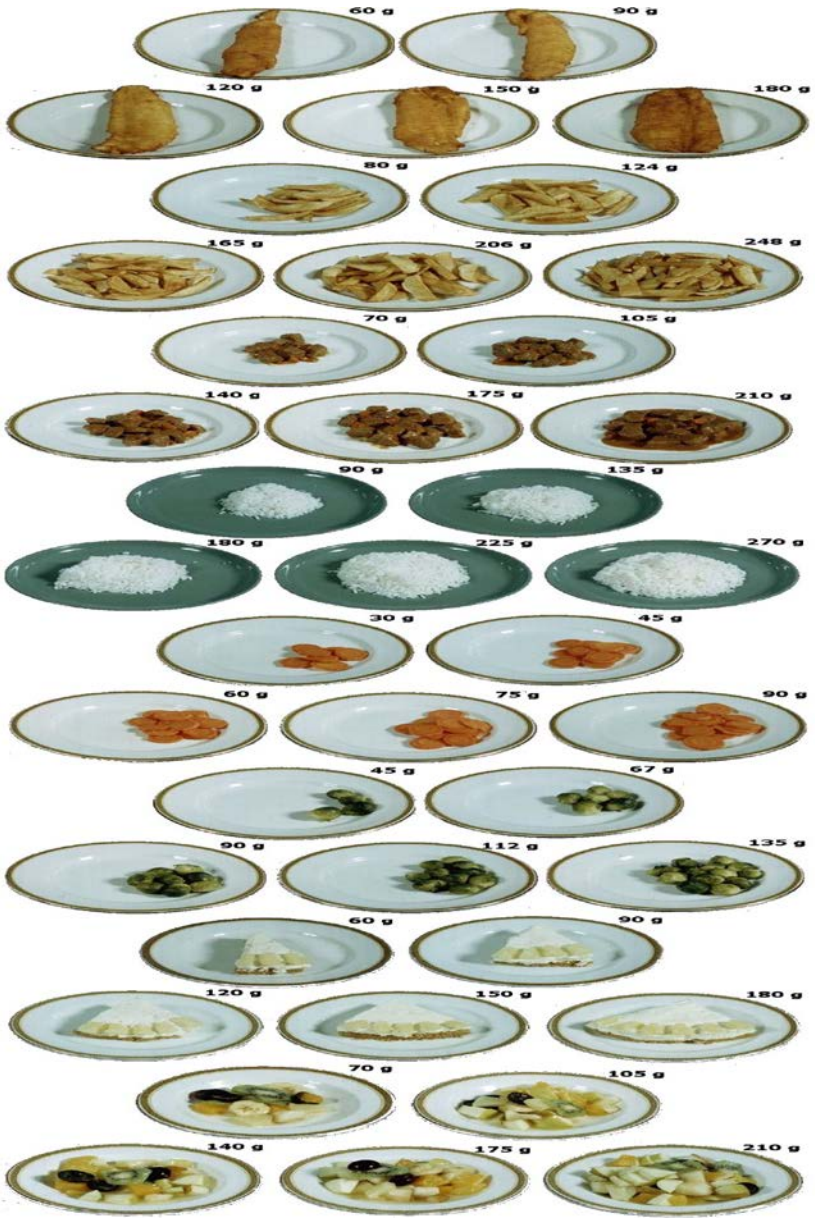


Step 4 – Volume is made upto 25 ml before analysis in Atomic absorption spectroscopy



Photographs of portion sizes used during 24 hour dietary recalls from the software WinDiets 2010 produced by Robert Gordon University

Eight different foods have been photographed with 5 different portion sizes; these foods provide a range of textures and colours and will help you to visualise what different weights look like. The weights (in grams) are indicated in the pictures for each food. The dinner plates were 10 inch and the dessert plates 7 inch in diameter.



24 hour recall format – for UK participants

Project Title: Comparative study of micronutrients in traditional South Asian diets in North West UK and North West Pakistan

24 Hour Dietary Recall

Form No. _____

Participant No _____ Age _____

Marital Status – Married / Single _____ Pregnant/Lactating _____

Please enter the following details

Date	Time of the recall	Mon	Tues	Wed	Thurs	Fri	Sat	Sun

Is this a regular day? Yes ☐ No ☐

If No then please describe your regular day

Are you taking any nutritional supplements in addition to regular diets?

Yes ☐ No ☐

If Yes, please give details

Name of the supplement - _____

Reason for consumption - _____

Is it consumed everyday or occasionally?

If it is consumed everyday then please specify the frequency of consumption during the day

_____

When it is generally consumed in a day _____

Amount consumed at a time _____

Is it a meal replacer _____

Please give details of the food consumed by you in last 24 hours

Please note :- There are no foods like `good foods and bad foods', so please feel free to identify all the foods consumed by you in the last 24 hours in detail

Time	Place (home, outside of the house)	Snacks / Breakfast (Name of dish)	Quantity consumed (Household measurement)	Ingredients used	Amount of ingredients (g)

Method of preparation of the recipes listed above

Time	Place (home, outside of the house)	Mid-morning snacks (Name of dish)	Quantity consumed (Household measurement)	Ingredients used	Amount of ingredients (g)

Method of preparation of the recipes listed above

Time	Place (home, outside of the house)	Lunch (Name of dish)	Quantity consumed (Household measuremen t)	Ingredients used	Amount of ingredients (g)

Method of preparation of the recipes listed above

Time	Place (home, outside of the house)	Afternoon snacks	Quantity consumed (Household measurement)	Ingredients used	Amount of ingredients (g)

Method of preparation of the recipes listed above

Time	Place (home, outside of the house)	Dinner	Quantity consumed (Household measurement)	Ingredients used	Amount of ingredients (g)

Method of preparation of the recipes listed above

Points to be covered during recall -

1. Type of milk consumed (cow's milk, buffalo's milk or any other please specify)
2. Type of water(plain, carbonated beverages, fruit juices please specify)

24 hour recall format – for PK participants

Please give details of the food consumed by you in last 24 hours

Please note: There are no foods like good foods and bad foods, so please feel free to identify all the foods consumed by you in the last 24 hours in detail

Food / beverages	Method of preparation (baked , fried, boiled caned etc.)	Amount eaten/ drank (cups, glass, plate, spoon, number etc)			Amount of ingredients (g)
		Day 1	Day 2	Day 3	
1. At morning (Breakfast)					
2. Between morning and noon (snacks)					
3. At noon (Lunch)					
4. Between noon and night (snacks)					
5. At night (dinner)					
6. Before going to bed (Snacks)					

Preparation of reagents used in phytate estimation

1. Phytate reference solution:

   The sodium salt of phytic acid, obtained from Sigma, Aldrich (P-8810), was used without further purification. Stock solution was prepared by diluting 0.15g sodium phytate in 100ml distilled, deionized water. The reference solutions were prepared by diluting stock solution with 0.2N Hcl in range of 2.22 to 17.76Mcg ml^{-1} (about 1- 8ml stock solution in 100ml)

2. Ferric solution:

   Ferric solution was made by dissolving 0.2g ammonium iron (ш) sulphate.12 H_2O in 100ml 2N Hcl and making up the volume to 1000ml with distilled water

3. 2, 2'- Bipyridine solution:

   Solution was made by dissolving 1 g 2, 2'-bipyridine and 1 ml of thioglycolic acid in distilled water and making up the volume to 100mls

Raw data of DDS for NW UK participants

Table 1Dietary diversity scores for NW UK

Participant number	D1WDDS	D2WDDS	D3WDDS
1	6	6	4
2	6	5	4
3	4	7	4
4	5	6	3
5	7	5	4
6	6	7	6
7	6	6	6
8	6	6	5
9	2	2	4
10	4	4	5
11	4	4	5
12	4	No recall	No recall
13	6	5	4
14	6	5	5
15	4	5	6

Table 2Dietary diversity scores for NW PK

Participant number	D1WDDS	D2WDDS	D3WDDS
1	2	4	1
2	4	3	3
3	2	3	2
4	2	2	2
5	2	2	2
6	4	2	2
7	5	4	3
8	3	2	2
9	3	3	3
10	3	3	2
11	2	2	2
12	3	3	2
13	2	3	3
14	4	3	4
15	2	3	3
16	4	4	4
17	2	2	2
18	4	4	3
19	3	3	3
20	4	2	3
21	4	3	2
22	1	4	1
23	3	3	1
24	4	3	3
25	3	3	3
26	4	2	3
27	2	2	2
28	2	3	3
29	4	3	4
30	3	3	3
31	4	4	4
32	2	2	2
33	3	3	2
34	3	3	3
35	4	2	3
36	5	4	3
37	2	3	2
38	3	2	2
39	2	2	2
40	3	3	3

Raw data of frequency of consumption of specific food groups for NW UK

Table 0 Consumption of starchy staples by participants in NW UK

Participant number	Day 1	Day 2	Day 3
1	1	1	1
2	1	1	1
3	1	1	1
4	1	1	1
5	1	1	1
6	1	1	1
7	1	1	1
8	1	1	1
9	1	1	1
10	1	1	1
11	1	1	1
12	1	No recall	No recall
13	1	1	1
14	1	1	1
15	1	1	1

0= not consumed on that day
1 = consumed on that day

Table 4 Consumption of starchy staples by participants in NW PK

Participant number	Day 1	Day 2	Day 3
1	1	1	1
2	1	1	1
3	1	1	1
4	1	1	1
5	1	1	1
6	1	1	1
7	1	1	1
8	1	1	1
9	1	1	1
10	1	1	1
11	1	1	1
12	1	1	1
13	1	1	1
14	1	1	1
15	1	1	1
16	1	1	1
17	1	1	1
18	1	1	1
19	1	1	1
20	1	1	1
21	1	1	1
22	1	1	1
23	1	1	1
24	1	1	1
25	1	1	1
26	1	1	1
27	1	1	1
28	1	1	1
29	1	1	1
30	1	1	1
31	1	1	1
32	1	1	1
33	1	1	1
34	1	1	1
35	1	1	1
36	1	1	1
37	1	1	1
38	1	1	1
39	1	1	1
40	1	1	1

0= not consumed on that day
1 = consumed on that day

Table 5 Consumption of vitamin A rich fruits and vegetables by participants in NW UK

Participant number	Day 1	Day 2	Day 3
1	1	1	0
2	0	0	0
3	0	1	0
4	1	1	0
5	1	1	0
6	0	1	1
7	0	1	1
8	1	1	1
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	1	0	0
14	0	0	0
15	0	0	0

0= not consumed on that day
1 = consumed on that day

Table 6 Consumption of vitamin A rich fruits and vegetables by participants in NW PK

Participant number	Day 1	Day 2	Day 3
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	1	0	0
7	0	1	0
8	1	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	0	1	0
17	1	0	0
18	1	1	0
19	0	0	0
20	1	0	0
21	0	0	0
22	0	1	0
23	0	0	0
24	1	0	0
25	0	0	0
26	1	0	0
27	1	0	0
28	0	0	0
29	0	0	0
30	0	0	0
31	0	1	0
32	0	0	0
33	0	0	0
34	0	0	0
35	1	0	0
36	1	0	0
37	0	0	0
38	1	0	0
39	0	0	0
40	0	0	0

0= not consumed on that day
1 = consumed on that day

Table 7 Consumption of other fruits and vegetables by participants in NW UK

Participant number	Day 1	Day 2	Day 3
1	1	0	1
2	1	1	1
3	1	1	1
4	1	1	1
5	1	1	1
6	1	1	1
7	1	1	1
8	1	1	1
9	1	1	1
10	1	1	1
11	1	1	1
12	1	0	0
13	1	1	0
14	1	1	1
15	1	1	1

0= not consumed on that day
1 = consumed on that day

Table 8 Consumption of other fruits and vegetables by participants in NW PK

Participant number	Day 1	Day 2	Day 3
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	1
5	1	0	0
6	0	0	1
7	1	0	1
8	0	1	0
9	1	1	1
10	1	1	0
11	1	1	0
12	1	0	0
13	0	0	1
14	0	0	0
15	0	0	1
16	0	0	0
17	0	0	1
18	0	1	1
19	1	0	1
20	1	0	0
21	0	0	0
22	0	0	0
23	1	0	0
24	0	1	1
25	1	0	1
26	1	0	0
27	0	0	1
28	0	0	1
29	0	0	0
30	0	0	1
31	0	0	0
32	1	1	0
33	1	1	0
34	1	1	1
35	0	1	0
36	0	1	1
37	0	0	0
38	0	0	1
39	0	0	1
40	1	1	1

0= not consumed on that day
1 = consumed on that day

Table 9 Consumption of meat and fish by participants in NW UK

Participant number	Day 1	Day 2	Day 3
1	0	1	1
2	1	1	1
3	0	1	0
4	1	1	0
5	1	1	1
6	1	1	1
7	1	1	1
8	1	1	1
9	0	0	1
10	0	0	1
11	0	0	1
12	1	0	0
13	1	1	1
14	1	1	1
15	0	1	1

0= not consumed on that day
1 = consumed on that day

Table 0-3Consumption of fish and meat by participants in NW PK

Participant number	Day 1	Day 2	Day 3
1	1	0	0
2	0	0	0
3	0	1	1
4	0	1	0
5	0	1	0
6	0	1	0
7	1	0	0
8	0	0	1
9	0	0	0
10	0	0	0
11	0	0	1
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	1	0	0
17	0	0	0
18	0	0	0
19	0	1	0
20	0	0	1
21	1	0	0
22	0	0	0
23	0	0	0
24	0	0	0
25	0	1	0
26	0	0	1
27	0	0	0
28	0	0	0
29	0	0	0
30	0	0	0
31	1	0	0
32	0	0	1
33	0	0	0
34	0	0	0
35	1	0	1
36	1	0	0
37	0	1	1
38	0	0	0
39	0	1	0
40	0	0	0

0= not consumed on that day
1 = consumed on that day

5.3 Appendix 3

a. Abstract accepted at Nutrition Society Postgraduate conference

b. Certificate of winning competition

c. Certificate of attendance

Comparative study of micronutrients (iron, zinc and vitamin A) in traditional south Asian diets in north west UK and north west Pakistan

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Micronutrient malnutrition continues to be the major public health problem globally, especially in South Asian (SA) countries including India, Pakistan, Bangladesh[(1,2)].Currently global statistics indicate a widespread increase in iron, zinc, vitamin A deficiencies among vulnerable populations[(1,3)]. Also, it is important to consider the bioavailability of micronutrients from the diet[(4)]. This is because cereals, which form the integral part of diets consumed in South Asian countries contain high amount of anti-nutritional factors, phytates, which inhibit the absorption of the micronutrients from such foods. Considering this background, the aim of the present study is to compare micronutrient intake and dietary sources in South Asian women living in England (NW UK) with those living in Baghbanan (NW Pakistan). In addition, the quantity of phytate in staple foods will be investigated in both the geographical settings. Furthermore, the relationship between dietary intake and biochemical indices of micronutrient status for Fe and Zn will be explored. For obtaining dietary data, 40 female participants in the age group of 18-30 years have been recruited in NW Pakistan and data has been obtained using three 24-hour dietary recalls. This data is being analysed using dietary analysis software (WinDiets version x, Robert Gordon University) and a dietary survey tool that can be used to generate a Dietary Diversity Score (DDS)[(6)]. Recruitment is currently being undertaken in NW UK. To investigate the quantity of phytate in the staples consumed, wheat flour samples have been obtained from both the geographical regions. These samples are being currently analysed for their iron and zinc content by Atomic Absorption Spectrophotometry (AAS) and for total phytate content by a spectrophotometric method (Haug and Lantzch method[(5)] at the laboratory of the University of Central Lancashire. Working towards the third part of the study, blood samples will be obtained for the participants in NW Pakistan and analysed for iron (haemoglobin) and plasma zinc concentration. In summary, this study will provide new information about the dietary intakes/sources of micronutrients of women living in Baghbanan, how these differ from 2ndgeneration Pakistani women living in England.

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