BONE HEALTH IN GAMBIAN WOMEN: IMPACT AND IMPLICATIONS OF RURAL-TO-URBAN MIGRATION AND THE NUTRITION TRANSITION

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DEDICATION

To the God of grace,

Who has given me Life!

&

To my parents Clifford and Cynthia,

who have always encouraged, guided, and supported my education, career, and life.

“Gracious words are like a honeycomb,

sweetness to the soul and health to the body.”

Proverbs 16:24

SUMMARY

Urbanisation and the associated nutrition transition have been linked with the recent rise in osteoporotic fragility fracture incidence in many countries. Predictions indicate that hip fracture incidence will increase 6-fold in Africa and Asia by 2050, partially attributed to demographic transition and population ageing. Differences in areal bone mineral density (aBMD) between rural and urban locations indicate that urban regions of high-income countries (HIC) have lower aBMD and a higher incidence of hip fracture. The few studies conducted in low and middle-income countries (LMIC) provide inconsistent results; in contrast to HIC, most have found higher aBMD in urban populations.

To investigate the impact of migrating to an urban environment, detailed studies of bone phenotype and factors affecting bone health have been conducted in two groups of pre-menopausal Gambian women: urban migrant (n=58) and rural (n=81). Both groups spent their formative years in the same rural setting of Kiang West, urban women were known to have migrated to coastal districts, concentrated in Brikama and Kanifing, when aged ≥16 years. Bone phenotype (bone mineral content (BMC); bone area (BA); aBMD, and size-adjusted BMC (adjusted for height, weight and BA) of the whole-body, lumbar spine and hip) was measured by dual energy x-ray absorptiometry (DXA), with further characterisation by peripheral quantitative computed tomography (pQCT). Data were also collected on anthropometry, body composition, food and nutrient intakes, physical activity, socio-demographic characteristics, vitamin D status, and 24hr urinary mineral outputs (Na, K, P, and Ca).

Mean age and height of rural and urban migrant groups were not significantly different ($p>0.05$). Urban migrant women were significantly heavier ($p<0.01$). Significant differences in BMC and aBMD were found between groups at all skeletal sites, with urban women having higher BMC and aBMD; BA was not significantly different. The greatest difference in BMC was found at the lumbar spine (8.5% ± SE 3.0, $p<0.01$), a meaningful difference, equivalent to 0.76 of rural SD. *T*- Scores

were also calculated using a young adult (white, female) reference population, mean *T*- scores were -1.03 and -0.22, for rural and urban groups respectively.

After adjusting for size, differences in whole-body and hip BMC were mostly attenuated ($p>0.05$), but difference in spine BMC remained significant (6.2% ± SE 2.1, $p<0.01$). These results indicate that rural-to-urban migration is associated with higher BMC; BA and height were similar, and difference in body weight could not fully account for higher BMC at the lumbar spine.

Calcium intakes were low in both groups, urban migrant 294mg/d (IQR: 235 to 385) and rural 305mg/d (IQR: 222 to 420). Urban women had significantly lower intakes of potassium, magnesium and dietary fibre ($p<0.01$), related to lower consumption of fruit, green leafy vegetables and groundnuts. 25-hydroxy vitamin D status was good in both groups, urban migrant 64.0 ± 14.2nmol/L and rural 68.3 ± 15.7nmol/L (*M* ± *SD*, $p>0.05$).

Implications for bone health of the nutrition and demographic transition, principally future fracture risk and other non-communicable diseases require further research in LMICs.

ORIGINAL CONTRIBUTION TO KNOWLEDGE

To my knowledge, this is the first study investigating the impact of rural-to-urban migration on bone health to be conducted in sub Saharan Africa. It is the first study of bone health and determinants of bone health in an urban population in The Gambia.

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aBMD	Areal Bone Mineral Density
BA	Bone Area
BMC	Bone Mineral Content
BMD	Bone Mineral Density
CDBH	Calcium, vitamin D and Bone Health group, sub-programme of Prof. Ann Prentice in The Gambia
CVD	Cardiovascular Disease
DALYs	Disability Adjusted Life Years
DINO	Diet In Nutrients Out
DRV	Dietary Reference Value
DXA	Dual Energy X-ray Absorptiometry
ENID	Early Nutrition Immune Development - study of rural women, from Kiang West, The Gambia used to form part of the rural WMS group
FAO	Food and Agriculture Organisation
FFQ	Food Frequency Questionnaire
FRAX	Fracture risk assessment tool
GamBAS	Study of rural born women, from Kiang West, The Gambia used to form part of the rural WMS group
GBoS	Gambia Bureau of Statistics
HIC	High Income Countries
KWDSS	Kiang West Demographic Surveillance System
LGA	Local Government Area
LMIC	Low and Middle Income Countries
LRNI	Lower Reference Nutrient Intake
MRC	Medical Research Council
MRCG	Medical Research Council: Unit The Gambia
MRCG at LSHTM	Medical Research Council: Unit The Gambia at the London School of Hygiene and Tropical Medicine

NaNA	National Nutrition Agency, The Gambia
NBH	Nutrition and Bone Health group of Prof. Ann Prentice
NCD	Non-Communicable Disease
NPNL	Non-Pregnant Non Lactating
Parity	The number of pregnancies that end in 20wk deliveries; the number of foetuses in pregnancy does not change the parity
pQCT	Peripheral Quantitative Computed Tomography
RNI	Reference Nutrient Intake
Rural group	Rural born women, from Kiang West, The Gambia
SA-BMC	Size - Adjusted Bone Mineral Content
SACN	Scientific Advisory Committee on Nutrition
SAMSON	Build a sub Saharan Musculoskeletal Network
SED food groups	Food groups developed by Sarah Elizabeth Dalzell
STEPs	STEPwise approach to surveillance, a WHO NCD risk factor survey
TFR	Total Fertility Rate: the average number of children that would be born to a woman over her 'reproductive' lifetime
UFS	Urban Feasibility Study - a feasibility study to trace migrants from Kiang West to urban coastal regions of The Gambia
UN	United Nations
Urban migrant group	Rural-to-urban migrant women, born in Kiang West, The Gambia and migrated to urban, coastal region of The Gambia after age 15 and lived at least 20% of life in urban area
UVB	Ultraviolet B
VAMU	Study of Food Vulnerability in the Urban Area of The Gambia
vBMD	Volumetric Bone Mineral Density
WHO	World Health Organisation
WMS	Women's Migration Study
YLDs	Years Lived with Disability

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1 INTRODUCTION

Global health concisely defined as "public health for the world", or more fully as:

> *"... the goal of improving health for all people in all nations by promoting wellness and eliminating avoidable diseases, disabilities, and deaths. It can be attained by combining clinical care at the level of the individual person with population-based measures to promote health and prevent disease. This ambitious endeavour calls for an understanding of health determinants, practices, and solutions, as well as basic and applied research concerning risk factors, disease, and disability." (Institute of Medicine, 2009)*

> *"Today, not only are health problems global, but lessons, insights, and fresh solutions regarding such problems flow in all directions." (Fineberg and Hunter 2013)*

These quotes come from the first of a series of articles published by the New England Journal of Medicine, and incorporated in the online course 'Readings in Global Health Harvard' (Harvard University, 2018).

Beginning with a global health perspective, provides a point of context, that the research I undertake is not done in isolation, but within the field of global health research and within the discipline of nutrition. The perspective of global health raises the following questions, what insights concerning bone health can we learn from other countries that have experienced extensive transition and urbanisation? Moreover, how might these lessons be used to promote bone health and prevent disease?

In 1996, Aspray et al. stated the following concerning bone health in The Gambia:

"The determinants of fracture risk rather than bone mineral status are important. In this community, where fractures are rare, such risk factors are unknown, but their identification may help to avoid changes likely to increase fracture rates. Lifestyle may be important." (Aspray et al., 1996)

To date, bone health research within The Gambia has been conducted primarily in rural populations, providing opportunities to develop an understanding of ethnic differences (Aspray *et al.*, 1996, Redmond *et al.*, 2014). This research has shown that rural Gambian populations have good vitamin D status, due to year round UVB sunshine, habitually low calcium intakes, and reportedly low incidence of fragility fracture; providing a contrasting perspective to the predominance of bone health research carried out in western, Caucasian populations.

1.1 Overarching purpose of the thesis

This PhD aims to explore whether rural to urban migration in Gambian women has an impact on bone health due to differences in potential risk factors of diet, lifestyle, and environment associated with the nutrition transition. Insights from this study will add to global understanding of urbanisation and bone health.

1.2 Structure of thesis

1. Introduction
2. Literature review I: Transition: Global and Gambian perspectives
3. Literature review II: Bone health
4. Setting and study design of Women's Migration Study (WMS)
5. Development of study design and methods
    a. Urban feasibility study
    b. Cross calibration
    c. Dietary assessment
6. Methods
7. Results I: Characteristics of study participants
8. Results II: Biochemistry, anthropometry, body composition, and bone phenotype
9. Results III: Food and nutrient intakes
10. Discussion
11. Conclusions
12. Training and skills development
13. References
14. Appendices

2 TRANSITION: GLOBAL AND GAMBIAN PERSPECTIVES

2.1 Transition: Global perspective

Environment, diet, and lifestyle are important determinants of health, including bone health. In the last century, the world's population has experienced significant transition in relation to these determinants, which vary geographically across and within nations.

In 2014, the United Nations (UN) published a report indicating that 54% of the world's population live in urban areas, a figure which is set to rise to 66% by 2050. Almost 90% of this predicted increase is concentrated in Asia and Africa, with 37% of projected population growth accounted for by India, China, and Nigeria (United Nations, 2014). In contrast to the current rapid urbanisation of low middle-income countries (LMICs), rates of urbanisation have now plateaued in the majority of high-income countries (HICs) (United Nations, 2014).

The aim of this chapter is to show how urbanisation, together with demographic, nutrition and epidemiological transitions, may be implicated in poor health. The chapter begins with an overview of urbanisation and health, followed by definitions and descriptions of the additional three processes of transition; firstly, from a broad global perspective, and then with particular focus on The Gambia, as the setting of this doctoral research. **Sections 2.2** to **2.5** provide further characterisation of the research context, including a brief history of the Medical Research Council (MRC) in The Gambia and current knowledge of the diet and lifestyle of the Gambian population. **Section 2.5** describes transition in The Gambia and **Section 2.6** considers the implications of transition for bone health.

2.1.1 Urbanisation and health

In March 2016, Nature Outlook published a series on the theme 'Urban health and well-being' (Hodson, 2016). Relevant topics, for example the challenge of sedentary lifestyles promoted by cities designed for cars, and how to improve public health by enabling people to walk and cycle more, are highlighted. Issues faced by expanding African cities are also explored, with an example from Nairobi, Kenya, where 'the average commuting distance increased from less than 1 kilometre in 1970 to 25 kilometres in 1998' (DeWeerdt, 2016). A greater dependency on vehicles not only encourages sedentary behaviour, but also leads to worsening traffic congestion and increased stress, while the resulting poorer air quality leads to an increase in respiratory conditions (DeWeerdt, 2016).

Despite the myriad perceived benefits of urban living e.g. improved access to services and employment opportunities, there is increasing evidence of significant detrimental implications, particularly in the context of rapid, uncontrolled urbanisation in LMICs, occurring without necessary infrastructures or policies in place. Urban environments have been associated with increasing non-communicable disease (NCDs) (Eckert and Kohler, 2014), (Vorster *et al.*, 2005), with a disproportionate burden among the poor (World Health Organisation, 2017). However the reality is complex, as shown by research published by Madise and Letamo (2017). In their review of thirty household surveys across Africa, they showed that women from wealthy, rural households were more likely to be overweight, than similarly wealthy urban women.

2.1.2 Demographic and epidemiological transition

In 1993, Professor Popkin aimed to provide a framework for considering historical worldwide nutritional patterns and transitions, helpfully expounding the inter-related concepts and impact of nutritional, demographic, and epidemiological transitions, and the influence of urbanisation and industrialising lifestyles.

The term demographic transition refers to 'the shift from a pattern of high fertility and high mortality to one of low fertility and low mortality (typical of modern industrialised countries)'

(Popkin, 1993). The result of these changes combined with improvements in health care is an increasing proportion of older people.

The epidemiological transition was firstly described by Omran (1971), and defined as 'the shift from a pattern of high prevalence of infectious diseases associated with malnutrition, periodic famine and poor environmental sanitation, to a pattern characterised by a high prevalence of chronic and degenerative diseases associated with urban-industrial lifestyles (Popkin, 1993).' One such degenerative NCD of ageing is osteoporosis and associated fragility fractures. Globally, NCDs account for 70% of deaths, with almost 40% occurring between the ages of 30 and 70 years. These deaths are described as 'premature', and over 80% of these deaths occur in LMICs (World Health Organisation, 2017). The majority of deaths in HICs (e.g. 90% in the UK) now occur because of NCDs, with little burden from infectious disease (World Health Organisation, 2017). In contrast, LMICs are experiencing a health transition characterised by a 'double burden of disease in which NCDs become more prevalent and infectious diseases remain undefeated' (Vorster *et al.*, 2005). Health transition is a term broadly encompassing the impact of both demographic and epidemiological transition (Vorster *et al.*, 1999).

In a review of the earliest publications on the nutrition transition, Popkin *et al.* (2012) concluded that it was clear that urbanisation was implicated with the rising global rates of obesity as well as the emergence of overweight and obesity in LMICs.

2.1.3 Nutrition transition

2.1.4 Defining the nutrition transition

Pelto et al. first described facets of the nutrition transition in their research on the 'delocalisation' of food production and distribution in 1983. They state that delocalisation has been key to the dramatic changes in worldwide dietary patterns over the past two centuries, and the majority of people no longer eat what they grow, or foods produced locally. These changes are associated with many positive improvements in the diversity of the diet, provided by more variety and increased availability out of season (Pelto and Pelto, 1983). The process of globalisation has promoted the development of a global food system, However, the current food system has been implicated in promoting 'unsustainable' and 'unhealthy' diets, which are reliant on only a few key crops (i.e. wheat, maize, and rice). Such diets are also associated with exhausting natural resources such as water and phosphorus. Growing concern for environmental sustainability of food systems is fuelling significant research, and receiving increasing political attention (Lang and David, 2012, Lang and Mason, 2017).

The development of interdisciplinary networks and institutional platforms in the last decade (i.e. Food Climate Research Network, Oxford; International Food Policy Research (IFPRI), Interdisciplinary Research Centre for Global Food Security, Cambridge; and Barilla Center for Food and Nutrition (BCFN)) demonstrates both the importance and commitment to this area of research. Literature from this field is outside the scope of the present research; however, a systematic review encompassing the determinants of dietary patterns, nutrition, and physical activity conducted by Prof. Hawkes provides a thorough introduction to the area (Hawkes *et al.*, 2006). The review encompasses environmental, economic, and political determinants, as well as socio-economic and cultural ones.

More recently, the nutrition transition has been described as the population-wide change in diet, from one that is indigenous, high in unrefined carbohydrates, and low in fat and processed foods, to a more 'western' diet higher in fat, sodium, and sugar, as well as higher protein due to an increase in animal foods e.g. meat and dairy products. The concept of 'nutrition transition' also encompasses a shift toward lower physical activity and overweight/obesity (Vorster *et al.*, 1999).

Figure 2:1 illustrates the five stages of the nutrition transition that populations tend to experience. The influence of urbanisation, along with shifts in the use of technology and the impact on demographic and epidemiological transitions are also indicated, particualrly for stage three: receding famine, stage four: degenerative disease, and stage five: behavioural change. In stage four, bone health problems are explicitly mentioned, indicating the association of several factors of the nutrition transition with various aspects of bone health. These will be described in more detail in **Section 2.6**.

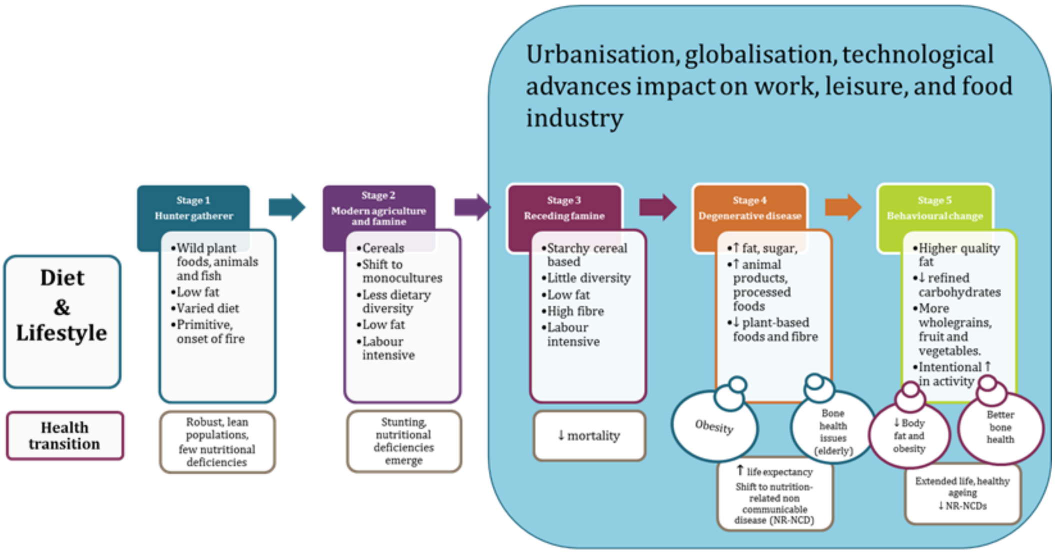


Figure 2:1 Stages of the nutrition transition (Figure based on Popkin, 2002)

Significant advances in technology have resulted in reduced physical activity in both work and leisure activities. The food industry in particular benefited and supported the development of refrigeration technologies, which enables the safe storage and efficient transport of food for many people. However, historically, the primary beneficiaries of such technology and wider food choice have been the upper social classes of western, industrialised nations. Today in HICs, there is comparatively little disparity in access to refrigeration and food choice is extensive. In contrast, in LMICs like The Gambia, much of the population rely on locally produced foods, and a large proportion of the population are subsistence farmers. There is little availability or access to labour saving devices and agricultural work is labour intensive. In resource-limited settings, struggles with poor and unreliable electricity infrastructure and inadequate access to fridges or freezers likely results in significant food spoilage (The Gambian Government, 2009).

2.1.4.1 Patterns of dietary transition

There is also accumulating evidence that dietary change in LMICs does not necessarily follow the same pattern of change that has occurred in HICs. In South Africa, transition in diet and lifestyle is not limited to only urban areas; levels of overweight and obesity are also rising in rural areas (Micklesfield *et al.*, 2014).

Critics of the nutrition transition concept, caution that "a one-size-fits-all" model has limitations, due to the complexity of interactions between local populations and wider global processes such as globalisation and urbanisation. Himmelgreen *et al.* (2014) examined the role and impact of globalisation and migration on dietary patterns and quality, and energy balance over a 20-year period. They conclude that using the concept of the nutrition transition more loosely will enable its application within a wider variety of settings. Professor Hawkes describes how globalisation processes can simultaneously result in complex homogenisation and differentiation of dietary patterns (Hawkes, 2006). This is an important consideration for research in The Gambia, as specific historical and cultural practices are likely to have a strong influence on the dietary choices of Gambians as they experience transition.

Research by Drewnowski and Popkin (1997) on the nutrition transition in HIC showed that increasing wealth was associated with increasing intakes of fat. However, the increased availability of cheap vegetable oil has increased the availability and accessibility of high fat foods to societies or parts of society with low incomes.

Comparing historic and modern patterns of transition, Adair and Popkin (2005) stated that 'the biggest difference with the developing world is that there is much more heterogeneity related to these patterns than in the developed world'. They compared patterns of snacking behaviour and out of home food consumption in China and the Philippines to the USA, stating that 'there is little snacking or away from home intake or fast food intake in China whereas the Philippines looks more similar to the US.' There is little evidence available from The Gambia on street food consumption, but currently, there are no large fast food chains in The Gambia. Before providing more detail of the situation concerning transition in The Gambia, **Section 2.2** provides a country overview.

2.2 Country overview: The Gambia

The Gambia is a small country situated in West Africa. A main geographical feature is the meandering river Gambia. Senegal surrounds the country on all borders, except the west, which has a coastline unto the Atlantic Ocean. The land area of The Gambia equates to ≈11,300km^2 with a population of 1,882,450 million. The resultant population density of 176 people/km^2 renders The Gambia one of the most densely populated countries in Africa (GBoS, 2014, GBoS, 2003b, GBoS and ICF International, 2014).

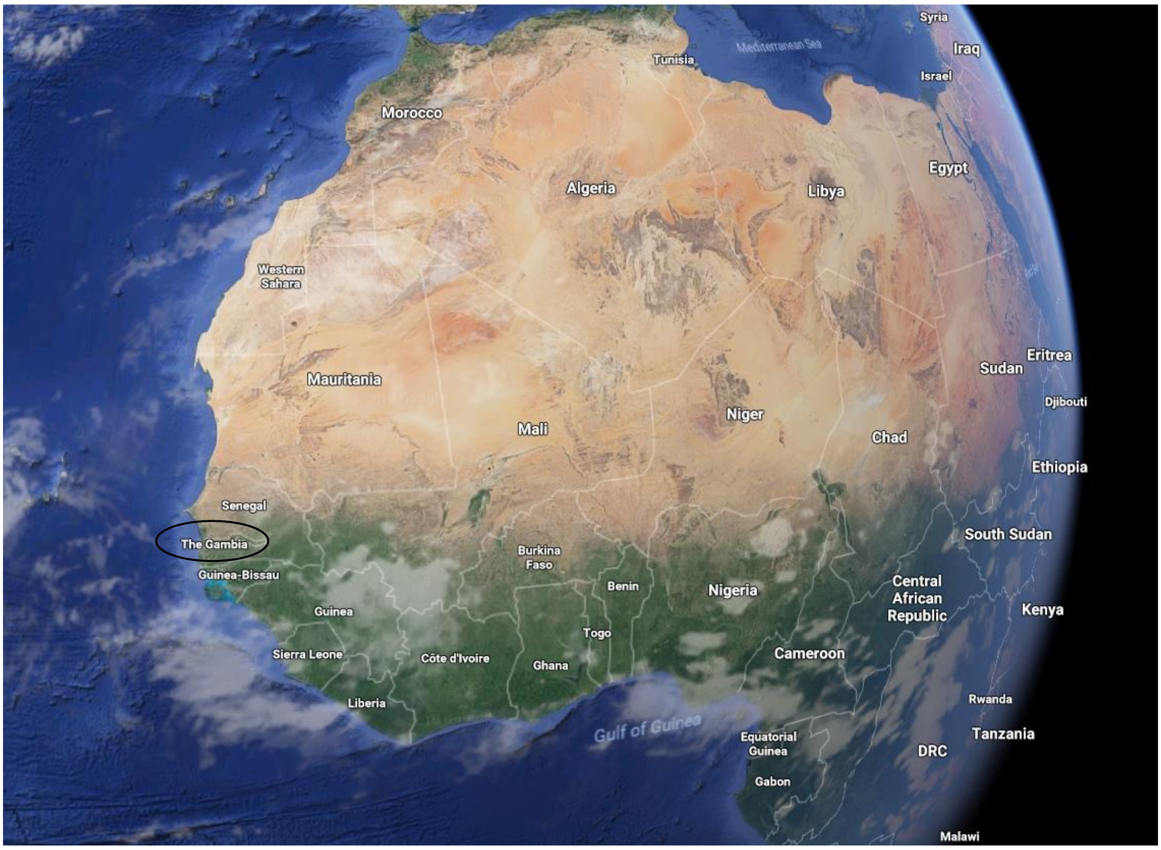


Figure 2:2 Map of West Africa Map data: Google, Landsat/Copernicus, Data SIO, NOAA, U.S. Navy, NGA, GEBCO

Currently ranked 175 out of 188 countries in the Human Development Index (HDI); The Gambia is classified as a least developed country. Improvements in recent decades include life expectancy at birth, which has increased from 52.1 years in 1990 to 60.2 years in 2014 (United Nations, 2016). Nevertheless, with an economy predominately reliant on agriculture and a short tourist season, the Gambian population is extremely vulnerable to increasingly erratic rainfall due to climate change (McSweeney *et al.*, 2010a), volatile food prices, and global financial situations.

Located at a latitude of 13°N, The Gambia has a tropical climate and experiences two main seasons: the wet season between July and September, and the dry season during the remainder of the year. Annual precipitation averages 67.6mm per month, with 216.7mm per month during the hot, wet season (McSweeney *et al.*, 2010a, McSweeney *et al.*, 2010b). In rural areas particularly, food availability and nutritional status are strongly influenced by season; the 'hungry season' coincides with the peak rains, usually the three months between July and September (Rayco - Solon *et al.*, 2004). The official language is English; however, ethnic groups, comprised mostly of Mandinka, Fula, Wolof, and Jola, each have their own languages. The majority of Gambians are Muslim, and polygamy is widely practised.

2.3 MRC Unit The Gambia: A brief history

Medical research in The Gambia has a long history, with MRC research dating back to 1947. Early research focussed on prevalent infectious diseases (e.g. malaria) and malnutrition; this continues up to today. The main research base is a 100-acre site, located in the urban area of Fajara, approximately nine miles from the capital city of Banjul. There are extensive laboratory and clinic facilities, as well as accommodation. The Keneba field station was established in 1949 by Professor Sir Ian McGregor, and later it became part of MRC Dunn Nutrition Unit (1973-98), and MRCG in 1998 [1]. Due to improvements in infrastructure (roads), Keneba is now ~2.5hrs by road from Fajara, in previous years it took more than 4hrs. A map indicating both urban and rural study locations, including MRC sites can be found in **Chapter 7**.

The Nutrition Theme is one of three unit-wide research themes. The Nutrition Theme has close links with both the MRC International Nutrition Group (ING) based at the London School of Hygiene and Tropical Medicine (LSHTM) and MRC Elsie Widdowson Laboratory, Cambridge. Research within the Nutrition Theme, includes Early Growth and Development; Iron, Infection and Anaemia; Nutritional genetics and epigenetics, and Calcium, Vitamin D and Bone Health (CDBH) (the MRC Nutrition and Bone Health programme at MRC EWL). The initial demographic and health survey established by McGregor covered three core villages: Keneba; Manduar; Kantong Kunda, and Jali from ~late 1950s, the villages are indicated in Figure 7:4. This has enabled accurate records of dates of birth in these villages since 1950 and close estimations of those now alive born before 1950 (Rayco ‐ Solon *et al.*, 2004). The survey area was expanded in 1989 to include the entirety of Kiang West (*N* ~14,000 across 36 villages). The predominant ethnicity of the district is Mandinka, although particular villages have a considerable number of people of Fula and Jola ethnicity. A more comprehensive overview of KWDSS was published in 2015 (Hennig *et al.*, 2015).

[1] In February 2018, MRCG transferred into LSHTM and is now known as MRCG at LSHTM.

2.4 Nutrition research in The Gambia

2.4.1 Description of typical rural Gambian lifestyle

The livelihoods of rural Gambian communities are based on subsistence agriculture, and women in particular play a central role in farming, gardening, and household tasks. Such activities have ensured that rural inhabitants are physically active, with significant time spent outdoors exposed to UVB sunshine, resulting in good vitamin D status, which is important for bone health (Aspray *et al.*, 2005).

2.4.2 Dietary intakes of rural Gambia

The diet in Keneba, Kiang West and surrounding villages has been described in significant detail, with dietary intakes, and specific food habits being particularly well characterised after many decades of nutrition and health research (Thompson, 1965, Hudson, 1995, Prentice *et al.*, 1993).

The typical rural diet is plant-based and comprises a staple cereal (mostly rice, limited use of millet or maize) and a variety of sauces, with small contributions of meat (goat, beef), poultry, and greater amounts of fish. Sauces are composed of several ingredients, primarily based on groundnuts, leaves, or vegetable oil. Tomatoes both fresh and as a paste are used in many sauces, and seasonings including dried baobab and hibiscus leaf, locust bean powder, chilli, and local salt.

Research in the late 1970s and 80s showed that most people prepared two meals per day, early afternoon and evening. Leftovers from the previous evening or porridge were often eaten for breakfast, in more recent years; bread (mostly plain) with tea is eaten occasionally.

The quote below is taken from a paper entitled 'Food intake in an African village' (Hudson, 1995), it highlights both the nature of dietary transition in the late 1980s in Kiang West and alludes to some of the processes behind the shift. "The diet in Keneba has changed considerably over a decade and continues to do so. The most striking change in the percentage frequency consumption of rice, which represented less than 50% of all cereals in 1981 but more than 90% in 1987 and 1988. This is a reflection of the year-round availability of aid rice and the villagers' ability to buy it. Findo, a cultivated grown known as 'hungry millet', has disappeared from the diet."

Previous research has shown that snacks are commonly eaten, and include handfuls of groundnuts or prepared foods, seasonal fruits (e.g. oranges, mangoes), cassava and roast maize cobs. As the majority of foods consumed are mostly local, they are also seasonal; therefore, nutrient intakes fluctuate across the year, especially vitamins and minerals (Bates *et al.*, 1994). Rice is an exception to the predominantly 'local' diet, which although grown particularly by Gambian women in rural areas, falls very short of the rising population's demand (FAO, 2018). Consequently, rice is imported into the country from Asia and South America. With a growing dependence on imported food comes vulnerability to price rises and political and market instability. Since 2002, the price of rice has quadrupled in The Gambia (FAO, 2014). Figure 2:3 shows an outline of the various seasons, activities, and foods associated with them. During the rainy season, food stocks from the previous year's harvest are low while physical activity increases as many women farm rice.

Jul Aug Sep Oct	Nov Dec Jan Feb Mar	Apr May Jun
Rainy Season	Dry: Post-Harvest Season	Dry: Pre-Farming Season (Hot)
Low stocks of cereals and groundnuts High agricultural work-loads High infection rates Weight loss * pregnant & lactating	Good supply of cereals and groundnuts Light farm work Improving nutritional status of mothers	Limited cereals and groundnuts Preparation of farms for rains
Plentiful wild leaves and fruits	Bush food limited Women's garden: onion, tomato, okra and chilli pepper	Plentiful mangoes Limited garden vegetables

Figure 2:3 Seasonal Diet and Activities of Kiang West (Prentice *et al.*, 1993)

2.4.2.1 Focus on calcium and phosphorus intakes

There are several aspects of nutrition relevant to bone health including intakes of bone forming minerals, particularly calcium and phosphorus. Other important nutrients include sodium, potassium, protein, and vitamins C and K; in addition, vitamin D status and physical activity are of significance. Implications of transition for bone health are described in more detail in **Section 2.6**; see **Section 2.6.4** specifically for dietary transition and bone health. As important bone forming minerals, calcium and phosphorus will be discussed in more depth, including dietary intakes and sources in The Gambia.

2.4.2.2 Important sources of calcium in The Gambia

Due to limited dairy consumption, intakes of calcium are very low (<300mg/d) (Prentice *et al.*, 1993, Jarjou *et al.*, 2013b). However, research in the late 1970s found several important 'hidden' sources, including baobab fruit and leaves, several varieties of other green leaves, fish (dried and fresh), cereals, groundnuts and local salt. **Figure 2:4** summarises the top ten foods in rural Gambian food tables (Prentice *et al.*, 1993). Also highlighted are the higher level food groupings 'Sections' with the greatest potential to contribute to calcium intakes in the current rural diet. Prentice et al. (1993) showed that milk was most often consumed as an addition to porridge, with 65% as fresh, powdered, or tinned milk and 35% as sour milk. The frequency of consumption was equivalent to once on 9% of study days during that period in the late 1980s and early 90s. Phosphorus in the diet mainly came from groundnuts and cereals.

Foods	Ca mg/ 100g	Section	Ca mg/100g
Furo/furundingo, dried	4000	Leaves	569
Baobab leaves, dried	1790	Fish	507
Milk, powdered, vegetable fat	1020	Milk and Eggs	240
Milk, powdered, full cream (Peak)	1020	Other Leaf Sauces	222
Okra, dried	825	Beans	153
Challo, dried	750	Kucha Leaf Sauces (without groundnuts)	136
Jambanduro leaves, fresh	705	Jambo Leaf Sauces (groundnuts, chilli pepper)	132
Dried fish (unspecified)	693	Nuts and Seeds	95
Locust beans, fermented (Tulingo)	449	Other Vegetables	70
Sardines, canned in oil	439	Water and Salt	64

Figure 2:4 Top ten richest sources of calcium in rural Gambian food composition tables, Left are individual foods, and right are sources by section. Dried furo is a small fish, eaten infrequently.

2.4.3 Evidence of declining calcium intakes

Jarjou *et al.* (1993) compared dietary intakes of lactating women between 1978-79 and 1990-91, they found that calcium intakes had reduced from 404±110mg/d to 283±119mg/d. This indicated that calcium intakes in this rural region had actually fallen and that this was due to changes in other aspects of the diet, particularly increased consumption of rice. They observed that rice displaced steamed millet, which usually included additions of dried baobab leaf during preparation, and which contributed to calcium and other micronutrient intakes. The change in staple also affected the types of sauces consumed, with fewer made from groundnuts and leaves, and more made from oil and flour, as these were considered complementary to rice. Notably, these are also usually less labour intensive dishes to prepare.

Thompson (1965) stated that 'Oil was available sporadically, but was used in particular at feasts for religious celebrations or for entertainment of visitors.' This reflected how the use of oil as a main component of sauces was a rarity. Nowadays, oil has increased quite significantly in both availability and consumption (Jaiteh, 2015).

More recently, a comparative study of rural Gambian boys and girls provided further indication of declines in both calcium and phosphorus (Jarjou *et al.*, 2013a). The authors proposed that this decline could be due to westernisation of the diet. If the traditional sources of calcium are being removed and alternative sources e.g. dairy products are not being introduced, then calcium intakes could also be lower in urban areas. However, the situation among wealthier individuals in the urban population may be different. Dairy products in The Gambia are expensive, as most have to be imported due to limited and often seasonal production locally. There is some evidence that more attention is being given to the marketing of dairy products in The Gambia, demonstrated by advertising on billboards. In addition, one study focussed on increasing dairy production and marketing in a cooperative in an urban area (Kombo North) of The Gambia (Somda *et al.*, 2003) (Somda *et al.*, 2004). Certain ethnic groups (e.g. Fula) are more traditionally associated with dairy farming in West Africa. Intakes of milk and milk products are unknown in the Fula population in The Gambia.

2.5 The Gambia in transition

The extent of urbanisation, demographic and epidemiological transition in The Gambia will be described in this section. Data are predominantly from research conducted through the Medical Research Council (MRC) in the Gambia, with the majority of nutrition research studies conducted in the rural region of Kiang West. The limited data available from the Gambian population in urban areas will also be reviewed.

2.5.1 Urbanisation in The Gambia

The entire population of The Gambia as surveyed in 2013, has increased by almost 40% since the last census in 2003, from 1.4 to 1.9 million (GBoS, 2014). In particular, peri-urban areas have experienced significant growth, with increases of 79.6% and 18.4% in Brikama and Kanifing Local Government Areas (LGA) respectively. The Brikama LGA includes the districts of Kombo North, South, and Central (**Figure 2:5: 30, 31, and 32**), the urban coastal area, where the urban component of the study presented in this thesis was conducted. Almost 60% of the total population of The Gambia lives in the three LGAs: Banjul City (the capital), Kanifing, and Brikama. Internal (rural-to-urban) migration has been suggested to be partly responsible for the increase in the urban population.

There is significant variation in the nature of urban environments and how 'urban' is defined. Definitions of rural and urban used in this thesis are described in **Chapter 3** (**Section 4.7.1**). For more detail on the patterns of urbanisation in West Africa particularly, see the Africapolis report on dynamics of urbanisation in West Africa across the period of 1950 to 2010 (Moriconi-Ebrard and Heinrigs). Although urban agglomerations in The Gambia are small compared to the expanding cities of Nigeria, the extent of urbanisation is highlighted by figures from the Africapolis report, showing the population of Serrekunda, the largest city in The Gambia, has grown from 84,322 to 603,000 across a thirty-year period (1980 to 2010). Data from the UN suggest that the total population will increase modestly, but the urban population of The Gambia will rise from 59% in 2014 to 71% by 2050 (United Nations, 2014).

2.5.2 Arising challenges of rapid urbanisation

Figure 2:5 illustrates the population density of The Gambia using district data from the 2003 census (GBoS, 2003a). This visual representation of the population highlights differences between districts, showing the clear distinction between the urban areas, Kanifing and Banjul, and more peri-urban Kombo districts. However, it is based on 2003 census data, and the most recent 2013 census shows that these peri-urban areas have become more densely populated in recent years.

Map of The Gambia removed for copyright reasons. Copyright holder is The Gambia Bureau of Statistics, Banjul, The Gambia

Figure 2:5 Maps of Local Government Areas and Districts in The Gambia, with lower figure indicating population density (GBoS, 2003a)

One challenge arising from the growth of urban Gambia's population is a reduction in land availability in urban areas. This has coincided with an increase in the cost of land, as it is progressively becomes a scarce commodity. Therefore, in earlier years, when urban areas were less densely populated, migrants may also have been more able to afford land and so continue to farm to a greater extent than today (Caprani, 1999). The only evidence characterising urban areas indicates that a quarter of people have no vegetation in their compound, 64% with a fruit tree and just over 1% had a food garden (NaNA, 2009). Horticultural groups may enable some urban dwellers to have access to more land for cultivation; however, with the very high population density, the available land would be insufficient for all urban dwellers. Community garden groups are estimated to contribute to 20-35% of produce in urban Gambia. Furthermore, in urban areas of The Gambia less than 10% of households were found to participate in horticulture (FAO, 2012).

In a small country such as The Gambia, improvements in transport and communication links between rural and urban areas are likely to result in the transfer of both favourable and unfavourable influencing factors of diet and lifestyle and wider influence of globalisation. Declining agricultural output, and greater access, due to the development of main roads between rural and urban areas within The Gambia has also encouraged rural-to-urban migration (FAO, 2018).

Nonetheless, as urban areas i.e. Banjul and Kanifing reach maximum capacity, the government is endeavouring to control the situation by encouraging people to live in the less densely populated districts further south and east, and by increasing the provision of housing schemes in the Brikama LGA, particularly in the district of Kombo North (GBoS, 2013).

Remittances from family members who have migrated to western countries may also contribute to improved standard of living for some families. A report published in 2017 showed that remittances sent by Gambian migrants overseas contributed 22% of GDP in 2016, and increased by 225.3% from levels in 2007. This ranks The Gambia as the second highest African country to have such a significant reliance on remitances for GDP (de Vasconcelos *et al.*, 2017). Income from remittances therefore often represents a large proportion of income for families, used towards food, schooling and housing costs.

Traditionally, several households, usually extended family, share one compound. For example, the 2013 census showed that rural areas have an average of 9.9 persons per household, compared to 7.1 persons in urban households (GBoS, 2013). While in the rural area captured by the Kiang West demographic surveillance system (DSS), there is an average of 16 (range: 1-170) people per compound (Hennig *et al.*, 2015). The challenge of rising cost of housing as demand grows in urban areas could therefore affect the nature of living arrangements.

2.5.3 Evidence of transition in The Gambia

The first demographic and health survey in The Gambia was conducted in 2013, results indicated differences in the total fertility rate (TFR) between urban and rural areas over the three year period before 2013, TFR= 4.7 in urban areas and 6.8 in rural areas. TFR is the average number of children that would be born to a woman over her 'reproductive' lifetime. Methods of family planning also showed a significant disparity between urban and rural women; contraception usage had a prevalence of 12% in urban areas and only 4% in rural areas (GBoS, 2014). As previously highlighted in **Section 2.2**, life expectancy has increased markedly, a key aspect of the demographic transition.

NCDs including cardiovascular disease (CVD), type 2 diabetes, and cancer, are now estimated to account for 34% of adult deaths nationwide (World Health Organisation, 2011). The increased prevalence is in addition to the ongoing burden of infectious disease and malnutrition, which still poses a major threat to the health of the Gambian population (Omoleke, 2013). At present, little is known about musculoskeletal health in sub Saharan Africa (Gcelu and Kalla, 2015), for example in The Gambia there are no available data on mortality after hip fracture. However, according to the global burden of disease 2016 study (GBD 2016), musculoskeletal disorders contribute significantly to chronic ill health and long-term disability, particularly in LMICs (Naghavi and Forouzanfar, 2013, Naghavi *et al.*, 2016). In The Gambia, musculoskeletal disorders are ranked the number one cause of years lived with disability (YLDs) and fourth for disability-adjusted life years (DALYs) in men and women aged 50-69 years (GBD, 2016). The very recent establishment of SAMSON: 'Build a Sub-Saharan African MuSculOskeletal Network' will assist in the launch of a new clinical fracture risk assessment tool (FRAX) and build collaborations across Zimbabwe, South Africa, The Gambia, and Uganda in the area of musculoskeletal health. This will also provide fracture incidence data for The Gambia (SAMSON, 2018).

In 2001, van der Sande et al. (van der Sande *et al.*, 2001) reported some of the earliest data on changes in cause of deaths (1942 - 1997) in the Gambian capital Banjul. The data demonstrated a significant rise in the incidence of NCDs in adults, and particularly a higher cause of death in men. Patterns of hypertension and CVD risk factors (obesity, diabetes and hyperlipidaemia) in both rural and urban areas of The Gambia were studied between October 1996 and June 1997 (n=5389). Data showed that prevalence of hypertension and CVD risk factors was higher in urban areas. However, a recently published survey has demonstrated that hypertension is higher in rural areas of The Gambia. Recent data from the Kiang West Demographic Surveillance System (KWDSS) shows that hypertension is also prevalent among the rural population; it is the most frequently diagnosed condition (27%) in the over 50s and ranks number four (5%) in those age 20-49years.

Indicative of the nutrition transition is the increase in the prevalence of overweight and obesity within The Gambia. The 1996/7 study showed that middle and older aged women had the highest prevalence of obesity (van der Sande, Ceesay et al. 2001). The World Health Organisation (2010) standardised STEPwise approach to surveillance (STEPS) is a population-based survey on chronic disease risk factors, providing a tool to monitor within country and facilitate cross country comparisons. The STEPs survey was conducted in early 2010 in Gambians aged 25-64years. Females were shown to have the highest percentage of obesity (16.5%), while 66% were also not engaging in vigorous activity. Overall, 42% of females aged 45-64years were found to have three or more risk factors, including low consumption of fruit and vegetables.

Awareness of the importance of monitoring the health and nutritional status of the Gambian population, led to the establishment of The Gambia's National Nutrition Agency (NaNA) in 2000. NaNA's involvement in a collaborative project entitled 'Food Vulnerability in the Urban Area of Banjul and Kanifing Municipality The Gambia (VAMU – French acronym)' has attempted to address the previous paucity of data on the health of urban Gambians. The publication of the VAMU report provided some necessary evidence of the reality of the nutrition transition within the Gambia (NaNA, 2009), showing that 85% of households surveyed in the urban area consumed fats and oils with even those with the least diversified diet consuming oils (NaNA, 2009) . Popkin (2010) highlighted that a significant trend of the nutrition transition is an increase in the consumption of edible oil, with similar trends also apparent for sugar and milk products. This is evident in The Gambia; the secular trend in supply of vegetable oil and quantity of oil imported in to The Gambia has seen substantial increases, from ~10,000 metric tonnes (MT) in 2000 to over 40,000 MT in 2012, a 300% increase in 12 years. The top five agricultural products imported into the country are rice, sugar, flour, onions, and oil (Jaiteh, 2015).

Anecdotal evidence (G Goldberg, personal communication) in The Gambia has also suggested that sodium intakes are increasing and could be higher in urban areas, with women often adding stock cubes e.g. Jumbo, Maggi, and Ajinomoto (mono sodium glutamate) when available, in addition to local salt traditionally harvested and used in rural areas.

There are limited data describing snacking behaviour among urban Gambians. One report in Gambian schoolchildren indicated that the preference for sugary snacks was increasing, along with greater accessibility (NaNA, 2009). This has also become more apparent in rural areas in recent years, although the trend is less pronounced.

2.6 Implications of transition for bone health

The background literature reviewed thus far has encompassed wide-ranging themes of transition both globally and in The Gambia. Having considered how processes of globalisation impact diet and lifestyles through influencing food systems, and affecting the day-to-day livelihoods of populations predominantly reliant on subsistence agriculture; what then are the potential implications of urbanisation and transition for bone health?

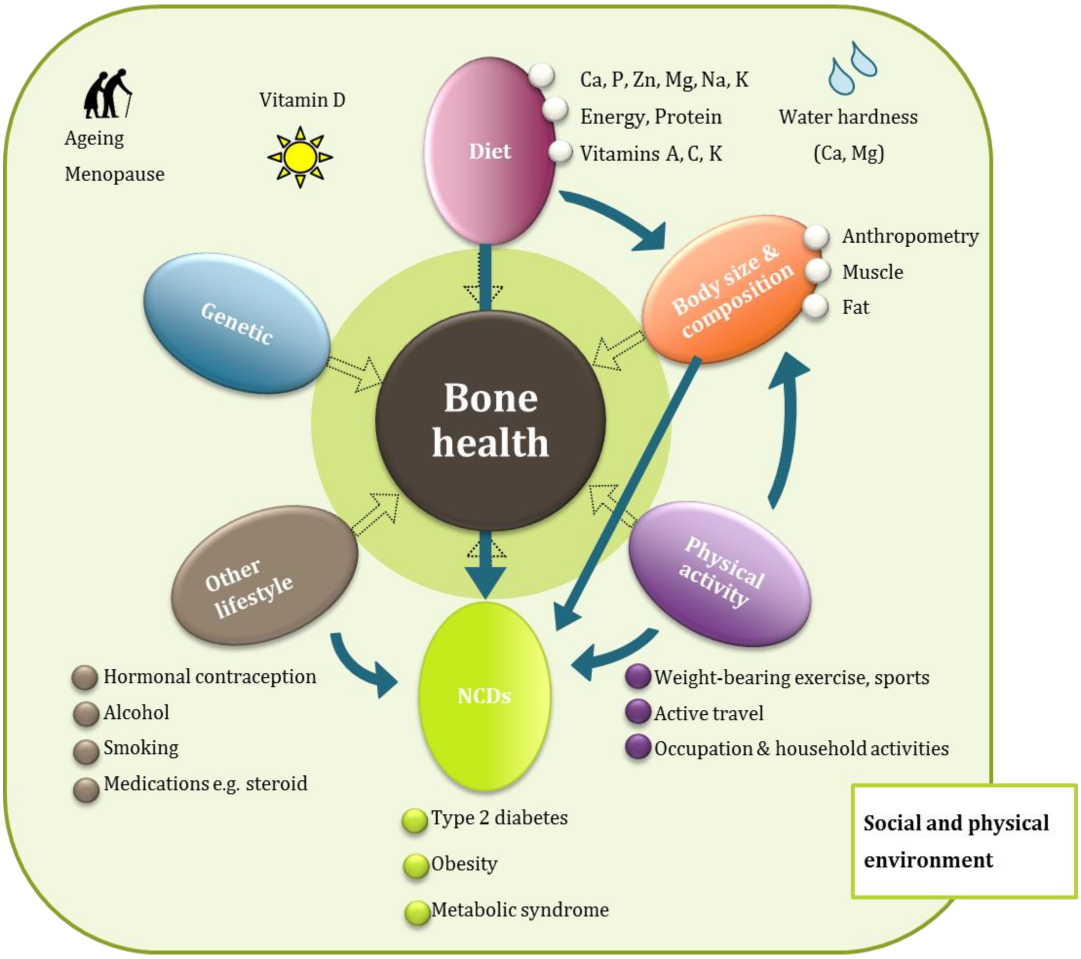


Figure 2:6 Conceptual framework of the impact of transition on bone health (based on evidence from several sources (Heaney *et al.*, 2000), (Walsh and Vilaca, 2017) and (Weaver *et al.*, 2016).

Figure 2:6 provides a framework to consider some of the contributing factors impacting bone health. It indicates the influencing environmental context and highlights other key factors associated with bone health and fragility fracture, which include diet (essential macro and micronutrients); physical activity (impacted by exercise, travel, occupation and household activities); body size and composition (encompassing muscle strength and function); NCDs (e.g. type 2 diabetes), and the metabolic syndrome (Compston, 2018) (Laurent *et al.*, 2016). This section will highlight several key aspects of diet and lifestyle and their potential implications.

2.6.1 Lower vitamin D status and physical activity

Vitamin D status (status marker, 25-hydroxycholecalciferol (25OHD)) is known to be an important determinant of bone health across all ages (Prentice, 2004). It is primarily synthesised by the skin through UVB exposure and dietary sources are limited e.g. oily fish, fortified margarine, and eggs. For bone health, 25OHD concentrations below 25nmol/L have been associated with an increased risk of under-mineralisation of the bone collagen matrix, and concentrations below 10nmol/l are likely to result in rickets in children or osteomalacia in adults. The UK Scientific Advisory Committee on Nutrition published recommendations in a recent review of Vitamin D and Health. The report suggested that vitamin D status could indirectly affect bone health and risk of fragility fracture through positive associations with muscle strength and function. They concluded that maternal vitamin D status was influential on infant bone mineral, but were unsure of the significance of this. In older adults, there was also some evidence for a beneficial effect of vitamin D supplementation on bone mineral status at some skeletal sites, and in combination with calcium for fracture prevention in older adults (Scientific Advisory Committee on Nutrition, 2016).

Physical activity, particularly when weight bearing, influences bone health through stimulating osteogenesis. Higher levels of physical activity are associated with increased bone mass and other aspects of bone strength related to structural parameters of bone. Physical activity also improves muscle strength and balance, which help to reduce the likelihood of fracture and falls (Weaver *et al.*, 2016).

Urban environments could encourage sedentary lifestyles due to the industrialisation of processes, which results in less physically demanding jobs, often more likely to be factory or office-based and thus also potentially reducing exposure to UVB sunshine and lowering vitamin D status. If safe and appropriate cycling and walking infrastructures are not put in place, then more vehicles are likely to result, which would not only reduce physical activity levels, but could result in higher levels of pollution which could further reduce UVB exposure. Therefore, the particular importance of providing clean, safe environments in LMICs should not be overlooked, as they could also provide a culturally appropriate way (especially for women) to be physically active.

2.6.2 Ageing populations and bone health

The demographic transition, related to lower fertility and mortality rates, and improved healthcare results in an ageing population. Higher use of contraception, particularly injectable hormone contraception e.g. Depo Provera, have been associated with loss of bone in users (Popoola *et al.*, 2016) (Berenson *et al.*, 2008). Ageing is not only associated with a reduction in the efficiency of vitamin D synthesis in the skin (MacLaughlin and Holick, 1985), but also reduced muscle strength and function, as well as bone loss (Ward, 2012).

Urban environments usually can also offer better access to medical care, which could be associated with extending life expectancy, particularly of the 'oldest old'. However, urban environments may propose fewer opportunities for outdoor activities, particularly in the most urbanised cities, which feature little green space. Depending on the climate, frail elderly populations may find it difficult to remain independent and active, thereby increasing bone and muscle loss, and increasing risk of falls and fragility fractures.

Shifts in social and family dynamics are also likely to have consequences on health. The increasing phenomena of rural-to-urban migration in societies, which have historically had strong family responsibilities for the care of elderly relatives, is likely to affect the context of ageing. As younger family members migrate to cities, older members are either left behind, or find they have to move later in life to an unfamiliar environment. In many contexts, this is less desirable than their familiar, rural home with social support and opportunities to continue gentle recreational activities such as gardening (Liu, 2014, Cook and Liu, 2016). However, the opposite may also be true, and it is definitely context and perhaps class specific.

2.6.3 Obesity, type 2 diabetes and metabolic syndrome

Globally, the nutrition transition, associated with an increasing prevalence of obesity is resulting in a growing burden of preventable disease. Of particular concern is the coexisting milieu of adverse metabolic characteristics, which affect some obese individuals, including low-grade inflammation, high leptin concentrations, and insulin resistance. This is hypothesised to be of relevance to the increased fracture risk and impaired bone quality observed in Type 2 diabetics (Viljakainen *et al.*, 2017).

However, higher body weight is also associated with higher aBMD, partially due to the increase in load, and this is related to a reduced fracture risk. Current evidence demonstrates that fracture risk (wrist, hip, and spine) is lower for obese populations, but higher for fractures at other sites (ankle, leg, and humerus) (De Laet *et al.*, 2005) (Premaor *et al.*, 2014).

A systematic review published by Sugimoto et al. aimed to elucidate relationships between 'lifestyle-related metabolic disorders, osteoporosis, and fracture risk in Asia.' The authors conclude that diabetes and atherosclerosis were associated with higher risk of fracture, but diabetes was not consistently associated with lower bone mineral density (BMD). They state that 'MetS is likely associated with osteoporosis or decreased BMD in men but not women.' These findings are somewhat different to studies in western populations, which have found that higher fracture rates occur in diabetics, with higher BMD. In 2017, Walsh and Vilaca (2017) published a review on obesity, type 2 diabetes, and bone in adults; the authors highlight the challenge faced in assessing risk of populations with higher BMD and high fracture incidence. There is some evidence that one of the detrimental mechanisms of type 2 diabetes on bone is through glycation of collagen, which reduces the quality of bone.

Fat distribution is also believed to have different actions on bone, for example, visceral fat produces cytokines, which are characteristically pro-resorptive; while high intramuscular fat has been associated with poorer muscle function and potentially increases the risk of falls. Higher subcutaneous fat may be positive through loading and the action of some adipokines (e.g.

adiponectin), but negatively impact bone through lowering vitamin D levels. Distribution of body fat varies by ethnicity, and this may be one explanation for disparity between diabetes and bone health in Asian populations and studies conducted in Caucasian populations.

2.6.4 Dietary transition and bone health

Determining the role of diet on bone health is complex. Several nutrients have been studied more extensively (e.g. Ca, protein) in relation to bone health. However, the interaction of biological systems, for example demonstrated by the implications of endocrine disorders such as type 2 diabetes with poor bone health, highlights that all nutrients essential for growth, development and maintenance of the body are of relevance, either directly or indirectly, to bone health (Prentice *et al.*, 2006).

Therefore, current dietary and lifestyle guidance (World Health Organisation, 2015) is likely to be appropriate for bone health, until further evidence is made available. This was summarised in a review published by Prentice et al. (2004), "Prudent dietary and lifestyle recommendations developed in respect of other chronic diseases may prove helpful in terms of fracture risk but firm evidence is lacking. These include increase physical activity, reduce sodium intake, increase consumption of fruit and vegetables, maintain a healthy body weight, avoid smoking and have moderate alcohol intake" (Prentice, 2004).

With this said, there are several nutrients which are key to bone health, these are bone-forming minerals (Ca, P, Mg, Zn); vitamins involved with Ca-P homeostasis and/or bone metabolism and structure (e.g. vitamins D, K and C), energy and protein (Prentice *et al.*, 2006) (Ward, 2012). While understanding the role and mechanism of individual nutrients is important, there are also many nutrient-nutrient interactions and phytonutrients worthy of consideration.

However, the question of how much of a particular nutrient is required to ensure optimal bone health is difficult, demonstrable by the disparity in current dietary reference values (DRVs) set for different countries. Calcium recommendations for example range from 700mg for UK adults to 1000mg for American adults. There is significant debate around methods to establish DRVs and assess the adequacy of diets and nutrient intakes (Prentice *et al.*, 2007). Needless to say "the disparity reflects difference in the methods, philosophies and assumptions used, plus the fact that the values selected are generally tailored for the diet composition of the population for which the references are formulated" (Prentice *et al.*, 2006).

For LMICs, such as The Gambia, no DRVs exist, and often WHO recommendations are used, despite evidence that national context and ethnicity are important determinants of requirements. This typically results in most Asian and African countries deemed to be in a weak position for bone health. For example, calcium intakes in many populations fall short of even the UK lower reference nutrient intake (LRNI) of 400mg; the amount estimated to meet the requirements of only 2.5% of the population. Where local diets do not typically include or are, low in dairy products this is common. However, evidence from populations with traditionally plant-based, low calcium diets have indicated that fracture incidence is lower than white populations; this is hypothesised to be due to ethnic and lifestyle differences which may protect bone health (Zengin *et al.*, 2015). This is also characteristic of the situation in The Gambia, as described by Aspray *et al.* (1996).

Using the China National Health and Nutrition survey, one research group has sought to question the notion that populations with a habitually low calcium diet, from a predominantly plant-based diet need to change their diet. They showed that the relationship between calcium intakes and fracture risk may be a U-shaped curve, concluding "The patterns of dietary calcium with fracture risk are U-shaped in men and possibly in women." They state that for women, Ca intakes below ~250mg were associated with an increased risk of fracture, while intakes above 650mg did not have any benefit in women, but were associated with an increased risk of fracture in men (Fang *et al.*, 2016). They also voice a specific concern for the sustainability of recommendations to consume more dairy foods, due to the environmental repercussions of such a decision. The balance of seeking to define nutritional adequacy within the ecological limits of the planet are described in two stimulating papers by Professor Lang (Lang and David, 2012) (Lang and Mason, 2017).

Therefore, caution should be exercised when assessing the adequacy of dietary intakes in the absence of nationally derived DRVs, as using the WHO recommendation although advocated for use globally, fail to consider these ethnic differences. Calcium supplementation studies conducted by Prof. Ann Prentice's group in The Gambia have shown some unexpected consequences of higher calcium in pregnant women and pre-pubertal children. These findings therefore caution against universal recommendations for calcium, in those accustomed to a low intake (Jarjou *et al.*, 2013b, Ward *et al.*, 2017, Ward *et al.*, 2014).

In light of this, and in the context of urbanisation, dietary transition and bone health, it is of most relevance to highlight particular foods and/or food groups associated with the nutrition transition, particularly those characteristic of African populations experiencing transition (Sodjinou *et al.*, 2007) (Bosu, 2015). Taking a 'whole-diet' approach to understand the role of foods and nutrients consumed together has been advocated and is likely appropriate when multiple aspects of diet shift concurrently (Ward, 2011).

Therefore, the remainder of this section will focus on briefly highlighting the implications for bone health of higher salt intakes, meat and animal products, sugar, oil and processed foods and lower intakes of fruits and vegetables, i.e. the shift to a western diet.

2.6.4.1 Higher consumption of meat and animal products

Meat and animal products provide rich sources of many nutrients (e.g. protein, Fe, Zn, vitamin B12); while dairy products contribute calcium, phosphorus, protein, B vitamins amongst others. Dairy products have been shown to have beneficial effects on bone, however the effects cannot be isolated to calcium alone, as both protein, and growth factors contained in these products may also play a role (Kouvelioti *et al.*, 2017).

On the one hand, urbanisation may be associated with a higher consumption of meat and animal products, and potentially increase the protein adequacy of the diet, thus prove beneficial for bone. However, detrimentally, diets high in meat and protein are also associated with an increase in endogenous acid and greater urinary calcium excretion, due to sulphur containing amino acids

(Nicoll and McLaren Howard, 2014). Nevertheless, to some extent such detrimental effects may be mitigated, depending on the source of protein in the diet. For example, other components e.g. Ca in milk or P in meat, and the overall acid-base balance of the diet could confer protective influences on bone health (Ginty, 2007).

In populations with historically low consumption of dairy products, migrating to an urban environment may increase access to these foods. However, this to some degree is likely to be associated with socioeconomic status, and the level of availability of these products.

2.6.4.2 Lower fruit and vegetables

Fruits and vegetables are rich in a variety of nutrients, particularly potassium and magnesium. A diet rich in fruits and vegetables has been associated with higher BMD and reduced bone loss in longitudinal studies. One of the beneficial effects on bone is thought to be mediated through their ability to buffer acid load, which can be produced from diets high in meat, as described above. A high acid load results in greater bone resorption, whereas high potassium intakes relate to a more alkaline urine and also increase the micronutrient content of the diet with implications for bone metabolism and formation (Prentice, 2004). For example, vitamin C is important for collagen formation and cartilage, while vitamin K is an essential cofactor for the synthesis of osteocalcin, a protein involved in bone formation. The relationship between dietary potential renal acid load (PRAL) and bone health is described more fully elsewhere (New *et al.*, 2000) (New, 2002, Remer *et al.*, 2003).

2.6.4.3 Higher salt consumption

As described in **Section 2.5.3**, anecdotal evidence indicates that salt intakes are high and perhaps rising in The Gambia, due to the combined use of salt and stock cubes in food preparation. Sodium has been suggested to adversely affect bone health, due to its effect on increasing calcium excretion (Prentice, 2004, Teucher *et al.*, 2008). There is a dependent relationship between sodium and calcium, whereby their movements through the kidney are intimately linked.

However, the extent to which dietary levels of sodium impact on calcium metabolism and consequently bone health are not fully elucidated, and not known in The Gambia.

2.6.4.4 Sugar, vegetable oil, and processed foods

The significant increase in the importation and likely consumption of sugar, oil, and white flour to The Gambia was described in **Section 2.5.3**. Higher consumption of sugar, oil, and processed foods could indirectly affect bone by increasing the risk of NCDs e.g. type 2 diabetes, and metabolic syndrome or by displacing the consumption of other locally sourced foods rich in micronutrients, especially bone forming nutrients. There is also evidence, which suggests that higher consumption of caffeine and alcohol may also be harmful for bone health, particularly at high levels increasing urinary calcium excretion (Prentice, 1997).

In contexts where the prevalence of micronutrient deficiencies is still high, the use of fortified stock cubes, vegetable oil, and flour may contribute to improving intakes of various nutrients. However, there is a danger for any population if their diet becomes reliant on a few fortified foods to meet their nutrient requirements.

For example, a diet that is primarily based on starchy refined carbohydrates, sugar, and vegetable oil (of often-unknown fatty acid composition), may meet energy requirements, but is likely to be low in fibre and many other nutrients. The overall composition of the diet will for instance modulate the composition of the gut microbiome and this in turn could have implications for calcium absorption (Whisner *et al.*, 2015), vitamin K synthesis, the immune system, and metabolic health in general (Kau *et al.*, 2011). Therefore, while there is likely a role for the fortification of certain food items, it is important that populations are aware that 'magic bullets' do not exist for nutrition, and consuming fortified stock cubes alongside a very nutrient poor, energy dense diet will certainly not resolve all nutritional problems. Instead, such dependency for nutrients from fortified foods could perpetuate the double burden of disease. This suggests the importance of dietary diversity, to ensure sufficient nutrient intakes from a wide variety of foods, and the need to promote nutrient dense sources.

Traditional foods sources such as leaves and indigenous fruits gathered from forests and the bush are still commonly consumed in many parts of Africa (Gockowski *et al.*, 2003) and Asia (Ickowitz *et al.*, 2016). The role and knowledge of these indigenous foods has been highlighted, including their potential to contribute to food security (Chivandi *et al.*, 2015) (Shackleton *et al.*, 2009). There is however vulnerability for local populations in situations where a global market emerges for nutritious indigenous foods such as baobab. Buchmann et al. (2010) describe the situation in West Africa in their paper entitled "The importance of baobab (Adansonia digitata L.) in rural West African subsistence - suggestion of a cautionary approach to international market export of baobab fruits" (Buchmann *et al.*, 2010).

In summary, there are several features of dietary transition implicated with bone health, these along with possible differences in physical activity and occupations could result in the erosion of many of the protective factors for bone health in The Gambia. Compared to rural areas, urban areas are likely to have better access to imported and processed foods. However, socio-economic status is a likely determinant to both lifestyle and food choice, as shown in South Africa (Micklesfield *et al.*, 2013, Madise and Letamo, 2017).

CHAPTER 2: SUMMARY

In this introductory chapter, various aspects of transition were explored and defined. First, the global perspective was considered, highlighting the effect of rapid urbanisation on LMICs with a focus on the extent of urbanisation and transition in The Gambia. The literature reviewed the diet and lifestyle of the rural Gambian region of Kiang West, underlining evidence of the nutrition transition over the last three decades. While considering shifts in patterns of food consumption, thought was also given to implications for NCDs, including the increasing prevalence of obesity and type 2 diabetes, and potential links to bone health. In the following chapter, the focus will be on bone health in the context of the NCD osteoporosis and fragility fractures.

3 BONE HEALTH

This chapter aims to define bone health and consider the impact of urbanisation and the nutrition transition on bone mineral status and incidence of fragility fracture. It begins with a brief overview of healthy bone development and maintenance. Determinants of bone health and risk factors of osteoporosis and fragility fractures are described. The various methods available to assess bone health are also explored, with a particular focus on dual energy x-ray absorptiometry (DXA) as the main method of measurement used in this research. An overview of global epidemiology of osteoporosis and associated fragility fractures highlight the current geographic variation, and potential reasons for this suggested.

3.1 What is bone?

Bone is a 'dynamic' organ, which provides structural support, organ protection and acts as a mineral reservoir of calcium for various functions throughout the body (Rosen, 2004). The three skeletal elements are bone cells, bone mineral, and bone matrix. There are three main types of bone cell, osteocytes, osteoblasts, and osteoclasts. Osteocytes are the primary strain sensing cells of bone tissue, detecting the need for change (i.e. bone formation or resorption), and consequently co-ordinating osteoblasts and osteoclasts, respectively. Osteocytes 'are former osteoblasts that remain within the mineralized bone matrix and form a network that senses mechanical and hormonal environmental cues' (Bellido *et al.*, 2014).

A matrix of collagen forms after production by the osteoblasts, and this undergoes mineralisation with inorganic mineral salts (hydroxyapatite), including calcium, phosphorus, and magnesium. There are two types of bone, cortical bone which is the more dense and hard outer layer, composed of many osteons, and trabecular bone which is the spongy layer that is more metabolically active and provides flexibility and strength, due to the formation of its component trabeculae (**Figure 3:1**). The composition of bone differs depending on the skeletal site, with varying proportions of the two types of bone (i.e. cortical and trabecular). The rate of turnover is much higher for trabecular bone compared to cortical.

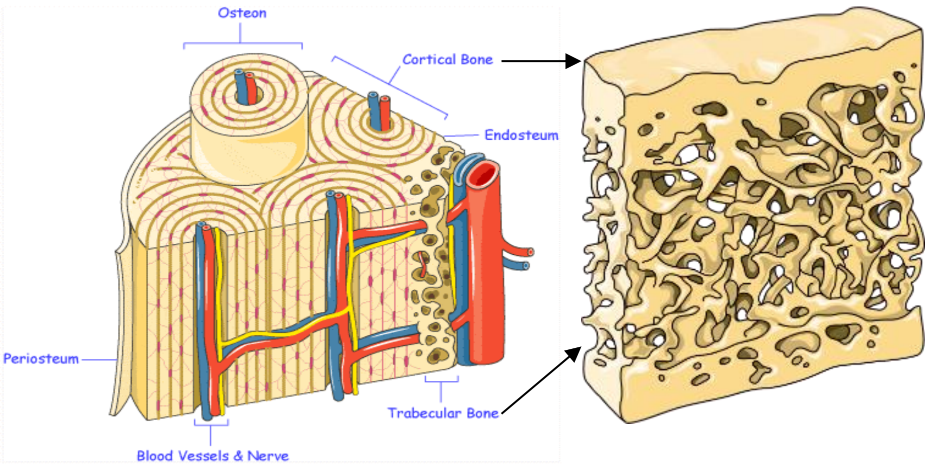


Figure 3:1 Bone structure (Servier, 2017)

Bone is 'dynamic' in that it must constantly adapt, and there are two key processes involved, bone modelling when 'either bone formation or bone resorption occurs on a given bone surface', and bone remodelling, a process which 'functions to renew the skeleton and involves sequential bone resorption and formation at the same spatial location' (Allen and Burr, 2014).

Bone modelling impacts on skeletal structure (i.e. size and shape) and composition (i.e. amount of cartilage, cortical and trabecular bone), and is most prominent during periods of growth, beginning in utero during the development of the foetus and continuing until epiphyseal fusion, which is typically by age 20.

Bone mass comprises collagen, mineral and extracellular fluid within the whole bone, and is accrued most rapidly during infancy and adolescence with the greatest increase occurring during puberty. Periods of rapid growth therefore represent one of the skeleton's most vulnerable times. **Figure 3:2** shows the trajectory of bone mass accrual and loss across the lifecourse in both men and women, and the steady state maintained until an imbalance of resorption and formation results in a net loss of bone. In addition to ageing, the menopause is a period (10-15years) of accelerated bone loss for women, due to a fall in the production of oestrogen. With ageing, both trabecular and cortical bone are lost. However, there is greater loss from the trabecular compartment (Compston, 1993). Loss of microarchitecture and strength conferred by the connectivity of trabeculae results in reduced bone strength and increased risk of fragility fracture.

During pregnancy and particularly lactation, there are additional demands for bone-forming nutrients, which the body achieves by adopting various physiological adaptations. However, many cycles of pregnancy and lactation may be associated with an accumulated burden on bone, meaning that higher parity could pose a greater burden on the skeleton and mineral 'reservoir' (Olausson *et al.*, 2012). Despite evidence that bone mineral decreases during pregnancy and lactation, research conducted by Sawo *et al.* (2013) found that bone mineral was replenished post lactation even in women on a very low calcium intake in The Gambia.

'Peak bone mass' is "generally thought of as the amount of bone gained by the time a stable skeletal state has been attained during adulthood" (Weaver *et al.*, 2016). However, the timing of peak bone mass and strength has been shown to vary both by skeletal site and bone compartment (i.e. cortical or trabecular). Therefore, current evidence advocates that behaviours, thought to be good for bone health should be sustained throughout life. Once growth has ceased, the accrued bone

Figure of bone mass accrual and loss across the lifecourse removed for copyright reasons. Copyright holder is Cambridge Unversity Press.

Figure 3:2 Development of bone mass over the life course (Ward, 2012).

mineral or 'peak bone mass' acts as a reservoir of mineral for later life. At this stage of development, several other facets of bone strength have also been determined, including the shape, size, and distribution of bone tissue within the periosteal envelope.

Numerous features of bone contribute to its strength; these include mass, density, microarchitecture, micro repair mechanisms and geometric properties (Weaver *et al.*, 2016). According to Ward (2012) "a healthy bone is one that is fit-for-purpose, has mechanisms for maintenance and repair, is responsive to changes in musculoskeletal environment, and will not fail during normal physiological activities". Muscle strength and function have been highlighted as important for bone development, as described in the Mechanostat model (Frost, 1987). This model describes how bone strength is modified to be 'fit for purpose' in response to changes in strain, which are primarily produced by the force that muscle exerts on bone.

In summary, it is during times of greatest growth (i.e. childhood and adolescence), and greatest bone loss (i.e. ageing), that musculoskeletal health is most likely to be determined, and when external factors such as nutrition could elicit either optimal or suboptimal bone development. For example, failure of the growing long bones to be mineralised, due to calcium and/or vitamin D deficiency results in rickets in childhood, while stunting occurs due to failure or delay in longitudinal growth. Osteomalacia, a condition characterised by a failure of mineralisation on all trabecular and cortical bone surfaces, can occur in children and adults causing soft and weak bones (Prentice *et al.*, 2006). The aetiology of osteoporosis and fragility fractures, defined below, which occur in older age, can also be understood from a lifecourse perspective of bone health. Factors likely to be beneficial are those that affect the achievement of peak bone strength in childhood and adolescence and minimise bone loss during ageing.

3.2 Defining bone health and its significance

Prentice *et al.* (2006) state that "in the context of public health, the term 'bone health' largely refers to the quality of being at low risk of three common skeletal disorders: (a) stunting; (b) rickets and osteomalacia; (c) osteoporosis and fragility fractures."

In the context of global shifts in population demographics and rapidly transitioning diet and lifestyle in low middle-income countries (LMICs), it is the third skeletal disorder osteoporosis and fragility fracture, which is of particular concern. The following section will define osteoporosis and highlight potential methods available to assess various aspects of bone health, with a focus on techniques on osteoporosis. The subsequent sections will then describe the global burden of osteoporosis and associated fragility fractures, highlighting geographical variation and current evidence of the impact of urbanisation from within country studies comparing rural-urban areas and the few published migration studies.

3.3 Defining osteoporosis

Osteoporosis is defined as "a multifactorial disease; a systemic skeletal disorder characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk" (World Health Organisation, 1994). The literal meaning of osteoporosis is porous bone, indicative of the characteristic deterioration in density and quality. **Figure 3:3** shows three images of bone with progressive porosity. The 'silent' nature of osteoporosis means that until a fracture occurs, there is no evidence of disease. Low trauma fragility fracture is considered the clinical expression of the disease, and typically occurs at the wrist, hip, or spine.

Osteoporosis can be referred to as primary, i.e. related to ageing including the menopause, or secondary, which occurs because of several conditions including hyperparathyroidism, hyperthyroidism, diabetes, or another chronic disease (World Health Organisation, 1994).

The definition of osteoporosis stated above was agreed upon at a consensus development conference in 1991. Shortly after this, a World Health Organisation (WHO) expert study group was commissioned to advise on: "Assessment of fracture risk and its application to screening for postmenopausal osteoporosis". They concluded that bone mass was one of the key measureable determinants of compressive and torsional bone strength, and bone loss was associated with an increase in the risk of osteoporotic fracture. Hence, they operationalised the definition of osteoporosis, as a bone mineral content (BMC) or bone mineral density (BMD) at or below 2.5 standard deviations (SD) of a young healthy adult mean, using appropriate population reference data, and measured using Dual Energy X-ray absorptiometry (DXA) (World Health Organisation, 1994). Clinically this is still the gold standard for diagnosing osteoporosis. The following section will outline various methods used to assess bone health, i.e. bone mineral status and bone turnover.

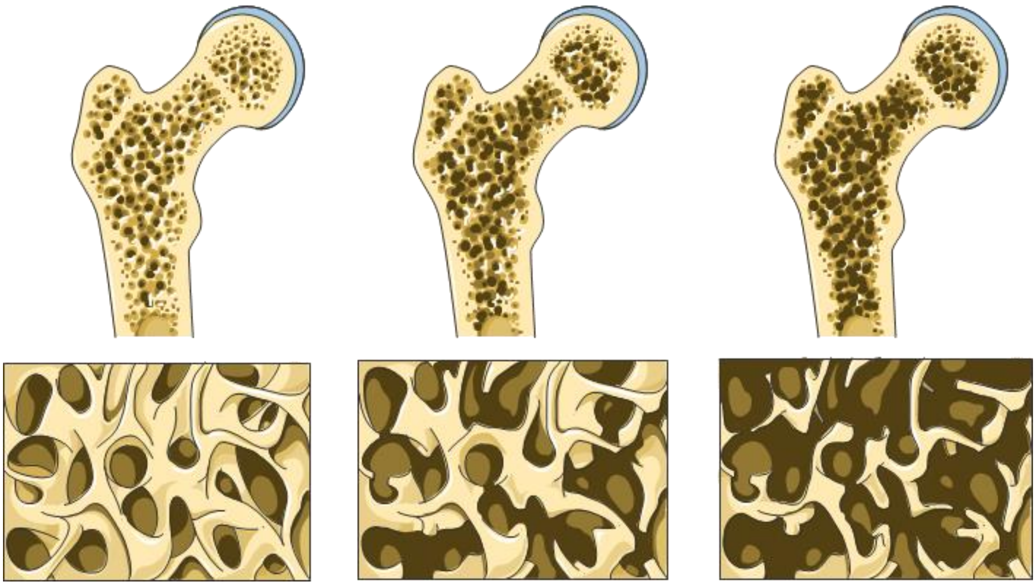


Figure 3:3 Images of bone ranging from healthy bone on the left to increasingly porous and osteoporotic bone on the right (Servier, 2017)

3.4 Overview: assessment of bone health

Bone health can be assessed in several ways, depending upon the particular aspect of interest. As described above, the diagnosis of osteoporosis using the WHO definition requires assessment of bone mineral status using DXA, although there are other bone densitometry techniques also used to assess status. These include (with earliest first):

- Single Energy X-Ray absorptiometry (SXA)
- Single photon absorptiometry (SPA)
- Dual photon absorptiometry (DPA)
- Quantitative ultrasound (QUS)
- Dual energy x-ray absorptiometry (DXA)
- Quantitative computed tomography (QCT)
- Peripheral quantitative computed tomography (pQCT)
- High-resolution pQCT (HR-pQCT)

The earliest instruments used to assess bone density were SXA, S/DPA; however, since the development of DXA over 30 years ago, they are no longer used. Quantitative ultrasound (QUS) is less expensive and portable instrument used at peripheral sites (e.g. Os calcis, radius, and tibia). It does not directly measure bone density, and cannot be used to diagnose osteoporosis; however, it is thought that the technique captures other parameters of bone structure and predicts individuals who later fracture. The machine provides a T-score, which correlates to the risk of fracture, however the measurement most commonly of the heel bone (Os calcis) is not a site associated with osteoporotic fracture.

DXA is now the most common bone-imaging technique used to assess bone mineral status. It provides measurements at both axial and appendicular skeletal sites. There are many advantages to DXA, including good precision, low radiation dose and its ability to measure sites prone to osteoporotic fracture e.g. hip and spine.

pQCT and HR-pQCT are more recent methods developed to assess the appendicular skeleton. They can be used to quantify bone mineral status as well as provide additional parameters related to bone strength (shape and dimensions). Importantly, they provide true volumetric density of bone mineral; a limitation of DXA derived areal density. HR-pQCT also provides information on trabecular and cortical microarchitecture. Advantages and limitations of DXA and pQCT techniques are described in **Table 3:1,** further details of DXA used in the work described in this thesis are included in the Methods chapter, **Sections 6.1.2**. Details of pQCT are included in **Appendix F**, as the data were collected in the overall study protocol, but have not been presented in this thesis as cross-calibration data were required to ensure validity.

Table 3:1 Advantages and limitations of DXA and pQCT

Densitometry technique	Advantages	Limitations
DXA	Non-invasive	Size dependent, provides areal density (g/cm2) not true volumetric density, cannot measure tissue thickness
	Quick scan time (approx. 10mins for total body)	Cannot differentiate a thin, high density bone from thick, low density bone
	Fast scan time	Affected by changes in body composition
	Good precision	Not mobile and must be kept in a stable environment
	'Gold standard' clinically	Unable to distinguish trabecular and cortical bone compartments
	Low radiation dose	Degenerative changes, aortic calcification and vertebral fractures can increase aBMD and bias results, particularly in older people
	Assesses clinically relevant skeletal sites e.g. hip and lumbar spine	Limited when comparing different populations
	Estimates body composition: lean and fat mass	Fan beam magnification can affect growing skeleton of children and young adults and effect estimated geometrical parameters e.g. hip axis length
	Reference data available for Caucasian, Asian and African-American	No reference data for black African populations
pQCT	Non invasive	Sensitive to movement
	Low radiation dose	Only measures peripheral skeleton
	Able to distinguish trabecular and cortical bone	Longer scan time: positioning and adjustments increase scan time
	Measures actual density (g/cm3)	Software less user friendly
	Size-independent – able to consider size and density	Significant variation in trabecular bone in the scan area, means slight shifts in positioning of ROI can alter BMD
	Measures body composition: fat and muscle	Acquisition and analysis protocols not standardised, differ in % sites, difficult to compare across studies
	Measures geometry e.g. CSA	Unable to assess microstructure of bone
	Measures bone strength, considers spatial distribution of mineral	
	Small and easily transported	
	Considers bone and muscle at same skeletal site	

(IAEA, 2010) and (Langton and Njeh, 2003)

3.4.1 Assessment of fracture risk: FRAX

More recent consensus among bone health researchers has resulted in a definition of bone health that centres on the importance of considering 'optimal bone strength', of which bone mineral content (BMC) is but one component (Ward, 2011, Bouxsein, 2005). This shift resulted in the development of the Fracture Risk Assessment Tool (FRAX®), by the University of Sheffield and WHO, and it is now under the auspices of International Osteoporosis Foundation (IOF). FRAX® estimates fracture risk of patients and combines DXA derived BMD at the femoral neck with other risk factors. Data from population-based cohort studies in Europe, North America, Asia, and Australia informed the development of the FRAX algorithm. Users are given a 10-year probability of hip fracture specifically, or a major osteoporotic fracture at any site. FRAX has not yet been validated for use in sub Saharan Africa; see **Section 2.5.3** for more details of the 'SAMSON' network and developments in this area. **Figure 3.4** highlights the FRAX risk factors and other lifestyle characteristics that are associated with an increased risk of osteoporotic fracture. Several other algorithms have been developed in addition to FRAX, these include the Garvan and QFracture (ISCD, 2015).

Screenshot of FRAX webinterface removed for copyright reasons. Copyright holder is Centre for Metabolic Bone Diseases, University of Sheffield, UK.

Figure 3:4 FRAX tool web interface for UK

3.4.2 Limitations of current osteoporosis definition

Based on the current WHO definition, available data indicate that the incidence and prevalence of osteoporosis varies worldwide, with apparent geographic and ethnic disparity (Cauley *et al.*, 2014). In 2004, Prentice (2004) published a comprehensive review outlining the evidence for diet and nutrition relating to osteoporosis. The review highlights the challenge of accurately determining the extent of osteoporosis worldwide, due to problems with definition and diagnosis. The requirement of specialist bone imaging equipment to diagnose osteoporosis, along with lack of expertise and trained operators, has resulted in very little data in Africa and many parts of Asia.

As previously mentioned, DXA is the most common bone-imaging technique used to assess bone mineral status; however, while other techniques have been developed, for example Qualitative Ultrasound (QUS) and Quantitative Computed Tomography (QCT), they cannot be used with the WHO definition of osteoporosis. Furthermore, the lack of population reference data for sub-Saharan Africa and specifically black African populations has made it impossible to determine the prevalence of osteoporosis in these regions based on this WHO definition. It is also important to clarify, that black African populations of sub-Saharan Africa differ significantly from African-Americans included in the USA reference data sets (Pettifor, 2015). Additionally, the WHO definition is not valid when applied to men or pre-menopausal women.

Unfortunately, focussing on a quantitative measurement of bone mineral can lead to an oversimplified understanding of the relationship between bone health, defined only in terms of bone mass, and fracture risk. This narrow definition disregards other qualitative components including bone shape and size, internal structure and metabolism, and loading conditions associated with bone strength (Prentice, 2004).

The use of BMC and BMD to compare populations has also been criticised, as both parameters are strongly influenced by body size, a factor that also differs between populations (Prentice, 2004). Evidence in more recent years has highlighted that not all populations with low bone mass are at high risk of fragility fracture, for example populations with shorter stature such as in Africa or

Asia, will also have lower bone mineral content. As a working definition within a population, it has been suggested that the WHO definition of osteoporosis is of some use, however, it is inadequate to compare across populations.

For this reason, fracture rate in older people has been advocated as most useful when comparing between populations. Hip fracture incidence is preferred, as many countries have hip fracture registers, which enable estimation of incidence. Other osteoporotic fractures, especially of the spine or wrist, may be asymptomatic and medical attention may not be sought. However, other challenges remain, including defining a low trauma, osteoporotic fracture and ensuring exclusion of traumatic fracture. Most challenging is the paucity of quantitative data from LMICs, and its potential unreliability, given the limited access to medical facilities, difficulty in ascertaining exact age and cause of fracture.

3.4.3 Assessment of bone turnover: biochemical markers of bone turnover and calcium homeostasis

To understand the rate of bone remodelling, biochemical markers can be measured in either blood or urine. Markers of bone turnover reflect bone resorption (osteoclast activity) and bone formation (osteoblast activity), but are not currently used to assess or diagnose osteoporosis. There are various markers available and they tend to be enzymes or proteins synthesised during bone remodelling, or breakdown products (e.g. N- and C- terminal telopeptides of collagen).

There are several markers of bone resorption available these include CTX (C-terminal telopeptide) and NTX (N-terminal telopeptide) of type I collagen, measured in urine or serum/plasma. A further example is tartrate-resistant acid phosphatase (TRACP 5b), an enzyme highly expressed by osteoclasts.

Markers of bone formation measured in plasma or serum include osteocalcin, bone alkaline phosphatase (BALP), and C and N-propeptide of type I collagen (PICP and PINP). Osteocalcin is a protein derived from osteoblasts (the cells responsible for bone formation), secreted in significant quantities in bone tissue (Bonjour *et al.*, 2014).

In addition to bone turnover markers, the measurement of parathyroid hormone (PTH), metabolites of vitamin D, and growth hormones IGF-1 and -2 can also provide further insight into bone metabolism, calcium homeostasis, and factors which modulate growth. However, there are several factors, which can influence the concentration of these markers including, age, skeletal and sexual maturity, growth velocity, and mineral accrual, as well as sex and ethnicity. Additional sources of variability include season, diet, exercise, kidney function, and in women, the phase of menstrual cycle and the use of oral/injectable hormonal contraceptives. Therefore, it is important that these factors are considered in the study design and adjusted for in statistical models. These make the interpretation of results more difficult, and the implication is that such biochemical markers do not translate directly to amounts of bone formed or loss, and therefore net bone balance. Markers of bone turnover are therefore best measured along with bone mineral status in order to provide a better understanding of mechanisms and overall bone health (Prentice *et al.*, 2006).

3.5 Current and future burden of osteoporotic fragility fractures

Both osteoporosis and associated fragility fractures are concomitant with severe morbidity and mortality in the older population, representing a significant burden on society with huge personal and economic impacts (Johnell, 1997). Disability adjusted life years (DALYs) provide a measure of overall disease burden; they represent the number of years lost due to ill health, disability or early death.

In LMICs, fewer resources and unplanned development of urban environments mean that the economic and social burden will likely be even more considerable, particularly in the context of the ongoing burden of prevalent infectious disease e.g. HIV.

3.5.1 Global differences in fragility fractures

Annually more than 8.9 million fractures occur because of osteoporosis (Johnell and Kanis, 2006); the highest incidence is among the elderly of western of HICs, with women experiencing the greatest burden in these countries. Worldwide estimates indicate one in three women and one in five men, over the age of 50 will sustain an osteoporotic fracture. Fragility fractures occur most often at the wrist, spinal vertebrae and hip, although they are possible at any skeletal site (Prentice, 2004). A comparison of the country with the highest rates of hip fracture to the lowest, suggest that age-standardised incidence rates of hip fracture vary >200-fold in women and >140-fold in men, see **Figure 3:6** (Cauley *et al.*, 2014). As previously mentioned, a particular challenge of reviewing global literature on bone health is the scarcity of quantitative data from LMICs. Limited access of these populations to medical facilities, especially in remote regions, also reduces the reliability of available data.

Map of world with hip fracture rates in different countries removed for copyright reasons. Copyright holder is International Osteoporosis Foundation and National Osteoporosis Foundation 2012

Figure 3:5 Hip fracture rates for women in different countries of the world categorised by risk.

Where estimates are available, countries are colour coded red (annual incidence >300/100,000), orange (200–300/100,000) or green (<200/100,000) (Kanis *et al.*, 2012).

Geographical variation in the incidence of hip fracture demonstrates that Africans are currently at lower risk than Caucasian populations (Nordin, 1966). One author refers to this as the 'African fracture paradox' (Hough, 2003). His statement is based particularly upon research conducted in South Africa, where vertebral fracture incidence in the Black population was found to be 10 times lower than in the White, Asian, or Mixed-race populations; despite all having similar BMD_{SPINE} (Daniels *et al.*, 1997). However, it is predicted that the incidence of hip fracture will increase 6-fold to approximately 6 million in Africa and Asia by 2050 (Cooper *et al.*, 1992). Figure 3:5 shows the hip fracture incidence for women. It is notable that the majority of Africa and significant regions of Asia do not have any data available.

The majority of research has been conducted in populations at high risk of osteoporotic fracture; risk factors investigated mostly within these populations have been described in **Section 2.6.** If, physically active lifestyles and diet are protective factors in developing countries then transitions occurring rapidly are likely to result in increases in fragility fracture.

Figure 3:6 Age-standardised hip fracture incidence rates in women and men accroding to country.

Countries are organised by continent or geographic region: Europe (pink); North America (green); Asisa (light blue); Middle East (brown); South America (purple); Oceania (dark blue); Africa (red). (Cauley et al., 2014)

3.5.2 Ethnic differences in bone health

Ethnicity is a social construct, and its definition can vary. However within bone health research it has generally been used to group individuals 'according to a mix of cultural and other factors including geography, language, diet, religion, ancestry, and physical features traditionally associated with race' (Zengin *et al.*, 2015). Differences between ethnic groups are likely to be due to several factors (Leslie, 2012); these have been suggested to be:

i. Genetics
ii. Skeletal size
iii. Body size and composition
iv. Lifestyle
v. Socio economic determinants
vi. Environmental exposure

However, Megyesi *et al.* (2011) challenge the extent to which genetics contribute to ethnic differences, as few studies have examined genetic data and lifestyle and other related factors could contribute to observed differences. The authors also comment on the significant variation in how ethnic groups are defined and categorised, which they suggest could limit interpretation and suitability to compare between studies and replicate findings.

A recent review on ethnic differences and bone health (Zengin *et al.*, 2015), highlighted that bone structure is a further aspect which differs between ethnicities. Gambian women were found to have shorter hip axis length (HAL), inferring a reduced risk of hip fracture, compared to white-British and Chinese women (Yan *et al.*, 2004, Dibba *et al.*, 1999). Prentice *et al.* (1991) commented that the notably lower fragility fracture incidence in Gambian women might be due to a 'better conservation of trabecular bone during ageing'; as the distribution of mineral within bone is an important aspect of bone strength, and an additional factor related to fracture risk (Ward, 2012).

This is currently being tested in a longitudinal study of bone ageing in The Gambia (GamBAS) (Zengin *et al.*, 2017).

3.5.3 Why low fracture incidence in Africa?

The low rate of fragility fracture in Africa is in part attributable to demography. However, despite the fact that African countries have a lower proportion of older people compared to HIC countries, age-adjusted models of fracture rates have shown that if the rate of fracture incidence in the USA (Caucasians) were to occur in The Gambia, it is unlikely to go unnoticed (Prentice *et al.*, 1991). Prior to the availability of bone imaging techniques in sub-Saharan Africa, the reason for reportedly low incidence of fragility fracture among black Africans was believed to be due to higher BMD, as had been found in African Americans. However, several studies in South Africa and The Gambia have shown that this is not the case (Nordin, 1966, Solomon, 1979, Bloom and Pogrund, 1982, Aspray *et al.*, 1996).

For example, studies in the late 1960s by Solomon et al. demonstrated that incidence of fracture of the femoral neck was low in the black South African population, and that bone density (of the metacarpal) was also lower compared to the white population (Solomon, 1968, Solomon, 1979). However, a review of ethnicity and bone in South Africa, highlighted that there are no data to confirm whether fracture rates of others skeletal sites demonstrate the same trend and relationship with BMD (Micklesfield *et al.*, 2011). Similarly, Aspray *et al.* (1996) demonstrated that Gambian women had a lower incidence of fracture compared to British women (independent of height and weight) and regardless of their lower BMC at the lumbar spine, hip, and radius (adjusted for size).

3.5.4 Fragility fractures in sub-Saharan Africa

In the study conducted in Johannesburg, South Africa (Solomon, 1979), incidence of femoral neck fractures were reported as 4.5 and 4.2 per 100,000 per annum in black men and women, respectively. Until recently, few other studies have been published; one study in Cameroon, conducted between 1996-98 (Zebaze and Seeman, 2003) which found similarly low incidence for hip fracture due to low trauma. The incidence was 4.1 in women and 2.2 in men per 100,000 people aged over 35 years. The authors suggest this is partly due to less than 1% of the population surviving beyond 65 years in Cameroon in 1997. Research conducted in the city of Ibadan, Nigeria, (1988-89) also showed a low incidence of hip fracture.

All these data are more than 20 years old and significant changes with regard to urbanisation and the nutrition transition are likely to have occurred across all these countries. In the last few years several papers have been published providing additional data to understand current rates of hip fracture in sub Saharan Africa.

From 2002-8, a study was conducted in the Owerri, Nigeria, a region comprised of one urban and two primarily rural local government areas. The aim was to determine causes of hip fracture. One hundred and six cases of hip fracture were identified from a register, and the majority (65%) occurred in males. Road traffic accidents were found to be the primary cause of hip fractures, particularly in the young and males. However, they also noted that among the elderly, falling from standing height was the major cause of low trauma hip fracture, and that these occurred more often in females (Onwukamuche *et al.*, 2013). They did not publish the incidence rate.

More recently published data from a study of hip fracture pattern at a major Tanzanian referral hospital has shown an annual increase of 2.34% over a 5-year period (2011-15). The majority of fractures occurred in men, similar to Nigeria. However, they found that most fractures were due to a low trauma incident and there was an equal burden of low trauma, fragility fractures among males and females (Tsabasvi *et al.*, 2017). They do mention that the tribe with the highest incidence may have been due to higher alcohol consumption, so it is uncertain whether these cases were classified as low trauma.

Moreover, a study of low trauma fractures in an older black South African population provides further indication that incidence of hip fracture in sub Saharan Africa may be increasing (Paruk *et al.*, 2017). The crude rate of hip fractures was found to be 12-fold higher than previously reported by Solomon (1968). They suggest that this may even underestimate the true incidence, as private medical facilities and those who did not seek medical care were not included. They also noted an age-related increase in the rate, and that higher incidence occurred earlier in men.

Data have also been published on prevalence of vertebral fractures in black African populations in South Africa (Conradie *et al.*, 2015) and The Gambia (Zengin *et al.*, 2017). Lateral vertebral assessment (LVA) scans in the Gambian Bone Ageing Study (GamBAS), showed that '9% of GamBAS participants had moderate or severe vertebral fractures (as defined by GE Lunar software), and 14% had spinal degeneration (osteophytes present).' These data are similar to the South African study, where prevalence assessed from radiographs was (9%) in black African women.

Overall, these few studies highlight the comparatively lower incidence of fragility fracture in Africa; however, they suggest the importance of considering the potential impact of urbanisation on bone health and fragility fracture risk.

3.6 Urbanisation and/or migrant studies

In the Introduction, the environmental determinants of health, with a particular focus on urbanisation were considered. Within this chapter on bone health, geographical disparity in fragility fracture prevalence has been described, and reasons for ethnic differences suggested. Current literature was also reviewed on fracture incidence in sub Saharan Africa.

This section will focus on the relationship between urbanisation and bone health; data on associations between urbanisation and fragility fracture, and other measures of bone health, including bone mineral status and bone turnover markers. Studies seeking to understand the impact of urbanisation have compared rural and urban populations, primarily using aBMD as a surrogate marker of bone strength and fracture risk, despite its limitations, as previously described. Secular trends in HICs are considered first, followed by studies of migrants from LMICs to HICs. Finally, the last two sections of the review will cover studies comparing bone health in rural and urban populations, primarily comparisons of bone mineral status, and data from rural-to-urban migration studies.

3.6.1 Association of urbanisation and fragility fractures

As described in the Introduction, urbanisation is strongly related to the nutrition transition; encompassing both increasingly westernised diets and sedentary lifestyles. **Section 2.6** described 'implications of transition on bone health' highlighting potentially detrimental characteristics of urban environments, diets and lifestyles in relation to bone.

3.6.1.1 Secular trends in high income countries (HICs)

It is notable, that western countries have not always had such a high incidence of hip fracture; today one third of British women (Caucasian) over the age of 50 are likely to suffer from a low-trauma fracture (Melton *et al.*, 1992). In 1983, Boyce et al. (Boyce and Vessey, 1985) compared the incidence of fracture at the proximal femur in the UK with cases 27 years before. While controlling for the rise in the number of elderly, their findings clearly showed that the incidence had doubled in both males and females, at all ages. This provides evidence that it was not just demographic change, i.e. increasing proportions of older people, which contributed to the rise in fracture incidence. The authors suggest that secular change may have been occurring due to a change in the aetiology of fractures. Therefore, if countries with a currently low fracture incidence are not to follow the same trend, it is important to understand the association between bone health and potential transitions in environment and lifestyle that may be related.

Significant increases in hip fracture rates in Hong Kong reflect a more recent occurrence of the earlier trend observed in the UK. Over a 30 year period (1960-1990), the incidence of hip fracture increased by 300%. Compared to other regions of China these rates are much higher (IOF, 2010). However, in recent years Hong Kong, has displayed a trend more similar to western countries, reaching a plateau and even a fall in the incidence of hip fractures (**Figure 3:7**). While in countries, still undergoing rapid development the trend in fragility fracture incidence is still increasing. In 2014, a review of global and secular trends in hip fracture more clearly elucidated the relationship between urbanisation and fracture rate (Ballane *et al.*, 2014b). **Figure 3:7** illustrates the secular trend in female hip fractures by continent, excluding Africa, due to a lack of data. The rapid increase and more recent fall in the rate of hip fracture in western, industrialised countries such as the UK and USA, is clearly demonstrated, while the consistent rise occurring in Asia is also shown. Despite this trend, the mechanisms that underpin the effect of urbanisation on hip fracture are not well understood. Proposed factors have included physical activity and an increase in hard surfaces, the latter associated with falls and fragility fracture.

Figure 3:7: Secular trends in female hip fracture by continent (% change per year) (Ballane *et al.*, 2014a)

3.6.1.2 Migrant studies (from LMIC to HIC)

In the USA, the rate of fragility fracture has fallen in recent years; however, an increasing number of ethnic minorities now contribute to fracture incidence (Wright *et al.*, 2012). This may be due to acculturation and the adoption of a more western diet and lifestyle.

Gong *et al.* (2006) sought to investigate bone health in a population of sub-Saharan African immigrants (Sudanese) living in the USA. The study aimed to understand whether change in environmental exposure related to aBMD (total body, hip, and spine). The findings confirmed other studies in black African populations, with Sudanese migrants having significantly lower aBMD compared with African Americans and Caucasians. The authors focus solely on the hypothesis that an increase in aBMD would lower fracture risk. Therefore, they do not comment on bone parameters other than aBMD, or consider additional factors believed to be protective in African populations (Prentice *et al.*, 1991).

Length of stay in the USA was positively correlated with aBMD $_{\text{TOTAL \& SPINE}}$ (p<0.015). The mean length of stay was 4.3 years (range 0.02 -24.8 years) for women and 5.0 (range: 0.05 to 11.8 years) for men. However, after adjusting for body weight, the association was no longer significant. Furthermore, although the authors describe the sample as recent migrants, the maximum number of years in the USA was 24.8 years for women and 11.8 years for men. The sample size was quite small with 88 women (premenopausal) and 55 men, and there was quite a lot of variation. aBMD $_{\text{HIP}}$ was positively correlated with milk intake (p<0.02), but this may be related to dietary habits and confounded by weight which was not adjusted for. Body weight appeared to explain some of the associations between BMD $_{\text{TOTAL \& HIP}}$ and length of stay in the USA, nevertheless it was a small sample and the author highlights that the sample may have been subject to volunteer bias.

Demeke *et al.* (2015) conducted a study of female Somalian migrants in Sweden; they also showed BMD_{SPINE} to be low compared to White and African-American reference populations. The sample (n= 67) was too small to look at length of stay in relation to BMD, and they did not adjust for body size. Vitamin D deficiency (<25nmol/l) was prevalent in the sample, with 73% below this threshold and highlights concern for the bone health of this population, particularly with regards to osteomalacia.

3.6.2 Urban-rural differences in bone health

Globally, little epidemiological data exist to compare fragility fracture incidence in rural and urban locations. One systematic review showed that the majority of studies suggest hip fracture incidence is higher in urban areas (Brennan *et al.*, 2010). In a further systematic review of global hip fracture incidence, Kanis *et al.* (2012) also commented that in addition to the large global variation, there is up to two-fold difference in hip fracture incidence within countries. Urban communities reported to have comparatively higher rates than rural communities, included Argentina (Wittich *et al.*, 2010), Turkey (Elffors *et al.*, 1994), Sweden (Jonsson *et al.*, 1992), Norway (Finsen and Benum, 1987); (Bulajic-Kopjar *et al.*, 1998, Kaastad *et al.*, 1998), Switzerland (Chevalley *et al.*, 2002), Croatia (Matkovic *et al.*, 1979) and USA (Jacobsen *et al.*, 1990, Madhok *et al.*, 1993).

The southern European, Mediterranean Osteoporosis Study (MEDOS) used a case-control design to compare hip fracture incidence across 14 regions, encompassing rural and urban areas of southern European countries. Portugal, Spain, France, Italy, Greece, and Turkey were included and the aim was to understand reasons for any difference in incidence (Elffors *et al.*, 1994). Overall, the study concluded that significant variation exists within countries.

In Turkey, the study was conducted from 1988-89 in two cities and three rural regions (Dilsen *et al.*, 1993). The investigators found that hip fracture rates were higher in rural compared to urban areas, even after adjusting for several confounders (e.g. age, BMI, and exercise). However, they concluded that primary reason was due to a higher incidence of high energy, traumatic fractures occurred in rural areas. They did not find any difference in incidence of hip fracture between urban and rural-to-urban migrants. They did show that in three rural areas of Turkey that there were no differences observed between the incidence in males and females.

Limited data from studies in developing and transitioning societies also demonstrate a rural-urban gradient in various parameters of bone health; however, there are very few studies and findings are inconsistent. In 2015, Matsuzaki et al. (Matsuzaki *et al.*, 2015) conducted a systematic review and meta-analysis comparing aBMD between rural and urban areas in both HICs and LMICs. The aim of the review was to determine whether 'urbanicity is consistently associated with lower BMD globally'. Several studies were included, with measurements conducted at multiple skeletal sites; each was included separately in the analysis.

Results from HICs showed that urban populations had lower BMD (**Figure 3:8**), thought to correlate with higher fracture observed in urban areas of HICs. The few available studies in LMICs provided inconsistent results and represented only three Asian countries, and none from Africa. The overall effect size indicated that urban populations had higher BMD. The data were presented as a ratio of means with 95% confidence intervals (CI), which due to the disparity between studies were wide. The authors concluded that 'Urban-rural differences of aBMD may be context-specific. BMD may be higher in urban areas in some low-income countries'.

Figure 3:9 shows the nine studies included in the LMIC model, representing three countries. The majority were conducted in females, with both China and Thailand featuring multiple times for measurements of different sexes and at different skeletal sites. In the following two sections, findings from several of the studies in the meta-analysis will be discussed, first for LMICs and then HICs. The discussion will also include several additional studies and other parameters of bone health measured, to provide additional insight.

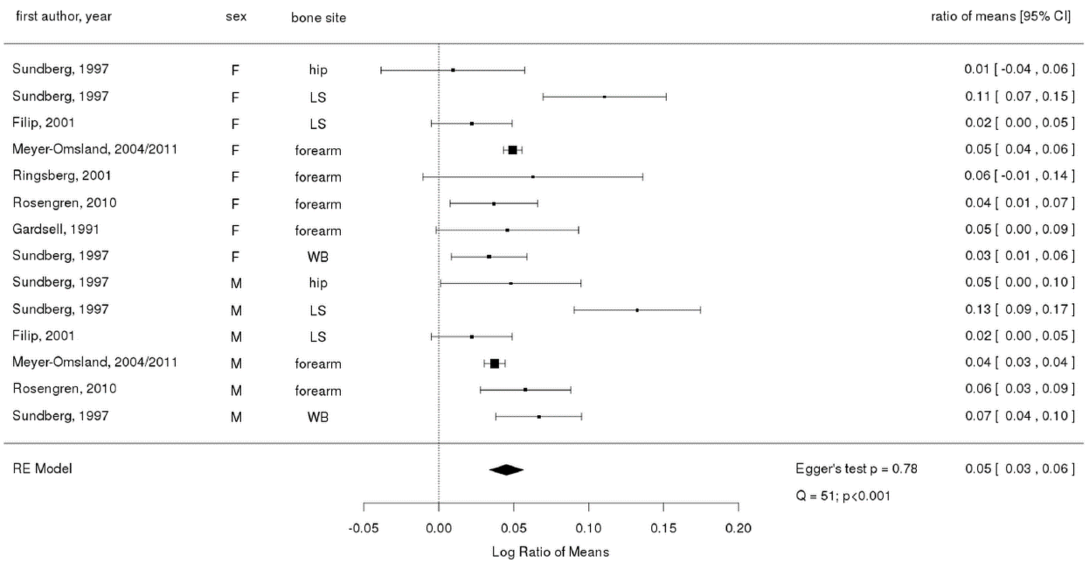


Figure 3:8 Ratio of means and 95% confidence interval for comparing bone mineral content or density in urban and rural populations in high-income countries.

Symbol sizes are proportional to sample sizes. The overall effect size was derived from a random-effects model. LS: lumbar spine. WB: whole body. F: female. M: male. (Matsuzaki *et al.*, 2015)

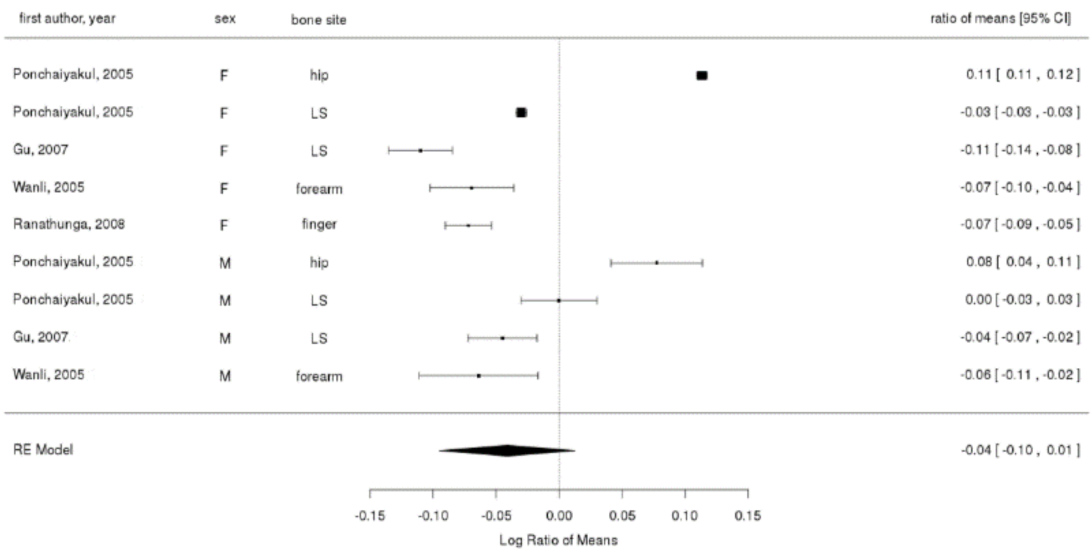


Figure 3:9 Ratio of means and 95% confidence interval for comparing bone mineral content or density in urban and rural populations in low and middle-income countries.

Symbol sizes are proportional to sample sizes. The overall effect size was derived from a random-effects model. LS: lumbar spine. WB: whole body. F: female. M: male (Matsuzaki *et al.*, 2015)

3.6.2.1 Low middle income countries (LMICs)

In China, economic growth and development has had considerable impact on the rate of urbanisation and therefore on the lifestyle of the growing urban population. Xia *et al.* (2012) published results demonstrating that over a period of four years (2002 to 2006) the rates of hip fracture had increased rapidly in Beijing, by 49% and 58% in men and women over the age of 50 years, respectively. Again, this demonstrates an association between urbanisation and osteoporotic fracture.

In order to investigate whether there were differences in bone mineral status between rural and urban populations in China, and whether lifestyle or body size differences could provide explanation, Gu *et al.* (2007) conducted a study in Shanghai as part of a population-based cross-sectional study. They defined urban and rural individuals according to the Chinese residential registration system, classifying those with an official urban residence who had lived for ≥20years in Shanghai as urban, or individuals of a county with agricultural residential registration as rural. The age range was 50-70 years and 1179 took part, 490 men, and 689 women; aBMD/C were measured at the lumbar spine (L1-L4) using DXA.

In comparison to studies in western populations, urban residence was associated with a higher aBMD/C and bone area (BA) in men and women. After adjusting BMC for size (height, weight and BA), differences between urban and rural men were no longer significant. Conversely, for women, body and bone size did not explain differences and none of the measured lifestyle variables including calcium and vitamin D supplement, physical activity, income, social activity, could explain the difference observed. Notably, frequent milk consumption was negatively associated with $aBMD_{SPINE}$ ($p<0.05$). The authors highlight that this may be due to those who have received a diagnosis of osteoporosis being more likely to alter their diet and follow recommendations to consume more dairy products.

Transitioning dietary patterns in China may be related to the differences in aBMD, with the consumption of higher more energy-dense diets. This study however did not measure dietary intake. Nonetheless in their discussion the authors refer to the National Nutrition survey conducted in 1959 (during the early life of subjects), which indicated that urban residents had a diet higher in soybeans and products, animal foods, fruit and vegetables, compared to rural residents. It is of particular interest that differences in bone or body size could explain differences in men but not females in this Chinese population. Perhaps suggesting that body composition, percent fat or lean mass in women is more significantly associated with bone mineral status.

Compared to the Chinese study (Gu *et al.*, 2007), which found higher BMD/C in an urban population, a study in Thailand found more similar findings to western countries. Again, a cross-sectional study was conducted of 411 urban and 436 rural Thai men and women (Pongchaiyakul *et al.*, 2005). Higher aBMD $_{\text{FEMORAL NECK}}$ was found in rural men and women, after adjusting for age and body weight; although the difference was not observed at the lumbar spine. They carried out further analyses, stratifying for sex, age group, and BMI category. In those with a higher BMI and less than 50 years, the differences between rural and urban became more pronounced. Subsequent publications from the same author, investigated the contribution of lean mass to the difference between urban and rural populations (Pongchaiyakul *et al.*, 2005). Their results indicate that lean mass accounted for more of the variation in femoral neck aBMD in men (23%) than in women (5%).

A study of urbanisation in black, South African women has been conducted in relation to bone health (Kruger *et al.*, 2011). Data from a subset of the PURE (Prospective Urban Rural Epidemiological) longitudinal study were analysed to understand risk factors between rural and urban women and bone health. This population is not entirely comparable to a female, Gambian population as they have a significantly higher prevalence of tobacco use, alcohol, HIV, across both urban and rural areas (GBoS, 2014), all factors associated with poorer bone health. In the absence of DXA measurements, a marker of bone resorption (c- telopeptide of type 1 collagen CTX) was measured in 658 women over 45 years. Rural women were found to have higher resorption, compared to urban women. Increased bone turnover is associated with increased bone loss, lower BMC and increased risk of fragility fracture in western populations (Biver *et al.*, 2012). However, higher bone formation and lower bone resorption observed in rural Gambian populations, has been hypothesised to remove fatigue damage and improve bone quality (Aspray *et al.*, 2005).

A study of Black and White South-African premenopausal females, compared indices of body composition including waist-to-hip ratio, percentage fat and fat free mass with aBMD (total body, femoral neck, total hip and lumbar spine) (Chantler *et al.*, 2012). In Black women, fat free mass, socio-economic status (SES) and injectable contraceptive use explained most variation in aBMD $_{\text{HIP and SPINE}}$. This is consistent with other studies, including a study in Brazil which found that muscle strength and fat free mass were positively associated with aBMD (p<0.05) (Lima *et al.*, 2009). However, in the white South African women, fat mass was associated with aBMD$_{\text{SPINE}}$. There may be some beneficial effect of increased body weight on bone density; nevertheless, recent research now indicates that inflammation associated with adipose tissue may impact negatively on bone mineral (Ward, 2012). Physical activity, which relates to body composition, is also likely to be a determinant of aBMD in this population. A further study in South Africans showed that physical activity in adolescence was associated with a higher aBMD later in life (Micklesfield *et al.*, 2003, Lloyd *et al.*, 2010), which is also supported by research in other ethnicities (Afghani and Goran, 2006, Morseth *et al.*, 2010).

These findings in South African women may be due to low dietary calcium intakes in rural (≈210mg/day) compared to urban groups (≈410mg/day) along with lower 25 OH vitamin D status. Nonetheless, evidence suggests that the effect of increased calcium is only transient (Ward, 2012). Higher levels of obesity in urban women may have contributed to lower vitamin D status, as some studies have suggested that it is sequestered into the adipose tissue (O'Connor *et al.*, 2013). However, research in younger South Africans indicates that rural areas are experiencing significant transition in diet and lifestyle, with perhaps less marked difference between urban and rural areas (Micklesfield *et al.*, 2014).

The focus of the paper was to see which variables were associated with risk of low bone mass, by assessing intermediary factors of bone mass. A key limitation of this study was that they were unable to measure bone mineral status and instead only considered one marker of bone resorption and reported fracture. Low or lower bone mass has not yet been associated with an increase in fragility fracture outcome in populations with low incidence (Prentice, 2004). More detail on other bone parameters such as cortical and trabecular density may have provided more evidence to substantiate findings. The inclusion of additional methods of bone imaging (e.g. pQCT) within a study design would enable further investigation of important aspects of bone phenotype; such as size, volumetric density, and trabecular bone architecture, which are associated with bone strength. They provide greater sensitivity to detect associations between bone phenotype in relation to nutrition or other factors (Ward, 2012).

Several additional studies of bone health have been conducted in African populations, including a group of 51 Fulani herdsmen in northern Nigeria (Laabes *et al.*, 2008). It was assumed that the diet was rich in calcium due to availability of dairy products and that physical activity levels were high, due to the demands of trekking with their animals. Unfortunately, these factors were not measured in the study. Ultrasonography was used to assess bone strength at the calcaneus. The ability of ultrasonography to predict fracture in this population is unknown, but evidence from other populations indicates that measurement of stiffness index, broadband ultrasound attenuation (BUA) and speed of sound (SOS) relate to fracture and bone strength. There was quite a range of ages from 16 to 49, with correspondingly varying weights and height; all age groups had low BMI, mean 19.9 ± 2.3 kg/m^2. The results were compared to a young, male Nigerian reference population, from a previous study of the authors. The derived T-score for the stiffness index was low (-1), which surprised the authors, who expected that access to high calcium foods and a very active lifestyle would result in higher bone strength parameters. However, there were wide confidence intervals, due to the small sample and wide variation in age and skeletal maturity. Whether fragility fracture is an issue for this population is unknown, and how these results relate to risk is also unknown.

As with all studies of bone mineral status, it is not appropriate to compare results reported from different studies, even using the same type of instrument, without conducting a cross-calibration. Measurements can vary significantly even from instruments from the same manufacturer. Ensuring that comparative studies have either used the same instrument for both groups, or conducted a cross-calibration is essential to ensure appropriate interpretation of findings.

3.6.2.2 High income countries (HICs)

Comparative studies of bone phenotype between rural and urban areas are designed based on geographical differences; however, it may be of additional interest to compare populations within a country, chosen based on their very different lifestyles. The effect of lifestyle on bone parameters measured by DXA and pQCT was studied in more detail in men and women (age 20-66 years) in the USA (Specker *et al.*, 2004). Three groups were compared: Hutterite (Anabaptist), rural (>75% lifetime farming) and non-rural (never worked on farm). The study objective was to understand whether lifestyle differences could account for differences in bone mass, size and geometry, by comparing groups with very different lifestyles in relation to diet and activity.

The authors found significant lifestyle differences ($p<0.001$) between females from the three groups; rural women spent significantly more time in vigorous activity and Hutterite women had higher grip strength. Calcium and vitamin D intakes were similar across the groups ($p>0.05$). However, in males, calcium intake differed significantly ($p<0.001$) with rural men consuming more calcium. Grip strength was higher in Hutterite men; grip strength acts as a measure of upper arm muscle strength as well as a proxy for physical fitness. Non-rural groups (both males and females) were not significantly heavier and did not have a higher percentage body fat. This finding contrasts data from studies in LMICs, where urban location has been associated with a higher prevalence of obesity. (Ziraba *et al.*, 2009). To model the effect of lifestyle on bone parameters, ANCOVA was used to adjust for age, height, weight and percent body fat (and oestrogen status in women). Between-group differences were found in adjusted models for both male and females. At most sites Hutterites had higher BMC and BA than both rural and non-rural subjects. Grip strength, BA_{TOTAL}, vitamin D intake, and BA_{SPINE}, were the only significant lifestyle-related factors associated with DXA measurements, which could account for groups differences (male and female). More lifestyle factors were significantly associated with pQCT bone measurements, particularly in women. With grip strength associated with all bone parameters in females except cortical vBMD, thickness at 20% radius and BMC_{TOTAL}, trabecular vBMD at 4% radius.

Similar research in Sweden (Gardsell *et al.*, 1991) comparing bone mineral status in rural and urban populations, found rural subjects to have higher BMC. They suggest that these differences in BMC were likely to relate to the lower prevalence of fracture in rural areas. The findings from the American Hutterite study (Specker *et al.*, 2004) also confirm these findings. However, the additional measures from pQCT indicate that differences in the majority of measures of bone size and geometry between rural and non-rural populations also exist. Bone strength has been shown to be in part determined by bone size, and particularly forces exerted by muscle. The loading of the bone perhaps due to more physical activity leads to an increase in periosteal expansion an important bone parameter of size. This was also demonstrated in a population of older Swedish men (Nilsson *et al.*, 2014). Nilsson et al. 2014 found that exercise in early life was related to periosteal expansion, which contributed to greater cortical bone size and that in older age, exercise could reduce bone loss in weight bearing bone.

A particular strength of the study by Specker et al. in the Hutterite population is that they classified subjects based on their lifestyle and not only geographical location; they did not assume that all rural individuals would be involved in farming. However, their strict criteria had to be extended in order to recruit a larger sample, in the end 21% of non-rural subjects had also lived on a farm for part of their lives. Early life differences in activity may have contributed to the differences seen between populations. Other studies also support this finding, indicating that pre-pubertal activity levels are perhaps more important in determining adult BMC and bone size than levels of activity later on in life (Bass, 2000, Bass *et al.*, 1998).

Gómez-de-Tejada Romero *et al.* (2014) published research on bone health of women in Gran Canaria, Spain. The objective was to study the prevalence of osteoporosis, vertebral fractures and hypovitaminosis D (<50nmol/l) in postmenopausal women in rural compared to an urban environment. A further objective was to understand how socio-economic status affected bone health. The study took place in 2008 with a sample of 1129 women, 390 from rural areas and 839 from urban areas. aBMD was measured at the lumbar spine (L1-L4) and proximal femur using DXA.

Rural women were more likely to be poor, and short in stature; furthermore, a higher proportion were obese, 40.1% and 27.6%, rural and urban respectively. Additionally, 36.5% of rural women were classified as osteoporotic compared to 28.7% in urban women. The number of individuals with a fracture was not significantly different, but a higher percentage of rural women had a vertebral fracture present. A higher percentage (40.8% $p<0.01$) of rural women had 25 hydroxy vitamin D status below 50nmol/l compared to 26.6% of urban women. This study also incorporated diagnoses of diabetes mellitus and hypertension, which have been associated with bone mineral status (Ferron and Lacombe, 2014, Garcia-Hernandez *et al.*, 2012, Hamann *et al.*, 2012). They again found that rural women had a higher prevalence of both NCDs. In regards to bone mineral status, $aBMD_{SPINE}$ (adjusted for age and BMI) was significantly lower in rural women ($p<0.05$).

These findings are in contrast to other studies in western populations, which found that rural residence was associated with higher aBMD (Gardsell *et al.*, 1991, Brennan *et al.*, 2010). There are several limitations with this study: firstly, the sample of urban dwelling women was much higher, and there were no data collected on physical activity, muscle strength, diet or body composition. **Section 2.6.3,** briefly describes how obesity, type 2 diabetes and the metabolic syndrome have been implicated with an increased fracture risk. It may be that other variables could explain why rural women had a lower aBMD at the spine.

The health of rural populations can vary significantly depending on their lifestyle; perhaps more deprived rural communities may have higher prevalence of risk factors detrimental for bone health. However, analysis of a British birth cohort (Hertfordshire Cohort study, *n*=3225) found no associations between social inequalities, osteoporosis and fracture incidence (Syddall *et al.*, 2012).

3.6.3 Rural-to-urban migration studies of bone mineral status

The reasons for the large variation in fracture risk globally are largely unknown, however it has been suggested that environmental factors are of greater significance than genetic. Kanis *et al.* (2012) comment on the usefulness of migration studies to determine the impact of environment on bone health, with examples of 'the higher fracture probabilities among Chinese living in Hong Kong and Singapore compared with mainland China', and the Japanese population of Hawaii compared to those in Japan. In addition to the previously discussed study of African migrants to the USA (**Section 0**), where the study population had migrated from an African country to a western country, there are several other possible migration study designs. For example, it is possible to compare rural-to-urban migrants with their rural counterparts, or to compare them to the urban-born population. However, there are very few published studies of rural-to-urban migration and bone health and none in sub-Sahara Africa.

In 2003, a study was conducted to determine prevalence of osteoporosis and fracture incidence in 1764 postmenopausal women who migrated from southern Italy to live in Milan, northern Italy (Varenna *et al.*, 2003). It is not a rural-to-urban migration study per se, but the authors comment that the environment and lifestyle of southern Italy differs significantly from northern. To be classified as migrants, participants had to have migrated more than 20 years ago and lived continuously in Milan for at least 15 years. They were compared with 4018 Milanese women of similar age. Bone mineral status was assessed at the lumbar spine using DXA.

The migrant population was found to have a lower lumber spine BMD and a higher prevalence of osteoporosis. The migrant group had a higher BMI, however this was not associated with higher

BMD as might have been expected based on other studies, which have suggested a positive relationship between BMI and BMD (Lloyd *et al.*, 2014, Ho and Kung, 2005). Moreover, these findings were not as expected from previously published data which indicated a lower prevalence of osteoporosis and mortality due to hip fracture in southern Italy (Heyse *et al.*, 1990). The authors suggest that perhaps like the study in Gran Canaria that socio economic status could be a factor influencing their findings between groups.

25 OH vitamin D was not measured in this study, and the authors recognise this as a limitation, especially as the migrant group from southern Italy are likely to have a darker skin pigment and this along with reduced sunshine associated with Milan's more northerly latitude could have led to a lower 25 OH D status in the migrants. The calcium intake, based on a semi-quantitative questionnaire included only three dairy products (milk, yogurt and cheese), was lower in the migrants ($p<0.01$) and if this was combined with a lower vitamin D status due to reduced skin synthesis then this could have resulted in reduced absorption and bioavailability of calcium and potentially lead to poorer mineralisation.

Finally, the most recent study investigating the impact of rural-to-urban migration on bone mineral status (aBMD) was conducted in Hyderabad, India (Viljakainen *et al.*, 2015). Skeletal sites measured included the lumbar spine and total hip using DXA. The study design was based on a comparison of a sibling-pair (n=185 sib-pairs). After adjusting aBMD for height, gender, age, and occupation, rural-to-urban migration was associated with higher BMD_{SPINE} and BMD_{HIP}. The authors suggest that 'differences in lean mass and to a lesser extent fat mass, largely explained the BMD differences' they observed. These findings are cross-sectional, and it is unknown whether this increased BMD, mainly due to differences in body composition, will translate into increasing fracture rate in later years.

SUMMARY

This section has highlighted interesting and apparent negative associations between urbanisation and bone health primarily defined in terms of higher fracture incidence and lower bone mineral status in HICs. Trends worldwide indicate the rapid and recent nature of the rise in fragility fracture incidence in many countries. This review has also underlined the complex myriad factors that affect bone health and their disparity within and across countries. Due to the paucity of data in sub-Saharan Africa, most of the literature is from non-African populations. However, it seems that potentially detrimental trends are beginning to emerge in some African countries, with higher fracture rates reported in regional studies in Tanzania and South Africa. With the predicted expansion of older populations across the continent, an increase in fracture incidence is likely; however, the implications of the nutrition transition combined with significant levels of rural-to-urban migration are not well understood in this context, and to date no studies have been conducted in The Gambia.

Literature reviewed in **Chapter 2** highlighted the available evidence suggesting that transition is occurring in The Gambia. Many aspects of rural lifestyle appear to be protective for bone health, but the impact of transition on bone health in The Gambia is unknown. The evidence suggests that the nutrition transition, associated with a more western diet and lifestyle are likely to be key contributory factors. The associated shift in body composition is likely to be important, along with the increasing prevalence of other NCDs.

4 Setting and Design of Women's Migration Study

This section comprises study objectives and hypotheses, the overall Women's Migration Study (WMS) design, and details outlining the two study locations. Information about study setup and logistics are also included.

4.1 Study aim

The main aim of the study was to investigate the impact of rural-to-urban migration and the nutrition transition on bone health of premenopausal Gambian women aged 35-50 years. In order to understand whether migrating to an urban environment in adult life would increase risk of potential fragility fractures in older age, due to differences in bone phenotype and determinants of bone health including diet, lifestyle, and environment, associated with urbanisation and the nutrition transition.

4.2 Research questions

- How does the bone phenotype of an urban migrant group compare to a rural group of similar women who never migrated from Kiang West?
- How are components of the nutrition transition associated with bone phenotype?
    - What are the nutrient intakes and patterns of food consumption of both groups, and do they differ?

4.3 Hypothesis

Urban migrant and rural women and will differ with respect to:

- Bone phenotype: size-adjusted bone mineral content (SA-BMC) by DXA

- Other characteristics e.g. diet, lifestyle, vitamin D status and body composition

4.4 Objectives

Primary objective

1. To compare bone phenotype (areal bone mineral density (aBMD), bone mineral content (BMC), bone area (BA), and size-adjusted BMC (SA-BMC)) at the lumbar spine, hip, femoral neck, and total body using DXA of urban migrant and rural groups.

Secondary objectives

2. To compare food and nutrient intakes from two-day weighed diet record of urban migrant to rural groups.
3. To compare body composition i.e. fat mass (total, android, and gynoid) and lean mass (total) using DXA of urban migrant and rural groups.

   To compare anthropometric measurements of urban migrant and rural groups.
4. To compare plasma 25OH vitamin D, plasma clinical chemistry (Ca, P, TALP, Cr, and Albumin), urinary electrolytes, and mineral outputs (Ca, P, Mg, and Cr) of urban migrant and rural groups.
5. To compare important aspects of 'traditional' rural physical activities (e.g. farming, gardening, household, and leisure activities) of urban migrant and rural groups.
6. To compare health and socio-economic status of urban migrant to rural groups.

NB: Additional parameters of bone phenotype assessed using pQCT were included in the overall protocol; however, the data are not presented within this thesis as cross-calibration data are required to ensure validity of the results. A description of the protocol used for the acquisition of pQCT data is described in Appendix F.

4.5 Outline of study design

CROSS SECTIONAL, COMPARATIVE OBSERVATIONAL STUDY

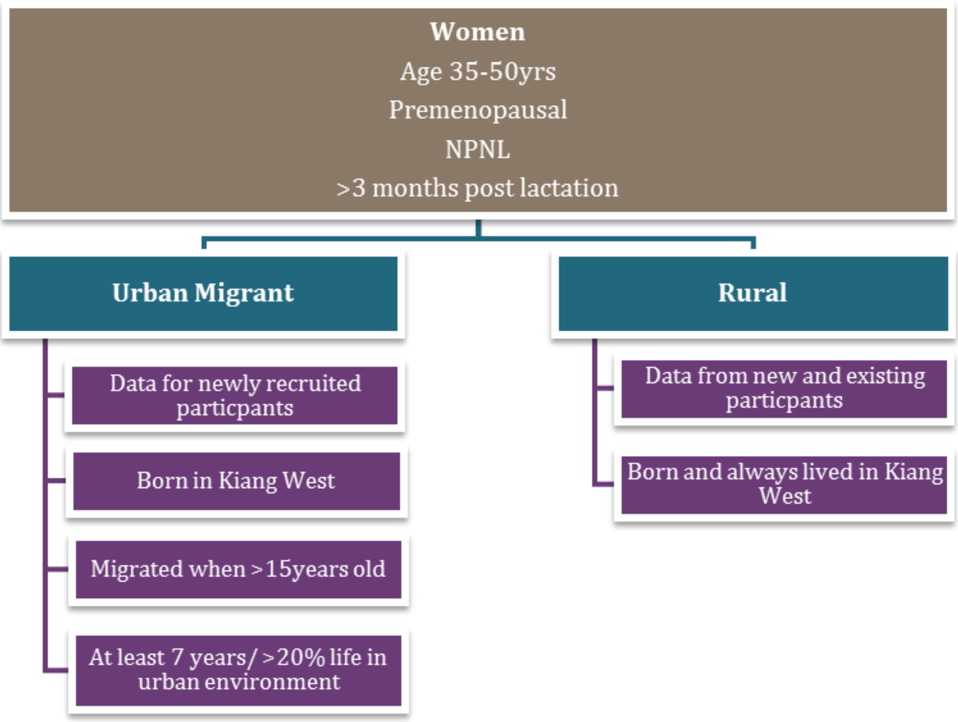


Figure 4:1 Overview of study design, NPNL: non-pregnant, non-lactating

4.6 Overview of data collection

Table 4:1 highlights the investigations included within the WMS study. Data were collected at home and clinic visits, these are detailed in the following sections. Clinic visits included bone imaging, anthropometry, blood collection, and health questionnaire.

Table 4:1 Summary of investigations in rural and urban areas

Data collected	Rural	Urban
DXA (hip; lumbar spine; total body; forearm)	✓	✓
pQCT radius and tibia	✓	✓
DXA body composition	✓	✓
Fasting blood sample	✓	✓
24hr urine sample	✓	✓
Dietary assessment	✓	✓
Anthropometry	✓	✓
Health and SES Q	✓	✓
Physical activity Q	✓	✓

4.7 Study setting

4.7.1 Defining urban and rural areas

Distinguishing between urban and rural areas is not straightforward; globally, there is substantial variation in the characteristics of what constitute urban areas, resulting in no universal definition. Research by demographers has aimed to ascribe appropriate definitions in order to conduct studies comparing different countries. A number of papers state that, in reality, a binary variable is over simplistic and a continuum would be more explanative; several scales of urbanicity have been developed for use in particular studies (Cyril *et al.*, 2013, Monda *et al.*, 2007, Novak *et al.*, 2012). However, for the purpose of the present research, the binary/dichotomous definition used officially in The Gambia to categorise rural and urban areas was considered to be adequate. According to the Gambian Bureau of Statistics (GBoS), the definition of an urban settlement is characterised by having most of the following criteria (in their absence an area is classified as rural), however these are subjectively measured (GBoS, 2003b) .

i. Commercial importance
ii. Institutional importance
iii. Majority of population should be non-agricultural in occupation
iv. Population should be 5,000 and above
v. Density should be high
vi. Some degree of infrastructural facilities should be available

4.8 Detailed study design

4.8.1 Scientific and logistic rationale for choosing the study population

The WMS study was designed to recruit middle-aged women prior to menopause between 35 and 50 years of age. **Section 2.5** showed that middle to older aged women may be particularly vulnerable to the nutrition transition; suggested by higher levels of obesity. Women in this age group are particularly stable, less likely to be pregnant or to have reached the more vulnerable, with regard to bone health, postmenopausal stage of life. Whereas peri and postmenopausal women have an increase in bone loss due to a reduction in oestrogen, which could confound any observed differences in bone mineral due to an urban environment. Limiting the number of villages to those with the most accurate dates of birth reduced error in the age of participant, and a narrow age range controlled for variation in age associated loss and related differences in bone mineral status.

4.8.2 Sample size

A difference of ½ SD in BMC is biologically and physiologically meaningful (Marshall *et al.*, 1996) (Gärdsell *et al.*, 1993). The sample size was calculated to detect a minimum between-group difference of ½ SD in size-adjusted BMC at the femoral neck (skeletal site measured by DXA with the lowest precision), with 80% power and 5% significance. Therefore, a minimum of 64 participants in each group were required. The calculations were based on data from Jarjou *et al.* (2010) .

Estimated sample sizes for a two-sample means test

t test assuming sd1 = sd2 = sd

H_o: m2 = m1 versus H_a: m2 != m1

Study parameters:

alpha = 0.0500

power = 0.8000

delta = - 0.2300

m1 = 4.5300

m2 = 4.3000

sd = 0.4600

Estimated sample sizes: N = 128 ∴ N per group = 64

m = mean; H_o = null hypothesis; H_a = alternate hypothesis

4.9 Eligibility criteria

A diagram summarising the study design and eligibility criteria can be found in Section 4.5 (Figure 4:1).

4.9.1 Inclusion criteria for all women

i. Female

ii. Healthy

iii. Born in Kiang West

iv. Resident (current or previous) of one of the following seven villages:

Keneba, Manduar, Kantong Kunda, Jali, Jiffarong, Bajana, or Kuli Kunda

v. ≥35.0 ≤ 50.9 years

vi. Not pregnant or lactating

vii. ≥ 3 months post lactation

viii. Premenopausal (defined as having regular monthly menses)

ix. Able to give informed consent

4.9.2 Additional inclusion criteria for urban women:

i. Lived until at least age 15years in Kiang West

ii. Migrated to Banjul, Kanifing or Brikama (LGA: Kombo North, South and Central)

iii. Migrated at least 7 years (>20% of life) ago and remained resident since

4.9.3 Exclusion criteria

i. Women unable to give informed consent

4.10 Scientific and ethical approvals

Approval for both studies (urban feasibility and WMS) was obtained from the Scientific Co-ordinating Committee (SCC) and the Gambian Government / MRC Joint Ethics Committee. Dr

Jarjou presented the studies at both internal scientific advisory meetings (SAM) in Keneba and to the SCC in MRCG Fajara.

The urban feasibility study (UFS) (SCC 1222 v2) (**Section 5.1**) was approved in August 2013 (**Appendix A**), and the main WMS study in August 2014 (**Appendix B**). This included the collection of both new data and the use of existing data from the two ongoing studies GamBAS (SCC1222v2) and ENID NPNL (SCC1126v2 add on L2009.66). A letter to amend the WMS study age range was submitted in January 2015, and approval was granted in March 2015 (**Appendix C**).

4.11 Study protocol

4.11.1 Village sensitisation

After SCC and ethical approval, (rural Gambian) villages are customarily informed about the commencement of a new study. Firstly, several key people (village heads, imams, and significant elders) are contacted and informed about the study. If they are content for the study to go ahead in their village, they are asked to organise a second meeting to which the entire village (men and women) is invited. For WMS this was arranged by Mr Michael Mendy and transport to villages outside of Keneba was arranged by myself. It is also considered polite to offer kola nuts as a gift for the elders, to thank them for their time. After discussions with local Gambian staff (Dr Landing Jarjou) who knew the area well, it was deemed unnecessary to conduct the same process in the urban area because the same traditional governance structures are not as significant in urban areas.

4.11.2 Informed consent

Details of how potential participants were identified are described below in **Section 4.11.6** (rural) and **Section 4.11.7** (urban). They were contacted at their homes and a detailed explanation of the project was carried out by CDBH staff. Explanations were in the participant's language, so that they were fully aware of the study objectives, the details of the procedures, and what measurements would be carried out if they decided to take part in the study. All potential rural participants were given an explanation to clarify that their right to free medical care at the MRC clinic in Keneba would not be affected if they chose not to take part in the study. Where possible written informed consent was obtained. However, due to high illiteracy levels, staff most often obtained a thumbprint, rather than a signature.

4.11.3 Anonymity

All data were managed under the UK Data Protection Act 1998, under which EWL (formerly HNR) and MRCG compliance falls. This included the anonymisation of participants.

It is common for Gambian people to have the same name (both given and surname); therefore, people are identified by multiple criteria including parents' names and DOB. All rural study participants lived within the region of Kiang West covered by the Demographic Surveillance System (DSS), therefore, their unique identification number (WKNO) was used initially and, once eligible, each participant was allocated a study specific ID.

All participants were allocated a WMS study ID (e.g. WMS0000X); rural women from the four core villages were allocated IDs beginning: WMS-6001X, -7001X, -8001X, and -9001X. The majority of urban women had already taken part in the UFS (urban feasibility study), (see **Section 5.1**) and therefore had an allocated study ID in a similar format: UFS-1001X, 2001X, 3001X, 4001X, and 5001X, based on 5 clusters, which approximate to government districts. UFS IDs were converted by changing the three-letter prefix from UFS to WMS. Newly identified women were allocated the next available number based on their urban location. The inclusion of a check letter provided an additional way to detect errors in identification.

Forms or files with identifiable data e.g. consent forms with study ID and participant's personal details, were stored in a locked office in MRCG Keneba.

4.11.4 Transportation

The MRC field station in Keneba has an excellent transport department headed by chief driver Mr Ebrima Jallow, each week a specific driver was allocated to the WMS study. The majority of field staff have an allocated MRC motorbike, used for day-to-day transport during fieldwork, and especially for dietary assessment.

Transport was an important requirement for several aspects of the study, including village sensitisation visits, recruitment (consenting visits), dietary assessment, and clinic visits.

Figure 4:2 Last day of urban recruitment with bone imaging operator Mr Michael Mendy, driver Mr Abdoulie Sanneh and a migrant woman from Kiang trying to help us find a few more participants.

During the rural phase of the study, to ensure the study team, vehicle and driver were available, several days in advance of a study visit, I prepared a call list and sent this via email to both the laboratory technician and the chief driver with subject's names, village and date of birth (telephone was included in urban site). This was also printed and placed in the DXA and pQCT rooms in the Bakary Dibba building in Keneba, to ensure that the identity of women were checked prior to each measurement. A similar process was in place in Fajara, the day before a visit, drivers were given the next day's list. The field assistant also called participants to arrange visits several days in advance of measurements.

The village assistant is a resident of rural villages within Kiang West DSS employed by the MRC to assist with communicating with study participants. During the WMS study, the village assistant was contacted a day prior to the study visit, their responsibility was to go and speak to the participant the evening before their visit, to confirm they were well and still able to attend the following morning for measurements; they also reminded the participant to remain in a fasted state. On average eight women came on each measurement visit.

Figure 4:3 Recruitment in Keneba (Late Mrs Fatou Manneh, died 2015 and Miss Sarah Dalzell)

In the urban area, the distance for study participants to travel was significantly greater than in the rural area, around 20 miles and up to 1 hr direct travel time. Initially a driver was booked through the main MRCG Fajara transport department. However, as participants needed to be collected early in the morning, it was not feasible for MRCG Fajara to continue providing transport. An alternative plan was made, and a driver from Keneba was sent for one week at a time. To enable a prompt start to measurements (~ 8 -9 am), the driver started very early (5 or 6am), firstly collecting the vehicle and then participants. On average 3 to 4 women came on each visit day. However, during the study design phase, I had been advised that due to work commitments with their market stalls etc. there would have to be greater flexibility in the schedule to accommodate urban women. Therefore, we also conducted measurements on Saturdays, or sometimes invited fewer women to enable a shorter time at Fajara.

Once all women had completed every aspect of the protocol, they often requested to see the clinic nurse; this was done while others had lunch together (arranged through the Bantabar onsite at Fajara and Mrs Fatou Colley, Keneba).

4.11.5 Recruitment

In both rural and urban areas, recruitment was carried out by a female field assistant and for the most part, I was able to be present. All recruitment took place during the dry season in both rural and urban areas. Previous experience in The Gambia has shown that disclosure of pregnancy is a sensitive topic in this culture (Stokes *et al.*, 2008). Therefore, it was decided that it would be appropriate for a female to ask the screening questions, to ensure that women would feel comfortable and able to be honest about their menstrual status. A female field assistant (Ms Isatou Camara) was stationed at Fajara for the entirety of the urban phase of the study. In Keneba, Mrs Fatou Manneh (late, died 2015) undertook the majority of recruitment, along with Ms Isatou Camara and Mrs Mariama Jammeh.

Recruitment proved to be one of the most challenging parts of the fieldwork, partly due to the unfamiliarity with conducting a study in an urban setting in The Gambia. The eventual success in completing recruitment was largely due to the dedication and enthusiasm of field staff and drivers from Keneba, who diligently used their local knowledge to locate potential urban participants.

4.11.6 Rural

Rural participants had to have been born and always lived in Kiang West. Two ongoing studies within the Calcium, vitamin D, and Bone Health group (CDBH) in MRCG Keneba had similar protocols to the main WMS study. This provided the option to use existing data, collected in the dry season to form part of the rural comparative group, without having to repeat measurements on the same women within a short timeframe. The two studies were the Gambian Bone Ageing Study (GamBAS) and a bone health sub-study of non-pregnant, non-lactating (NPNL) women who were eligible for the Early Nutrition and Immune Development (ENID) trial.

The WMS study comprised of two groups, one rural and one urban migrant group. The initial aim was to recruit women born in villages with the earliest recorded dates of birth, referred to as 'core villages', Keneba, Manduar, Kantong Kunda and Jali.

All rural women resident in core villages, within the age criteria, not already participating in other bone studies were identified using the KW DSS and were invited to take part in the study. They were then screened to ensure they met additional study criteria (NPNL, 3 months PW, premenopausal and healthy). Unfortunately, there were not enough women from these four villages who met the criteria, so existing data were used from two bone studies with similar protocols, ENID NPNL and GamBAS.

ENID NPNL was a bone health sub study of the ENID randomised control trial, which invited all women of reproductive age (18 to 45 years) registered in Kiang West DSS (36 villages) to take part. Once consented to take part in ENID, women were asked each month whether they had had their menstrual period. The ENID trial aimed to recruit women early in pregnancy (after missing two periods) and additional bone measurements were conducted for lactation and post weaning time points. However, in order to have a non-pregnant group to follow-up in during pregnancy, an additional study was conducted called 'ENID NPNL'. These women were recruited from the main ENID trial, and were at least 3 months post-lactation, reported to be have had a recent period and therefore were not pregnant or premenopausal by definition. In order to create a list of ENID NPNL participants potentially eligible to take part in WMS, only women measured in the dry season, who also met the age and village criteria were included. See flow diagram of rural sample in Results I (**Section 7:1**).

GamBAS is an ongoing longitudinal study of bone and muscle ageing, with participants aged 40 year and older living in Kiang West, including the core villages. Women who were known to be pregnant from clinic records, or those who had delivered within the last 3 months were also excluded. Additional screening was also done at recruitment to exclude those who were not NPNL, or who were not 3 months PW. In order to create a list of GamBAS participants potentially eligible at time of measurement for WMS, a list of all participants from the data office and filtered to only include women aged 40 to 50 years, measured in the dry season.

The data office in Keneba provided a list of potential study participants from the two studies. Data included their study ID, mother, and father's name, village, birth, and measurement date, enabling me to calculate their age and time since the last measurement.

These women were then consented for inclusion in WMS and screened for retrospective eligibility, primarily to ascertain if at the time of their measurement, they were premenopausal (all women already had to be NPNL for these particular studies). Time since measurement was between 2.4 and 4.8 years, therefore based on response to questions regarding current menstruation I made a decision on whether to include them and follow-up with additional health and activity questionnaires, unique to WMS.

Recruitment of new rural participants commenced from early November 2014 and finished in early January 2015. The Kiang West DSS enabled selection of women of the required age range, and excluded women who were pregnant or had recently delivered (and therefore likely to be breastfeeding) from potential subject lists. This resulted in fewer exclusions for these reasons. Recruitment (including screening) of participants from existing GamBAS and ENID studies was conducted between January 2015 and November 2015. In order to minimise between-group differences in age and proportion from each rural village, I waited until the urban group recruitment was complete before deciding which participants to include.

4.11.7 Urban

Participants for the urban migrant group had to meet the same criteria as rural women, including they had to be aged 35-50 years old, apparently healthy, premenopausal females, non-pregnant and non-lactating (NPNL), born in Kiang West. The exception in criteria was that they had to have migrated in adolescence or young adulthood to urban and peri-urban regions of The Gambia (the Kombos (North, South, and Central), Kanifing, or Banjul). Migration criteria were specified; participants had to have spent their formative years (until at least age 15) in rural Kiang West, controlling for significant differences in early life determinants. For those who had migrated in adulthood, participants had to have lived a minimum of 20% of their lives in urban area, as this would provide sufficient time for significant bone turnover and the potential impact of migration to an urban area to be observed in the skeleton.

Urban recruitment was a more labour-intensive phase of the study, lasting from mid-January to end of May 2016. The location of many Kiang West migrants in the urban coastal region had already been identified through the feasibility study (UFS) and for many, there was a current mobile number, described in **Section 5.1**.. However, as there is no postal code system in The Gambia, address was recorded at village level and the location mostly recalled by the field staff. Mobile numbers were sometimes no longer in use, or phones were not charged, which slowed progress initially. Nevertheless, through consistent and combined effort, 174 women were traced, of whom 65 were screened and found eligible. The original plan was to restrict recruitment to those who originated from the four core villages. However, this was not possible, as there were insufficient eligible women in the urban area originating from these villages. Therefore, the villages of Bajana, Jiffarong, and Kuli Kunda were also included, because they were most similar to the core villages.

A flow diagram of the urban migrant and rural sample frame, indicating participants inlcuded and excluded in the analysis is presented in Results 1 (**Section 7.1, Figure 7:1**).

4.12 Visits to MRCG Keneba and MRCG Fajara

4.12.1 Home Visits

Dietary assessment was conducted over two consecutive days; 24hr urine was collected during the first day. Home visits normally occurred within the two weeks of the measurement visit to MRCG Keneba or Fajara. However, as recruitment took longer than anticipated in the urban area, and when Ramadan and the wet season were both approaching, dietary intakes and 24hr urine measurements were attempted in the two weeks prior to DXA measurements.

4.12.2 Field staff

In the rural area, the CDBH field team were involved in the collection of study data (**Table 4:2**). Two experienced CDBH field staff (Mr Musa Sanyang and Mr Mustapha Jarjou) were stationed in Fajara for carrying out home visit measurements in the urban group. They had also been involved in the feasibility study and were most familiar with the area where the urban participants lived.

Figure 4:4 Urban recruitment by Miss Sarah Dalzell and Mrs Isatou Camara

4.13 Study team and responsibilities

Table 4:2 Study personnel in The Gambia

Role	Contact	Institution
Study PI	Miss Sarah Dalzell	MRC EWL
Supervisor	Dr Gail Goldberg	MRC EWL
Co-investigators	Prof. Ann Prentice	MRC EWL
	Dr Kate Ward	MRC EWL & MRC LEU
	Dr Landing Jarjou	MRCG Keneba
	Dr Rita Wegmuller	MRCG Keneba
Head of clinical services	Dr Ian Head	MRCG Keneba
Head of clinical services	Dr Suzanne Anderson	MRCG Fajara
Lab technician	Mr Alansana Saidykhan	MRCG Keneba & MRCG Fajara
	Dr Shailja Nigdikar	
	Mrs Ann Laidlaw	
Laboratory analysis	Mrs Janet Bennett	MRC EWL
	Mrs Tabasum Makhdoomi	
	Mr Abhilash Krishnankutty	
	Mr Mohammed Ngum	
Data management	Mr Lamin Darboe	MRCG Keneba
	Mr Ebrima Comma	
Biostatistician	Dr Ivonne Solis-Trapala	MRC EWL
Bone imaging operators	Mr Michael Mendy Mr Mustapha (Tapha) Ceesay	MRCG Keneba
Blood collection	Mr Tapha Ceessay Mr Alansana Darboe	MRCG Keneba
Field assistant supervisor	Mr Lamin Jammeh	MRCG Keneba
Field assistants	Ms Isatou Camara, Mr Buba Ceesay, Mrs Fatou Manneh, Mrs Mariama Jammeh, Mr Ebou Jarjou, Mr Fabakary Bajo, Mr Malang Jammeh, Mr Musa Sanyang, Mr Mustapha Jarjou, Mr Kissima Sawo, Mr Mustapha Jarjou and Mr Musa Sanyang	MRCG Keneba
Cooks	Bantabar (On-site catering)	MRCG Fajara
	Mrs Fatou Colley and team	MRCG Keneba

5 DEVELOPMENT OF STUDY DESIGN AND METHODS

5.1 Urban feasibility study (UFS)

In late 2013, an Urban Feasibility Study (UFS) was conducted, to characterise men and women aged 40 years and over from Kiang West, who had migrated to the urban coastal districts of The Gambia. Key informants (staff at MRCG Keneba) from the core villages went to all compounds of their respective village and asked the whereabouts of any family members over the age of 40 years who had migrated. This stage provided details of 665 individuals eligible to be followed up. Over a six month period (Nov 2013- April 2014), a total of 435 male and female migrants originating from the four core villages of Kiang West were traced and gave informed consent. They were asked to complete a questionnaire providing details of their age, migration history (dates, reason), names, parent's names, telephone numbers, and occupation. Additional questions relating to food purchasing and preparation were included to assess the appropriateness of current dietary assessment methods. These data were presented at the end of my first year and formed part of my first year report and upgrade.

Results from UFS indicated that 160 of the 216 females appeared to be in the eligible age range (initially 40-55 years); sufficient women for the proposed WMS study. Based on the UFS data a list of potentially eligible women was created. However, due to uncertainty of the ages provided, crosschecking was completed before beginning recruitment for the urban group of WMS, using record cards held at MRC, which exist for residents of the four core villages. I worked with a local office administrator in MRCG Keneba using names and parents' names to identify potential participants and record their accurate dates of birth.

After finishing this process, it was clear that the number of women within the eligible age range was lower than expected (n=116). After beginning rural recruitment, we also quickly realised that women between 50-55years were already peri-menopausal and none were eligible. Therefore, a decision was made to lower the age to 35-50years and an amendment was submitted for ethical approval (**Section 4.10**). In consultation with the data office, alternative methods were used to find additional migrant women aged 35-50 years.

Learning from the UFS study were incorporated into the main Women's Migration Study (WMS) study, particularly regarding questionnaire design. For example, date of birth was found to be more useful than age, and when asking about time of migration, it was realised that some people remembered the year they migrated (e.g. 1975), others the age at migration (e.g. 16 years old) and others how many years ago it was (10 years ago). Therefore, the design of the main WMS study questionnaires included multiple response options for these questions.

5.2 Preparation for WMS in The Gambia

5.2.1 Orientation visits to The Gambia

During my first year, I made two orientation visits, both combining several days in rural and urban areas of The Gambia; this greatly aided my familiarisation with Gambian culture and MRCG staff and settings. In January 2014, I visited most of the urban coastal area with MRCG field staff and Dr Gail Goldberg, which helped me to characterise the urban environment. I spent time in Keneba, where I was able to meet the CDBH team, discuss the feasibility study, and meet with data office staff to discuss both UFS and early plans for WMS. I shadowed dietary assessment methods in the rural area and visited village gardens, to see the activities of rural women and the kinds of vegetables they grow. This trip provided the opportunity to engage with local staff and begin discussions of the requirements for my project.

Another trip in April 2014 provided opportunities to shadow urban dietary assessment of a recent migrant followed up for another study. By this stage, I was able to discuss with the data management team my requirements for WMS and they advised me on the development of a data dictionary. During this trip, I was accompanied by senior scientist Dr Kate Ward, as she was overseeing the installation of the new iDXA scanners in Fajara and Keneba. This provided time for her to begin my training in scan acquisition and analysis. I also arranged for a meeting with dietary field staff to discuss potential challenges for data collection, particularly for the dietary assessment in the urban area. This information was used to make minor alterations to the dietary analysis software, described in **Section 6.5** this involved increasing fields for more foods.

5.2.2 Refinement of research questions and study design

At MRC EWL in Cambridge, meetings with Dr Gail Goldberg and Prof. Ann Prentice assisted in focussing my idea development, research questions, and interests. Initially, I explored the possibility of incorporating questions requiring qualitative methods and this was followed up with meetings and advice from staff in the Department of Health Services Research and Policy at the LSHTM, particularly Drs Sophie Hawkesworth and Mary-Alison Durand. However, during my first year upgrade process, it was decided that focusing on the main quantitative questions for my PhD would be most sensible, as they would provide sufficient data; furthermore, scientists with relevant qualitative expertise were not based at MRC EWL or MRCG.

5.2.3 Setting up bone imaging facilities in MRCG Fajara

Prior to the commencement of my PhD, the bone group had anticipated an urban study to be based at Fajara. Preparations were made, which involved combining two rooms in the Platt building, where the Nutrition Theme is based, to form one larger space, able to accommodate both a new DXA, and a pQCT no longer required in Cambridge. During my second orientation visit in April 2014, I was able to shadow the installation and setup of the new DXA.

Once based in The Gambia, I co-ordinated the set-up of IT facilities; ensured all necessary equipment was available and functioning in the lab, including a pH meter, acid washing facilities, urine containers, cool boxes , dietary scales, temperature monitoring in place, and adequate freezer space. Field assistant, Mrs Isatou Camara and I assembled a screen to provide privacy between the DXA and pQCT scan areas.

Figure 5:1 Setup of Platt building at MRCG Fajara

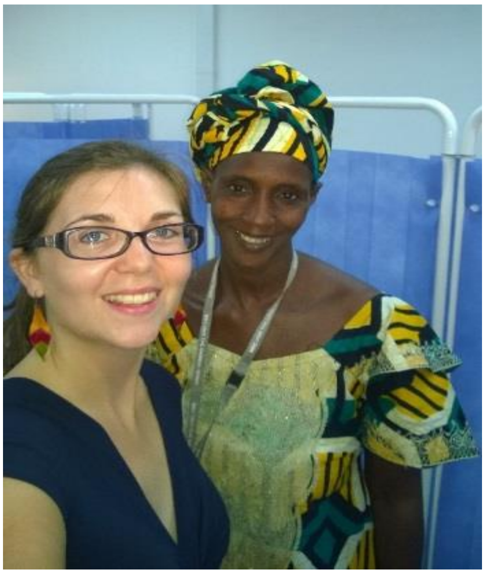


Figure 5:2 Sarah and Mrs Isatou Camara at Fajara setting up the room with equipment and screens.

5.2.4 Study manual

A study manual for WMS was written and developed before the study commenced. It included details of the background to the study, people involved, a flow diagram of the project and all protocols and SOPs required.

The previously described orientation visits and meetings with my supervisor and group leader helped to refine research questions and possible study designs, during my first year. The applicability of current protocols were then assessed and potential adaptations were discussed primarily with my supervisor and Gambian senior scientist Dr Landing Jarjou. The manual was disseminated to all staff involved in the study. Throughout the study, revisions to the manual were documented and updated versions issued.

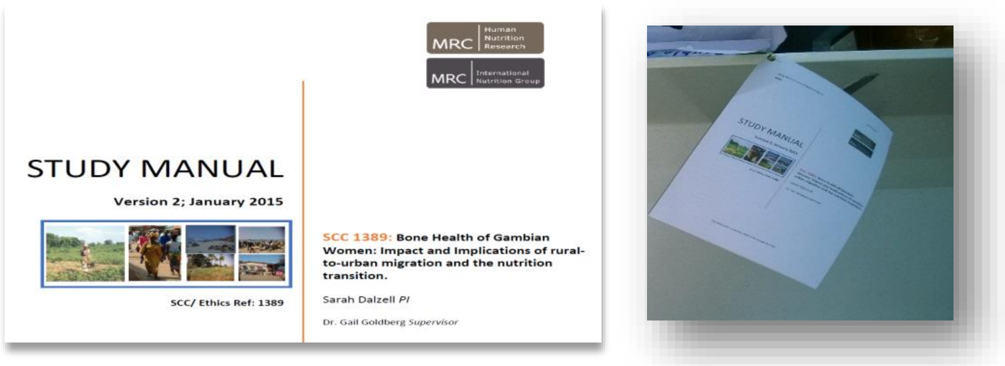


Figure 5:3 Study manual displayed in study room, MRCG Fajara

5.2.5 Training field team

CDBH field staff involved in WMS were already familiar with the majority of the protocols e.g. bone imaging (DXA and pQCT), dietary assessment and questionnaires.

I held several meetings with the field team to go over the protocols, and during the study fieldwork phases I was present during as many aspects of data collection as possible.

Training for the consenting and screening questionnaire was done with female staff. We were able to discuss the sensitivity of questions related to menstrual status, and specifically how these questions would be asked, and answered. I was present for the majority of recruitment visits.

5.2.6 Data management and planning

The Keneba data office is very familiar with studies conducted in the rural Kiang West district. This area is covered by the Kiang West DSS; therefore, the availability of data on potential participants can be filtered for example by: village, age, and date of most recent child. This enables the creation of a very specific list of potential study participants, who are essentially pre-screened. Mr Lamin Darboe, Mr Mohammed Ngum, Mr Bakary Sonko, and Mr Ebrima Comma, were the main members of the data team involved with the design of the WMS database.

Study forms were designed in MS Word and submitted along with the SCC and ethics applications. All forms were then re-designed in MS Access and during the first three months of study setup, I was able to spend a lot of time in the data office, initially shadowing, but eventually actively involved in the process.

The urban arm of WMS required more planning, concerning several aspects of data management including form printing, data entry, and remote database management. In the urban area, data could not be entered in real time, and forms were sent at the end of all measurements. I recorded necessary recruitment screening data using MS Excel, to enable tracking of participant eligibility.

5.2.7 Piloting

The physical activity questionnaire (PAQ) was based on a questionnaire developed and validated for use in sub-Saharan Africa (Sobngwi *et al.*, 2001). I adapted it specifically for use with Gambian women. I held several meetings to further develop and tailor the questionnaire with the local Gambian CDBH team, who are familiar with farming and gardening seasons as well as the daily life of local women.

Both health and activity questionnaires were piloted in the rural area with female housekeepers working in MRCG Keneba who could speak the local Mandinka language but not English, and therefore were similar to potential participants.

5.3 Cross Calibration of bone imaging equipment

5.3.1 Introduction

To achieve the required sample size for the rural group, existing data from two other studies conducted in Kiang West were included (**Section 4.10**). Protocols were the same; however, scans were acquired using the earlier DXA model, GE Lunar Prodigy.

An *in vivo* cross-calibration between the GE Lunar Prodigy and iDXA was carried out, according to recommendations of the International Society for Clinical Densitometry (ISCD) (ISCD, 2012). Bone mineral and body composition parameters were included in the analyses.

This cross-calibration study was essential to ensure that conclusions from the present WMS study were not affected by potential instrument bias. A systematic bias between the two instruments may be greater than potential biological differences between groups.

The aims of the cross-calibration were:

i. To assess the agreement between GE Healthcare Lunar (Madison, WI, USA) Prodigy and iDXA narrow-angle fan beam densitometers

ii. To produce cross-calibration equations to enable Prodigy data to be transformed to iDXA equivalent for the WMS study and other ongoing CDBH studies.

5.3.2 Methods

5.3.2.1 Subjects

Subjects were recruited from two ongoing cohort studies, including both healthy adolescents (PSC) and older adults (GamBAS), both sexes. Combining participant's data from both studies resulted in greater variation in body (and bone) size and composition, and thus ensured the cross-calibration to be as representative as possible of the participants that are studied by CDBH at the MRC facilities in Keneba and Fajara, including WMS. The sample size of 125 was adequate, exceeding the recommendation set by ISCD (Shepherd *et al.*, 2006).

Prodigy and iDXA scans were acquired on the same day except for 10 pairs of data, which were completed within a few weeks. Scientific and ethical approvals for the cross calibration study using participants already enrolled in GamBAS and PSC were obtained from the MRC SCC and Gambia Government/MRC Joint Ethics Committee (EC) (**Appendix D**).

5.3.2.2 Bone Densitometers

See main methods **Section 6.2.1** for descriptions of the two instruments (GE Lunar iDXA and GE Lunar Prodigy Advance DXA) and scanning procedures. EnCORE™ software 2015, Version 15.20.002, GE Healthcare Lunar, Belgium was used for analysis of scans.

5.3.2.3 Inclusion and exclusion criteria

Men and women had to be currently enrolled in GamBAS or PSC; all women had to be NPNL. Participants for cross-calibration were approached and consented to take part when contacted for their next follow-up visit. They were informed of the additional time and small increase in radiation that participating would involve.

5.3.2.4 Scan acquisition and analysis

Standard operating procedures (SOPs) were adhered to for both acquisition and analysis of scans. The following separate sites and bone parameters were included: Total body, lumbar spine, total hip and femoral neck, areal bone mineral density (aBMD), bone mineral content (BMC), and bone area (BA), and total body lean and fat mass. Software version enCORE™ 2015, Version 15.20.002 was used for analysis of all scans.

Scans were visually evaluated by myself and a trained research assistant at MRC EWL (~50% each). This involved checking for appropriate positioning of both the participant and ROI lines, and whether artefacts were present such as high-density areas or geophagy. Further details of scan image analysis and scanning positions are included in **Section 6.2.4**.

General details of statistical analysis of bone data are included in **Section 6.8.5**. Cross-calibration equations were produced using linear regression, where X (independent variable) was the

Prodigy (old scanner), and Y (dependent variable) the iDXA. Differences between machines were analysed using paired t-tests and overall bias with Bland Altman limits of agreement **Figure 6:19.** The validity of the resulting equations was confirmed by transforming the Prodigy data and running another paired t-test.

5.3.3 Results

Results are presented separately for each skeletal site. **Table 5:1** includes descriptive statistics of measured bone parameters (BMC, BA, and aBMD) and body composition (total body lean and fat mass), with both absolute and percentage differences. Linear regression results are shown in **Tables 5:2, 5:4, 5:6**, and **5:8**. Results of paired t-tests of transformed prodigy data to iDXA are in Table 5:9. Final cross-calibration equations for all sites are presented in **Table 5:10.**

Initially 125 individuals consented to take part (70 PSC and 55 GamBAS); however, we did not have complete pairs of scans for five subjects. One subject was excluded, as the total mass (TMass) was significantly different between the instruments and therefore a mistake was possibly made at the scanning stage. For the lumbar spine, three subjects did not have the region of interest (ROI) L1-L4 within the scan image, as the scan started too far down; therefore, 116 subjects were included in the analysis for the lumbar spine. Finally, the study was conducted in 119 (65 female, 54 male) aged 15-94years.

BMC (g)	Bone mineral content
BA (cm^2)	Bone area
aBMD (g/cm^2)	Areal bone mineral density

Some individuals were highlighted as outliers in scatter plots; however, they did not significantly affect the final equation (see **Section 6.8.5** for details of statistical analysis). It was decided to include these participants, as higher BMC for example was due to high-density areas (osteophytes) and other biological phenomena.

5.3.3.1 Lumbar spine

Only BMC was significantly different between instruments (mean ± SE, 0.38 ± 0.23%, $p<0.05$). There were no significant differences for parameters at the lumbar spine after prodigy data were transformed using the cross-calibration equations. See **Table 5:1** and **Table 5:2. Figure 5:4** shows scatterplots and regression lines after the prodigy data were been transformed

Table 5:1 Measured parameters at the lumbar spine (L1 to L4)

	Prodigy	iDXA	Difference	*p*-value	% Difference
BMC (g)	45.24 ± 1.20	45.47 ± 1.24	0.22 ± 0.11	0.05	0.38 ± 0.23
BA (cm²)	47.54 ± 0.59	47.67 ± 0.60	0.13 ± 0.07	0.08	0.26 ± 0.15
aBMD (g/cm²)	0.940 ± 0.018	0.941 ± 0.018	0.001 ± 0.001	0.4	0.07 ± 0.07

Values presented are mean ± SE, n=116; mean difference (d): iDXA - Prodigy; formula for relative mean difference (d%): [(iDXA – Prodigy) x 100/Prodigy] *p*-values from paired t-tests.

Table 5:2 Linear regression coefficients for lumbar spine (L1 to L4)

	Intercept	*p*-value	Slope	*p*-value	r^2
BMC (g)	-0.95 ± 0.40	**0.02**	1.03 ± 0.01	**0.0001**	99.2
BA (cm²)	-0.36 ± 0.56	0.5	1.01 ± 0.01	**0.0001**	98.5
aBMD (g/cm²)	-0.011 ± 0.007	0.1	1.014 ± 0.008	**0.0001**	99.4

Values presented are mean ± SE, n=116. *p*-values from linear regression.

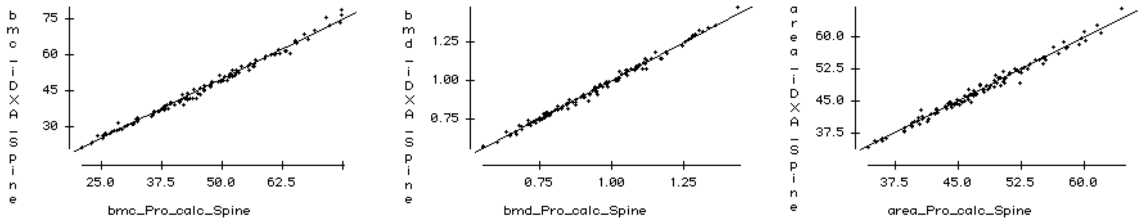


Figure 5:4 Scatterplots and regression lines of transformed prodigy (x) and iDXA (y) for lumbar spine, left to right BMC, BMD, and Bone Area.

5.3.3.2 Total body

There were no significant differences between instruments for BMD, BMC and BA. Total fat mass differed significantly between the Prodigy and iDXA (*p*=0.02), but not lean mass. After transformation using cross calibration equations, mean differences were small. See **Table 5:3** and **Table 5:4.** **Figure 5:5** and **Figure 5:6** show scatterplots of iDXA parameters compared to transformed prodigy.

Table 5:3 Measured parameters for total body

	Prodigy	iDXA	Difference	*p*-value	% Difference
BMC (g)	2005 ± 38	2001 ± 40	-4.32 ± 3.18	0.2	-0.36 ± 0.16
BA (cm²)	2022 ± 20	2019 ± 20	-2.80 ± 3.05	0.4	-0.12 ± 0.15
aBMD (g/cm²)	0.985 ± 0.012	0.983 ± 0.012	-0.002 ± 0.002	0.3	-0.229 ± 0.165
Fat mass (kg)	12.19 ± 0.55	12.08 ± 0.54	-0.11 ± 0.05	**0.02**	-0.61 ± 0.50
Lean mass (kg)	37.18 ± 0.67	37.21 ± 0.69	0.04 ± 0.06	0.5	0.03 ± 0.17

Values presented are mean ± SE, n= 119. mean difference (d): iDXA - Prodigy; formula for relative mean difference (d%): ((iDXA – Prodigy) x 100)/Prodigy. *p*-values from t-tests.

Table 5:4 Linear regression coefficients for total body

	Intercept	*p*-value	Slope	*p*-value	r^2
BMC (g)	-70.96 ± 14.25	**≤0.0001**	1.03 ± 0.01	**≤ 0.0001**	99.5
BA (cm²)	29.76 ± 28.25	0.3	0.98 ± 0.01	**0.0001**	97.7
aBMD (g/cm²)	-0.026 ± 0.012	**0.03**	1.025 ± 0.012	**≤0.0001**	98.4
Fat mass (kg)	0.13 ± 0.10	0.2	0.98 ± 0.01	**≤0.0001**	99.3
Lean mass (kg)	-0.77 ± 0.31	**0.01**	1.02 ± 0.01	**≤0.0001**	99.3

Values presented are mean ± SE, n= 119.

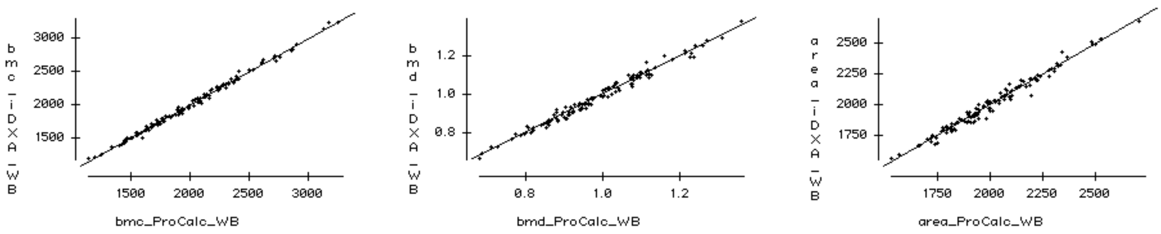
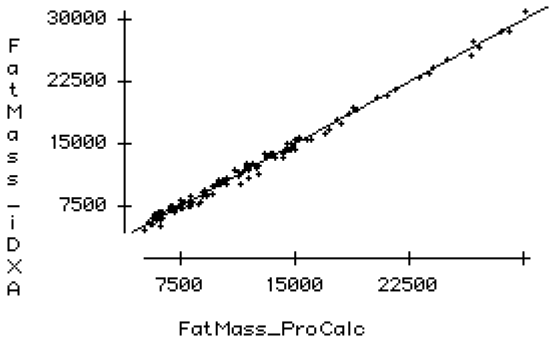


Figure 5:5 Scatterplots and regression lines of transformed prodigy (x) and iDXA (y) whole body bone parameters, left to right BMC, BMD, and Bone Area.



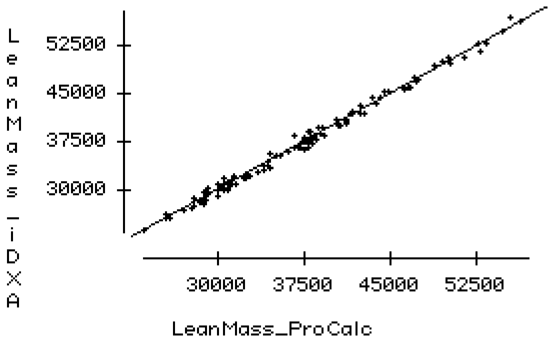


Figure 5:6 Scatterplots and regression lines of transformed prodigy (x) and iDXA (y) whole body composition parameters, left to right fat mass and lean mass

5.3.3.3 Total hip

There were significant differences between all parameters for the total hip ($p<0.05$). BMC had the greatest difference (1.82 ± 0.28%, $p<0.0001$), with iDXA higher than Prodigy for all parameters. After transforming Prodigy data with cross-calibration equations, there were no significant differences between iDXA and Prodigy parameters. See **Table 5:5** and **Table 5:6. Figure 5:7** shows a scatterplot of iDXA parameters compared to transformed prodigy data.

Table 5:5 Measured parameters for total hip

	Prodigy	iDXA	Difference	*p*-value	% Difference
BMC (g)	27.70 ± 0.64	28.13 ± 0.63	0.43 ± 0.07	**0.0001**	1.82 ± 0.28
BA (cm²)	29.25 ± 0.32	29.38 ± 0.33	0.13 ± 0.05	**0.02**	0.42 ± 0.19
aBMD (g/cm²)	0.943 ± 0.018	0.955 ± 0.017	0.012 ±0.001	**0.0001**	1.40 ± 0.18

Values presented are mean ± SE, n=119. Mean difference (d): iDXA - Prodigy; formula for relative mean difference (d%): ((iDXA – Prodigy) x 100)/Prodigy

Table 5:6 Linear regression coefficients for total hip

	Intercept	*p*-value	Slope	*p*-value	r^2
BMC (g)	1.07 ± 0.26	**0.0001**	0.98 ± 0.01	**0.0001**	99.0
BA (cm²)	-0.36 ± 0.45	0.5	1.02± 0.02	**0.0001**	97.4
aBMD (g/cm²)	0.024 ± 0.007	**0.001**	0.987 ± 0.008	**0.0001**	99.3

Values presented are mean ± SE, n=119.

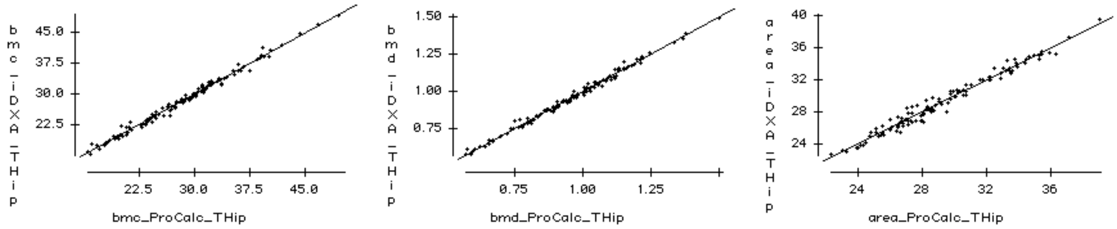


Figure 5:7 Scatterplots and regression lines of transformed prodigy (x) and iDXA (y) total hip bone parameters, left to right BMC, BMD, and Bone Area.

5.3.3.4 Femoral neck

All parameters were significantly different between scanners (p<0.05). BMC was higher for iDXA compared to prodigy, while bone area was lower. There were no significant differences after applying cross-calibration equations (p≈1). See **Table 5:7** and Table 5:8. **Figure 5:8** shows a scatterplot of iDXA parameters compared to transformed prodigy.

Table 5:7 Measured parameters for femoral neck

	Prodigy	iDXA	Difference	*p*-value	% Difference
BMC (g)	3.97 ± 0.10	4.00 ± 0.10	0.03 ± 0.01	**0.006**	1.13 ± 0.34
BA (cm²)	4.34 ± 0.06	4.31 ± 0.06	-0.03 ± 0.01	**0.007**	-0.61 ± 0.24
aBMD (g/cm²)	0.912 ± 0.018	0.927 ± 0.018	0.015 ± 0.002	**≤ 0.0001**	1.756 ± 0.260

Values presented are Mean ± SE, n=119. Mean difference (d): iDXA - Prodigy; formula for relative mean difference (d%): ((iDXA – Prodigy) x 100)/Prodigy

Table 5:8 Linear regression coefficients for femoral neck

	Intercept	*p*-value	Slope	*p*-value	r^2
BMC (g)	0.14 ± 0.04	**0.001**	0.97 ± 0.01	**0.0001**	98.7
BA (cm²)	0.02 ± 0.06	0.7	0.99 ± 0.01	**0.0001**	97.6
aBMD (g/cm²)	0.028 ± 0.011	**0.01**	0.986 ± 0.012	**0.0001**	98.4

Values presented are Mean ± SE, n=119.

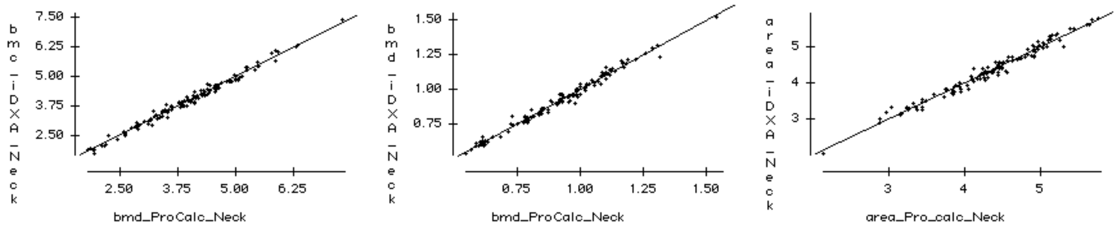


Figure 5:8 Scatterplots and regression lines of transformed prodigy (x) and iDXA (y) femoral neck bone parameters, left to right BMC, BMD, and Bone Area.

5.3.3.5 Results of t-tests for cross-calibration

Table 5:9 shows the confirmatory results from t-tests comparing iDXA measured parameters, and Prodigy parameters that have been transformed using cross calibration equations, derived from linear regression models.

Table 5:9 Measured parameters of transformed prodigy data compared to iDXA

	DXA parameter	Transformed prodigy	*p*-value
Lumbar spine			
	BMC (g)	45.47 ± 1.24	0.9998
	BA (cm2)	47.67 ± 0.60	0.9991
	aBMD (g/cm^2)	0.941 ± 0.018	0.9997
Total body			
	BMC (g)	2001 ± 40	0.9979
	BA (cm^2)	2019 ± 20	0.9998
	aBMD (g/cm^2)	0.983 ± 0.013	0.9975
	Fat mass (kg)	12.08 ± 0.54	0.9999
	Lean mass (kg)	37.21 ± 0.68	0.9992
Total hip			
	BMC (g)	28.12 ± 0.63	0.9999
	BA (cm^2)	29.38 ± 0.32	0.9988
	aBMD (g/cm^2)	0.955 ± 0.017	0.9912
Femoral neck			
	BMC (g)	4.00 ± 0.10	0.9999
	BA (cm^2)	4.31 ± 0.06	0.9998
	aBMD (g/cm^2)	0.927 ± 0.018	0.9998

Values presented are mean ± SE, n=119, except at the lumbar spine n=116. *P*-value from paired t-test, difference was less than 0.00001 for all parameters.

5.3.3.6 Cross-calibration equations for all sites

Table 5:10 shows the cross-calibration equations derived from linear regression models.

Table 5:10 Cross-calibration equations for all skeletal sites

	Cross calibration equations
Lumbar spine	
	bmc_iDXA= (1.026*bmc_pro) - 0.946
	ba_iDXA = (1.010*ba_pro) - 0.356
	bmd_iDXA = (1.014* bmd_pro) - 0.011
Total body	
	bmc_iDXA = (1.033*bmc_pro) - 70.958
	area_iDXA = (0.984*area_pro) + 29.760
	bmd_iDXA = (1.025*bmd_pro) - 0.026
	fat_mass_iDXA = (0.980* fat_mass_pro) + 0.133
	lean_mass_iDXA = (1.022* lean_mass_pro) – 0.768
Total hip	
	bmd_iDXA= (0.987* bmd_pro) + 0.024
	bmc_iDXA= (0.977*bmc_pro) + 1.067
	area_iDXA = (1.017*area_pro) - 0.358
Femoral neck	
	bmc_iDXA = (0.973* bmc_pro) + 0.140
	area_iDXA = (0.988*area_pro) + 0.0239
	bmd_iDXA = (0.986*bmd_pro) + 0.028

n=119, except at the lumbar spine n=116

5.3.4 Summary

After applying the cross-calibration equations, there were no significant differences between instruments for any parameter across all sites. These equations have been used in the WMS study to transform DXA data from participants measured on a Lunar Prodigy; reducing measurement error introduced by the use of two different DXA scanners.

5.4 Development of dietary assessment for WMS

This section describes background to the dietary assessment in The Gambia and developments of new food codes and food groups for analysis of WMS data. The methods used in the study are more fully described in the main methods chapter (**Section 6.5**)

5.4.1 Gambian dietary assessment and food composition

The accurate assessment of nutrient intake depends upon the accuracy of food composition data. A dietary assessment method has been developed over many years in The Gambia. Dietary data collection began in the 1970s in The Gambia (Prentice *et al.*, 1981), at which time the availability of African food composition tables were restricted to raw ingredients. Between 1974 and 1990, significant progress was made to directly analyse samples of raw and prepared dishes, consumed in the rural Gambian district of Kiang West. The variety of foods chosen for analysis was varied, ensuring analysis of highly seasonal foods. Local knowledge was obtained on important foods within the diet, through interviews with women residing in the study villages. There are more foods now in the food tables than in the printed version, including new foods and recipes added during WMS. After several decades of research, the number of foods and nutrients analysed has grown. In 2011, food tables were published, compiled by Celia Prynne and Alison Paul at MRC EWL, formerly MRC Human Nutrition Research (HNR). In addition to macronutrients, Ca, P, Zn, phytate, and carotene (Prentice *et al.*, 1993, Prynne *et al.*, 2002) were analysed in key foods. Fe, Mg and K were added using literature values.

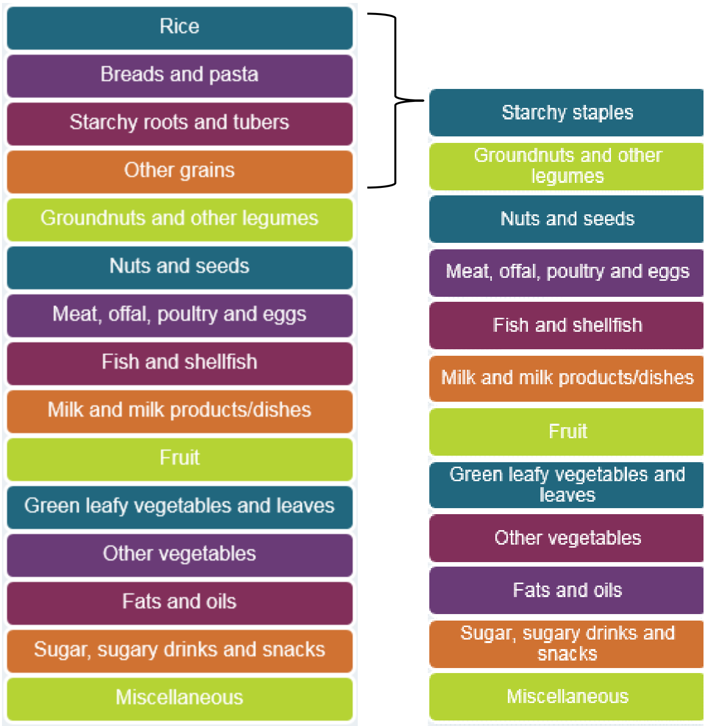
Figure 5:9 Gambian food composition tables front cover

5.4.2 Software for coding and analysis of dietary data

Diet in Data Out (DIDO) software was developed at the Dunn Nutrition Unit and then HNR, Cambridge to enable efficient coding and analysis of Gambian dietary data; but was initially developed for British diets. Details of the Gambian DIDO system and the recipes of sauces have been published (Prynne *et al.*, 2002).

In 2005, Diet in Nutrient Out (DINO) software was developed by Mr Darren Cole at HNR, now EWL, replacing DIDO; thus providing integration of dietary data entry and nutrient analysis. More details of the development and flexibility of DINO have also been published (Fitt *et al.*, 2015).

5.4.3 Description of SED food groups



15 SED groups **12 SED groups**

In order to compare rural and urban food consumption in WMS, SED food groups were developed, as the existing food groupings were not appropriate for the purposes of the WMS study. AEB food groups were comprised of one hundred and forty four separate groupings originally developed for analysis of specific nutrients. They were too detailed for the purposes of understanding overall food consumption. While the seventeen broader food sections used in the tables did not provide sufficient detail, for example, cereals were one group, yet drinks and sugars were separate groups, despite several commonly consumed drinks having a high sugar content. Therefore, the choice of fifteen main 'SED' after (Sarah Elizabeth Dalzell) food groups (see list on left) was based on WMS dietary data, while taking into consideration others commonly used e.g. FAO food groups. For some analyses the starchy staple food groups were combined to form one food group 'Starchy staples', while for dietary diversity scores (detailed in **Section 5.4.4**), ten SED food groups were used, including the combined starchy staples as one group, and excluding the sugars and miscellaneous groups.

5.4.4 Dietary diversity methods

Dietary diversity scores (DDS) provide a method to assess overall diet quality; diets incorporating a wide variety of food groups also include a variety of nutrients. There are several possible methods including quantifying total number of foods consumed, or the number of food groups consumed. Gambian food tables contain many composite dishes, thus it is difficult to quantify intakes of individual foods. Therefore, comparing the number of SED food groups consumed was decided to be a more useful method. As described in **Section 5.4.3** there are several versions of SED groups; exploratory analyses were conducted using the fifteen, twelve and ten. For the DDS calculated in WMS, the ten groups were used, combining the starchy staples, and excluding the sugary foods and miscellaneous. This was based on evidence that a higher DDS may not necessarily mean a healthier diet, for example, if more sugary foods or drinks are consumed then that will add diversity in an unhealthful way. It was also the method used by (Sodjinou *et al.*, 2007) in Benin.

DDS was calculated by summing the total number of food groups consumed over the course of the two days of dietary assessment. Each food group only counted once to the score, so even if starchy staples were consumed multiple times they were only counted once.

5.4.5 Developing new food codes

An established protocol is in place at MRC EWL if a food in a diet record is not included in the composition tables (Prynne and Paul, 2011). The suggested options are:

i. Find a closely related food e.g. mono for sato, nyelengo for serengo

ii. Combine two foods, or add another ingredient to an existing food.

iii. If a mixed dish, obtain the information on the amounts of ingredients and calculate the composition

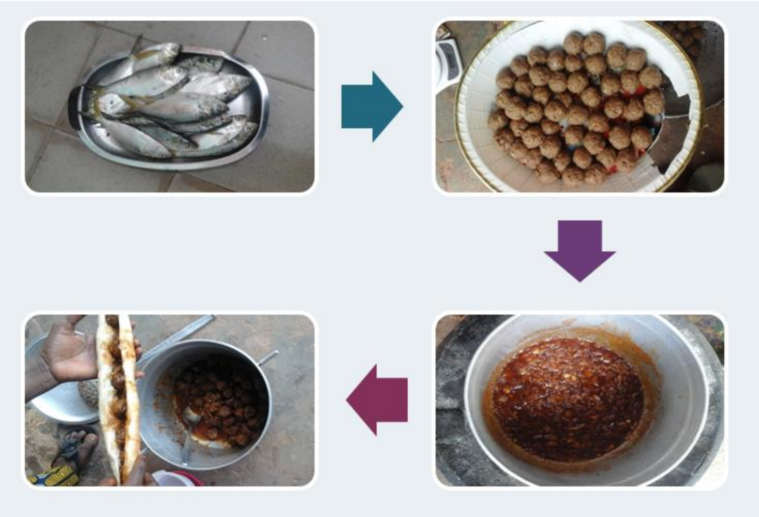


Figure 5:10 Bread, fish balls, and oil sauce: preparation for creation of new food code

Within DINO, there is a section to enter portions, for when a food was eaten outside of weighed mealtimes. Several new codes or new portion sizes were needed (~40), including new data for portions of mango, several varieties of sandwiches on two types of bread rolls, and fish balls with stew. Discussions about new recipes were conducted with Dr Gail Goldberg, Mr Musa Jarjou in The Gambia, Dr Landing Jarjou; as well as with Miss Nida Ziauddeen and Dr Birdem Amoutzopoulos, based in the MRC EWL UK dietary assessment team.

SUMMARY

This chapter has included an overview of the urban feasibility study (UFS) designed to trace migrant population in preparation for future urban studies, including WMS. I oversaw this and analysed the data. This chapter also included aspects of dietary assessment that were developed or tailed to WMS, including the development of SED food groups and creation of new food codes. Further details of the dietary assessment methodology used in the WMS study are described more fully in **Section 6.5.1.**

6 METHODS

6.1 Bone imaging techniques

6.1.1 Principles of absorptiometry

The non-invasive bone imaging techniques used in this study rely on principles of absorptiometry and the interaction of X-ray photons with bone and soft tissue. Early bone imaging techniques such as Single Photon Absorptiometry (SPA) had limited use due to the assumption they made of constant tissue thickness. Imaging was restricted to the peripheral skeleton and therefore common fracture sites such as the spine or hip could not be scanned, due to regional variation in soft tissue thickness.

The term attenuation is defined as 'the gradual loss in intensity of any kind of flux through a medium. For instance, "sunlight is attenuated by dark glasses, X-rays are attenuated by lead, and light and sound are attenuated by water" (Denman, 2017). Attenuation coefficients are specific to different materials, and are affected by both physical (e.g. thickness, density) and chemical properties (e.g. atomic number). Therefore, bone, fat and lean tissue have different attenuation coefficients. The interaction of photons with electrons in atoms and molecules in the body is dependent upon their photon energy.

6.1.2 Dual energy X-ray Absorptiometry (DXA)

6.1.2.1 Background to DXA

DXA uses ionising radiation with two (high and low) photon energies. Figure 6:1 depicts the key aspects and functioning of DXA. Dual energy (high and low) X-rays are produced and photons are filtered through a collimator. The slit collimator produces a fan beam of photons, which travel towards the participant, while reducing the potential for scatter, and ultimately result in an improved image.

However, the fan beam system does have a limitation, which relates to the position of the participant between the X-ray tube and the detector. If the object or participant to be scanned is further away from the x-ray source, both bone mineral content (BMC) and bone area (BA) can appear lower. This magnification mostly affects the geometrical parameters for example at the hip e.g. hip axis length.

6.1.2.2 Theoretical assumptions of DXA

According to the International Atomic Energy Agency (IAEA), DXA has three fundamental assumptions (IAEA, 2010):

i. Transmission through the body of the X-rays within the two energy windows can be accurately described by a mono-exponential attenuation process

ii. Individual image pixels of the human body can be described as a two component system, i.e. soft tissue and bone mineral, or when bone is not present, fat and lean mass. Thus, DXA is described as a three component model for body composition.

iii. The soft tissue overlaying the bone in the image has a composition and X ray properties of the tissue near but not overlying the bone.

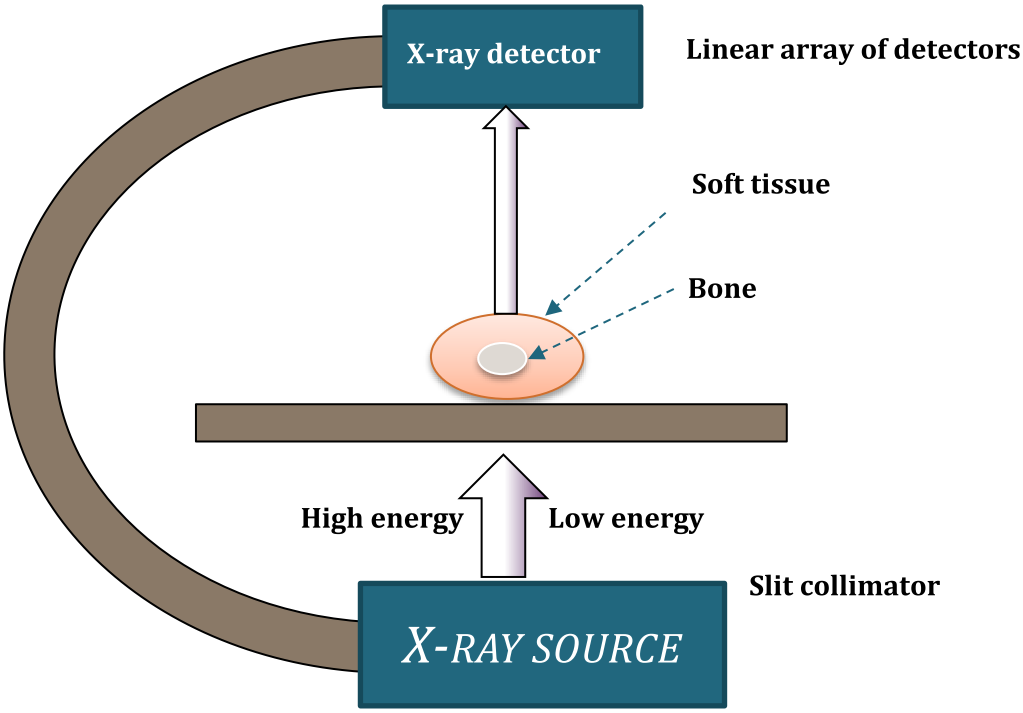


Figure 6:1 Principle of DXA (IAEA, 2010)

6.1.2.3 DXA measurement sites

DXA is optimised to measure bone mineral, and derived bone parameters include areal bone mineral density (aBMD), BMC, and BA at several regions of interest (ROIs), including lumbar and lateral spine, total body, proximal femur (hip), and forearm. In adults, the lumbar spine and hip are of most clinical relevance, as they are often the location of osteoporotic fragility fractures. Research studies commonly include these sites in order to determine associations between aBMD and fracture risk. Both the spine and hip are rich in trabecular bone and are therefore more susceptible to increases in bone turnover, for example, during menopause.

At present, DXA-derived aBMD forms the WHO diagnostic criteria for osteoporosis. Clinically, it is used to predict fracture risk; however, this predictive ability is limited to western white older adults, where a one SD decrease in aBMD is associated with a 2.6-fold greater fracture risk (Marshall *et al.*, 1996).

In addition to bone parameters, the total body scan provides body composition parameters: lean and fat mass as total (kg) or percentage of total mass. Regional analysis of android and gynoid fat is derived as total (kg) or percentage of total soft tissue. Fat-free mass refers to the sum of BMC and lean mass. Langton and Njeh (2003) explain technical aspects of how these body composition parameters are derived: 'in those areas of a total body scan where the X-ray beam does not intersect bone, it is possible to use the 'R-value (ratio of the attenuation at the two energies) to estimate separately the masses of fat and lean tissue.'(Langton and Njeh, 2003)

6.1.2.4 Scan image formation for DXA

An algorithm is used to ascertain bone edges. The summation of pixels within bone edges provides the total projected area; this is termed bone area (BA). aBMD is calculated as the 'mean BMD over all the pixels identified as bones' (Njeh and Shepherd, 2004). BMC is aBMD multiplied by the projected BA.

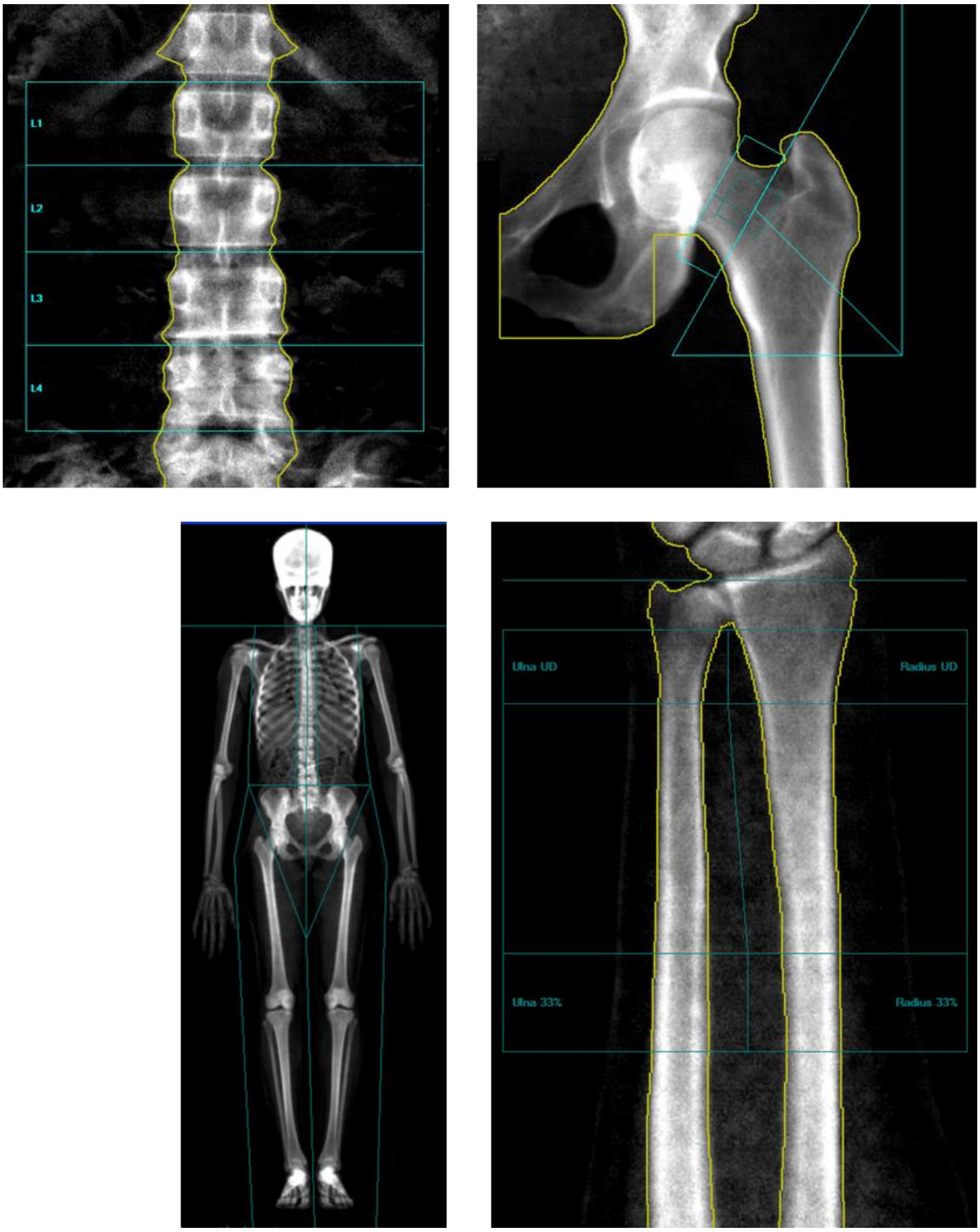


Figure 6:2 Scan images of regions of interest (ROI) of WMS study, including a) lumbar spine (L1-L4); b) total body c) hip d) forearm (GE Medical Systems, 2014)

6.2 Details of DXA bone density measurements

6.2.1 Study equipment

Two versions of DXA scanner were used in the study. All urban participants measured in MRCG Fajara (n=58), were scanned using a GE Lunar iDXA (enCORE™ software 2015, Version 15.20.002, GE Healthcare Lunar, Belgium). All rural participants were measured at MRCG Keneba, thirty-one rural participants were scanned on the iDXA (**Figure 6:3, A**) and fifty-one rural participants using a GE Lunar Prodigy Advance DXA (**Figure 6:3, B**) (enCORE™ software 2015, Version 15.20.002, GE Healthcare Lunar, Belgium). In order to reduce systematic difference in the two scanners, a cross calibration study was conducted (see **Section 5.3**).

Image of the two GE Lunar DXA scanners used in the study removed for copyright reasons. Copyright holder is GE Medical Systems.

Figure 6:3 GE Lunar DXA scanners (A) IDXA and (B) Prodigy (GE Medical Systems, 2014)

6.2.2 DXA scan acquisition

Standard operating procedures (SOPs) were adhered to for both acquisition and analysis of scans. Two fully trained and experienced bone imaging staff, Mr Michael Mendy and Mr Mustapha Ceesay, from the CDBH group in The Gambia were responsible for the bone imaging of WMS study participants in both Keneba and Fajara; I was present for the acquisition of almost all new rural scans.

Scan acquisition mode is automatically determined by the software, and relates to trunk thickness. There was no difference between the patient thickness limits of the two instruments (Prodigy and iDXA). During the cross-calibration scans, occasionally there were differences in scan mode selected, resulting in fewer subjects scanned in thin mode on the iDXA than Prodigy, particularly for lumbar spine and hip; and more for total body.

The identity of each participant was confirmed and their details (study ID, date of birth, height and weight) were entered into the software. Women were asked to confirm they were not pregnant. Participants could wear light clothing (without zips, metal fixings, jujus etc...), but all jewellery, or anything metal (e.g. keys, coins) had to be removed; these would attenuate the X-rays and affect bone density measurements. The scanning procedure was explained to the participant, highlighting the importance of staying as still as possible. The total effective dose of radiation that participants receives was less than 21 microsieverts.

6.2.2.1 Total body scan protocol

The participant had to lie in a supine 'flat' position on the DXA table; ankles (and knees if required) strapped together with a Velcro strap (**Figure 6:5**). Care was needed to ensure that head, arms, and feet were kept within the scanning field, particularly for large participants. Guidance lines on the table pad helped to keep the total body central, with arms pronated (palms face down), and kept separated from the thigh, except where this would result in extending beyond the scan field limits. In case of this, hands were placed just under the thighs. The implications of this were limitations on regional measures and on body composition.

6.2.2.2 Proximal femur (hip) scan protocol

The participant had to be positioned flat on the bed. Feet were strapped to a positioning aid which assists in providing the correct rotation of the femur (**Figure 6:6**). The red 'positioning' light was moved to the correct place, indicated by using a hand span from the greater trochanter (approximately 7cm down the mid shaft of the femur). Arms were crossed over the chest, to ensure they were not within the ROI.

6.2.2.3 Lumbar spine scan protocol

To enable good positioning, i.e. straight spine alignment, a soft foam block was placed under the legs, supporting the knees, allowing for separation of the vertebrae and alignment with the X-ray beam (**Figure 6:4**). The positioning light was placed about 2cm (two-finger widths) below the navel. Identification of anatomical markers in the scan image should include the top of the pelvis and the 12th rib, L5 is usually characterised by its M or butterfly shape (Njeh and Shepherd, 2004).

Image of positioning for AP spine scan removed for copyright reasons. Copyright holder is GE Medical Systems.

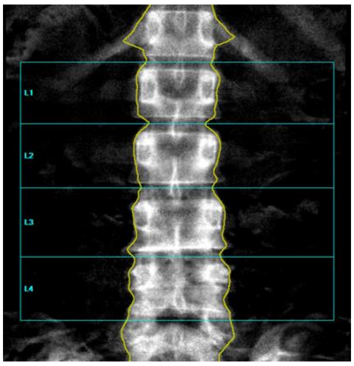


Figure 6:4 Anterior posterior (AP) L1-4 lumbar spine positioning (GE Medical Systems, 2014).

Image of positioning for hip scan removed for copyright reasons. Copyright holder is GE Medical Systems.

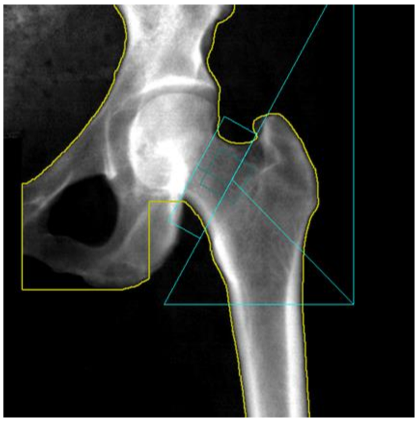


Figure 6:6 Hip scan positioning (GE Medical Systems, 2014).

Image of positioning for total body scan removed for copyright reasons. Copyright holder is GE Medical Systems.

Figure 6:5 Total body positioning (A) Space above head; (B) Hands placed flat, unless insufficient space in scan area, in which case they are placed flat slightly beneath the hips; (C) Velcro straps used to keep ankles and sometimes knees together (GE Medical Systems, 2014).

6.2.3 Quality Assurance (QA) and Quality Control (QC)

Throughout the study, the GE QA calibration block (**Figure 6:7**) was used to perform daily checks; calibrating the machine's functioning and assessing accuracy and precision of measurements. The software provided a detailed report, which indicated the pass/fail of the DXA. A printout of the QA report was checked and filed in the scanning room; any problems were reported. This process enabled monitoring of both the scanner's performance and precision during the study.

In addition, an aluminium spine phantom (**Figure 6:7**) was scanned once or twice per week in Keneba and daily in Fajara. The aBMD measured was monitored over time to ensure there was no drift. Both QA and QC checks remained stable throughout the study for all DXAs. QA checks were also conducted immediately after scan acquisition, which involved inspecting the image to ensure good positioning, no movement and that there was no need to repeat the scan before the participant left.

The room temperatures were monitored in accordance with the manufacturers recommendation, QA checks were repeated if the temperature changed by more than 5°C.

Image of QA block and spine phantom removed for copyright reasons. Copyright holder is GE Medical Systems.

Figure 6:7 Calibration block consisting of tissue-equivalent material with three bone-simulating chambers of known mineral content (GE Medical Systems, 2014).

6.2.4 DXA scan image analysis (after acquisition and participant left)

Firstly, sets of scans were crosschecked to ensure that the acquisition date was the same as the date recorded on their visit form. Discrepancies were investigated, and when resolved, scans were visually compared by a trained research assistant and myself at MRC EWL, (approximately 50% each). This involved checking for appropriate positioning of both the subject and ROIs; and whether artefacts were present such as high-density areas (e.g. fracture, osteophytes), jewelry, jujus, or geophagy.

Automatic detection and positioning of ROIs by the software sometimes required adjustment to ensure inclusion of the appropriate area and consistency between pairs of scans (for the cross calibration). For hip scans, the neck box dimensions were 1.5 x 6 cm, except when subjects had a short femoral neck, or poor positioning resulted in insufficient rotation.

The software setting 'enhanced analysis' (EA) was used for Prodigy total body scans; according to GE, this EA enables improvements in point typing (i.e. bone edge detection, particularly for younger and smaller individuals) and the soft tissue composition model is more comparable to iDXA, which uses EA.

Calcification of the aorta could lead to overestimation of BMD as it can lie within the Spine ROI, this was checked and density values checked. The software automatically labels the ROI L1-L4, but this must be checked, along with detection of bone edges and definition of tissue region. Spine BMD should increase progressively from L1 to L4. **Table 6:1** details the grading protocol used at MRC EWL and MRCG Keneba. Scans were graded by trained researchers, including myself i.e. not by machine, from 1 to 3, with 1 being very good and 3 indicating that part or all of a scan was unusable.

Table 6:1 Grading criteria for DXA scans

Grade	Scan quality	Analysis quality
1	Good No movement, correct positioning (minor tilting) no artefacts	Little or no adjustment to automatically positioned ROI lines
2	Acceptable Artefact or movement	Adjustments made to ROI lines
3	Abnormal bone detection Unusable area of interest (one or more) Area of interest outside scan field Artefact over area of interest, affecting BMD Poor bone detection Abnormal T-scores in spine (≥3) Femoral neck box ≤ 1cm	Grading not required

Source: MRC EWL DXA grading protocol

6.3 Precision of DXA and pQCT

In order to distinguish small differences between groups or change over time, densitometry techniques need to be precise. Precision of in-vivo bone imaging is defined as the scanner's ability to repeatedly give the same result when measuring the same parameter on the same participant at the same skeletal site. Precision is normally expressed as standard deviation (SD) or coefficient of variation (CV). Several factors can impact precision, for example hardware or software issues, when different equipment or new software may result in changes to algorithms. During scan acquisition, poor positioning can lower precision, as can inadequate soft tissue, artefacts in scan field, and change of weight in longitudinal studies. The analysis of scans can also impact precision due to incorrect placement of ROI or the presence of fracture (Njeh and Shepherd, 2004). Poor positioning is one of the most common factors resulting lower precision.

6.4 Anthropometry

Anthropometric measurements were made during the clinic visit following SOPs in MRCG Keneba. Anthropometry and blood samples were the first measurements taken, as this ensured all women were in a fasted state, of particular importance for weight and bloods. Anthropometric measurements were also necessary to carry out pQCT and DXA measurements. Weight, sitting and standing height, and waist and hip circumference measurements were measured in triplicate. Between each measurement, participants were repositioned and if necessary, the equipment was reset. Measuring in triplicate reduces measurement error, with the mean of the three measurements used in analysis.

6.4.1 Measurement of tibia and forearm length

The left tibia was measured while the participant was sitting with feet flat on the floor and leg at 90° to the thigh. The distance between the tibial plateau and midpoint of the medial malleolus was measured to the nearest 0.5mm. The tibial plateau is found between the patella and the tibia.

The left forearm was measured to the nearest 0.5mm using a non-stretchable tape measure. The lower arm was positioned at a right angle to the humerus, and the distance measured between the olecranon (tip of elbow) and the 'midpoint' of the ulnar styloid process (prominence at wrist) (**Figure 6:8**).

6.4.2 Measurement of standing and sitting height

A stadiometer (Brand: Marsden Leicester) was used to measure height in triplicate. A stick of known length was used to check the instrument each morning and when the stadiometer was repositioned for sitting height. In MRCG Keneba, there were two stadiometers, one for standing, and one for sitting height. However, in MRCG Fajara, after all standing heights were measured, the stadiometer was adjusted to measure sitting height; a wooden stool was commissioned to specifications, identical to the one in Keneba. Participants were required to remove sandals and headwear. Typical clothing worn by Gambian women includes long skirts and dresses. This often meant that it took longer to ensure that feet were in the correct position and that they did not move when any adjustments were made.

Participants were asked to stand with their heels, buttocks, and shoulders against the stadiometer. Knees had to be kept straight and as far back as possible, sometimes requiring relaxing of the shoulders. In order to measure sitting height, participants had to sit with their buttocks and shoulders against the stadiometer. Their feet placed together and resting flat on the footrest, with knees together. Their head was positioned in the same way for both sitting and standing height measurements, kept in Frankfort's plane, the position when the lower of the left orbit and upper margin of the external auditory meatus are horizontal. To achieve this position the participant looked forward and had level shoulders (**Figure 6:9**). The measurer would also apply pressure to the mastoid process (behind the ear) to try and stretch the participant upwards, ensuring that feet were not moved in the process. The measuring slide was then lowered toward the head, just enough to touch. The measurement was recorded to the nearest mm.

6.4.3 Measurement of weight

Weight was measured in triplicate to the nearest 0.1kg, using a digital weighing scale (Tanita HD-314). The equipment was zeroed before each participant was asked to step on; weight was recorded when both feet were balanced evenly. All women wore light clothing after removing their sandals, small purses, jujus (traditional tied charms) and any mobile phones. Prior to each study visit, weighing scales were calibrated with a weight of known mass; replacement batteries were available if needed.

6.4.4 Measurement of waist and hip circumference

Waist and hip circumference were measured to provide an indication of central adiposity, with the ratio of waist to hip circumference providing an index of body fat distribution (World Health Organisation, 2008). A tape measure (non-stretchable) was used to measure both circumferences, either immediately before or after DXA measurements. Female field assistants made all hip and waist circumference measurements. To enable my training, if the participant was happy, the measurement was repeated by me, although the data were not used. Participants were asked to stand and place their arms across their chest. The tape was held close to the skin, but not tight enough to cause an indent. The waist circumference measurement was made at the minimum circumference between the bottom of the rib cage and the iliac crest. The hip circumference measurement was made at the largest circumference between the waist and the top of the thighs.

Figure 6:8 Measuring forearm length

Image of positioning for height measurement removed for copyright reasons. Copyright holder is John Wiley & Sons, Inc.

Figure 6:9 Positioning of Frankfort plane for height measurement (Madden and Smith, 2014)

6.4.5 Calculated variables

Additional variables were calculated from the anthropometric measurements. These were: BMI = weight (kg) / height (m^2)

Waist: Hip ratio = waist (cm)/ hip (cm)

6.5 Dietary assessment

6.5.1 WMS dietary assessment methods

Since the late 1940s, dietary assessment methods have been developed for use in research. Dietary assessment was conducted for two reasons, primarily to estimate intakes of nutrients important for bone e.g. calcium and phosphorus. Secondly, to investigate differences in overall food and nutrient intake in order to compare aspects of the nutrition transition in rural and urban areas. For example, differences in proportions of macronutrients contributing to energy intakes, and intakes of specific food groups e.g. fat, sugar, and dairy products. A description of typical dietary intakes in rural Gambia can be found in **Section 2.4.2.**

The primary methods of dietary assessment used in WMS was a two-day weighed diet record along with a calcium specific food frequency questionnaire (Ca-FFQ). Several important sources of Ca in rural Gambia are seasonal and indigenous (Prentice *et al.*, 1993); therefore, the Ca-FFQ provides a means to estimate Ca intake across the year, or whether particularly rich sources but infrequently consumed foods are consumed at all e.g. milk. The gold standard in dietary assessment is a prospective 7-day food record, including both week and weekend days, which in a western setting typically vary in both food and nutrient content. In rural Gambian studies two days has been decided to be sufficient at a group level to estimate intakes.

6.5.2 Prospective two day weighed diet record

In The Gambia, food is consumed from a shared bowl, which makes it difficult to estimate an individual's intake. However, due to the long history of dietary research in Keneba, an acceptable method to measure food intake has been developed. This requires a field assistant to visit the participant at each mealtime, approximately 4-5hrs intervals throughout the day to weigh and record the details. The majority of women in both rural and urban study populations were illiterate and therefore a field assistant was required to conduct these.

6.5.2.1 Logistics for dietary assessment

Arrangements for the dietary assessment schedule were discussed with the senior field assistant Mr Lamin Jammeh. I was advised that each field assistant allocated to the study could manage to see three participants concurrently in the rural villages. However, I was advised that due to the wide geographical distribution of study participants in the urban area, only one to two could be conducted at a time, depending on their proximity.

Figure 6:10 Dietary assessment and co-ordinating with field team

Food intake was recorded on a form routinely used for dietary studies by the CDBH group. It is specifically designed to capture details of the recipe as well as the consumption of snacks outside of meal times. The participant was asked to designate a food bowl that she could use throughout the study; this was weighed using electronic weighing scales, to the nearest g. The scale was zeroed and the participant then proceeded to serve herself the meal. The weight of the food bowl was measured and recorded after the addition of each food item; the first item is usually a cereal e.g. boiled rice, followed by sauce, vegetables and fish or meat if being consumed. A comments box provides additional space for specific ingredients in the sauces to be recorded, for example whether dried leaves or locust bean were added during cooking. Once the participant indicated that they were finished the meal, the remaining food was weighed in the bowl and recorded. If there were leftovers, participants were asked if they planned to eat the remainder later, if yes, the field assistant would return to weigh the final leftovers, and record this on the same form. Each field assistant was provided with six forms, one for each potential mealtime over the two consecutive days (**Figure 6:11**). Food eaten between main meals was recalled and recorded in the comments box on the form.

6.5.2.2 Adaptation for WMS

For the WMS study, several extra questions were included, relating to amount of oil and sugar added to the cooking pot and the number of people the meal was prepared for. **Figure 6:10** shows an urban participant's lunch being weighed by field assistant (MS). Due to the distance and transport restraints in the urban area, I was unable to join field staff to observe dietary assessment. However, I was able to observe several participants in the rural area, both during the study and on prior orientation visits to The Gambia.

Figure 6:11 Left: Front cover of the Gambian food composition tables. Right: a field assistant weighing food and recording weights on the form.

6.5.3 Assigning food codes

As previously described in **Section 5.4** a variety of composite codes have been developed in The Gambia, covering the spectrum of commonly consumed dishes. For example rice and basic sauce, or with additional ingredients, commonly added to sauces if available. This enables coding of reheated food, where the individual foods (e.g. rice and sauce) can no longer be weighed separately. The proportions of rice to the six most common sauces were previously established, by taking the mean of both items from weighed food records. Providing a percentage of sauce to cereal, which can be used to derive other recipes. Several new codes were also developed, these are described along with the process in **Section 5.4.5.**

6.5.4 Dietary analysis: entry, coding and export of data

Gambia Diet in Nutrients Out (DINO) is software specifically tailored for Gambian dietary assessment. It is a system, which combines the ability to record and analyse dietary data. DINO was originally developed at EWL by Mr Darren Cole for the UK National Diet and Nutrition Surveillance (NDNS), and has been adapted for analysis of Gambian records in 1997 (Fitt *et al.*, 2015).

Coding of dietary data is not straightforward data entry, and requires training and experience. It is a complex and important stage of dietary assessment; small discrepancies in the choice of a code to represent what was actually eaten can make an impact on final nutrient intakes, e.g. sauces with or without leaves, will contribute to intakes of calcium, as well as other micronutrients. I coded all dietary data and chose to recode the diet records from rural participants of ENID and GamBAS. This re-entry ensured the coding decisions were consistent across the study, for both rural and urban groups.

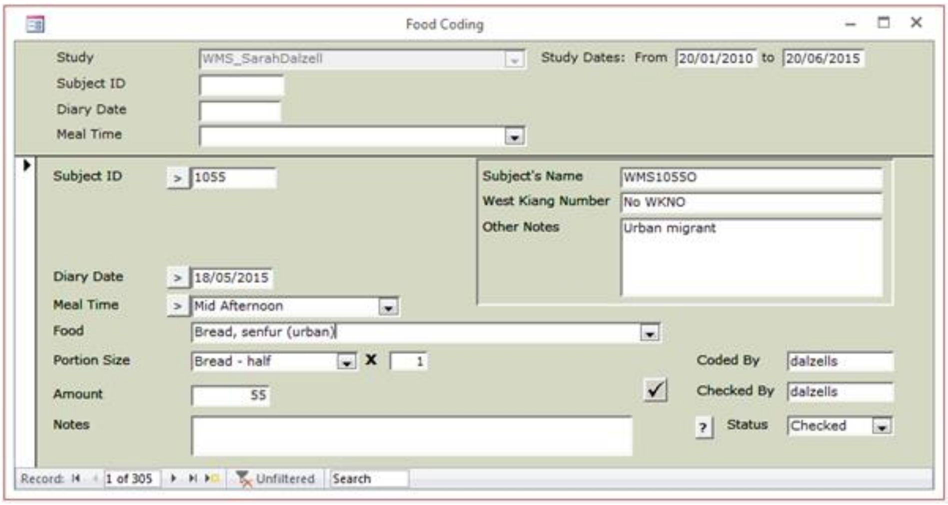


Figure 6:12 DINO entry screen for foods recorded as portions

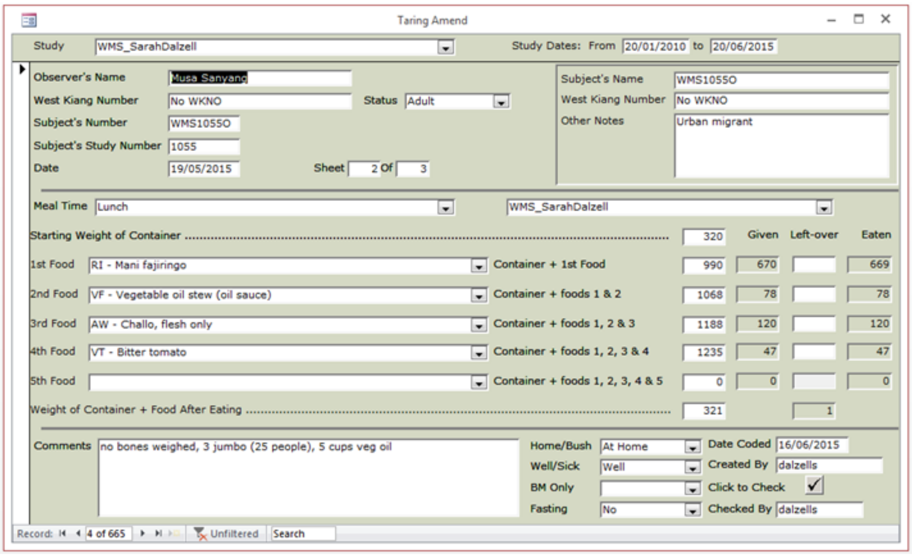


Figure 6:13 DINO entry screen for taring, the first food: Mani fajiringo (boiled rice) followed by oil stew, challo fish, and bitter tomato.

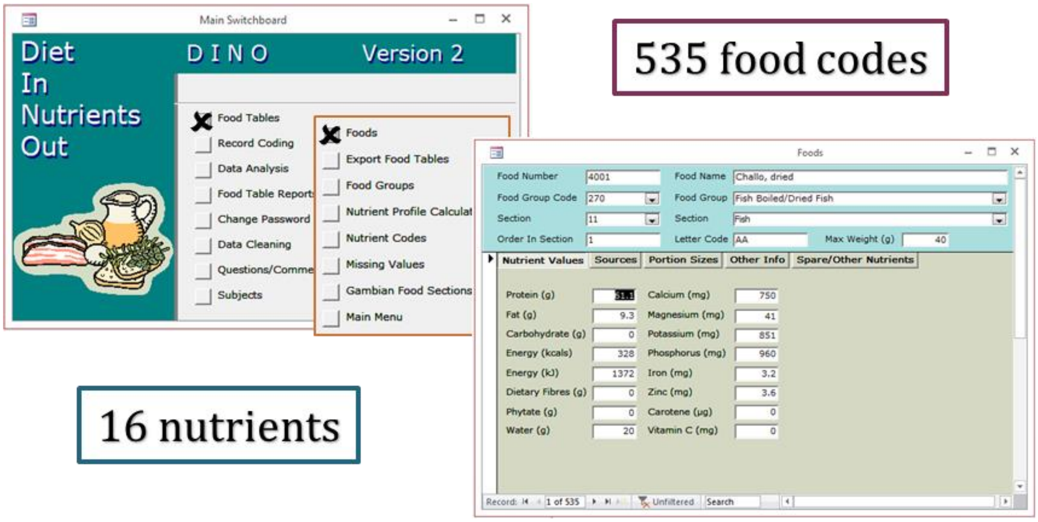


Figure 6:14 DINO main screen and food tables within software, used for the calculations of nutrient intakes

6.5.5 Calculated variables

6.5.5.1 Percentage contributions of macronutrients to total energy

Percentage energy contributed by macronutrients (carbohydrate, protein and fat) were calculated based on mean intakes in grams, where 1g carbohydrate = 17kJ; 1g protein = 17kJ; 1g fat = 37kJ, divided by the total energy from the sum of all.

6.5.5.2 Salt intakes

Salt intakes were calculated by converting urinary sodium excreted during a 24-hr period, the preferred method, which captures approximately 95% of recent sodium intake. A single 24-hr urine collection is valid to estimate mean intakes of a group, but not individuals, as there are large variations in day-to-day intake. 24hr urinary sodium excretion was converted to salt (sodium chloride) intake by dividing by 17.1, based on the following equation: 17.1mmol of sodium =1g salt. This assumes that dietary intake of sodium is equal to the 24-hour sodium output in urine, and that all sodium in the diet comes from salt.

6.5.5.3 Dietary diversity scores

These are described in Section 5.4.4.

6.5.6 Misreporting in dietary assessment

To determine the plausibility of the mean energy intake of the study population, confidence limits (cut offs) were calculated using the following equation (Black, 2000):

Equation for calculating Goldberg cut-offs (95% confidence limits) +/- 2 SD

$$\boldsymbol{EI}_{\mathbf{rep}}\mathbf{:BMR} > \boldsymbol{PAL} \times \boldsymbol{e}^{\left[-2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

$$\boldsymbol{EI}_{\mathbf{rep}}\mathbf{:BMR} < \boldsymbol{PAL} \times \boldsymbol{e}^{\left[2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

BMR = Basal Metabolic Rate

$\boldsymbol{EI}_{\mathbf{rep}}$ = mean reported energy intake

S is the factor accounting for variation in intake, BMR and energy requirements, calculated:

$$\boldsymbol{S} = \sqrt{\frac{\boldsymbol{CV}_{\boldsymbol{wEI}}^2}{\boldsymbol{d}} + \boldsymbol{CV}_{\boldsymbol{wB}}^2 + \boldsymbol{CV}_{\boldsymbol{tP}}^2} \;=$$

$\boldsymbol{CV}_{\boldsymbol{wEI}}$ = within-subject coefficient of variation of variation in intake = 23% (calculated as 25.3% in WMS)

d = number of days of dietary assessment = 2

$\boldsymbol{CV}_{\boldsymbol{wB}}$ = coefficient of variation of repeated BMR measurements / precision of estimated compared with measured BMR = 8.3%

Between subject coefficient of variation in physical activity (CV_{tP}) = 15% (average used based on literature)

$\boldsymbol{CV}_{\boldsymbol{tP}}$ = coefficient of variation derived from the mean and standard deviation of a study, including true between-subject, an element of within-subject variation, and methodological errors = 15%

Within subject daily variation in energy intake (CV_{wEI}) = 23%

Day-to-day variation is high within individuals, 23% has been provided from the literature as a suitable average to substitute into the Goldberg equations.

An estimation of misreporting in WMS is included in **Appendix E**, along with calculations and a brief discussion.

6.6 Biological sample collection

Biological samples included fasted bloods (~20ml) taken at the time of the measurement visit, and a 24hr urine collection during the first day of dietary assessment. After the completion of the study, samples were packaged and shipped to the UK.

6.6.1 Fasted blood collection

Fasted blood samples were obtained from all subjects (n=141) and taken by a trained phlebotomist, either the laboratory technician (Mr Alasana Saidykhan) or bone imaging operator (Mr Mustapha Ceesay), most of the time I was present. Taking a fasted blood sample helped to standardise the collection. Details relating to the collection of the blood sample were recorded on the measurement form. All participants were asked if they had eaten anything during the morning. The laboratory in Keneba and Platt building in Fajara have a designated area for sample collection. Women were able to sit comfortably, while a vein was identified and blood collected into three types of collection tubes: lithium heparin (LiH), potassium ethylenediaminetetraacetic acid (EDTA) and a plain serum tube. Samples were initially placed in a basin of ice, then analysed and stored at -20°C, according to the standardised protocol detailed in the study manual. Figure 6:17 provides an overview of the blood collection processing.

Figure 6:15 Blood collection in Platt building laboratory, Fajara, MRCG

6.6.2 Urine (24hr collection)

The 24hr urine collection was conducted at the participant's home or place of work, beginning on the first morning of their dietary assessment. Participants received two acid washed containers along with a compact freezer box with frozen ice packs. Most participants did not have access to electricity, or a fridge/freezer, therefore these provisions ensured urine could be kept cool, despite high ambient temperatures. All equipment (e.g. measuring cylinder and containers) were acid washed according to the SOP; containers were labelled with the study ID and the date. Equipment used for titratable acidity measurements were not acid washed, as this would interfere with the measurement.

Before starting the collection participants were asked to void their bladder; the field assistant recorded this time on the designated form. The participant was asked to collect all subsequent urine into the containers over the 24hrs. The field assistant would visit at meal times during the day, for dietary assessment purposes. This enabled the replacement of ice packs and the harvesting of full urine bottles, which could then be stored in the laboratory fridge at the MRC Keneba or Fajara. After 24hrs, the collected urine was pooled for each participant and the total volume calculated, and recorded on the form. After mixing well, four aliquots of 2ml were pipetted into cryotubes, two for a non-acidified sample, and two for acidified (included 20μl of hydrochloric acid (HCL)). Figure 6:18 provides an overview of urine collection and processing. Titratable acidity was measured by pipetting 10ml of urine into a glass beaker and incrementally adding 0.025M NaOH until pH 7.4 using a burette, a calibrated pH meter, and a magnetic stirrer. The mmol/l of acidity is calculated using the following formula:

$$\frac{\mathbf{0.025}\ \boldsymbol{M\ x\ Volume\ of\ NaOH\ used\ in\ ml\ x}\mathbf{1000}}{\boldsymbol{Volume\ of\ urine\ taken\ in\ \ ml}\ (\mathbf{10}\boldsymbol{ml})}$$

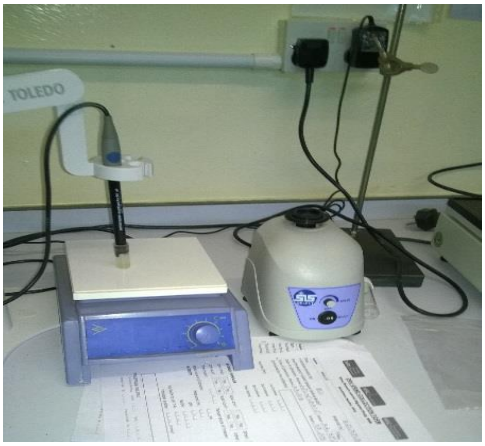


Figure 6:16 pH meter and stirrer

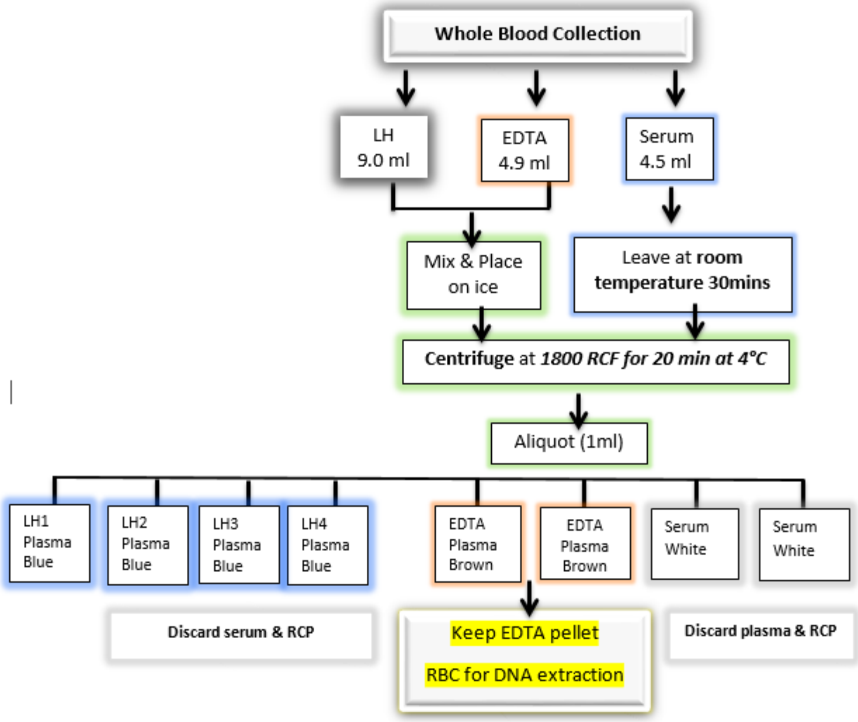


Figure 6:17 Flow diagram of blood collection and processing

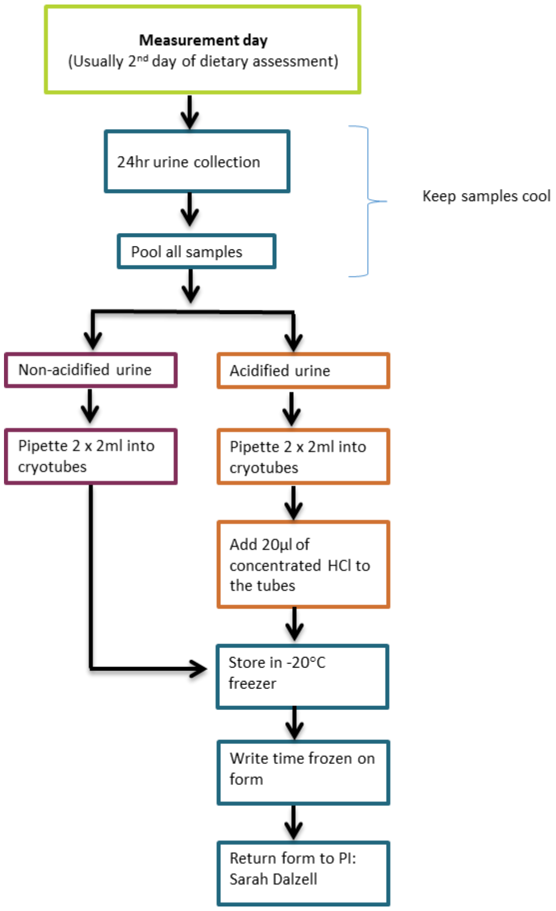


Figure 6:18 Flow diagram of urine collection and processing

6.7 Biological sample analysis

Biochemical analyses of blood and urine were carried out at MRC EWL, Cambridge. Clinical chemistry (plasma and urine) and plasma 25 OH Vitamin D (25OHD) were analysed by the NBH group, specifically Mrs Janet Bennet, Mrs Ann Laidlaw, and Dr Shailja Nigdikar. Urinary electrolytes (Na and P) and urea (U), and creatinine (Cr) were analysed by the Nutritional BioAnalysis (NBA) team, by Mrs Tabasum Makhdoomi, Mr Abhilash Krishnankutty, and Mrs Lorna Cox. Both labs are part of quality assurance schemes, with samples routinely analysed as part of the Vitamin D External Quality Assessment Scheme (www.deqas.org) and the National External Quality Assessment Scheme (www.ukneqas.org.uk).

Sample entry and management was carried out using Item Tracker 3.5.7 (ItemTracker Software Ltd., Ipswich, UK). Existing rural samples were located using Item Tracker and analysed concurrently with new samples. I observed the first run of all sample analysis.

6.7.1 Plasma 25-hydroxyvitamin D

LiH plasma samples were analysed to measure 25(OH) D. The DiaSorin LIAISON 25 OH Vitamin D TOTAL assay (DiaSorin, Stillwater, MN, USA) is a chemiluminescent immunoassay (CLIA) technique used to determine quantities of 25(OH) D in serum, LiH plasma, or EDTA plasma. CLIA works by emitting light during a chemical reaction, when an antigen-antibody complex occurs. The concentration of 25(OH) D is inversely proportional to relative light units (RLU), measured by a photomultiplier.

6.7.2 Plasma clinical chemistry: Ca, P, Mg, Cr and TALP

Clinical chemistry included the analysis of Ca, P, Mg, Cr, and total alkaline phosphatase (TALP), using a Konelab 20i clinical chemistry analyser platform (Kone, Espoo, Finland), in heparinised plasma samples.

6.7.3 Urine clinical chemistry: Ca. P, Cr, Na, and K

Urinary Ca, P, Mg, and Cr were analysed with the same instrument as used for plasma analysis, Konelab 20i clinical chemistry analyser platform (Kone, Espoo, Finland).

6.7.4 Urinary electrolytes: Na and K

Electrolytes (Na and K) were also measured in 24hr urine samples, using the Siemens Dimension® ® Xpand clinical chemistry system with the QuikLYTE® module.

6.7.5 Calculations

6.7.6 Estimated glomerular filtration rate (eGFR)

In order to investigate kidney function and determine whether any particpants had chronic kidney disease (CKD), eGFR was calculated. The CKD-EPI equation was used, where:

eGFR = 141 * min(Scr/κ,1)α * max(Scr/κ, 1)-1.209 * 0.993Age * 1.018 [if female] * 1.159 [if black]

Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

This formula has not been validated in a Gambian population; however, following South African guidelines, the Black factor was not used. The results will therefore be more conservative (i.e. lower) than leaving it in. GFR was also calculated from the 24hr urine outputs, however, although there was little discrepancy between eGFR and calculated GFR it is likely that eGFR gives a more robust diagnosis of CKD, as 24hr urine collections are quite likely to be incomplete for some participants, a well known issue.

6.8 Data management, statistical methods and analysis

6.8.1 Data management

All completed study forms were checked for both completeness and ambiguity, by either myself or Mr Michael Mendy (CDBH) at the end of each study day. Field assistants and lab technicians were asked to clarify any issue. Forms were given to the assigned WMS data manager Mr Lamin Darboe at the data office in Keneba, who had developed the database and co-ordinated the double entry process. Data were entered by a team of data entry clerks, under the supervision of Mr Darboe. Any discrepancy between the two entries was checked against the hard copy. Forms were scanned to create an e-copy, and stored as a pdf, which could be accessed upon return to Cambridge. The results of sample analysis conducted at MRC EWL in Cambridge, were checked by the lab staff, and provided to me as an MS Excel spreadsheet.

In order to help familiarise myself with Gambian foods and the study population, I decided to enter the two-day weighed intakes for WMS, and re-enter the dietary data for those participants from existing studies. Further details of dietary entry, coding, and management of data can be found in the previous **Section 6.5.4.**

The majority of data entry was complete prior to my departure from The Gambia in July 2015. All tables in the database were exported in MS Excel and transferred to my encrypted laptop to bring back to the UK. Data were initially cleaned in MS Excel and tables imported into MS Access, enabling more advanced management, query design and linking of data.

A data analysis plan was developed before the study began, detailing the main variables of interest and process of analysis. This was used to guide the analysis.

6.8.2 Merging datasets from multiple studies

MS Access was used to merge datasets; this was particularly useful when combining participants from existing studies to one main WMS data table.

The statistical software used for analysis requires the data to be in a 'relation', that is a rectangular dataset; each column representing a variable e.g. height, and each row a case, or participant e.g. WMS6001X. To ensure data for each participant were in the correct row, I designed a query in MS Access with a 'spine template' containing the maximum number of participant study IDs, and therefore the maximum number of rows, sorted in alphabetical order. Each time a variable needed to be added to the main relation, it could be added to the query, which connected all variables to the 'spine' list of IDs, and finally copied and pasted into the statistical software.

6.8.3 General statistical analysis

Statistical analyses were conducted using the statistical software Data Desk 6.3.1 (Data Description Inc., Ithaca, NY). GraphPad Prism 6 and MS Excel were used to produce figures.

Statistical significance was set at a probability value (*p*-value) ≤0.05. If the null hypothesis can be rejected (i.e. that there is no difference between groups) then $p \leq 0.05$, indicating that the likelihood of this result occurring by chance is less than or equal to one in twenty. For all continuous variable, scatterplots and histograms helped to initially identify the distribution and obvious outliers. Several plots (Leverages, E-studentized residuals and DIFFITs) were inspected for normality, identifying potential outliers and their overall effect on the regression equations, see **Figure 6:19** for examples from the cross calibration of the two DXA instruments.

Descriptive statistics of normally distributed variables are presented as mean ± standard deviation (SD). If variables were positively skewed, data were normalised by transforming to natural logarithms (Ln). For non-parametric data the median and IQR are presented as the 25th and 75th percentile; within which 50% of values fall. The mean of the study sample, provides an estimate of the true population mean.

The standard deviation is a measure of the variability of data points around the mean. One SD includes 68% of the distribution of values. If the SD is high relative to the mean then there is a lot of variation. It is calculated by:

$$\boldsymbol{SD} = \sqrt{\frac{\sum(\boldsymbol{x}-\bar{\boldsymbol{x}})^2}{\boldsymbol{n}-\mathbf{1}}}$$

$$\boldsymbol{SE} = \frac{\boldsymbol{SD}}{\sqrt{\boldsymbol{n}}}$$

$$\mathbf{CV} = \left(\frac{\mathbf{SD}}{\bar{\mathbf{x}}}\right) \times \mathbf{100}$$

The standard error (SE) is used when comparing the difference between the means of two groups, and indicates the precision of the mean i.e. how well the sample mean predicts the population mean. A large SD results in a larger SE and a larger sample size results in a smaller SE and therefore a more precise estimate of the true mean.

To measure linear associations between continuous variables, correlation coefficients were calculated using Pearson's product moment correlation. If one or more of the variables was categorical then Spearman's rank correlation was used.

6.8.4 Significance tests

Between-group differences in categorical variables, i.e. variables that can include several different, distinct categories e.g. marital status and food groups, were calculated using Chi-squared tests. For normally distributed continuous variables, i.e. a variable with a quantitative value, measured using a defined scale and unit e.g. weight (kg), unpaired t-tests or Scheffé post hoc tests from ANOVA were used to determine significant differences between rural and urban groups. If data were non-parametric or not normally distributed, comparisons of group means were done using the Mann-Whitney U test.

6.8.5 Statistical analysis of bone data

In accordance with recommendations from International Society for Clinical Densitometry (ISCD), bone data are presented as follows: bone mineral density (BMD) to 3 decimal places, bone mineral content (BMC) and Bone Area (BA) to 2 decimal places.

Data Desk contains linear model software, ANOVA with Scheffé post hoc tests were used to look at the difference between means of size adjusted BMC (SA-BMC), by including the binary rural/urban variable in the model as an independent variable.

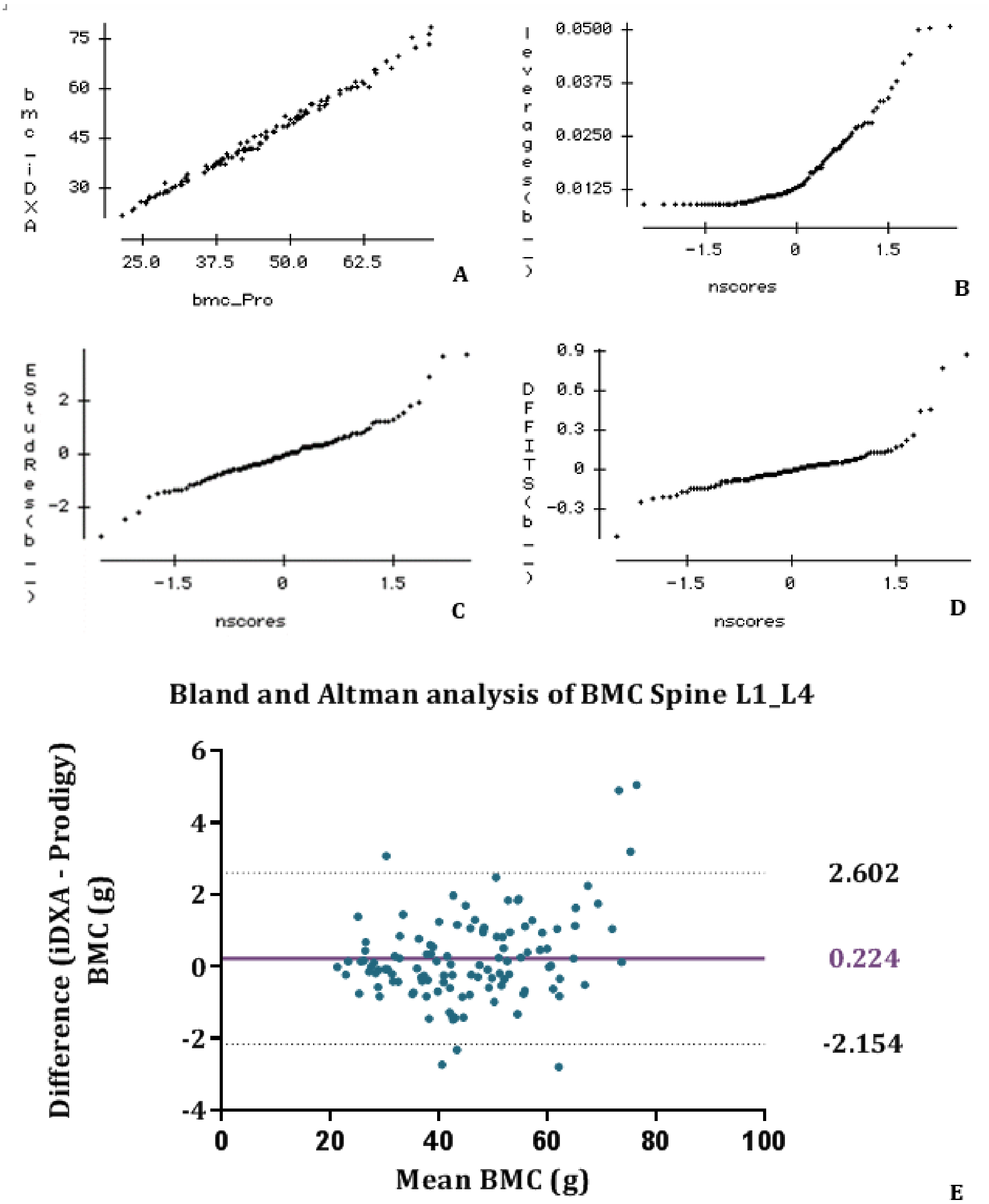


Figure 6:19 Example plots from lumbar spine BMC. Scatterplot of iDXA and Prodigy (A) and diagnostic plots for linear regression analysis: leverages (B), E-Studentized residual (C), DFFits (D), and Bland and Altman plots (E) showing mean bias and upper and lower limits of agreement (± 2SD).

6.8.6 Calculating size–adjusted BMC using linear model

A significant limitation of DXA is that it is unable to provide a true volumetric density measure of bone or tissue depth, and therefore only ever provides an estimation of bone mineral within a projected bone area. Density is calculated by dividing mass by volume. The DXA parameter areal BMD (aBMD) is derived by dividing BMC by BA, therefore assuming that both variables are directly proportional. This assumption would mean that a 1% increase in BA would result in a 1% increase in BMC. However, often this is not the case and there is no theoretical basis for this assumption.

To obtain the correct power relationship, variables can be converted to natural logarithms (ln) and the power coefficient calculated by regression analysis of BMC on BA. In their critique of aBMD, Prentice et al. (Prentice *et al.*, 1994) state that there are variations in the power relationships between population and skeletal site. In addition, there are associations between aBMD with body size. They proposed the use of multiple linear regression analysis with BMC as the dependent variable and BA, weight and height as independent variables; the authors refer to this as 'size-adjusted BMC', in order to compare individuals of different bone and body size. The use of logged variables enables the interpretation of proportional relationships between independent variables e.g. weight and dependent variables e.g. BMC. The regression coefficients can be read as a percentage change in the independent variable, for example using the analysis presented below in **Figure 6:20 (A)**, a 1% change in BA_{SPINE} is associated with a 1.2% increase in BMC_{SPINE}. Additionally, the use of logged variables enables results from the Scheffé post hoc analysis to be interpreted as a sympercent.

The WMS study involves the comparison of rural and urban groups, **Figure 6:20 (B)** shows results of the multiple linear regression for SA-BMC_{SPINE} as an example. The difference between urban and rural groups can be expressed as 6.2 % ± 2.1 p<0.004, calculated by multiplying the coefficients by 100 and the difference assumed to be symmetrical (Cole, 2000).

A

DESIGN

Dependent variables

Name	**Code**
ln-bmc-spine2	l-2

Type of analysis: OLS ANOVA

Factors

Name	**Code**	**Nested in**	**F/R**	**Kind**
ln-Weight	l-W	()	Fix	Cont
ln-ba-spine2	l-2	()	Fix	Cont
ln-Height	l-H	()	Fix	Cont

Partial (Type 3) Sums of Squares

Interactions up to 1 - way

No Modifications

RESULTS

General Results

140 total cases of which 4 are missing

ANOVA

Analysis of Variance For **ln-bmc-spine2**
No Selector
140 total cases of which 4 are missing

Source	**df**	**Sums of Squares**	**Mean Square**	**F-ratio**	**Prob**
Const	1	2158.2	2158.2	158.76e3	≤ 0.0001
l-W	1	0.362569	0.362569	26.671	≤ 0.0001
l-2	1	0.779657	0.779657	57.353	≤ 0.0001
l-H	1	12.01e-6	12.01e-6	883.49e-6	0.9763
Error	132	1.79439	0.0135939		
Total	135	4.11364			

Results for factor l-2

Coefficients

Coefficients of: ln-bmc-spine2 on l-2

Covariate	**Coefficient**	**std. err.**	**t Ratio**	**prob**
l-2	1.205	0.1591	7.573	≤ 0.0001

B

RESULTS

General Results

140 total cases of which 4 are missing

ANOVA

Analysis of Variance For **ln-bmc-spine2**
No Selector
140 total cases of which 4 are missing

Source	**df**	**Sums of Squares**	**Mean Square**	**F-ratio**	**Prob**
Const	1	2158.2	2158.2	168.04e3	≤ 0.0001
l-W	1	0.220247	0.220247	17.148	≤ 0.0001
l-2	1	0.858163	0.858163	66.816	≤ 0.0001
l-H	1	0.00260887	0.00260887	0.20312	0.6530
R-U	1	0.111863	0.111863	8.7096	0.0038
Error	131	1.68253	0.0128437		
Total	135	4.11364			

Results for factor R-U

Coefficients

Coefficients of: ln-bmc-spine2 on R-U

Level of R-U	**Coefficient**	**std. err.**	**t Ratio**	**prob**
Rural	-0.03098	0.0105	-2.951	0.0038
Urban	0.03098	0.0105	2.951	0.0038

Expected Cell Means

Scheffe Post Hoc Tests

	Difference	**std. err.**	**Prob**
Urban - Rural	0.0619555	0.02099	0.00375159

Figure 6:20 Example of SA-BMC using a linear model in Data desk

6.8.7 Deriving summary statistics of size-adjusted BMC from linear model

This process was followed with rural and urban data combined.

i. Calculate the mean lnBMC

ii. Set up linear model for size adjustment of lnBMC (lnBA, lnWt, lnHt), as described in **Section 0**.)

iii. Compute residuals from the model and 'evaluate' to make a new variable

iv. Residuals are in natural logs, so must be transformed

v. Set up a derived variable of mean lnBMC + residuals

vi. Evaluate this to obtain lnSABMC for each individual

vii. Antilog the lnSABMC (e^ lnSABMC)

viii. Conduct summary statistics using this new variable

6.8.8 Precision and accuracy

Table 6:2 provides an overview of the terms precision and accuracy. Precision of DXA and pQCT are described in Section 6.3.

Table 6:2 Overview of precision and accuracy

	Precision	Accuracy
Definition	The degree to which a variable has nearly the same value when measured several times	The degree to which a variable actually represents what it is supposed to represent
How to measure	Comparison among repeated measurements	Comparison with a reference standard
Significant to study	Increase power to detect effects	Increase validity of conclusions or diagnosis
Types of errors	Random errors (caused or contributed by the observer, the subject, and the instrument)	Systematic errors (contributed by the observer, the subject, and the instrument)

(Njeh and Shepherd, 2004)

In order to improve precision several methods are generally used:

i. Standardisation of measurements: a detailed protocol with specific instructions ensures uniformity in the measurements made.

ii. Training operators/observers: will improve the consistency of techniques used.

iii. Refine the instruments: variability can be reduced by refining the instrument

iv. Automate the instrument: devices or software can be automated in order to improve precision, compared to human observer. It is important to confirm that the automatic process is relatively precise.

v. Repetition: Random error can be reduced by repeating measurements and using the mean of all measurements made.

Similar methods to those described above for precision, are used to improve accuracy, in addition to:

i. Blinding: an example of a double blind experiment is where neither the observer nor the subject know (Njeh and Didier, 2004)

ii. Calibrating the instrument: accuracy of instruments, particularly those that are mechanical or electrical can be improved by regular calibration using a gold standard (Njeh and Didier, 2004).

6.9 Questionnaires

All staff involved in the study spoke both English and several of the local languages. This enabled questions to be asked in the participant's local language and translated back to English to be recorded on the form. For the screening and health questionnaires, if I was present I usually asked the questions and the field assistant translated the question and response for me. I held two training sessions, one with staff administering the health questionnaire and one with the dietary field staff who were administering the physical activity questionnaire (PAQ). They attempted to complete the PAQ during the first day of the dietary assessment and this then provided me with time to review it and send it back to be completed or to follow up a particular question if there was any uncertainty. Both were then piloted with several local women.

6.9.1 Health and SES questionnaire

The health and socio-economic questionnaire was based primarily on the routinely used questionnaire included in other nutrition and bone studies in The Gambia. Additional questions were included to capture behaviours related to urbanisation; these were based on previous research in The Gambia by Siervo et al. (Siervo *et al.*, 2006). The questionnaire included demographic data (age, migration details), socio-economic data (education, occupation, and western influence), general health and reproductive history, supplement use and fracture. These were administered during the measurement visit to MRCG Keneba or Fajara.

6.9.2 Physical activity questionnaire (PAQ)

The PAQ was based on the validated sub Saharan Africa physical activity questionnaire (Sobngwi *et al.*, 2001). It is a tool that was developed and validated in both rural and urban Cameroon populations, against objectively measured activity assessed using an accelerometer and heart rate monitor.

During a process of discussions with local field staff in The Gambia it was refined and adapted to capture occupational, farming and gardening activities in the wet and dry season, household, and leisure time activities. A list of typical activities common for Gambia women also provided additional guidance (Lawrence *et al.*, 1985).

7 RESULTS I

Results are presented in the following three chapters. **Results I** comprises demographic, socio-economic and lifestyle characteristics of 139 (81 rural and 58 urban migrant) participants. Subsequent results chapters then address the two main hypotheses. Results II focuses on urban-rural differences in bone phenotype, anthropometry, body composition, and plasma and urinary biochemistry (including vitamin D status). Results III encompasses detailed analysis of food and nutrient intakes.

CHARACTERISTICS OF STUDY PARTICIPANTS

Consort diagrams (**Figure 7:1**;**Figure 7:2; Figure 7:3**) detail the study flow from potential participants to those included in the final analyses. Details of eligibility criteria and the recruitment process are summarised in **Section 4.9** and **Sections 4.11.6** (rural) and **4.11.7**. (urban)

7.1 Location and distribution of study participants

Figure 7:4 shows a map of The Gambia with approximate urban and rural study areas, and details of the number of participants living within each district in the urban area, and village in the rural area. The urban study location encompassed four districts (**Table 7:1**). The majority of participants were from three urban districts: Kanifing Municipal Council (KMC), Kombo North (KN), and Kombo Central (KC). The rural study area included five villages in Kiang West; four of which are classified as "core villages", having been involved in demographic and research studies since 1950.

Table 7:1 Details of local government areas and districts included in the study

Local Government Area	District Name	District Population	Main towns of participants
Kanifing	Kanifing Municipal Council (KMC)	382,096	Tallingding & Fagikunda
Brikama	Kombo North (KN)	344,756	Busumbala & Brufut
	Kombo Central (KC)	142,831	Brikama & Kembujeh
	Kombo South (KS)	108,773	Tanjeh & Yundum
Mansakonko	Kiang West (KW)	14,953	Keneba, Manduar & Jali

KMC, KN, and KC are the most urban areas, with high population densities and amenities.

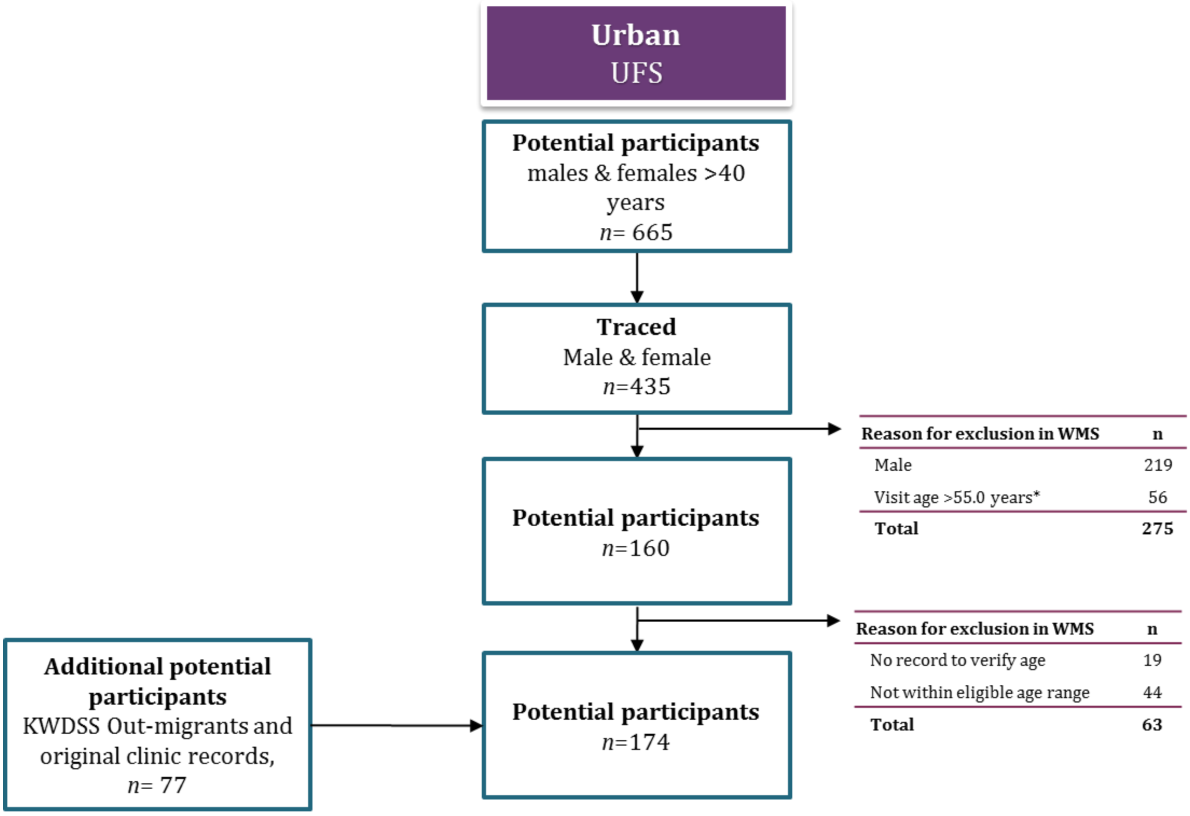


Figure 7:1 Flow diagram of WMS sampling frame for the urban migrant group.

Included and excluded paritipcants primarily identified through the 'UFS' feasibility study. Key informants identified potential urban migrants from every household of 'Core' villages and attempted to trace them; additional potentials were added due to insufficient numbers from out migrants more recently capture in the KWDSS, and also from participants contained in old clinic record database but no longer in KWDSS, these included potentials from three additional villages to the 'Core' villages. KWDSS, Kiang West Demographic Surveillance System; UFS, urban feasibility study; WMS Women's Migration Study (PhD project).

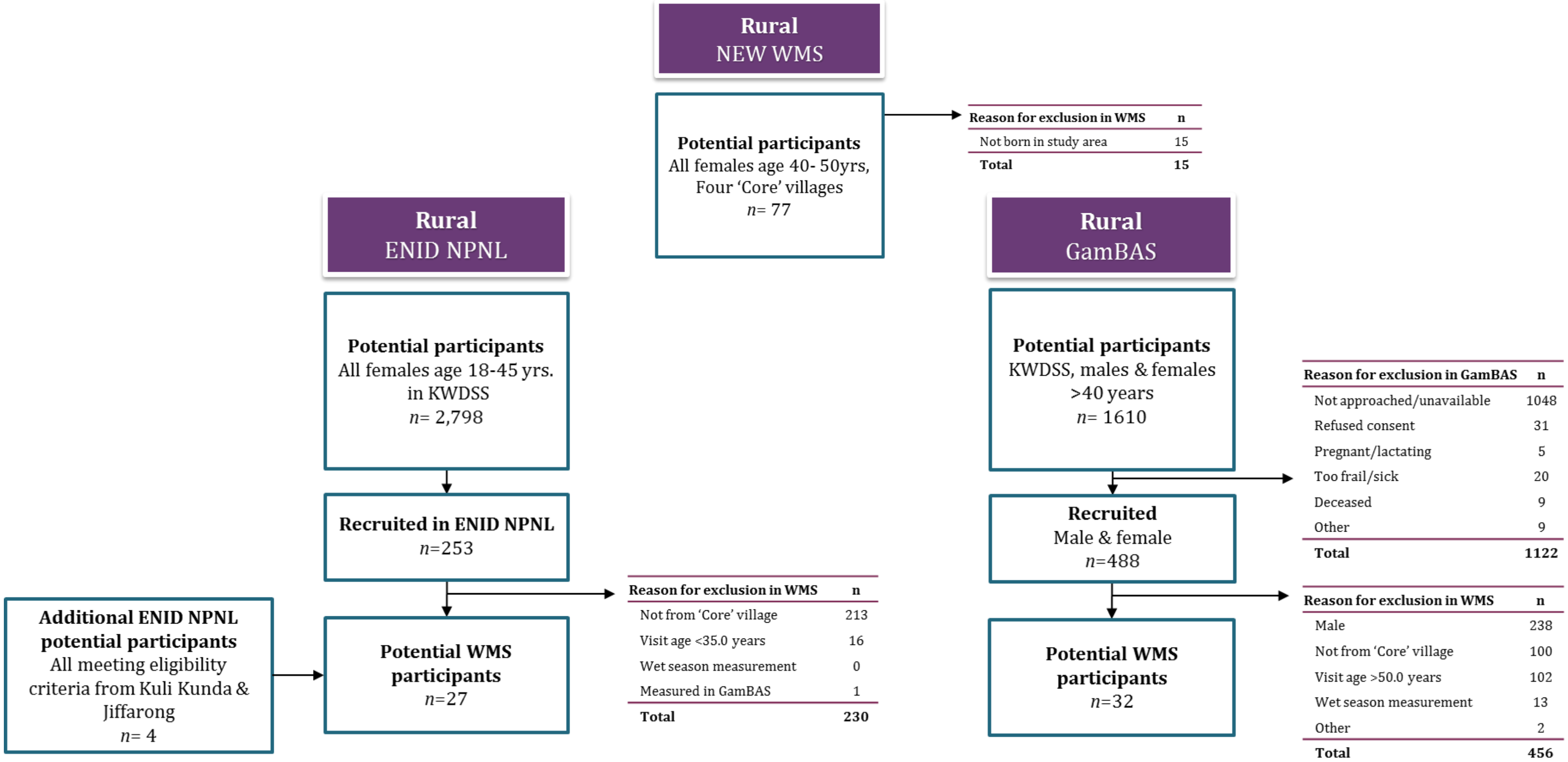
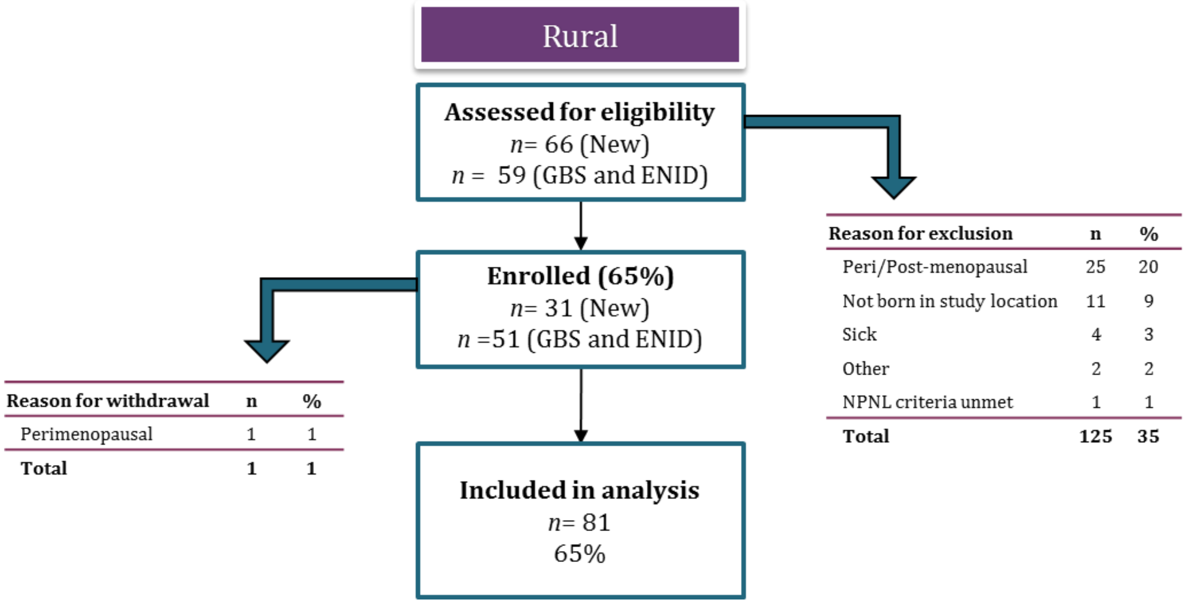


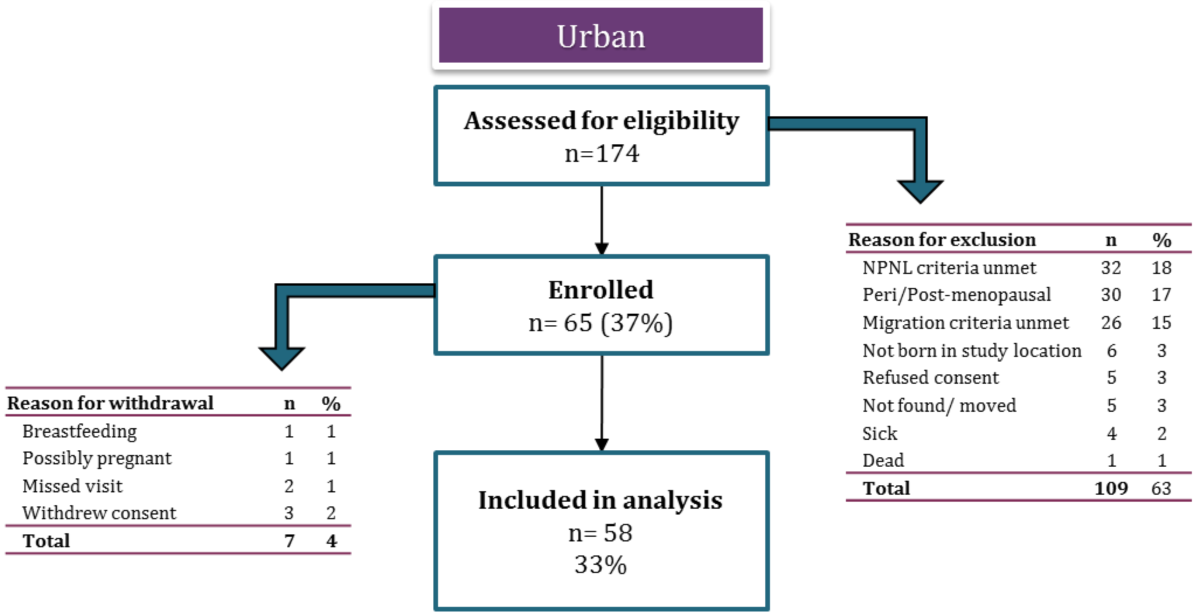
Figure 7:2 Flow diagram of WMS sampling frame for the rural comparison group.

Included and excluded paritipcants from two studies ENID NPNL and GamBAS, in addition to a new sample of potential rural women. ENID NPNL, Early Nutrition and Immune Development non-pregnant non-lactating; GamBAS, Gambian Bone Ageing Study; KWDSS Kiang West Demographic Surveillance Survey; WMS Women's Migration Study.



Reason for exclusion	n	%
Peri/Post-menopausal	25	20
Not born in study location	11	9
Sick	4	3
Other	2	2
NPNL criteria unmet	1	1
Total	**125**	**35**

Reason for withdrawal	n	%
Perimenopausal	1	1
Total	**1**	**1**



Reason for exclusion	n	%
NPNL criteria unmet	32	18
Peri/Post-menopausal	30	17
Migration criteria unmet	26	15
Not born in study location	6	3
Refused consent	5	3
Not found/ moved	5	3
Sick	4	2
Dead	1	1
Total	**109**	63

Reason for withdrawal	n	%
Breastfeeding	1	1
Possibly pregnant	1	1
Missed visit	2	1
Withdrew consent	3	2
Total	**7**	**4**

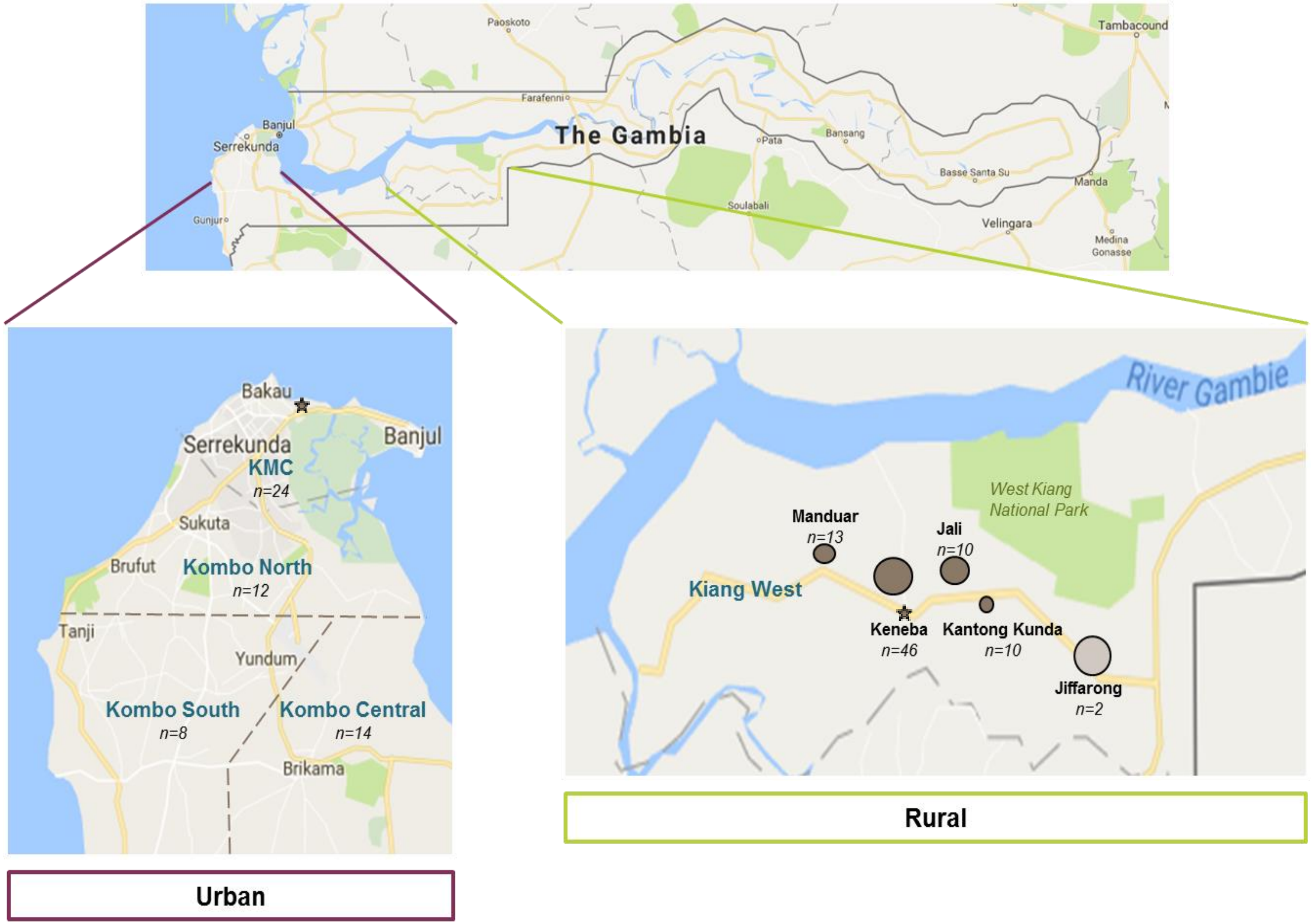
Figure 7:3 Consort diagram: flow of participants in rural and urban groups

Figure 7:4 Map of study locations. Number and distribution of study participants at district level in urban area and village level in rural area.

Circles indicate the size of the village.

● = Core villages, where health and demographic studies have been conducted since 1950.

★= MRCG Fajara (urban) and MRCG Keneba (rural)



7.2 Demographic and socioeconomic characteristics

All women were premenopausal as this was a criterion of participating in the study. Women were asked at screening about current menstruation; several questions were used to ascertain whether amenorrhea was due to pregnancy, lactation, or having reached the menopausal transition. Questions were repeated within the health questionnaire on the measurement day, to reconfirm status. Several women were excluded at the time of the clinic visit, as their response to these questions changed. **Figures 7:1; 7:2, 7:3** provide an overview of reasons for exclusion and withdrawal from the study. Although not a specified criterion, the majority of participants were of Mandinka ethnicity, a result of choosing villages known to be predominantly Mandinka.

There was no significant difference in age between groups (p=0.3). The median age at migration for urban women was 18.5 years (IQR: 16.5 to 21.9), with an average of 23.8 years (IQR: 18.9 to 28.6) spent in the urban environment. The majority of women had at least one co-wife; a significantly higher proportion of urban women had no cowives (48.3%), compared to rural women (18.5%) (p<0.002).

Table 7:2 Summary of age, migration details, and marital status

	Rural n=81	Urban n=58	*p*-value
Age (years)	43.5 (41.3 to 45.5)	44.9 (39.5 to 47.0)	0.3
Age at migration (years)	NA	18.5 (16.5 – 21.9)	NA
Urban years (years)	NA	23.8 (18.9 to 28.6)	NA
Currently married	96.3%	94.8%	0.7
Co-wives			
None	18.5%	48.3%	**0.002**
One	48.1%	34.5%	**0.002**
Two	28.4%	12.1%	**0.002**
Three	4.9%	5.2%	**0.002**
Live with husband	90.1%	89.7%	0.7

***p*-values from Mann Whitney non-parametric test for skewed data and chi-squared tests**

Almost 10% (n=13) of rural women had more than 6 years of Quranic education, compared to only 4% (n=3) of urban women ($p<0.002$). More urban women had at least one year of government primary school education ($p \leq 0.0001$), but the median years was very low for both. Both groups had family members overseas, and a similar proportion (~40%) received remittance support. Rural and urban women had similar levels of employment (~18% $p=0.8$), the majority of urban women were self-employed as street vendors and one in a groundnut factory. Rural women were mostly either cleaners or cooks. Urban households (defined as those sharing food) tended to be larger (15 IQR: 10 to 20.5) compared to rural (9 IQR: 7 to 15, $p<0.007$).

Rural compounds were more likely to have fruit trees; similar proportions of rural and urban compounds had a garden. The majority of rural compounds owned animals (76.5%), with goats, and hens the most commonly owned. Of those who had animals, 20% owned donkeys and 27% owned cows. Fewer urban compounds owned animals (42.1%), comprised mostly of hens; in contrast to rural compounds donkeys were not owned by any urban compound.

There were several significant differences in household assets; more urban households had electricity, fridge, freezer, television, radio, or car, compared to rural ($p<0.05$). Mobile phone ownership was similar between groups (~80% $p>0.05$); only two urban women reported access to the internet.

There were no significant differences in age at menarche, parity or number of children breastfed ($p>0.05$). Median parity was rural 8 and urban 7 ($p>0.05$). A greater proportion of rural women were currently on, or had previously used hormonal contraception. Neither rural nor urban women reported smoking or drinking alcohol. Similar numbers of women (~20%) stated that they had received a diagnosis for hypertension. While more urban women (5%) said they had been diagnosed with diabetes, compared to rural (n=1%).

Table 7:3 Education, income and occupation

	Rural n=81	Urban n=58	*p*-value
Government school education (≥1year)	3.7%	27.6%	**0.004**
Years of government education	0 (0 to 0)	0 (0 to 1)	**≤0.0001**
Quranic education	67.9%	37.9%	**0.002**
Quranic education (years)	3 (0 to 5)	0 (0 to 3)	**0.0007**
Paid employment	17.3%	19.0%	0.8
Family overseas	59.3%	74.1%	0.07
Remittance support from family overseas (n %)	40.3%	40.7%	0.96

Values presented are median and (IQR); *p*-values from Mann Whitney non-parametric test for skewed data and chi-squared tests.

Table 7:4 Description of households, compounds, and assets

	Rural n=81	Urban n=58	*p*-value
Household size	9 (7 to 15)	15 (10 to 20.5)	**0.007**
Fruit trees in compound	98.8%	86.2%	**0.003**
Number of fruit trees	5 (3 to 9)	4 (2 to 8)	**0.4**
Garden in compound	25.9%	32.8%	0.4
Animals owned by compound	76.5%	42.1%	**≤0.0001**
Electricity	4.9%	69.0%	**≤0.0001**
Fridge	3.7%	24.1%	**0.0003**
Freezer	1.2%	22.4%	**≤0.0001**
Television	3.7%	62.1%	**≤0.0001**
Mobile phone	76.5%	82.8%	0.4
Internet	0%	3.5%	0.09
Radio	67.9%	84.5%	**0.03**
Cart	46.9%	5.2%	**≤0.0001**
Bicycle	64.2%	74.1%	0.2
Motorcycle	6.2%	13.8%	0.1
Household car/truck	7.4%	25.9%	**0.003**

Values presented are median and (IQR); responses are presented as %. *p*-values from Mann Whitney non-parametric test for skewed data and chi-squared tests

Table 7:5 Reproductive history, lactation, and health

	Rural n=81	Urban n=58	*p*-value
Age at menarche	14.6 ± 1.1	14.9 ± 1.0	0.08
Parity	8 (6 to 9)	7 (6 to 9)	0.2
Number of children breast fed	7 (6 to 9)	7 (5 to 8)	0.05
Current hormonal contraception use	8.6%	8.8%	0.98
Past hormonal contraception use	43.2%	24.1%	**0.02**
Current or past use of injectable contraception	35.9%	19%	**0.03**
Smoking	0%	0%	NA
Alcohol	0%	0%	NA
Diagnosed hypertension	22.5%	17.2%	0.5
Diagnosed diabetes	1.3%	5.2%	0.2
Diagnosed Tuberculosis	2.5%	1.7%	0.8

Values presented are mean ± SD, or median and (IQR). *p*-values from Mann Whitney non-parametric test for skewed data and chi-squared tests.

Table 7:6 Comparison of farming and gardening activities

	Rural n=81	Urban n=56	*p*-value
Farmed in last 12 months	89.4%	10.6%	**≤0.0001**
Farm for own consumption	93.7%	62.5%	**0.003**
Shelling groundnuts	46.9%	23.2%	**0.005**
Gardened in last 12 months	82.7%	67.9%	**0.04**
Gardened at home	11.1%	25.0%	**0.03**
Collected leaves	63.0%	64.3%	0.9
Washed salt	83.7%	0%	**≤0.0001**

***p*-values from chi-squared tests**

Results presented in **Table 7:6** and **Table 7:7** are a comparison of farming and gardening activities. A significantly lower proportion of urban women had farmed in the previous 12 months, 11% compared to 89% of rural women (p<0.0001). The majority of rural women were farming rice (94%) and to a lesser extent sorghum and beans. Most crops were produced for own consumption, although the proportion was higher in the rural group (94%), compared to urban (63%).

Table 7:7 Crops, leaves and vegetables grown

	Rural n=81	Urban n=56	*p*-value
Crops			
Rice	93.8%	5.4%	<0.0001
Maize	6.2%	3.6%	0.5
Groundnuts	8.6%	7.1%	0.8
Beans	13.6%	7.1%	0.2
Sorghum	22.2%	1.8%	0.0007
Millet	3.7%	1.8%	0.5
Leaves			
Kereng-kerengo (Corchorus olitorius Linn.)	34.6%	35.7%	0.9
Kucha (Hibiscus sabdariffa Linn.)	33.3%	13.3%	**0.01**
Morongo (Amaranthus caudatus Linn.)	45.7%	37.5%	0.3
Nana (Mentha spicata)	0%	19.6%	**<0.0001**
Vegetables			
Aubergine	29.6%	30.4%	0.9
Bitter tomato	30.9%	32.1%	0.9
Cabbage	21.0%	14.3%	0.3
Carrots	6.2%	0%	**0.06**
Cassava	17.3%	12.5%	0.4
Chilli pepper	8.6%	12.5%	0.5
Cucumber	1.2%	1.8%	0.8
Okra	50.6%	28.6%	**0.01**
Onions	80.2%	44.6%	**<0.0001**
Pepper	14.8%	12.5%	**0.7**
Pumpkin	6.2%	1.8%	**0.2**
Tomato	37.0%	32.1%	**0.6**

***p*-values from chi-squared tests**

A significantly lower number of urban women were gardening compared to rural. Both groups grew several varieties of vegetables; the most common were onions, okra, tomato and several types of leaves. Urban women grew less kucha leaf (sorrel), but more nana (mint) leaf. Nana leaf can be found in the bush, and is likely to be foraged by rural women. It is typically added to the green tea 'attaya', which the urban women consumed more of. The majority of gardening took place in communal gardens and not at home, although a significantly higher proportion of urban gardening took place in home-based gardens (25%, p=0.03). Approximately two-thirds of rural and urban women reported collecting leaves over the past 12 months; in addition, 84% of rural women stated that they washed

local salt; this activity was not done by anyone in the urban group.

The results of several questions about leisure activities are presented in **Table 7:8**, the data highlight that urban women were more likely to watch videos (urban 77%, rural 9%, $p\leq0.0001$) and listen to music (urban 43%, rural 19%, p=0.002). Other popular activities in both groups were meeting friends, drinking Attaya (sweetened green tea) and plaiting hair. Walking for leisure was mentioned by the majority of rural women (62%) and a significant number of urban women (48%, p=0.1). Only one rural woman reported doing sport.

Table 7:8 Leisure activities

	Rural n=81	Urban n=58	*p*-value
Watch videos	8.6%	76.8%	**<0.0001**
Listen to music	18.5%	42.9%	**0.002**
Traditional dance	17.3%	16.1%	0.9
Sport	1.2%	0%	0.4
Leisure walking	61.7%	48.2%	0.1
Drink Attaya	69.1%	66.1%	0.7
Brew Attaya	25.9%	35.7%	0.2
Plaiting hair	42.0%	55.4%	0.1
Crocheting	24.7%	19.6%	0.5
Reading or writing	33.3%	32.1%	0.9
Teaching at home	23.5%	10.7%	0.06
Tie dye	3.7%	1.8%	0.5
Soap making	29.6%	19.6%	0.2
Meeting friends	74.1%	87.5%	0.06
Singing	4.9%	3.6%	0.7
Play musical instrument	0%	5.4%	**0.04**

***p*-values from chi-squared tests**

SUMMARY OF PARTICIPANT CHARACTERISTICS

Both groups were of a similar age and had comparable socio-demographic backgrounds (e.g. education level and literacy); however, urban women lived in larger households and had greater access to electricity. Significantly, fewer urban women farmed in the past twelve months, perhaps indicating lower physical activity. They also reported lower household ownership of livestock. Both rural and urban groups grew a variety of vegetables and leaves; rice was the predominant cereal grown, and this was mostly by rural women.

Parity was similar in both groups, but the urban group reported a lower use of hormonal contraception. The prevalence of diagnosed hypertension was similar around ~15-20%.

8 RESULTS II

BONE MINERAL STATUS, BIOCHEMISTRY, ANTHROPOMETRY, AND BODY COMPOSITION

8.1 Bone mineral status (DXA)

Table 8:1 shows results for bone parameters measured by DXA and **Figure 8:1** urban-rural differences. The urban group had significantly higher BMC and aBMD at all skeletal sites ($p<0.05$); BA was not significantly different. The greatest difference in BMC was found at the lumbar spine, mean ± SE (8.5 ± 3.0 %, $p<0.0005$), a biologically meaningful difference, equivalent to 0.76 of rural SD. *T*- Scores were also calculated using a young adult (white, female) reference population, mean *T*- scores were -1.03 and -0.22, for rural and urban groups respectively.

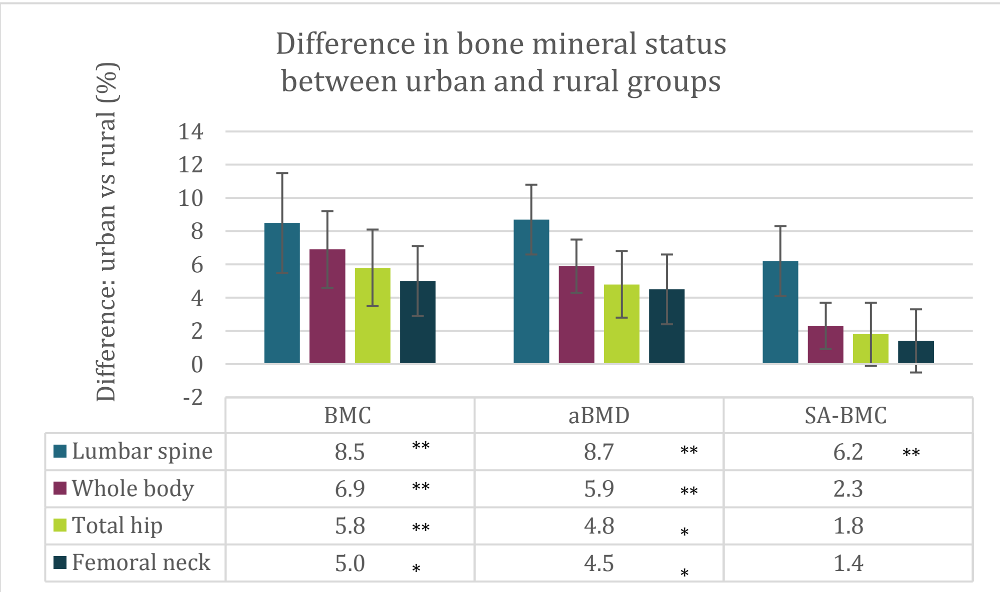


Figure 8:1 Bone mineral status measured by DXA (* $p \leq 0.05$, ** $p \leq 0.01$)

After adjusting for size (height, weight and BA), group differences in whole-body and hip BMC were mostly attenuated (p>0.05), but the difference between urban and rural spine BMC remained significant (6.2 ± 2.1 %, *p*<0.01).

Table 8:1 Summary statistics of DXA-derived bone densitometry data

	Rural n=81	Urban n=58	% difference ±SE Urban - Rural	*p*-value
Total body ǂ				
BMC (g)	2116 ± 244	2277 ± 341	6.9± 2.3	**0.003**
Area (cm^2)	1998 ± 126	2020 ± 155	1.0 ±1.2	0.4
aBMD (g/cm^2)	1.059 ± 0.093	1.125 ± 0.116	5.9 ± 1.6	**0.0004**
SA-BMC (g)	2151 ± 165	2194 ± 174	2.3 ± 1.4	0.1
Lumbar spine*				
BMC (g)	52.77 ± 8.97	57.18 ± 9.18	8.5 ± 3.0	**0.005**
Area (cm^2)	49.88 ± 4.48	49.78 ± 4.44	-0.2 ± 1.6	0.9
aBMD (g/cm^2)	1.051 ± 0.127	1.147 ± 0.140	8.7 ± 2.1	**0.0001**
SA-BMC (g)	52.83 ± 5.79	55.83 ±6.60	6.2 ± 2.1	**0.004**
Total hip				
BMC (g)	28.16 ± 3.68	29.87 ± 4.07	5.8 ± 2.3	**0.01**
Area (cm^2)	28.75 ± 1.94	29.04 ± 1.80	1.1 ± 1.1	0.3
aBMD (g/cm^2)	0.979 ± 0.109	1.028 ± 0.123	4.8 ± 2.0	**0.02**
SA-BMC (g)	28.58 ±2.96	29.03 ±3.07	1.8 ± 1.9	0.4
Femoral neck ¥				
BMC (g)	4.40 ± 0.48	4.63 ± 0.57	5.0 ± 2.1	**0.02**
Area (cm^2)	4.59 ± 0.28	4.61 ± 0.32	0.4 ± 1.2	0.7
aBMD (g/cm^2)	0.961 ± 0.102	1.006 ± 0.123	4.5 ± 2.1	**0.04**
SA-BMC (g)	4.43 ± 0.87	4.73 ± 0.99	1.4 ± 1.9	0.5

Values presented are mean ± SE, percentage difference is sympercent difference %

Differences between groups were tested with Scheffe post hoc tests from ANCOVA models with continuous variables in natural logarithms

SA-BMC= size-adjusted BMC (derived by including height, weight and bone area in the loge- loge linear model; evaluating the residual for each participant; adding the residual to lnBMC for each participant; evaluating the mean and calculating the antilogarithm).

***rural: n=80 urban: n=56; ǂ urban: n=56; ¥ rural: n=63 urban=56**

8.2 Anthropometry

There was no significant difference in standing height between groups, however rural women had a lower sitting height (*p*<0.001). Urban women were significantly heavier (*p*≤0.001) with a between group difference of 13.6 %. Over half (56%) of urban women were classified as overweight or obese according to their BMI; 11% of rural women were underweight and 3% of urban women. Both waist and hip circumferences were significantly greater in the urban group ($p \leq 0.0001$). **Table 8:2** shows summary statistics for anthropometry and derived variables including the proportions of participants within each BMI category. The majority of both groups had normal BMI; however, a large proportion were overweight or obese; 30% of rural participants and 56% of urban, of those classified as obese, 3% and 7%, were severely or morbidly obese (BMI ≥35), rural and urban respectively.

Total fat mass (from DXA) was significantly higher in the urban group, 27.0 (18.3 to 35.4) kg $p < 0.0001$ compared to rural 17.4 (13.7 to 23.2) kg (**Table 8:2**). Fat mass in the android region showed the greatest between-group difference; urban women had significantly greater android fat (54.1 ± 11.6 %). There was no significant difference in total lean mass between groups ($p > 0.05$). Difference in body weight could be attributed to higher body fat in urban women, reflected in greater central adiposity indicated by both DXA derived fat mass and measured waist circumference.

Table 8:2 Summary of anthropometry

	Rural (n= 81)	Urban (n= 58)	Difference (U-R) S % ± SE	*p*-value
Height (cm)	160.6 ± 5.8	162.0 ± 6.1	0.8 ± 0.6	0.2
Weight (kg)	58.3 (51.6 to 67.3)	67.7 (55.3 to 79.4)	13.6 ± 3.4	**0.0005**
BMI (kg/m^2)	22.1 (20.2 to 26.0)	25.6 (21.7 to 30.9)	11.8 ± 3.3	**0.0007**
Underweight	11%	3%		0.1
Normal	59%	41%		**0.04**
Overweight	21%	28%		0.4
Obese	6%	21%		**0.01**
Severely or morbidly obese	2%	7%		0.2
Waist circumference (cm)ǂ	72.8 ± 8.9	81.8 ± 12.0	11.3 ± 2.2	**≤0.0001**
Hip circumference (cm)*	87.2 ± 9.3	97.2 ± 10.7	10.9 ± 1.9	**≤0.0001**
Waist: Hip ratio (cm/cm)	0.83 ± 0.06	0.84 ± 0.06		0.5
Sitting height (cm)	80.2 (77.5 to 82.3)	82.1 (79.7 to 84.4)		**0.0004**
Sitting: Stand height (cm)	0.50 (0.48 to 0.51)	0.51 (0.50 to 0.52)		**0.002**

Values presented are Median (IQR), *p*-values from Mann Whitney non-parametric test and chi-square for proportions of underweight BMI <18.5; overweight BMI ≥25.0; obese ≥30.0; severely obese BMI ≥35.0; morbidly obese BMI ≥40.0. Significant difference between groups is shown by a bold *p*-value (p≤0.05). ǂ *n*=57 urban; 81 rural * *n*= 57 urban; 72 rural

Table 8:3 Summary of body composition measured by DXA

	Rural (n=80)	Urban (n=56)	*p*-value	Difference (U – R) S % ± SE	*p*-value
Fat mass (kg)	17.4 (13.7 to 23.2)	27.0 (18.3 to 35.4)	**≤0.0001**	34.7 ± 6.8	**≤0.0001**
Body fat (%)	30.3 (26.1 to 36.3)	39.2 (33.7 to 44.3)	**≤0.0001**		
Lean mass (kg)	38.1 (34.6 to 41.6)	39.4 (35.5 to 42.6)	0.1	3.4 ± 2.3	0.1
Android fat mass (kg)	0.8 (0.5 to 1.4)	1.6 (0.9 to 2.6)	**≤0.0001**	54.1 ± 11.6	**≤0.0001**
Gynoid fat mass (kg)	3.4 (2.7 to 4.5)	4.3 (3.4 to 5.8)	**0.0003**	24.7 ± 6.3	**≤0.001**

Values presented are Median (IQR). Significant difference between groups is shown by a bold *p*-value (*p*≤0.05). Differences between groups were tested with Scheffe post hoc tests from ANOVA models.

8.3 Vitamin D status

There was a trend toward lower vitamin D status in the urban group, although not statistically significant. All participants had concentrations of 25 OHD above 25nmol/L, while 11% and 14% of rural and urban women respectively, had concentrations <50nmol/L. There was no significant relationship between BMI or percent body fat and vitamin D status (*p*=0.4).

Table 8:4 Plasma 25 OH vitamin D status

	Rural n=81	Urban n=57	*p*-value	Range
25 OH vitamin D (nmol/L)	68.3 ± 15.7	64.0 ± 14.2	0.1	32.4 – 106.5
< 50 (nmol/L)	11%	14%	0.6	45.5 – 122.0

Values are Mean ± SD

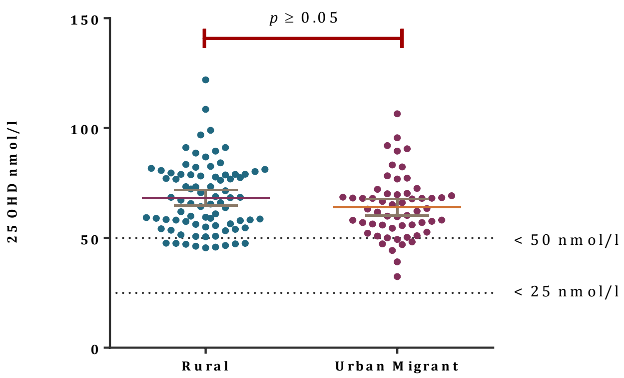


Figure 8:2 Comparison of plasma 25 OH vitamin D status

8.4 Plasma and urine biochemistry

Results of biochemistry analyses are presented in this section, including plasma and urinary clinical chemistry (**Table 8:5** and **Table 8:6**) and vitamin D status (**Table 8:4** and **Figure 8:2**). There were no significant differences in plasma biochemistry, except for total alkaline phosphatase (TALP), which was higher in rural participants (*p*=0.04). Estimated glomerular filtration rate (eGFR) calculated from plasma creatinine, was not different between rural and urban groups, with all values lying within the normal range. Urinary creatinine was higher in the urban group (*p*=0.03), while magnesium and potassium and excretion was higher in the rural group ($p \leq 0.0001$, adjusted for creatinine). Sodium excretion was not significantly different, and is also analysed and presented as salt (sodium chloride) in **Section 9.3.4**. The ratio of sodium to potassium was significantly higher in the urban group ($p<0.01$). While there was no difference in urine pH.

Table 8:5 Plasma biochemistry

	Rural *n*=81	Urban *n*=57	*p*-value
Calcium (mmol/l)	2.22 ± 0.08	2.23 ± 0.08	0.6
Phosphorus (mmol/l)	1.09 ± 0.15	1.06 ± 0.15	0.2
Creatinine (μmol/l)	66.6 ± 8.40	67.5 ± 8.01	0.5
TALP (mmol/l)*†	89.1 (69.8 to 101.1)	78.1 (64.5 to 92.4)	**0.04**
Albumin (g/l)	33.9 ± 2.5	34.0 ± 2.9	0.9

Values are mean ± SD for normally distributed data; values for skewed data (denoted by *) are median (IQR); † rural n=80. *p*-values from Mann Whitney non-parametric test for skewed data.

Table 8:6 24hr urinary electrolyte and mineral excretion

	Rural *n*=71	Urban *n*=58	*p*-value
Creatinine (mmol/24h)*	5.4 (4.3 to 7.3)	6.3 (5.4 to 8.0)	**0.03**
Calcium (mmol/24h)*	0.74 (0.32 to 1.55)	1.05 (0.53 to 1.68)	0.1
Calcium: Creatinine (mol/mol)*	0.13 (0.06 to 0.27)	0.16 (0.11 to 0.28)	0.4
Phosphorus (mmol/24h)*	9.18 (5.72 to 11.7)	9.73 (7.84 to 12.5)	0.2
Phosphorus: Creatinine (mol/mol)	1.62 (1.30 to 1.98)	1.57 (1.32 to 2.02)	0.95
Magnesium (mmol/24h)	2.50 (1.57 to 3.5)	1.81 (1.23 to 2.71)	**0.01**
Magnesium: Creatinine	0.43 (0.33 to 0.62)	0.30 (0.22 to 0.41)	**≤ 0.0001**
Potassium (mmol/24h)	17.9 (12.0 to 28.1)	14.9 (12.4 to 19.8)	**0.02**
Potassium: Creatinine (mol/mol)*	3.51 (2.5 to 5.01)	2.46 (2.11 to 2.98)	**≤ 0.0001**
Sodium (mmol/24h)*	98.0 (62.6 to 133.5)	112.3 (72.4 to 145.7)	0.2
Sodium: Creatinine (mol/mol)*	17.7 (12.9 to 26.5)	17.5 (14.0 to 21.1)	0.5
Sodium: Potassium (mol/mol)	5.8 ± 3.0	7.0 ± 2.2	**0.003**
eGFR (mL/min)#	99.3 (88.8 to 105.2)	99.6 (89.7 to 103.4)	0.5
Urine pH	6.20 ±0.62	6.21 ± 0.47	

Values are mean ± SD for normally distributed data; values for skewed data (denoted by *) are median (IQR); *p*-values from Mann Whitney non-parametric test for skewed data. # Estimated Glomerular filtration rate was calculated using CKD-EPI formula, see Methods 6.7.5 for equation.

SUMMARY

Both groups were of similar height and had good vitamin D status. Urban women were much heavier, with significantly higher body fat but similar lean mass, as measured by DXA. After adjusting for body size (weight, height and bone area), the urban-rural difference was no longer significant at the hip and whole body. Difference in SA-BMC ranged from 1.4 to 1.8% at the hip and femoral neck, 2.3% for the whole body; and a significant difference of 6.2% in SA-BMC was found at the lumbar spine. Bone area and weight explained the most variance in BMC at the spine. Adjusting for percent body fat did not attenuate the urban-rural difference, but increased it to 7.2%.

9 RESULTS III

Food and nutrient intakes

9.1 Introduction

Dietary data collected from a prospective two-day weighed intake assessment are presented in this chapter, along with salt intakes calculated from 24hr urinary sodium excretion. **Section 9.2** explores dietary intakes, comparing rural and urban groups in terms of food consumption (food groups, sauces, and staples), overall dietary diversity, and frequency and portion size of foods, especially those related to the nutrition transition (dairy products, meat, bread, fat, and sugar). **Section 9.3** presents data on nutrient intakes, focussing on foods contributing important bone forming nutrients (Ca, P, Mg, and K). A description of SED food groups is included in the development of the dietary assessment in **Section 5.4.3**.

9.2 Dietary intake patterns: Food intake and dietary diversity

9.2.1 Overview of food consumption

Table 9:1 shows a summary of SED food groups consumed by both urban and rural participants, ranked in order of frequency. **Figure 9:1** presents the proportion of participants consuming each of the twelve food groups; unsurprisingly, all participants consumed starchy staples. In order to describe individual foods consumed in each food group, the four staple SED food groups ('Rice', 'Breads and pasta', 'Starchy roots and tubers', and 'Other grains') were combined to one group, 'Starchy staples', and these are described in detail in **Sections 9.2.3**. A breakdown of the foods consumed in the remaining eleven food groups is in **Section 9.2.6**. (**Figure 9:6**; **Figure 9:8**; **Figure 9:7**; **Figure 9:9**). As the diets of both urban and rural groups are largely rice-based with various sauces, the frequency of sauces consumed is also presented in **Section 9.2.5**.

Table 9:1 Ranking of major food groups according to frequency of consumption

	Rural	Urban
1	Starchy staples	Starchy staples
2	Groundnuts and other legumes	Sugar, sugary drinks and snacks
3	Fish and shellfish	Fish and shellfish
4	Fats and oils	Fats and oils
5	Green leafy vegetables and leaves	Other vegetables
6	Sugar, sugary drinks and snacks	Groundnuts and other legumes
7	Fruit	Green leafy vegetables and leaves
8	Other vegetables	Miscellaneous
9	Miscellaneous	Milk and milk products/dishes
10	Nuts and seeds	Nuts and seeds
11	Milk and milk products/dishes	Fruit
12	Meat, offal, poultry and eggs	Meat, offal, poultry and eggs

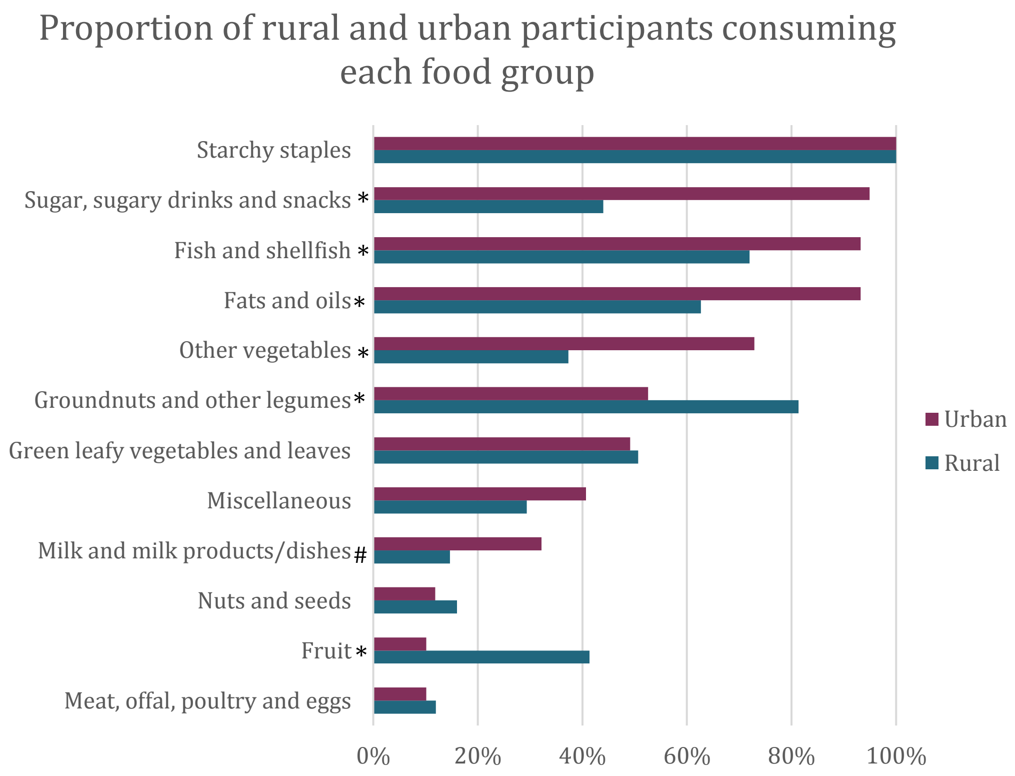


Figure 9:1 A comparison of participants in rural and urban groups consuming food groups across 2-day dietary intake. Staple food groups are combined. * p ≤ 0.01, # p ≤ 0.05 from chi-squared tests.

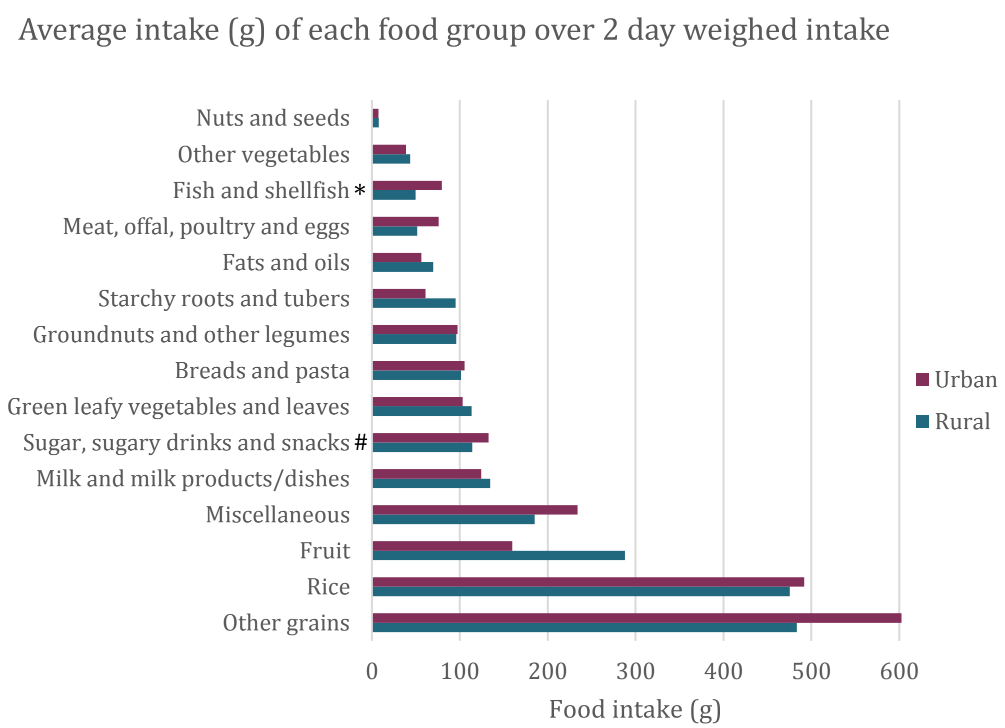


Figure 9:2 A comparison of mean intakes of 15 food groups, staples are shown separately. * p ≤ 0.01, # p ≤ 0.05 from Mann Whitney tests

9.2.2 Dietary diversity score

Figure 9:3 shows the frequency of dietary diversity scores. The mean dietary diversity score was similar in both groups, rural 4.9 and urban 5.3 (*p*=0.06). However, a larger proportion of rural participants consumed four or less food groups across the two-day dietary assessment, rural 37% and urban 24%. A larger proportion of urban participants (40%) consumed six or more food groups, compared to rural (32%). The mode was five food groups in both urban and rural areas.

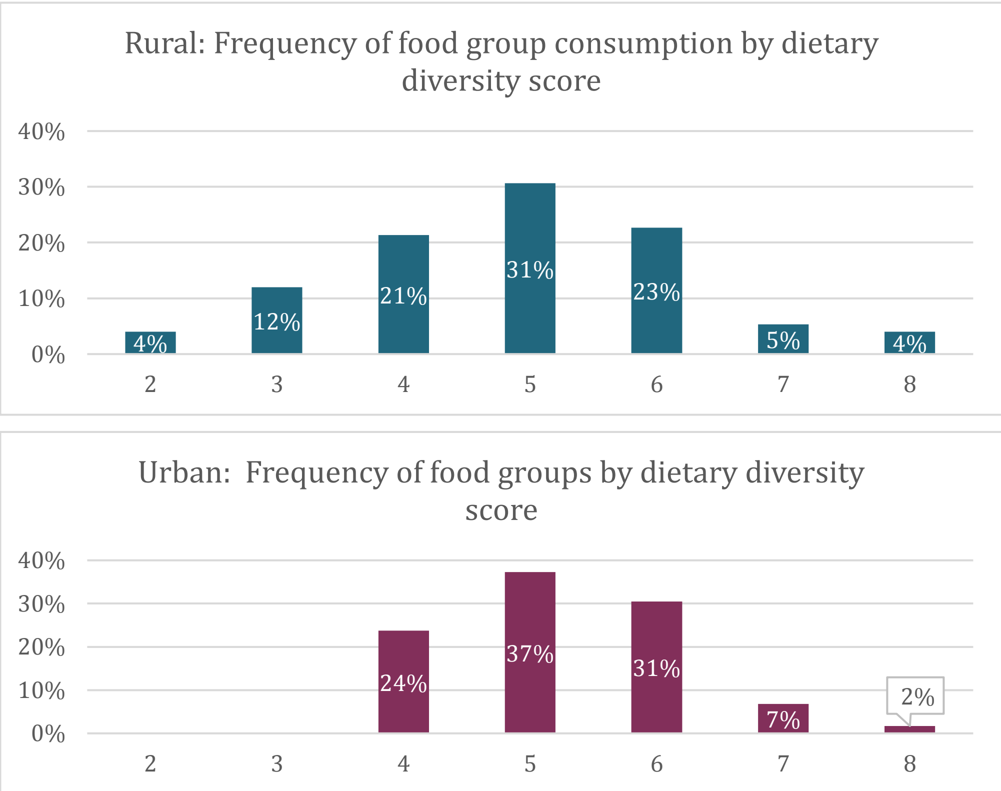


Figure 9:3 A comparison of the frequency of dietary diversity scores in rural and urban groups, based on the consumption of ten SED groups (including staples as one group, and excluding sugar and miscellaneous food groups).

9.2.3 Staple consumption

Rice was the most commonly consumed food, eaten by all participants on both days of dietary assessment. The average portion was approximately 500g; this was similar in both areas, rural 473g and urban 492g (**Figure 9:2**). **Figure 9:4** describes the percentage of participants consuming each staple, and frequency of consumption. Rice was most frequently consumed as boiled rice. Consumption of 'Other grains', comprised mostly of millet, was similar, rural 43% and urban 41%. Starchy roots and tubers, typically cassava was consumed by 33% of rural participants, and 27% of urban. Bread consumption was much higher among urban participants, with 71% of urban migrants consuming bread, versus 23% of rural women.

9.2.4 Porridge consumption

Figure 9:4 shows the frequency of consumption of different porridges. Fifty three percent of urban participants consumed porridge and 59% of rural. Millet was the most commonly consumed porridge in the urban area, comprising 57% of porridges, while 35% of porridges were based on rice. In contrast, rice-based porridges were most common in the rural area, comprising 63% of porridges consumed, while millet represented 35% of porridges. Maize and wheat porridges were less frequently consumed; wheat accounted for 2% of rural porridges, while maize and wheat, each accounted for 4% of all porridges eaten by urban participants.

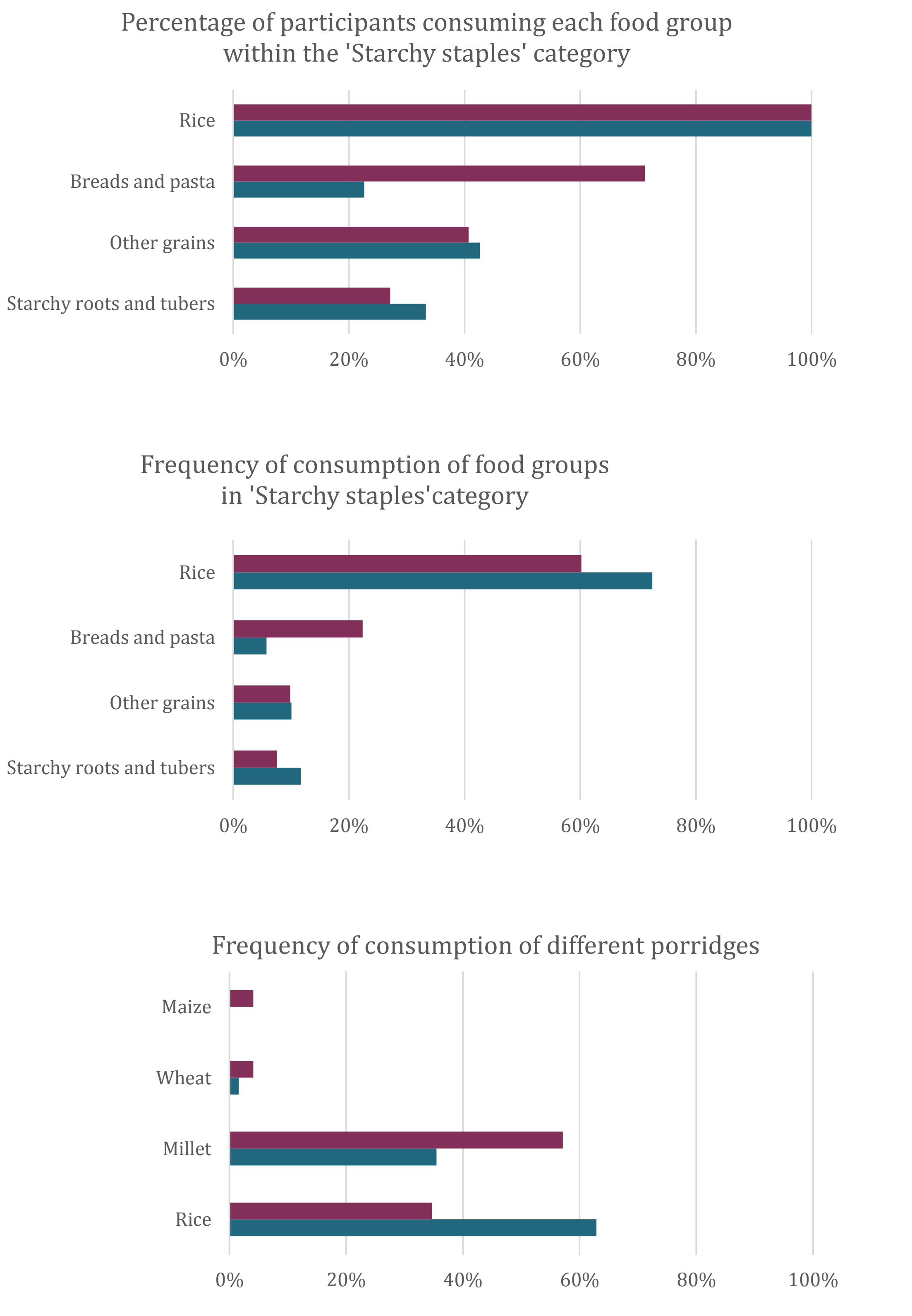


Figure 9:4 Percent frequency of staple foods

9.2.5 Frequency of sauce consumption

Oil stew (palm or vegetable oil) was the most frequently consumed sauce in both rural and urban groups, yet they represented a greater proportion of sauces consumed by urban participants (55%) compared to rural (34%). Groundnut sauces were eaten almost as frequently as oil stews by rural participants (32%), but only represented one fifth of all sauces consumed by urban participants.

Almost one quarter of sauces eaten by rural participants were based on leaves, while these only comprised 10% of sauces consumed by urban participants. The least frequently consumed sauces were flour and others. Other sauces included an okra and palm oil sauce (super kanjo), a rice dish with rice cooked in oil stew (benechin), a watery sauce made from stock (dajiwo), and a fish and palm oil soup (ebbeh).

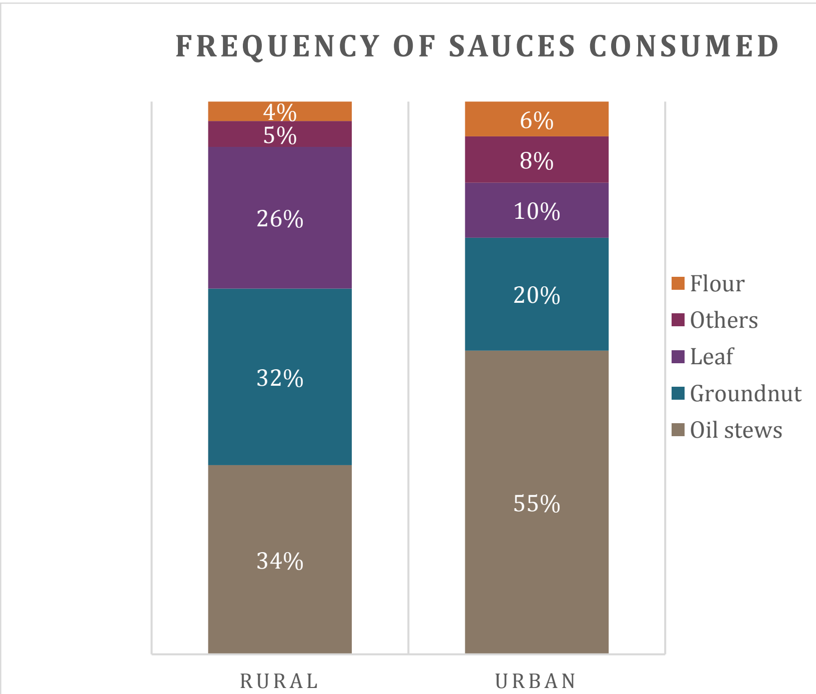


Figure 9:5 Frequency of sauces consumed by rural and urban groups

9.2.6 Food consumption within each SED food group

Figures 7:6 to **7:9** present a breakdown of foods consumed within each of the twelve food groups, except for starchy staples, which have already been described in **Section 9.2.3**. Due to the number of food items within the food group 'green leafy vegetables and leaves', this group has been collapsed to sets of similar sauces, and not to individual food items.

9.2.7 Groundnuts and legumes; nuts; fruits and vegetables

'Groundnuts and other legumes' includes a variety of groundnut sauce (locally called 'Tia Durango'), as well as groundnuts consumed as a snack. Fewer urban participants (53%) consumed foods from this group, compared to 81% rural (p=0.0004).

Nuts were consumed infrequently, with 16% of rural participants consuming kola nuts (*Sterculiaceae cola vera*) and 12% of urban participants. Only one rural participant reported consuming cashew nuts.

Fruit consumption ranked higher among rural participants, while the 'Other vegetables' group featured more frequently among the urban women. The proportions of rural and urban women consuming 'Green leafy vegetables and leaves' were similar; however, individual foods eaten within the group were different. A variety of green leaves are often added to sauces. Rural participants were more frequently consuming groundnut sauce with added leaves, whereas a greater number of urban women had a separate portion of cooked leaves served alongside their meal, typically hibiscus 'Kucha'. There are also leaf-based sauces 'Jambo' and 'Kucha Durango'; rural participants more commonly ate these. A detailed comparison of sauces consumed follows after results of staple food consumption are presented.

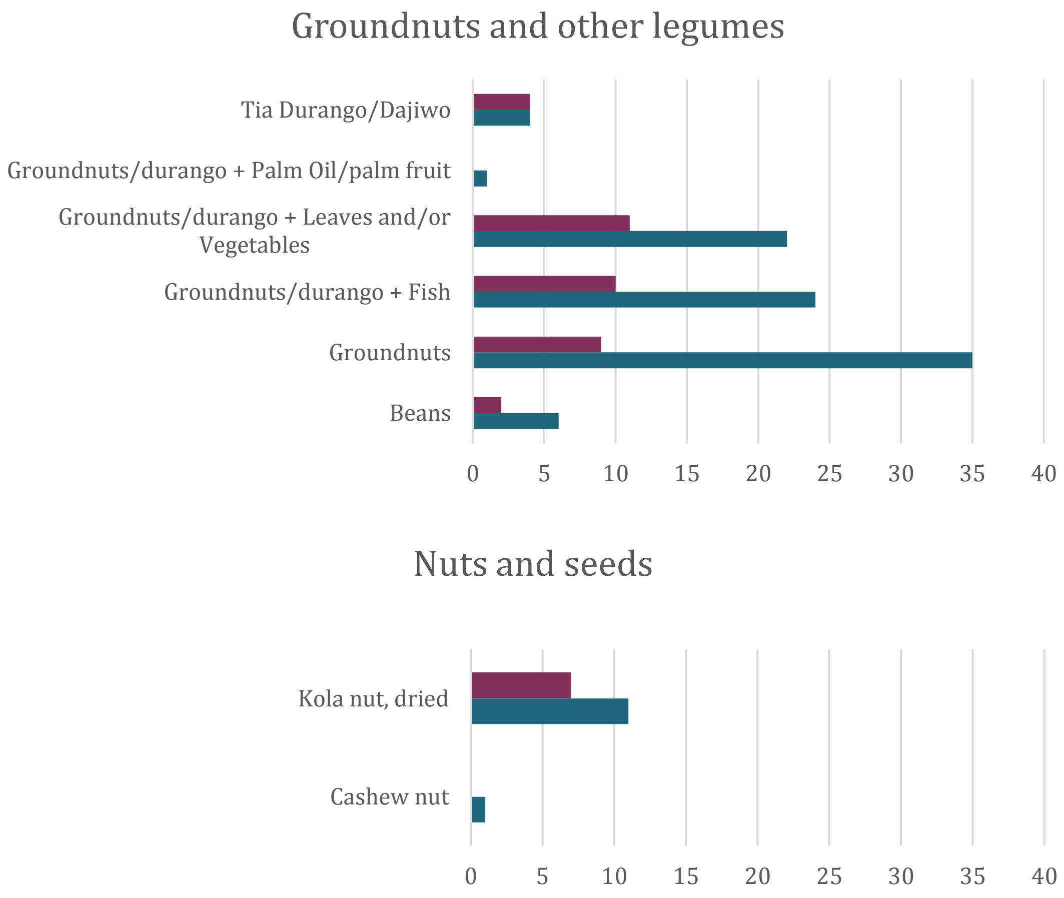


Figure 9:6 Distinct count of participants consuming food items within food groups

■ Urban ■ Rural

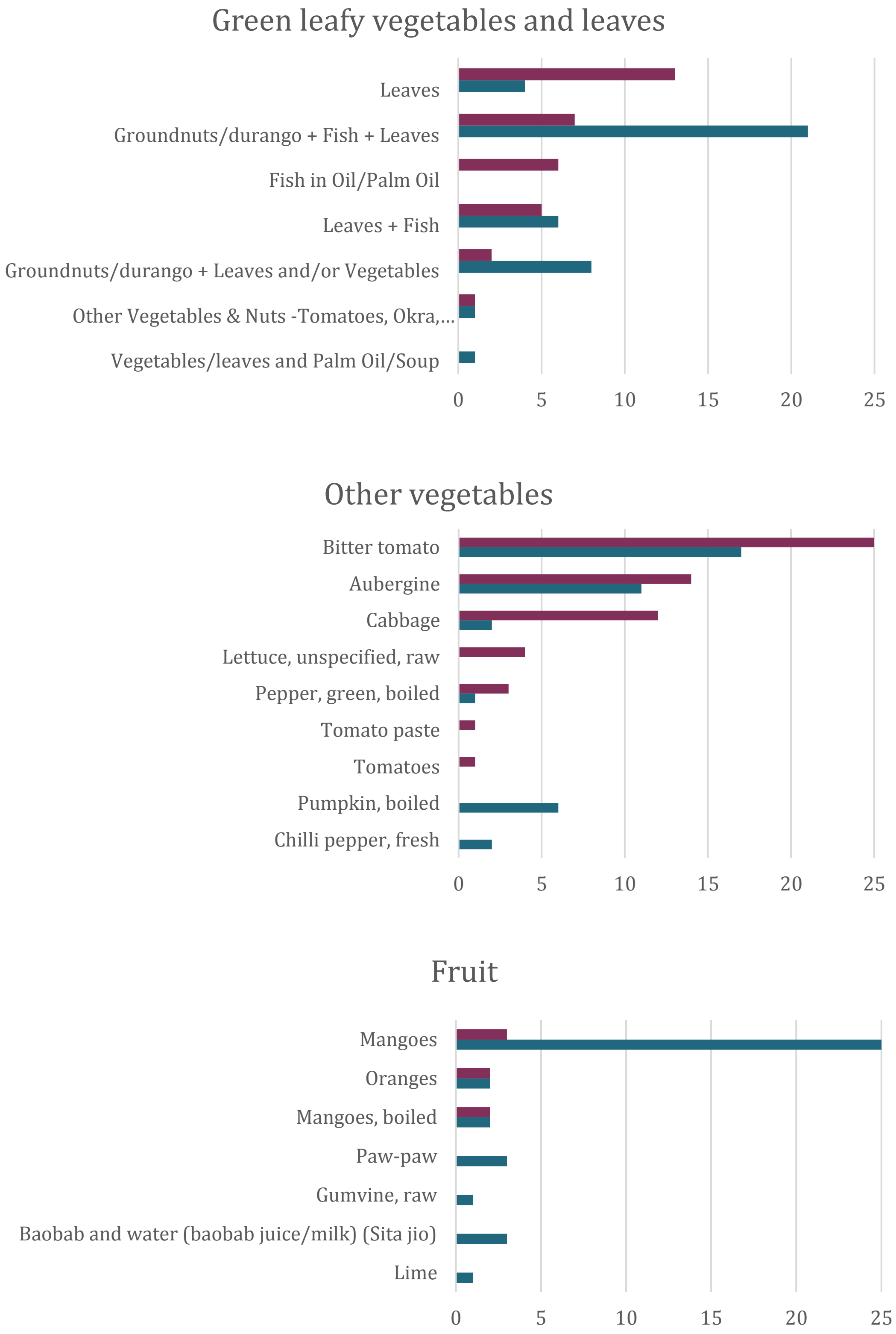


Figure 9:7 Distinct count of participants consuming food items within food groups

■ Urban ■ Rural

9.2.8 Fish, and animal foods and products

'Meat, offal, poultry and eggs' was the least consumed food group in both rural and urban areas, with 12% and 10% respectively eating small portions of chicken or meat (goat and beef). On the other hand, fish were frequently consumed. The food group 'Fish and shellfish' includes mostly varieties of fish coded as a separate item, fish were typically added to various sauces, particularly groundnut sauces, see **Figure 9:6.** Across both groups, the majority of women consumed fish as a separate item, 95% of urban women and 72% of rural women (p=0.002). The portion size of fish and other food items is explored in more detail in **Section 9.2.10**.

'Milk and milk products/dishes' were consumed by a greater proportion of urban women, 32% versus 15% of rural (p=0.02). Fresh milk was only consumed on one occasion in both rural and urban groups. Sour milk was most popular for both groups, typically added to porridge. Milk, either dry or tinned was consumed with tea. Only one rural participant consumed sweetened condensed milk. However, as previously mentioned, sugar is always added to tea, if available. For a breakdown of foods consumed in this food group, see **Figure 9:8.**

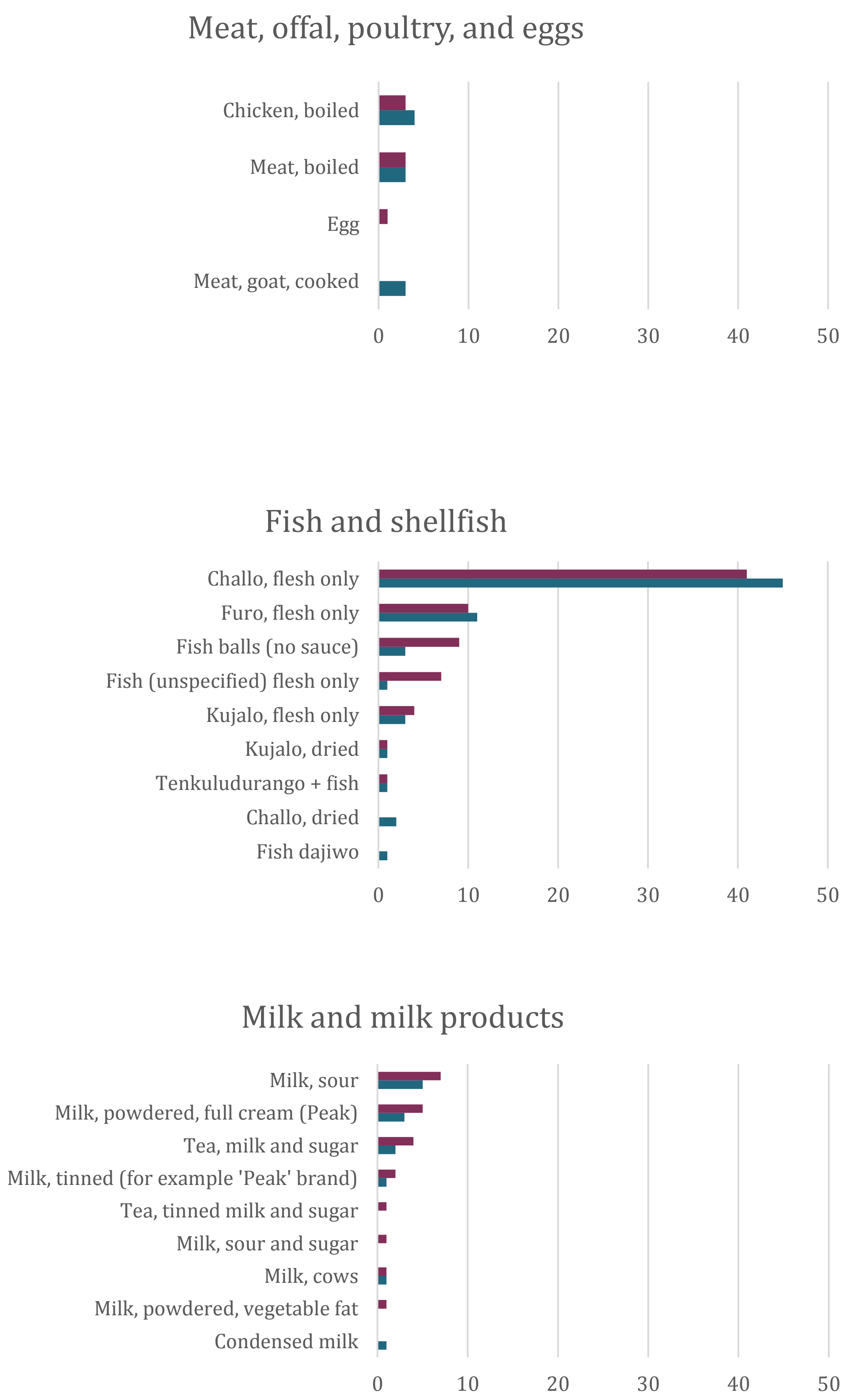
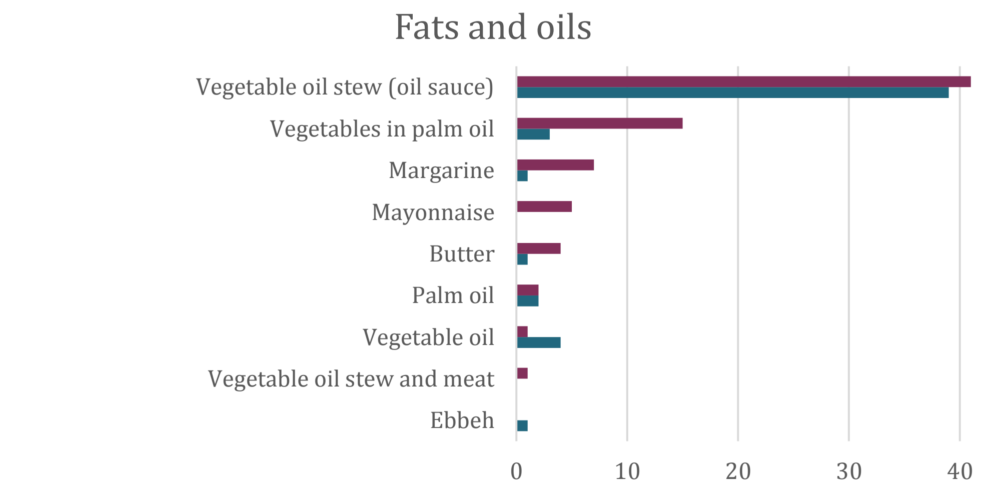


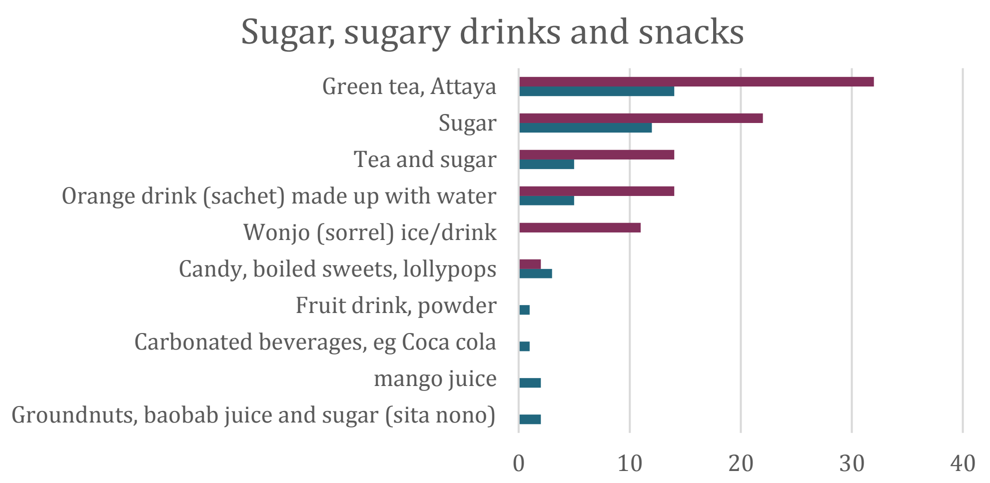
Figure 9:8 Distinct count of participants consuming food items within food groups

Urban Rural

9.2.9 Fats, oils and sugar

Ninety-five percent of urban participants consumed 'Sugar, sugary drinks and snacks', compared to 44% of rural ($p \leq 0.0001$). The most frequently consumed foods were green tea (locally called 'Attaya'), typically brewed with a lot of sugar; and sugar added to black tea and porridge. **Section 9.2.6** (**Figure 9:9**) includes a breakdown of foods consumed. A greater proportion of urban women consumed 'Fats and oils' ($p \leq 0.0001$), 93% compared to 63%; reflected in the types of sauces that were most frequently consumed, described in **Section 9.2.5** (**Figure 9:5**).





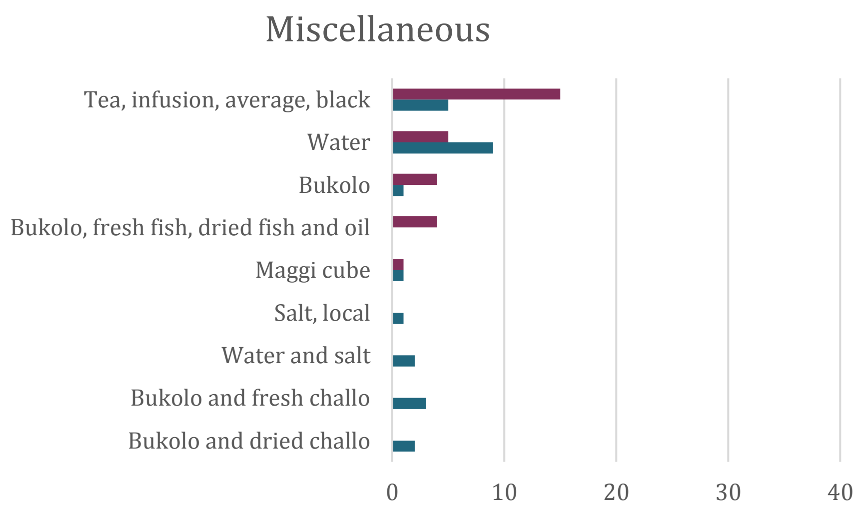


Figure 9:9 Distinct count of participants consuming food items within food groups

■ Urban ■ Rural

9.2.10 Portion size comparison

A selection of foods characteristic of the nutrition transition are highlighted in **Table 9:2.** The urban group had larger portions of fresh fish, almost double the mean rural portion. The number of occasions that fish, meat, and poultry were eaten were similar in both groups, with urban portions larger. Although, it is important to consider that the urban group had fewer participants, therefore the number of occasions eaten relative to the number of participants is higher for urban than rural. Portions of sour milk tended to be larger in the rural group, but not frequently consumed in either group.

Table 9:2 Comparison of foods associated with the nutrition transition, frequency (count of occasions eaten) and portion size

	Rural		Urban	
	Count	**Portion (g)**	**Count**	**Portion (g)**
Fish and shellfish				
Challo, flesh only	78	48 ± 25	70	83 ± 36
Furo, flesh only	15	47 ± 25	13	77 ± 39
Meat, offal, poultry and eggs				
Chicken, boiled	4	61 ± 33	5	70 ± 36
Meat, boiled	3	32 ± 6	4	93 ± 40
Milk and milk products/dishes				
Milk, powdered, full cream	3	20 ± 14	7	18 ± 15
Milk, sour	6	171 ± 63	8	125 ± 45
Milk, cows	1	150	1	56
Sugar, sugary drinks and snacks				
Green tea, Attaya	22	63 ± 31	56	81 ± 15
Sugar	14	36 ± 17	34	42 ± 19
Wonjo (sorrel) ice/drink	0	-	13	161 ± 28

Portion presented as mean ± SD, where count equals one the mean is presented only.

SUMMARY OF DIETARY INTAKES

Rice featured as the predominant staple, eaten by all rural and urban participants on both days during the dietary assessment. There was a marked difference in bread consumption, with much higher intakes among urban participants. Urban migrants also consumed more oil-based sauces, primarily more palm oil stew. Alongside this, urban participants consumed more sugar-containing foods, particularly sugar added to green tea 'Attaya' and black tea. More urban participants consumed dairy foods, and this was supported by data from the FFQ, not presented here. Urban participants more frequently consumed fish as an individual item, and the portion size of fish was much larger; meat consumption was rare in both groups. The rural group consumed more fruit and vegetables, the sum of the three groups: 'Fruit', 'Other vegetables', and 'Green leafy vegetables and leaves' was rural 445g and urban 302g.

9.3 Nutrient intakes

Nutrient intake results include urban-rural comparisons of energy, macronutrients (carbohydrates, protein, and fat), micronutrients (calcium, phosphorus, iron, zinc, vitamin C, carotene, potassium, and magnesium), calcium to phosphorus ratio, dietary fibre, and phytate. Data are presented in tables and figures below; **Table 9:3** includes an overview of energy and macronutrient intakes, and shows percentage contributions of macronutrients to energy intake. **Table 9:4** includes intakes of micronutrients, dietary fibre, phytate, and salt.

9.3.1 Energy and macronutrients (fat, protein and carbohydrate) intakes

Energy intakes were low in both groups, urban 1763 ± 525 kcal and rural 1709 ± 481 kcal. Carbohydrates contributed most to energy intakes, urban 67% and rural 65% ($p>0.05$); fat was the second largest contributor, with urban slightly higher than rural ($p>0.05$). Intakes of protein were also higher in the urban group, 55.4 ± 18.4g/d compared to 51.4 ± 14.9g/d, although did not reach statistical significance ($p=0.2$).

Table 9:3 Daily intakes of energy and macronutrients from two-day weighed intake

Nutrients	Rural n=75	Urban n=59	*p*-value
Energy kJ/d	7153 ± 2012	7385 ± 2199	0.5
Energy kcal/d	1709 ± 481	1763 ± 525	0.5
Carbohydrate g/d	304 ± 83	305 ± 94	0.9
% of total energy	68 ± 7	65 ± 6	**0.01**
Fat g/d	40 ± 20	45 ± 17	0.1
% of total energy	20 ± 6	22 ± 5	**0.02**
Protein g/d	51 ± 15	55 ± 18	0.2
% of total energy	12 ± 1.5	13 ± 2.3	0.08

Values presented are mean ± SD. Differences between groups were tested with Scheffe post hoc tests from ANCOVA models. Significant difference at p≤0.05 is denoted by a bold *p*-value.

Table 9:4 Intakes of micronutrients, dietary fibre, phytate from two-day weighed intake and salt from 24-hr urinary excretion of sodium

Nutrients	Rural n=75	Urban n=59	*p*-value
Calcium (mg/d)	305 (222 to 420)	294 (235 to 385)	0.5
Phosphorus (mg/d)	707 ± 211	697 ± 225	0.8
Ca:P (mg/mg)	0.45	0.44	0.6
Potassium (mg/d)	2154 ± 693	1823 ± 518	**0.002**
Magnesium (mg/d)	432 ± 143	364 ± 116	**0.003**
Iron (mg/d)	25 ± 12	22 ± 10	0.2
Zinc (mg/d)	7.9 ± 2.6	7.2 ± 2.2	0.1
Carotene ?	2150 (512 to 6579)	1442 (291 to 9838)	0.1
Vitamin C (mg/d)	29 (15 to 72)	21 (11 to 54)	0.1
Dietary fibre (g/d)	38 ± 12	32 ± 11	**0.005**
Phytate (g/d)	1.1 ± 0.4	0.8 ± 0.8	**0.0001**
Salt * (g/d)	6.3 ± 3.4	6.6 ± 2.7	0.6

Normally distributed data presented as mean ± SD, otherwise values presented are median and (IQR). Differences between groups were tested with Scheffe post hoc tests from ANCOVA models. Significant difference at *p*≤0.05 is denoted by a bold *p*-value. *Salt intake (g) = (Urinary Na mmol/24hrs) /17.1

9.3.2 Bone forming minerals: Ca, P, Ca: P, Mg, K

Calcium intakes were low in both groups urban 294mg/d (IQR: 235 to 385) and rural 305mg/d (IQR: 222 to 420); all intakes were inadequate compared to international recommendations. The appropriateness of this particular recommendation will be described in the discussion (**Section 10.4.2**). Phosphorus intakes were similar between groups, with average intakes of 700mg/d; approximately 30% of rural and urban groups had intakes below the EAR (IOM recommendation). The ratio of calcium to phosphorus was also comparable, ~0.45.

Magnesium and potassium intakes were significantly different between groups, with rural intakes higher than urban ($p<0.01$). The majority of participants in both groups consumed intakes above the EAR. The recommendation for potassium has recently increased, based on growing evidence of benefits to reduce hypertension. Only 3% (*n*=2) of rural women met this level; no urban women met the specified 3510mg/d. Higher intakes of groundnuts and legumes may have contributed to differences in the intakes of these nutrients.

9.3.3 Other nutrients: Fe, Zn, Vitamin C, Carotene

Mean intakes of iron were 22 ± 10mg and 25 ± 12mg, for urban and rural groups respectively (p>0.05). Intakes of zinc (mean ± SD) were urban 7.2 ± 2.2mg and rural 7.9 ± 2.6mg. Intakes of both vitamin C and carotene largely reflect intakes of fruit, vegetables, and palm oil; the urban group had lower intakes of both nutrients. Vitamin C intakes (median and IQR) were, urban 21.6mg (11.4 to 53.8) and rural 29.1mg (14.8 to 71.9), p=0.139. Carotene intakes (median and IQR) were urban 1442mg (291 to 9838) and rural 2150 mg (512 to 6579), p=0.09. Few foods in the Gambian diet are rich in carotene, and there is large variation in the content of these foods as described in previous research in the Kiang West region of The Gambia, therefore their presence or absence makes a marked difference between individuals and groups.

9.3.4 Fibre, phytate and salt (from urinary sodium excretion)

Dietary fibre intakes were significantly lower in the urban group, urban 31.8 ± 11.5g and rural 37.8 ± 12.2g, p=0.005. The urban group also had lower intakes of phytate, 0.8 ± 0.8 compared to rural 1.1 ± 0.4g (p<0.001). There was no difference in mean salt intake estimated using 24hr Na output. Median (IQR) intakes were urban 6.5 (4.2 to 8.5) g and rural 5.7 (3.7 to 7.8) g. However, the intakes of both groups were higher than current recommendations; 55% and 42% of urban and rural participants respectively, had intakes higher than 6g per day.

	Rural	Urban migramt
>4g	51 (71.8%)	45 (77.6%)
>6g	30 (42.3%)	32 (55.2%)
>9g	12 (16.9%)	10 (17.2%)

9.3.5 Potential Renal Acid Load

Preliminary analysis was undertaken to investigate differences in dietary potential renal acid load (PRAL) and net acid excretion (NAE). Reported intakes of protein, phosphorus, potassium, magnesium, and calcium were used to calculate PRAL, estimated NAE (NAE_{ES}), protein: potassium ratio (Pro: K), and renal NAE (RNAE) using validated formulae derived by (Remer *et al.*, 2003) and (Frassetto *et al.*, 1998). Values were not adjusted for energy. The rural group had lower predicted values compared to urban for all formulae ($p \leq 0.001$).

Table 9:5 Predicted dietary PRAL and NAE

	Rural	Urban Migrant	*p*-value
PRAL (mEq/day)	-9.3 ± 11.7	1.1 ± 7.0	**≤ 0.0001**
NAE_{ES} (mEq/day)	29.2 ± 12.2	42.7 ± 8.3	**≤ 0.0001**
Pro:K (g/mEq)	1.2 ± 0.2	1.4 ± 0.2	**≤ 0.0001**
RNAE (mEq/day)	53.9 ± 13.7	68.5 ± 11.5	**≤ 0.0001**

Values are mean ± SD

Rural: n=75, Urban: n=58

Remer equations

PRAL (mEq/day) 0.49 x protein (g/day)

+ 0.037 x phosphorus (mg/day)
- 0.021 x potassium (mg/day)
- 0.026 x magnesium (mg/day)
- 0.013 x calcium (mg/day)

Total urinary NAE_{ES} PRAL + Organic Acid production $_{ES}$

(Where OA = Body Surface Area- x 41/1.73)

Frasetto equation

RNAE (mEq/day) -10.2 + 54.5 (pro/K)

9.4 Foods contributing to intake of bone forming nutrients

There are several key nutrients that have been highlighted as important for bone health; these include calcium, phosphorus, magnesium, potassium, and protein. Analyses were conducted in order to understand and compare which food groups contribute to these nutrients in both urban and rural groups. Estimated intakes of vitamin D could not be calculated from the dietary data, as currently, there are no values of vitamin D in the Gambian Food Composition Tables. However, food sources of vitamin D are limited and generally contribute little to 25OHD concentrations.

9.4.1 Calcium

In order to compare sources of calcium to previous data collected in the late 1970s and early 1990s, detailed analyses were undertaken of individual foods contributing to total calcium intakes. These included rice, other cereals, fish, leaves, groundnuts, milk, fruits, and others; it was also decided to separate the contribution from vegetables, as it represented a significant amount of the others group. Calculations of the percentage of different foods within mixed/composite dishes had been developed for previous studies, and these were adopted for the current analysis. **Figure 9:10** shows percent contribution of these foods to total calcium intakes.

Fish was the largest contributor to calcium intakes in both rural and urban groups, 26%, and 31%, respectively. Rice provided the same proportion (16%) in both groups, while groundnuts contributed more to calcium intakes for rural participants, 13% compared to only 5% for urban. Milk contributed more to intakes of calcium among urban participants, 12% compared to 5% in rural. Other notable differences were the contribution of leaves, rural 19%, and urban 13%.

Table 9:6 highlights the contributions of the different SED food groups, a more crude analysis compared to the above based on calculations of individual foods from composite dishes. The contribution of rice is overestimated, particularly in the rural group, 29% versus 16%, perhaps where there were more (61) occasions when rice and sauce were coded together. In the urban group, there is less difference in the contribution to calcium estimated from rice and rice dishes as a food group versus rice as an individual food; there were also fewer occasions when rice and sauce were coded together.

Table 9:6 Top 3 SED food groups contributing to calcium intakes

Rank	Rural	%	Urban	%
1	Rice	29	Green leafy vegetables and leaves	21
2	Green leafy vegetables and leaves	22	Fish and shellfish	20
3	Groundnuts and other legumes	11	Rice	19

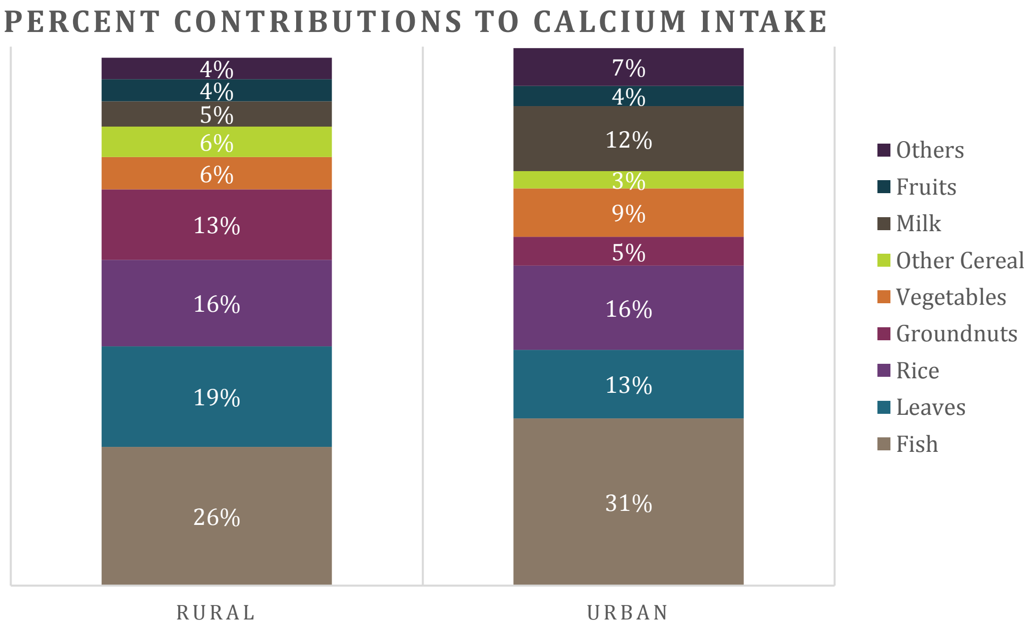


Figure 9:10 Percent contributions to calcium intakes

9.4.2 Contribution of dried and fresh leaves to calcium intakes

Leaves contributed 13 and 19% of calcium intakes in urban and rural groups, respectively. In rural areas of the Gambia it is common for dried baobab leaf 'naa' to be added to sauces and steamed cereals; in order to separate out the calcium from dried baobab leaf compared to other leaves, previous studies have compiled ratios. The same ratios are applicable to the current study; this analysis will enable more detailed comparisons between groups and to existing data.

Earlier research in rural Gambia has shown that two groups of sauces 'Groundnuts + leaves' and 'Groundnuts + leaves +fish' account for a significant proportion of calcium intakes. In this study, these two groups accounted for ~63%, of the rural group's total calcium from leaves; but only ~37% of the urban groups. Therefore, to provide a more representative investigation of sources of calcium from leaves, a breakdown of contributions from baobab leaf within cereals, leaves alone (e.g. as a side dish), and all other leaf sources is shown in **Table 9:7**. The proportions assumed to be from baobab leaf show a greater shift in urban areas, when including other groups. The percent contribution of dried baobab to total calcium from leaves was much higher in the rural group, (between 51-66% depending on how many sub groups were included). In the urban group, fresh leaves contributed more to calcium intakes, representing 65-87% of calcium from all leaves.

Table 9:7 Comparison of the percent proportion of dried baobab and fresh leaves to total calcium from leaves

Number of sub food groups	Dried baobab leaf		Fresh leaves	
	Rural (%)	**Urban (%)**	**Rural (%)**	**Urban (%)**
2: 'Groundnuts + leaves' and 'groundnuts + leaves + fish'	56	27	44	73
3: Same as 2 + cereals including dried baobab	66	35	34	65
4: All groups contributing toward total leaf calcium	51	13	49	87

9.4.3 Phosphorus

Intakes of phosphorus did not differ between groups; rice was the main contributor in rural and urban groups, 51% and 41% respectively. Fish and shellfish contributed 10% and 22% in the rural and urban groups respectively.

Table 9:8 Top 3 contributing food groups to phosphorus intakes

Rank	Rural	%	Urban	%
1	Rice	51	Rice	41
2	Groundnuts and other legumes	11	Fish and shellfish	22
3	Fish and shellfish	10	Breads and pasta	9

9.4.4 Potassium

Potassium intakes were lower in the urban group; rice was the main contributor in both groups, 40% and 38%, in rural and urban groups respectively. Groundnuts and other grains made a greater contribution to rural (16%), than urban intakes (9%).

Table 9:9 Top 3 contributing food groups to potassium intakes

Rank	Rural	%	Urban	%
1	Rice	40	Rice	38
2	Groundnuts and other legumes	16	Fish and shellfish	12
3	Other grains	10	Groundnuts and other legumes	9

9.4.5 Magnesium

The urban group had significantly lower intakes of magnesium, the main contributor in the urban group was 'Rice'; the other two food groups contributed less. The top three contributing food groups to rural intakes were 'Rice', 'Groundnuts and other legumes' and 'Other grains'.

Table 9:10 Top 3 contributing food groups to magnesium intakes

Rank	Rural	%	Urban	%
1	Rice	54	Rice	55
2	Groundnuts and other legumes	16	Other grains	10
3	Other grains	12	Groundnuts and other legumes	10

9.4.6 Protein

Absolute protein intakes were similar between groups; rice was the greatest contributor in both, however it represented a greater proportion of protein in the rural group, 52% compared to 39% in the urban group.

Table 9:11 Top 3 contributing food groups to protein intakes

Rank	Rural	%	Urban	%
1	Rice	52	Rice	39
2	Groundnuts and other legumes	13	Fish and shellfish	27
3	Fish and shellfish	12	Bread and pasta	10

Summary of Results III: Food and nutrient intakes

A comparison of diet, foods, and nutrient intakes has been presented in this chapter. There were no significant differences in energy intakes, although the urban group tended to have higher protein and fat intakes. The majority of energy was from carbohydrates, predominately rice; although the urban group had significantly greater bread consumption.

Calcium intakes were low in both groups, while salt intakes were high in both groups. Magnesium, potassium, fibre, and phytate were lower in the urban group, compared to rural.

Several food groups consistently feature in the top three contributors. The significance of rice consumption to nutrient intakes is highlighted, as well as the implication of higher consumption of 'groundnuts' in the rural group. Higher fish consumption in the urban group was reflected in its greater contribution to protein, potassium, phosphorus, and calcium.

10 DISCUSSION

10.1 Summary of research and hypotheses

The aim of this research was to investigate the impact and implications of rural-to-urban migration on bone health in premenopausal Gambian women. The main research question was 'How does the bone phenotype of an urban migrant group of premenopausal women compare to a rural group of similar women who never migrated?' Hypothesising that there would be differences in bone phenotype measured by DXA (primary outcome) and pQCT (secondary outcome, not presented), due to differences in diet, lifestyle, and overall environment. Therefore, rural-to-urban migration was also hypothesised to be associated with differences in factors important for bone health including nutrient intakes and patterns of food consumption, anthropometry, body composition, vitamin D status, lifestyle, and physical activity.

In this study, rural women were born and had always lived in the Kiang West region of The Gambia. In contrast to the paucity of data available in sub Saharan Africa, bone health and factors important for bone health have been particularly well characterised in this population, due to many years of research conducted by the Nutrition and Bone health group led by Prof. Ann Prentice. Therefore, in order to determine the impact of migration on bone health, a group of urban migrant women originating from this well-characterised rural region was identified, and a detailed study of bone phenotype and factors related to bone health conducted. Both groups spent their formative years in the same rural environment and the urban group migrated at an average age of 18.5years.

This chapter aims to integrate results presented in **Chapters 7 to 9**. The objective is to interpret the findings of the primary outcome bone phenotype, and secondary outcomes factors related to bone health, and to discuss their implications in the context of current literature.

Few data are available comparing bone phenotype, i.e. bone mineral status measured using DXA, in urban and rural populations in LMICs and even fewer in migrant populations. The most appropriate data to compare these results to include findings from the South African arm of the Prospective Urban Rural Epidemiological (PURE) study (Kruger *et al.*, 2011) and the Hyderabad Indian migration study (Viljakainen *et al.*, 2015). However, other studies included in the literature review will also be referred to when appropriate.

10.2 Discussion of Results I

CHARACTERISTICS OF RURAL AND URBAN WOMEN

10.2.1 Comparison of study population characteristics

Several household characteristics differed between groups; firstly urban women were more likely to be in a monogamous marriage (48.3%) compared to 18.5% of rural women. Siervo et al. conducted a study on urbanisation and obesity in The Gambia; they reported that only 6% of urban women aged 35-50 years were in a polygamous marriage (Siervo *et al.*, 2006). The most recent national demographic survey showed that, on average, 68.2% of urban women do not have co-wives. The survey findings indicated that older women and those with lower educational attainment are more like to have at least one co-wife (GBoS and ICF International, 2014).

Marital status may influence household size, composition, and overall dynamics; for example, the number of dependants may be lower if the household head, usually male, has only one wife. The household head takes responsibility to provide food, shelter, and security and, therefore, is key to decision making. Social shifts in the household could influence decision-making and resource allocation, including food provision and distribution. However, interestingly, household size (defined in this study as 'sinkiro' those cooking and eating together) was greater in the urban group (15 compared to 9). This suggests that more than just one nuclear family were eating together, as median parity in both groups was similar, 7 and 8 urban and rural respectively. It is well known that children of rural families often migrate to urban areas of The Gambia for secondary education and they frequently stay with family members; this may be one explanation for the greater number of people eating together. Furthermore, in urban areas it is more likely that housing is rented and families may share a compound and cooking arrangements with a number of unrelated families (GBoS, 2013).

Other factors that could potentially influence lifestyle and dietary habits include having access to a fridge/freezer and electricity. The present study reported that 69% of urban women had electricity, compared to only 5% of rural; while almost a quarter of urban women had a fridge and/or freezer, compared to 1-3% of rural. This enables safer storage of food, potentially reduces food waste, and could increase the types of foods prepared at home, thus influencing the diversity of the diet, discussed in **Section 10.4.6**.

Current or past use of hormonal contraception was significantly lower in the urban group, compared to rural. This may be due to better access to healthcare for the rural population, as all Kiang West residents can freely attend the MRC clinic in Keneba. Injectable hormonal contraception has been associated with increased bone loss, particularly at the spine (**Section 2.6.2.**). However according to a Cochrane review in 2014, evidence is of moderate quality and the current consensus is that after cessation, bone mineral may be recovered (Lopez *et al.*, 2014). Kruger et al. reported higher use of 57% younger urbanised women, they did not publish urban-rural differences (Kruger *et al.*, 2011).

Tobacco and alcohol were not reported in either group; the previously mentioned study by Siervo et al. (2006) also found that all women were classified as non-smokers (Siervo *et al.*, 2006). The WHO STEPS survey, conducted in 4,111 Gambian men and women survey also reported that only 1% of females currently smoked tobacco and 99% identified as lifetime abstainers from alcohol. This was in contrast to men, who while mostly identifying as lifetime abstainers from alcohol, were more likely to smoke tobacco (~30%, smoking on average 10 cigarettes per day). Smoking tobacco is a known risk factor for osteoporosis and fracture, included in the FRAX risk assessment. It has been associated with increased resorption, and potentially reduced calcium absorption due to interference with oestrogen production (Yoon *et al.*, 2012). The low use of tobacco is unsurprising, in The Gambia, as a majority Muslim country.

These results for Gambian women are significantly different to a study on urbanisation and bone health in South African women, which reported that approximately half of both rural and urban women <50years currently smoked (Kruger *et al.*, 2011) . In addition, they also reported mean daily alcohol intake of 6.3g in rural and 9.4g in urban women; 8g is the equivalent of one unit of alcohol. As we have demonstrated, Gambian women are less likely to undertake these behaviours, which is likely to be protective for bone health.

10.2.2 Farming, gardening and physical activity

In contrast to the sparsely populated rural regions, urban areas have substantially less land available for growing food, resulting in smaller horticultural activities, and/or home gardens near compounds. In the present study, urban migrant women were less likely to have farmed or gardened in the last 12 months compared to rural, and the majority of those who had farmed grew rice. Farmed produce was grown primarily for own consumption, as would be expected in an area of subsistence agriculture, and in a culture where it is men who generally farm cash crops e.g. groundnuts. Urban women were more likely to garden in their compound, which may be on a smaller scale to the communal gardens available in Kiang West. There was quite a large variety of vegetables and leaves grown by both rural and urban women and these represent an important source of micronutrients, including calcium. Consumption of leaves and leaf sauces are described in **Section 9.2.6**, but is known to be higher in the wet season, when more leaves are available. The present study was conducted in the dry season.

The majority of both groups had fruit trees within the compound, but more urban women reported not having any fruit trees (~14%); the median number of trees in the compound was similar in the rural and urban areas at 4-5. It is unknown whether fruit would be used for household consumption or income generation, although with many urban women working as market vendors, it is possible that they could generate income from selling fruit. The potential role of fruit-bearing trees to improve food security in sub Saharan Africa has been described by Chivandi *et al.* (2015).

Farming and gardening contribute significantly to physical activity levels of rural women, and although total physical activity has not been calculated in either group, the overall pattern was that urban women were less likely to do moderate/vigorous activities. Car ownership (household level) was higher in the urban group, and this, along with access to local transport e.g. taxis for travel to market, could reduce the amount of walking and overall physical activity.

There are very few data on physical activity levels in sub-Saharan Africa, and studies have used a wide variety of mostly subjective measures such as questionnaires. One study in Cameroon compared objectively-measured activity in rural and urban populations; it showed that urban residence, obesity, and higher educational levels were important correlates of physical activity. The present study showed very little difference between rural and urban education levels, with most having less than 1 year of government education.

The 2010 WHO STEPS Survey in The Gambia assessed levels of physical inactivity along with other risk factors of chronic disease. They reported that 66.0 (58.8 -73.3)% of women were not engaging in any vigorous activity, classified as activities that require hard physical effort and cause large increases in breathing or heart rate, and that over a quarter of women had low levels of activity (World Health Organisation, 2010). Based on observation it might be assumed that many farming and gardening activities conducted in a setting with little to no farming mechanisation would be classified as moderate or vigorous activities. However, research conducted by Lawrence et al. (1985) in Kiang West showed that the majority of activities undertaken by women were classified as light (<3.5kcal/min). Moderate activities (≥3.5 < 5.4 kcal/min) were mostly related to farming including bending or standing digging, and one activity in the home, pounding grain (Lawrence *et al.*, 1985). However, the direct benefits of physical activity for bone and overall musculoskeletal health is not in relation to energy expenditure, but via the impact that weight-bearing activity can have on bone, stimulating osteoblast (bone forming cells) activity, and on improving muscle strength and function, which translates to better balance and reduced likelihood of falls (Gregg *et al.*, 2000).

10.3 Discussion of Results II

10.3.1 Bone mineral status and influence of body size

The primary outcome of the present research was to determine urban-rural difference in bone phenotype measured by DXA. This is the first study to assess bone mineral status in an urban population in The Gambia, and the first to investigate the impact of rural-to-urban migration on bone mineral status in sub Saharan Africa.

In the present research, a significant difference in bone mineral status between urban and rural premenopausal women was shown. Urban women had higher BMC at all skeletal sites (whole body, hip and spine), with the greatest difference at the spine. Urban and rural women were of similar height and had similar bone area (BA); however, weight was significantly greater in urban group.

In the Indian Migration Study, rural-to-urban migrants, from now on referred to as urban migrants, were compared to rural-non-migrant siblings, with 185 sibling pairs included in the analyses. Siblings did not have to be of the same sex, and results were not presented separately, making comparison of data with the present study more difficult, particularly of anthropometric measurements as there are likely to be differences in fat distribution between men and women.

Compared to the present study, both rural and urban migrant groups were older, and interestingly more of the rural group had occupations classified as non-manual, this may be because a larger proportion of the rural group were male and urban migrants were employed in a factory. Physical activity was also higher in the rural group; therefore indicating that this population is somewhat different to the Gambian study presented, where rural women consist of mostly subsistence farmers, with moderate to high levels of activity. 25OHD was not included in the analysis, which may have been expected to be lower in urban migrants, due to working indoors, and more pollution in the city reducing UVB sunshine exposure.

Similar to the present study, urban migrants had a higher BMI than the rural group, primarily due to higher fat mass, although lean mass was also significantly higher in the urban group. The greatest difference in fat mass was in the android region, and this is likely to increase risk of type 2 diabetes, which could increase risk of fracture (**Section 2.6.3**). In The Gambia, the present study has shown that DXA measured lean mass was higher in the urban group, but not significantly. Analysis of appendicular lean mass and of pQCT parameters of body composition may provide further understanding of differences in fat and lean mass.

It is important to note that ethnic differences between Indian and Gambian women are likely to be significant. Other data from the Indian Migration Study (IMS) (presented in a poster abstract) has shown that abdominal fat mass was significantly lower in rural versus both urban-born and urban-migrant men, but that there was no difference among women (Kuper *et al.*, 2011).

In the present study, the magnitude of the urban-rural difference in unadjusted aBMD was larger than found in IMS, which showed a 3.1 - 3.7% difference at the femoral neck and total hip, with urban higher than rural. While the present study showed an unadjusted difference in aBMD of 4.5 - 4.8% at these sites; the greatest difference in aBMD between urban and rural Gambian women was at the lumbar spine (8.7%). Yet in IMS, the difference at the spine only became apparent after adjusting for sex, height, age, and occupation type. There are limitations to this method of size adjustment as previously described by (Prentice *et al.*, 1994) and in **Section 6.8.5**. The difference at the spine was attenuated after also adjusting for smoking, alcohol consumption, moderate and vigorous physical activity (MVPA), lean, fat mass and insulin.

In the present study, size adjustment of BMC (SA-BMC) involved adjusting for bone area, height, and weight. Urban women were much heavier, with significantly higher body fat but similar lean mass, as measured by DXA. After adjusting for body size (weight, height and bone area), the urban-rural difference was no longer significant at the hip and whole body. Difference in SA-BMC ranged from 1.4 to 1.8% at the hip and femoral neck, 2.3% for the whole body; and a significant difference of 6.2% in SA-BMC was found at the lumbar spine. Bone area and weight explained the most variance in BMC at the spine. Adjusting for percent body fat did not attenuate the urban-rural difference, but increased it to 7.2%.

Difference in weight between the groups was primarily due to significant differences in fat mass, as lean mass was not significantly different. Exploratory analysis was done using fat and lean mass instead of weight, and this showed that fat mass was the significant determinant of spine BMC; however, when the binary urban-rural variable was included, neither fat or lean mass remained significant.

Other studies of bone mineral status are only available from one hundred and forty four of the urban African women included in the PURE study, described in **Section 3.6.2.1**. aBMD at the lumbar spine and hip was measured using DXA in this group of older (>50 years), black, urban, south African women. To my knowledge, these data have not yet been published, but have been presented in a poster at the European Calcified Tissue Conference in 2016 (Kruger *et al.*, 2016). The main PURE study published data on CTX, a marker of bone resorption, in a subset of 658 women >45 years (Kruger *et al.*, 2011). Despite claiming that urbanisation may increase risk of low bone mass due to high bone turnover, they showed that CTX was higher in the rural women, as was PTH, similar to previous findings from The Gambia (Aspray *et al.*, 2005). Bone turnover markers and PTH, a regulator of calcium homeostasis, have not yet been analysed in the present study. The authors also describe vitamin D status as low in the older, urban group; however, levels for rural and urban 45 - 50 year olds were similar ~75nmol/L, while for the older groups, levels were approximately 64nmol/L, similar to the present study. These levels are higher than both the UK population protective level (25nmol/L) and USA (50nmol/L) recommendations, suggested as adequate for bone health.

10.3.2 Compared to other urban-rural studies

In the present study, urban women were much heavier, with significantly higher body fat but similar lean mass. After adjusting for body size (weight, height and bone area), the urban-rural difference was no longer significant at the hip and whole body. However, urban women still had significantly higher BMC at the spine, after adjustment for size. Bone area and weight explained the most variance in BMC at the spine. Adjusting for percent body fat did not attenuate the urban-rural difference.

Higher SA-BMC in urban compared to rural women in The Gambia is in contrast to what has been found in most high-income countries (HICs), but is more similar to rural-to-urban studies of bone mineral status in LMICs as shown in the meta-analysis conducted by Matsuzaki *et al.* (2015). The studies in the meta-analysis of LMICs represented three Asian countries, and included the Chinese study discussed below.

This finding is similar to an epidemiological study conducted in Shanghai, China; aBMD was measured using DXA at the lumbar spine in a sample of 50-70 year old men and women. Overall, 318 urban and 371 rural women were measured; their data showed that urban women had higher BMC, aBMD, and BA, compared to rural. However, after adjusting for size, using the same method as the present study, SA-BMC remained significantly higher in women, but not in men. Similar to the present study, urban women were also heavier, and had a higher BMI and waist circumference, however the Chinese, urban women were taller. Whereas there was no reported difference in height in the present study.

Studies comparing aBMD in rural and urban areas of HICs have mostly shown that aBMD is higher in rural populations. In contrast to this, one urban-rural study conducted in 1229 post-menopausal women in Gran Canaria, Spain found significantly higher spine aBMD (adjusted for age and BMI) in urban women that was not related to size, as rural women were shorter, heavier, and a higher proportion were obese. While the urban group were comparatively healthier and younger. In the rural group, lower spine aBMD was associated with a higher reported number of vertebral fractures, as might be expected in a Caucasian population; however, the rural group were also older. There were no significant differences in either unadjusted or adjusted aBMD (age, BMI) at the femoral neck or total hip; however, the mean was higher in the rural group. This is the opposite of the lower aBMD shown at the spine. This maybe because the hip is a weight-bearing site and the higher weight of the rural group may have affected this site more than the spine.

A higher proportion of the rural women also reported diabetes indicating metabolic disturbance. The data were not self-reported, but accessed from medical records. The authors did not distinguish between type 1 and 2 diabetes. Both have been associated with an increased risk of fracture (Janghorbani *et al.*, 2007), however, people with type 1 generally have lower aBMD, a known risk factor for fragility fracture, while people with type 2 diabetes tend to have a higher aBMD, due to the occurrence of type 2 in obese populations, and the known relationship that weight has on bone. However, this was not seen in the Gran Canaria study, urban women were lighter, and had higher aBMD at the spine compared to rural women, who were heavier. Type 2 diabetes has also been associated with an increase in vertebral fractures in a Latino population (Kilpadi *et al.*, 2014), but there are inconsistencies.

As previously described, there are limitations to adjusting BMD for BMI (Prentice *et al.*, 1994). There are several other limitations to the study; they did not ask about physical activity, hormonal contraception use, smoking, alcohol consumption, or dietary intake. However, they did assess vitamin D status, and found rural women to have lower 25OHD concentrations (p <0.05). It is perhaps the impact of diabetes, and other unmeasured factors that are related to lower spine aBMD in this rural population.

Urban-rural studies in HICs have predominantly come from northern Scandinavian countries, where perhaps rural inhabitants are more active and 'healthier' than urban. Comparatively, the study in Gran Canaria and perhaps other rural populations may be less physically active, and this may correlate with lower socio economic status.

One migration study has been conducted in relation to bone health in a HIC (Varenna *et al.*, 2003). The study was a cross-sectional comparative study of 1764 postmenopausal women from southern Italy who had migrated to northern Italy (Milan). The comparison group comprised 4018 northern women, who were from Milan. The urban migrant group had lower aBMD at the spine compared to the urban group, and were also heavier, shorter and had a higher percentage who were classified as overweight. Again, despite higher body weight they did not have higher bone mineral status, compared to urban women. The authors suggest that the migrant population had not assimilated local dietary habits, and therefore consumed less calcium, they may also have had lower vitamin D status due to slightly darker skin colouring and perhaps working indoors, although this was not measured. They were less physically active during leisure time; however, a greater proportion of migrant group had manual occupations.

There appear to be significant ethnic and sex differences in the impact of migrating to an urban environment. This may be due to some genetic predisposition or differences, or potentially urban lifestyles adopted by men and women may be different, in different cultures. Studies have shown that, for a given BMI, African Americans (both men and women) have lower visceral fat, compared to white and Hispanic Americans. The same is also true of the Indian population were insulin resistance and percent body fat is higher at lower BMI (Yajnik, 2018). Obesity has also increased more in the male Chinese populations, compared to female (Song *et al.*, 2016). This is in contrast to African populations, where obesity is more prevalent in urban female populations.

Therefore, the results from the present study are consistent with the few studies conducted in LMICs, showing that urban residence is associated with higher BMC, which is not attenuated after adjusting for size. The magnitude of the urban-rural difference is most similar to the study comparing rural and urban Chinese populations (Gu *et al.*, 2007).

10.3.3 Anthropometry and body composition

Body size and composition are important factors that influence skeletal size and development. Previous rural-to-urban migration studies have demonstrated the association between overweight/obesity and living in an urban environment. However, analysis of 30 African cross-sectional national household surveys showed a complex association between rural/urban residence, household wealth, and overweight in women (Madise and Letamo, 2017). Instead of considering rural-to-urban migration as a socioeconomic driver of overweight, Madise and

Letamo suggest the use of the term urbanism, referring to the adoption of ‘urban’ lifestyles, which can also occur in rural areas.

The present study has shown that overweight and obesity are prevalent in both rural and urban women, but at higher proportions in the urban group. The small study of urban Gambian women previously cited by Siervo et al. (2006) reported that 34% and 50% of 35-50 year olds were overweight or obese, respectively, compared to 28% and 21% in the present study. This may be because the urban area included in this study also included more peri-urban areas, while the study area of Siervo et al. recruited women in an overtly urban area.

Exploratory analysis comparing weight by district showed that in the present study, participants living in the most urban area (KMC, Kanifing Municipality) tended to be the heaviest, followed by Kombo Central and North (KC and KN); Kombo South (KS) had the lowest weight. Although differences were not significantly different between districts ($p>0.05$), maybe reflecting the small numbers in each group, this does suggest that women in ‘more urban’ areas are likely to have a higher body weight and prevalence of overweight and obesity. Body composition data are discussed alongside bone mineral status data in **Section 10.3.1**.

10.3.4 Biochemistry

All participants had good kidney function as would be expected for this age group, none were classified with chronic kidney disease, indicated when eGFR < 60mL/min/1.73m^2 (Renal Association, 2018). Total alkaline phosphatase activity (TALP) was lower in the urban group compared to the rural; however, there were no other significant differences in plasma biochemistry and all values were in the normal range.

Urban women had lower 24hr urinary Mg, K outputs and Na:K compared to rural. These differences may reflect different dietary intakes of these nutrients; these are discussed in **Section 10.4.1**. Urinary creatinine was higher in urban women and this may be due to higher body size or higher consumption of animal protein. The importance of Mg, K and Na:K for bone health are further discussed below in **Section 10.4.1**.

10.3.5 Vitamin D status

There was no difference in vitamin D status as measured by plasma 250HD concentrations between rural and urban groups, although both values were lower than previously reported in The Gambia, which showed rural Gambian women to have higher concentrations (80-100nmol/L), compared to the present study (64-68nmol/L) (Prentice, 2008). If the Institute of Medicine (IOM) concentration levels recommended for sufficiency (<50nmol/L) is used then 11% of rural women and 14% of urban women had values below this level (Institute of Medicine Food and Nutrition Board, 2011). No woman had a 25OHD concentration lower than the UK population protective level of 25nmol/L (Scientific Advisory Committee on Nutrition, 2016).

Section 2.6.3 describes the potential for obesity and higher percentage body fat to lower vitamin D concentrations. Therefore analyses were done to look at the relationships in the present study. When groups were analysed together, there was no association between vitamin D status and percent body fat. However, when analysed separately, 25OHD was lower with increasing percent body fat in the rural group, while in contrast, the same analyses in the urban group showed no association, and the trend was in the opposite direction. George et al. (2014) also found no association between total body fat or fat distribution and 25OHD concentration in their study of urban, black South African women (George *et al.*, 2014).

Kruger et al. published data on vitamin D status as part of the PURE study in rural and urban black South Africans; in the youngest women aged between 35 to 50years, they reported no difference in 25OHD concentrations (~77nmol/L). However, with increasing age, urban women were more likely to have significantly lower levels, compared to rural (Kruger *et al.*, 2011).

10.4 Discussion of Results III

There were several key findings in terms of food and nutrient intakes between urban and rural women; of particular interest were the lower intakes of Mg and K, and high intakes of salt. These results are presented in **Chapter 9 'Results III'**, while the impact of dietary transition on bone health were described in more detail in the literature review, **Section 2.6.4.** This is the first attempt to analyse consumption of food groups using weighed diet records in The Gambia.

10.4.1 Intakes of bone forming nutrients (Ca, P, Mg, K)

Urban women had significantly lower intakes of K and Mg; differences were also reflected in urinary excretion of K and Mg, which was lower in urban women ($p<0.05$). Urinary potassium is a more reliable biomarker compared to magnesium, however both are used to reflect intake. Further analyses of foods contributing to Mg intake indicate that lower consumption of 'Groundnuts and other legumes' was likely related to lower intakes of Mg. While the higher intakes of fish contributed more to urban intakes of K, and again 'Groundnuts and other legumes' were contributing less. This may be one reason for the lower nutrient intakes, along with lower intakes of fruit.

Ca intakes from the weighed food record showed no difference in intakes; however, Ca excretion was higher in urban women, but not significantly. Urinary calcium is very weakly correlated with intake and therefore not a good biomarker of intake.

Food group analysis showed that fish was actually the most important contributor to calcium intake in both groups, representing just over one quarter of rural and almost one third of urban calcium intakes. Milk contributed 12% to urban intakes, but only 5% of rural, while leaves contributed more towards rural intakes.

Intakes of calcium in The Gambia have fallen in recent decades (as described in **Section 2.4.3**). If other factors, dietary or otherwise e.g. dietary PRAL, salt intake, vitamin D status, which affect absorption and utilisation of calcium, change in the future without an associated increase in calcium, then the intakes reported here could become a cause for concern. A longitudinal study in China, in a population consuming a predominantly plant-based diet has reported that intakes below 250mg were associated with an increased risk of fracture (Fang *et al.*, 2016).

10.4.2 Nutrient intakes in reference to requirements

10.4.3 Overview of dietary reference values

Currently, The Gambia has not set nutrient requirements for their population; therefore, in order to provide an estimate of whether intakes are relatively high or low, nutrient intakes in the present study have been compared to dietary reference values (DRV) set by the UK government's Scientific Advisory Committee on Nutrition (SACN). DRVs are defined as "a series of estimates of the energy and nutritional requirements of different groups of healthy people in the UK population. They are not recommendations or goals for individuals" (BNF, 2017). There are four types of DRV, for the purposes of the present study, reference nutrient intakes (RNI) are most appropriate. The RNI is the amount required to ensure that the nutrient needs of 97.5% of the population are met. **Table 10:1** shows nutrients analysed in the present study and whether mean rural and urban intakes are higher or lower than the UK RNI.

This highlights that there were no noticeable differences in relation to recommendations for either urban or rural groups. Overall reported energy intake was low, with a higher proportion of carbohydrate contributing to energy, while the proportion of fat contributing was lower than the reference. Protein intake was shown to be higher than the reference for relative rural and urban body size. Ca was lower; however, P, K, Mg, Fe, and Zn were higher. The higher fibre and phytate in the diets of both groups may however inhibit the absorption of several nutrients, particularly Ca, Fe, and Zn. As has been previously shown, the seasonality of the diet reflected the low intakes of vitamin C and vitamin A, which are primarily higher in the diet when mangoes are in season (Bates *et al.*, 1994). Preformed vitamin A (retinol) in the diet is low due to low meat, dairy and oily fish consumption. Carotene tends to come from palm oil, mangoes, oranges, and pumpkins.

Table 10:1 Comparison of rural and urban nutrient intakes in relation to dietary reference values

Nutrient	RNI (female, adult)	Rural	Urban Migrant
Energy (kcal/d)	2103	Lower	Lower
Carbohydrate#	50%	Higher	Higher
Fat#	< 35%	Lower	Lower
Protein*	43.7g or 50.8g	Higher	Higher
Calcium (mg/d)	700	Lower	Lower
Phosphorus (mg/d)	550	Higher	Higher
Potassium (mg/d)	1600	Higher	Higher
Magnesium (mg/d)	270	Higher	Higher
Iron (mg/d)*	14.8	Higher	Higher
Zinc (mg/d)#	7	Higher	Higher
Vitamin A (RE) (µg/d)*	600	Lower	Lower
Vitamin C (mg/d)	40	Lower	Lower
Dietary fibre (g/d)	30	Higher	Higher
Salt (g/d)	4	Higher	Higher

RNI: Reference Nutrient Intakes for healthy female adults have been used and compared to average intakes as presented in Section 9.3. # As percent of total energy *Protein RNI = 0.75g per kg of body weight ∴ rural 58.3kg*0.75= 43.7g and urban 67.7kg*0.75 = 50.8g. *Vitamin A (RE) 1µg = 6µg beta carotene ∴ rural= 2150/6= 358 µg and urban=1442/6= 240µg

The overall potential renal acid load (PRAL) was also shown to be less favourable in the urban compared to rural group, based on urban dietary intakes, which were higher in protein and lower in Mg and K. Although PRAL was still a lot lower than high income countries, consuming a 'western' diet.

10.4.4 Salt intakes

As shown in **Section 9.3.4** average intakes of salt in the present study were higher in both rural and urban groups, compared to the UK RNI (4g). Intakes in the UK are significantly higher than the RNI, and therefore SACN have advised a maximum intake of 6g per day. Data presented below indicate that the majority of both groups had intakes over 4g and approximately half over 6g, with many significantly higher. The more recent survey did not publish for men and women separately.

High salt intake, and therefore high sodium intake is of concern in relation to its calciuric effect and association with lower bone mass (Prentice, 2004, Teucher *et al.*, 2008).

Salt (24hr Na urinary outputs):		Rural	Urban
	>4g (UK RNI)	72%	78%
	>6g (UK Max)	42%	55%

There are few recent data available on urinary sodium in African populations (Oyebode *et al.*, 2016); previous studies conducted in rural Gambia (Kiang West) reported sodium excretion of 124.2mmol/d in 25-44 year old women who were non-pregnant and non-lactating. This is the equivalent of 7.3g of salt (Aspray *et al.*, 2005). A study of 24hr urinary sodium excretion in an urban South African population (males and females) reported 117mmol/d (Maseko *et al.*, 2006), similar to the present study and previously reported data from The Gambia. Salt intakes published in the PURE study in South Africa using dietary intake data showed urban intakes to be significantly higher than rural, 3.6g and 1.3g respectively (Kruger *et al.*, 2011). This is much lower than what was reported in the previously cited study in The Gambia, or the present study. However, this method is unlikely to capture added salt, and the varying amounts in different processed foods.

In the UK, the majority of sodium in the diet is not from added salt at the table, but from the consumption of shop-bought processed foods. This was not the case in the present study, as dietary assessment identified very low amounts of purchased processed foods. Instead, salt and sodium-rich stock cubes are routinely added to all sauces and staples when cooking, except some of the mono style porridges.

Urban women did not report washing/harvesting local salt, a rich source of minerals (Ca and P) (Prentice *et al.*, 1993), while the majority (84%) of rural women did; although we do not know whether this was consumed by rural women or sold. The rural study area is located near to a tributary of the river Gambia, therefore has relatively easy access to salt flats, thus enabling this activity. The kind of salt used and consumed by urban women is currently unknown. Ubiquitous use of additional stock cubes or sachets, such as Maggi, Aja, or TAC, is also likely to have contributed to the high levels of sodium excreted in both groups. It is well known that women typically add several kinds of seasoning to a single dish; these were recorded on the dietary assessment forms but have not yet been coded or analysed. However, from observation of the dietary forms, there were multiple kinds of seasonings added by women in both groups.

10.4.5 The nutrition transition implications for bone health

10.4.6 Dietary diversity score

The data in the present study were an estimate of habitual intake at a group level. **Figure 10:1** highlights the variation in dietary diversity scores (DDS) in the primary urban districts of The Gambia (NaNA, 2009), ranging from 6.7 to 10.1. The present study found that median urban DDS was 5.3, lower than the NaNA 2009 estimate. Perhaps this is due to the use of a weighed dietary assessment method in the present study, compared to a food frequency questionnaire, which tends to overestimate consumption. A further possibility is that migrants tend to retain elements of the rural diet compared to the urban-born population.

Although a contributing factor to nutritional status, dietary diversity is not particularly useful as an overall indicator of dietary quality. Research in Benin found that a more diversified diet was actually associated with a lower health score, with similar observations in a Mexican study (Ponce *et al.*, 2006). One contributing factor is likely to be the inability of dietary diversity to take into account the quantity consumed of each food group.

Ideally conducting dietary assessment on non-consecutive days would allow for investigation of day-to-day variability, as it is likely that the same food may be consumed two days in a row, which is often observed in studies of dietary intakes in rural Gambia. The habit of consuming leftovers the following day reduces the variation that is likely experienced by that individual. One study in

Malawi demonstrated that 'in order to estimate true individual intakes within an error range of ±20% required 8-23 days for energy, protein, carbohydrate and fibre; and 95-213d for micronutrients' (Nyambose *et al.*, 2002).

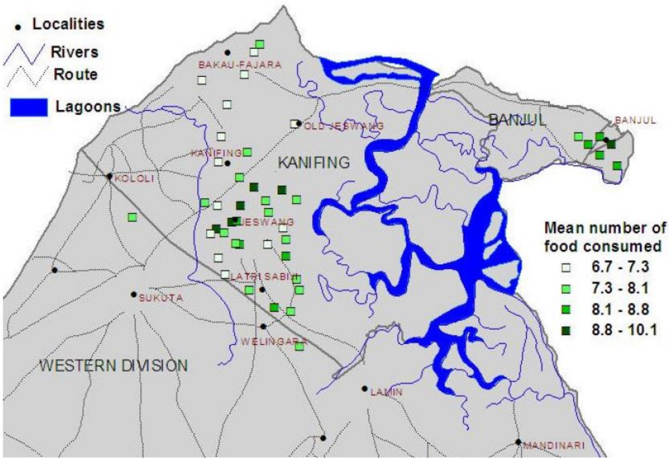


Figure 10:1 Dietary diversity in Banjul and Kanifing Municipality (NaNA, 2009)

10.4.7 Staples, porridge and bread

Prentice et al. (1991) presented data describing the frequency of consumption of major food categories by pregnant women between 1978 and 1979. Rice dishes represented 57% of staples eaten, followed by findo, which was sometimes provided as an aid food, along with rice during the 'hungry' season July- September. In the late 1980s, Hudson et al. published data showing the marked increase in the consumption of rice, compared to other staples.

Hudson (1995) commented that between 1981 and 1988 a significant shift occurred in the consumption/significance of rice, compared to other cereals/ staples in the diet of Keneba and two other nearby villages (core villages). In 1981, rice represented less than 50% of all cereals, by 1987 and 1988, this figure shifted to 90%. The increase in the availability of aid rice was the explanation provided by the authors, in addition to its affordability for the community. Findo, or 'hungry millet', disappeared from the diet.

In comparison to these data, the present study found that rural women had a similar preference/reliance on rice, with 72% of dishes based on rice. Urban women had a lower proportion of dishes from rice (~60%) and instead bread consumption was much more common, around four times more frequently consumed than by rural. An increase in the consumption of bread is a typical feature of transition, (Otter, 2012).

10.4.8 Patterns of dietary intake

As previously described by Jarjou *et al.* (2013) shifts in cereals have resulted in changes in other aspects of the diet, including types of sauce consumed. These secular trends observed previously in The Gambia were shown to be related to lower calcium intakes. In the present study, higher bread consumption was reported in the urban group, and this may displace dishes with sauces rich in important bone-forming minerals (Jarjou *et al.*, 2013a). Intakes of milk products were higher in the urban group, but did not result in higher intakes of calcium. Research in an urban area of Benin, West African found both a 'traditional' and 'transitional' dietary pattern; neither pattern was high in fat; similar to the present WMS study. However, the transitional diet was more diversified with a higher proportion of vegetables, fat and milk products. Yet, milk and milk product intakes were very low in both patterns although higher in the transitional pattern. Their results also suggest that higher socio-economic status and having an urban birthplace influence dietary patterns toward a 'transitional' pattern, compared to length of time in urban area (Sodjinou *et al.*, 2007).

The present study found that urban women were heavier, despite intakes of energy being similar in rural and urban groups. There are several suggestions for this finding; firstly, the urban women may have richer foods not captured by the current food composition data, based on rural recipes. Secondly, urban women may have consumed more snacks or unrecorded foods, and thirdly, the diet of the urban women may be more diverse and therefore two days were not enough to capture habitual intake.

10.5 Strengths and Limitations of the WMS study

10.5.1 Strengths

As the study participants were born in the Kiang West area included in the demographic surveillance, dates of birth, and therefore age are accurate for most participants, particularly in comparison to other similar populations studied in LMICs. Thus, there is confidence that both groups are reliably of a similar age and urban-rural comparisons are therefore reliable. Participants were studied during the same season. Season is well known to impact nutritional status, food availability (amount and type), physical activity levels, infectious disease, and weight change.

The study used detailed DXA-derived measurements of musculoskeletal parameters, and biochemical markers of nutrient status and nutrient intakes. In addition, in-depth background social, demographic and lifestyle data along with food consumption dietary and lifestyle assessment were collected. This comprehensive investigation has added significant, new knowledge and additional understanding of the impact of rural-to-urban migration on important determinants of health, and specifically bone health. An in-vivo cross calibration was conducted to enable appropriate comparison of DXA data acquired on a prodigy scanner to data acquired on an iDXA. A further strength of the research is that pQCT data were also collected as part of the study protocol, providing additional parameters of bone phenotype. pQCT is described further under limitations, as it has not been inlcuded in the present PhD thesis.

As fasted bloods were also collected, vitamin D status could be assessed, there are also future plans to analyse markers of bone turnover and metabolism, providing for example P1NP (bone formation) and CTX (bone resorption). These data would demonstrate whether there are differences in overall bone turnover; a higher calcium intake for instance could result in a reduced PTH, as has been found in South Africa (Kruger *et al.*, 2011). This enables further assoications with bone phenotype to also be conducted in this data set.

Dietary intake data are based on objective weighed intakes and therefore do not rely on subjective recall of participants. The methodology is culturally appropriate and uses Gambian food composition data. A noteworthy strength is the development of SED food groups as part of this PhD and these will enable more translatable and detailed analysis of Gambian diets. Additional analyses using SED food groups for several other Gambian projects with dietary data have already been conducted or are in the planning process. The additional urban food codes developed as part of this research also provide an excellent foundation to build and expand the Food Tables for use more broadly in urban populations in The Gambia. However, because the majority of foods in the food tables are composite dishes, it is difficult to separate the components and this is a current limitation, as all ingredients could not be disaggregated. For example, the fish food group only relates to individual portions of fish consumed and does not account for the often considerable amount of fish consumed within recipes for sauces.

Because household socio-economic data were collected, there is potential to explore the relationship between socio-economic status and outcomes including dietary intakes, body size and composition, and bone phenotype. Two possibilities have been identified, creating either a wealth index, used in research on nutritional status of Gambian adolescents (Juwara *et al.*, 2016), or using scores used by Siervo et al. in The Gambia (Siervo *et al.*, 2006). The relationship between SES and urbanism has been reported by Madise and Letamo, along with the complexity of using a rural and urban dichotomy. Both rural and urban populations can demonstrate urbanism 'urban lifestyles', and they suggest that "overweight patterns in rural and urban areas change as countries progress with globalisation and urbanisation" (Madise and Letamo, 2017). There are plans for this analysis to be conducted with WMS data as part of Sarah Dalzell's post-doctoral research, and to explore the potential for wider application in other Gambian datasets of the Nutrition and Bone Health research group.

10.5.2 Limitations

The cross sectional design is a limitation of the present study, as it is not possible to infer causality. The ideal study design, particularly in this study population would be to identify and measure individuals prior to migration and then follow them up longitudinally, along with a rural sibling who has not migrated, though such an investigation would take many years to conduct. However, the two groups, although not related, were from a well-characterised population, of the same ethnicity, and therefore grew up in similar environments. It is possible that the migrant women were 'healthier' before migration. However, we found no difference in height, which, if different, would have suggested better nutritional status in early life. Sitting height was found to be slightly higher in the urban group, which could suggest a potnetial difference in growth, however, this could possibly be due to large hips raising the participant higher when seated.

As previously mentioned, these DXA-derived bone data provide important and novel bone density assessment of an African population, as well as comparison of two well defined rural and urban migrant groups. Due to the impact of overall body size on DXA-derived measurements, between group analysis of BMC was adjusted for height, weight and bone area. Thus enabling a conclusion to be made of whether urban migrant women had higher BMC for their overall size. The limitation of this choice of analysis, is that body weight and composition are part of the impact of migration on bone health, it could be argued that by adjusting for these factors it alters the interpretation. However, literature reviewed in the background chapters also demonstrate that in some populations, despite higher weight, BMD is lower. Further and more advanced statistical analysis could be conducted to disentangle relationships between body size and bone in this study, and one identified possibility is the use of mediation analysis.

Cross calibration was not conducted for the two iDXA scanners (rural and urban). However, published cross-calibration data show little difference for bone parameters across scanners of the same model (i.e. two iDXA scanners), BMD and BMC were within 2%, but do suggest a bias in body composition (Krueger *et al.*, 2016). This *in vivo* cross-calibration conducted in the USA showed a mean bias of -833 g, +860 g, and 1.1% for lean, fat, and percent body fat. It is of course not ideal to extrapolate these findings to a Gambian population. The between group difference observed in this study is, however, significantly greater than these findings.

Additional bone and muscle parameters acquired using a pQCT scanner also formed part of the wider protocol for the WMS study, which is a strength of the overall protocol, as noted previously. However, the data could not be finalised at the time of writing this thesis, which is a limitation of the work presented here. This is due to the lack of cross calibration data to enable appropriate comparisons between groups measured on different machines. The manufacturers do not consider there to be a need to cross calibrate their equipment, however, researchers have noted differences previously, particularly of the cortical parameters. There are few appropriate phantoms that could be used as an alternative to invivo cross calibration, however, one option suggested by a PhD student in my group was to scan a cortical phantom on both machines. After some consideration, this would only be limited solution, and ultiamtely an in vivo comparison would be the most appropriate solution. In order to overcome this, I am working with colleagues in my research group at MRC EWL to develop a protocol for an invivo cross calibration in The Gambia.

The inclusion of pQCT parameters of bone and body composition would have complemented the DXA measurements and provided a more complete assessment to compare bone health of urban migrant and rural populations.

In recent years there has been growing recognition that low aBMD should be viewed as a risk factor and not a disease itself (Curtis *et al.*, 2017). Despite low aBMD identifying individuals at increased risk of fracture, data suggest that most fragility fractures actually occur in those with either normal aBMD or just below normal aBMD. Although these data are not from African populations. A meta-analysis of the predictive ability of bone density on occurrence of osteoporotic fracture, found that aBMD could predict osteoporotic fracture in Western, white older adults, with a 1SD decrease in aBMD leading a 2.6 fold greater fracture risk (Marshall *et al.*, 1996). However, it is also clear that more than half of older women who sustain a fragility fracture, would be classified as having a normal aBMD, according to WHO definitions (Aspray, 2015). Prediction of fracture using aBMD in individuals with diabetes has also been shown to be unreliable (Walsh and Vilaca, 2017).

According to the census, there are more men living in the urban LGAs, particularly Banjul (54% men). The present research has focussed on women; however, lifestyles of Gambian men are potentially more detrimental to bone health, for example higher tobacco use and lower physical activity, particularly men employed in office jobs, who are less physically active. Yet, very few bone health studies have been conducted in male populations in sub Saharan Africa; however, the Gambian Bone Ageing Study (GamBAS) study described earlier is characterising bone health in both older men and women (Zengin *et al.*, 2017). A study is currently planned to investigate musculoskeletal health in a large urban sample; this will complement the rural GamBAS study.

A larger study of both urban born men and women would enable further relationships to be better understood between physical activity, bone health and other factors important for bone health e.g. body size and composition and muscle strength.

Colleagues working on bone health in The Gambia have been involved in the establishment of SAMSON: 'Build a Sub-Saharan African MuSculOskeletal Network'; which will assist in the launch of a new clinical fracture risk assessment tool (FRAX) and build collaborations across Zimbabwe, South Africa, The Gambia, and Uganda in the area of musculoskeletal health. This will also provide fracture prevalence data for The Gambia. During the WMS study, this tool was not available, therefore it was an unavoidable limitation at the moment.

Grip strength was measured for some participants as a measure of muscle strength, however, there was a problem with the instrument, and the data could not be analysed.

10.5.3 Limitations in dietary assessment

We were unable to capture the location of where meals were eaten, in the rural setting this is typically at home or in the fields during the rainy season. It would have been valuable to know whether sandwiches and other items were purchased and where foods were consumed, in both rural and urban groups.

The majority of foods in the current food composition tables are based on rural Gambian recipes, and these may differ to recipes used by urban women. However, new recipes were created for WMS, including a large variety of urban and rural sandwiches. Further analyses of contemporary rural recipes have been conducted in order to explain the low energy intakes; these are presented in **Appendix E**.

The Gambian food composition tables do not currently provide a breakdown of carbohydrates to total and individual sugars and starches, or total fats to constituent fatty acids (i.e. saturated, mono and polyunsaturated and trans), thus limiting the level of analyses. However, one recent study did undertake analyses of foods from rural Gambia for several additional nutrients known to be 'methyl donors', including B vitamins. There are plans for these data to be incorporated into the food composition tables. A more significant limitation for the present study with consideration of nutrients important for bone health is the absence of vitamins D, vitamin K and silicon in the food composition tables.

10.5.4 Questionnaires

Physical activity questionnaire data have not been reported as total physical activity levels; however, the collected data could be analysed to look at MET (metabolic equivalent) using available data for activities in The Gambia (Lawrence *et al.*, 1985), which is a notable strength of the data, utilising previously published work in this area. Challenges faced when measuring physical activity levels using either questionnaires or objective measures in Sub Saharan Africa have been described in a commentary by Wareham (2001). The physical activity questionnaire was originally developed and validated for use in both urban and rural African settings, however as it was adapted of use in the present study (see **Section 6.9.2**) it cannot be classified as a validated tool. Adaptation for the present study involved tailoring for use in a Gambia context; i was reviewed by Gambian staff, and adaptations were based on previous physical activity research conducted in The Gambia (Lawrence *et al.*, 1985).

A recent questionnaire has now been published to assess the reliability of a questionnaire on built environment for physical activity in seven African countries (Oyeyemi *et al.*, 2016). Measuring features of urban environments that facilitate physical activity may have been a useful addition in the present study to characterise variation in the urban environments of participants.

A potential limitation is related to questions on fracture, disease, and menopausal status, which were self-reported. To minimise any misreporting of menopausal status this question was asked at both time of screening and at the measurement visit in the health questionnaire. One participant was excluded later due to reporting peri-menopausal status. For rural participants, medical records in Keneba were used to confirm the number of children, but this resource was not available for urban women. It has been suggested that due to cultural preference for African women to be larger, that some resort to using steroid medication, as a tablet or injection. These could potentially interfere with bone metabolism, however, a question on use was not asked, although it is unclear if such information would have been willingly disclosed.

10.6 Future Directions

10.6.1 Additional analysis of WMS data

10.6.2 pQCT bone phenotype and bone turnover markers

In the present study, bone phenotype has also been measured using pQCT; however, cross-calibration is required and once this has been conducted the data will be readily comparable between rural and urban migrant groups. Analysis of bone turnover markers (bone formation – P1NP; bone resorption – CTX, and PTH) will be conducted, and this will enable a more detailed understanding of the consequences of rural-to-urban migration on bone health in the WMS study.

Although there was no significant difference between groups for whole body lean mass measured by DXA, there is potential to look at appendicular lean mass by DXA and body composition parameters measured by pQCT, which include muscle cross sectional area and density, and intramuscular fat. This may indicate regional differences in body composition, and overcome the relative inaccuracy of trunk soft tissue estimation. It would also be possible to look at differences between urban and rural groups and relationships with bone health.

10.6.3 PRAL and bone

It would be of interest to investigate the validity of potential renal acid load (PRAL) equations for a Gambian population, i.e. how well the published formulae predict actual biochemical measures calculated using 24-hour urine samples collected in The Gambia. Look at associations between PRAL and bone, and biochemical parameters in WMS.

Further analysis of associations between SED food groups in relation to DXA and pQCT bone parameters and biochemical markers of bone turnover are also planned.

11 CONCLUSIONS

Rural-to-urban migration was associated with a higher SA-BMC, particularly at the spine, and greater weight, but no difference in bone area or height. Weight explained most of the difference in BMC at weight-bearing sites e.g. hip, but not the spine. The absence of a difference in height between groups suggests similar early life nutritional environment. Body size and composition varied between groups, with urban women having significantly higher fat mass and greater central adiposity, thus predisposing to metabolic conditions, such as type 2 diabetes, which could relate to a higher risk of fracture, including vertebral fracture, even with higher BMD.

Vitamin D status was good in both groups, although there was a slight trend to lower levels in urban group. There was no significant relationship between vitamin D status and body fat. The 25OHD concentration in both areas was lower than historical data, which may reflect shifts in lifestyle, or could also relate to different analytical methods.

Intakes of bone-forming nutrients highlighted similarly low intakes of Ca in both groups, but lower intakes of Mg, K, fibre, and phytate in urban group. These nutrient intakes were also reflected in the urban group having a higher PRAL, related to higher protein, and lower Mg and K intakes. This has not been studied in an African population before and could represent a greater burden, due to the low Ca intakes and a potential increase in demand from the mineral reservoir in the skeleton. However, the lower fibre and phytate intakes of urban women could also potentially increase Ca absorption and the higher aBMD in the absence of type 2 diabetes or other metabolic conditions is likely to be protective. Yet, how aBMD relates to fracture risk is unknown in this population.

11.1 Contribution to knowledge

To my knowledge, this is the first study investigating the impact of rural-to-urban migration on bone health to be conducted in sub Saharan Africa. It is the first study of an urban population in The Gambia; and it is the first study measuring bone mineral status in urban sub Saharan African women using both DXA and pQCT.

12 Overview of training and Skills Developed through the PhD

Nutrition Theme retreat 2015, MRCG Keneba, The Gambia

Students and staff from qualitative and mixed methods course (QMM) 2016, MRCG Fajara, The Gambia

Throughout my doctoral studies, I have maintained an interest in understanding both how and why diet and lifestyles are transitioning in LMICs, recognising the importance of context when designing interventions, and/or making policy recommendations. Therefore, I sought to gain skills in the use of qualitative and mixed methods; most notably, I undertook a highly regarded 4-week course 'Qualitative and mixed methods in international health' in The Gambia, through the Institute of Tropical Medicine, Antwerp. In addition to extensive reading of the literature in the field of nutritional anthropology, dietary transition, and sustainable food systems, I took opportunities to attend relevant day seminars, webinars, and local conferences in Cambridge (e.g. Cambridge Institute of Public Health (CIPH), Global Food Security Network and the recent Cambridge Strategic Research Initiative for the Sustainable Development Goals (SDGs). In 2017, I awarded funding from the MRC Flexible Fund to spend 5 months with a research group based at University of East Anglia, who form part of the Centre of Diet and Activity Research (CEDAR). Their research is broadly based around investigating environmental determinants of diet and activity; I was primarily involved in the evaluation of a physical activity intervention in Hertfordshire.

I attended a data management course at the University of Cambridge during my first year, which helped me to consider the various aspects involved in managing different data.

I have been able to consolidate my learning by continuing to use MS Access to manage my study data, and to further my skills by undertaking furhter training. During the rural phase of my fieldwork, I was trained practically in how to correctly position participants and operate the DXA. By the time of the urban phase, I was able to position independently, under the supervision of the local staff.

Further training, conferences and presentations are outlined in below.

General skills
Project management
Working in different cultural contexts
Oral and written communication to different audiences (academic and general audience)
Statistical analysis
Good clinical practice (GCP)
Ionizing radiation
Bone imaging techniques, scan analysis, cross calibration
DXA training
Shadowing in lab
Shadow in data office
Developing new food codes and recipes

Conferences, presentations and other training opportunities
MRC funded interdisciplinary placement at University of East Anglia (5-months)
Qualitative and mixed methods in international health (4 week course)
Cambridge Institute of Public Helath (CIPH) – poster presentations (x2)
European Nutrition Leadership Platform (ENLP) 2016
Nutrition Society Postgraduate meeting oral presentation, 2015
Nutrition Society attendance and presentations 2017 (oral and poster)
Nutrition Society Winter meeting attendance
British Nutrition Foundation meeting on fibre
International Medical Geography Conference 2017, during UEA placement
Global food security conferences, early career networking events
Cambridge GradSchool – team building and communication skills training
Presentations at MRCG Fajara and Keneba during fieldwork
Placement with Gambian Government nutrition dept. (National Nutrition Agency)
Global challenges intiative, University of Cambridge
Cambridge Africa events, at University of Cambridge
Science meetings in Keneba, other nutrition topics from the theme – malaria, infectious disease
Science meetings MRC EWL
Regular propect updates to resarch group
MRCG Nutrition Theme Scientific retreats 2015 and 2018 – oral presentations
Wellcome trust centre bid

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14 APPENDICES

APPENDIX A: ETHICAL APPROVAL FOR FEASIBILITY STUDY AND CROSS-CALIBRATION (GAMBAS)

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
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3 October 2013

Dr Kate Ward
MRC Human Nutrition Research
Elsie Widdowson Laboratory Fulbourn Road
Cambridge CB1 9NL

Dear Dr Ward

L2013.56, Requests to (1)conduct a study to determine the feasibility of adding an urban cohort to GamBAS and (2) to perform cross calibration of scanners to introduce updated technology into existing protocol

Thank you for submitting your letter dated 19 August 2013 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 27 September 2013.

Our Committee is pleased to approve your request.

With best wishes

Yours sincerely

Mr Dawooda Jagne
Acting Chairman, Gambia Government/MRC Joint Ethics Committee

Additional documents submitted for review:-

- Participant Information Sheet (cross calibration), Version 1.0 – 15 August 2013
- Consent Form (cross calibration), Version 1.0 – 15 August 2013
- Participant Information Sheet (feasibility), Version 1.0 – 15 August 2013
- Consent Form (feasibility), Version 1.0 – 15 August 2013

APPENDIX B: ETHICAL APPROVAL FOR WMS

PARTICIPANT INFORMATION SHEET

Version 1 Date 11th July 2014

Study Title: Bone Health in Gambian Women: Impact and Implications of Rural-Urban Migration and The Nutrition Transition

SCC:	1389	Protocol:	

Sponsor: MRC HNR Cambridge

What is informed consent?

You are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of regular medical care is to improve one's health. The purpose of a research study is to gather information. It is your choice to take part and you can stop any time.

Before you decide you need to understand all information about this study and what it will involve. Please take time to read the following information or get the information explained to you in your language. Listen carefully and feel free to ask if there is anything that you do not understand. Ask for it to be explained until you are satisfied. You may also wish to consult your spouse, family members or others before deciding to take part in the study.

If you decide to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study.

Why is this study being done?

It is really important to have healthy bones, to prevent our bones from breaking. Good bone health enables us to live active, independent lives within our families and communities. There are many factors which contribute to bone health; including our environment, diet and lifestyle. In recent years significant numbers of people from West Kiang have migrated to the coast; a more urban area of The Gambia. We are interested to investigate bone health in two groups of women: one in West Kiang (rural) and one urban group (migrants from West Kiang). This will enable us to explore important determinants of bone health in women living in different environments.

The results of the study will be made available to your community.

You are being invited to take part in this study because:

You have previously taken part in a feasibility study and indicated that you would be happy to be contacted again for future studies, **or** you are currently resident in West Kiang (in Keneba, Jali, Manduar, or Kanton Kunda) and have always lived in West Kiang, **or** you are currently taking part in GamBAS, or ENID-Bone and we would like to ask you to have a new spine scan and ask you some additional questions.

What does this study involve?

The following will be required of you if you agree to take part in the study:

Firstly, we will ask you a few questions to see if you are eligible for the study. Also we cannot do DXA scans in pregnant women, and we will ask you again on the day of measurements if you are or could be pregnant, or prefer not to say.

If you live in the Kombos a visit to MRC Unit The Gambia at Fajara will be arranged for you. If you live in West Kiang a visit will be arranged at MRC Keneba. When you arrive we will measure your height, weight and waist circumference. Bone health will be examined by doing two sets of scans using DXA and pQCT scanners. During this visit a small blood sample (20ml; about 4 teaspoonfuls) will be taken, a jumping test will be done and questionnaires on lifestyle will be completed.

We will then agree which days a fieldworker will come to your compound to measure the food you eat for 2 days. During the days when we are measuring your diet we will ask you to collect your urine over 24hrs, you will be provided with the necessary containers.

If you have taken part in GamBAS or ENID-bone studies we will ask you to have a spine scan on the iDXA at MRC Keneba (this is new equipment and was not previously available) and ask you some questions about your life and lifestyle.

After the main part of the study is complete, we hope to be able to do an additional study to find out more detail about your diet. You will be asked if you are happy to be contacted again to consider taking part in this further study.

In case the investigator discovers you are sick and decides that you cannot participate in the study because of that, you will receive immediate care at the study site and then be referred to the appropriate health facility.

If the research study needs to be stopped, you will be informed and you will have your normal medical care.

What will happen to the samples taken in this study?

The collected samples of blood and urine will be processed in our research laboratory in MRC The Gambia at Fajara or Keneba. The processed samples will then be sent overseas to MRC HNR Cambridge, UK for analysis, as we don't have the ability to assess all the factors we are investigating within The Gambia. We will also store the samples for further analysis. The analyses may include genetic testing.

What harm or discomfort can you expect in the study?

There are minimal risks associated with taking part in this study.

Collection of blood can cause minor discomfort, but will not cause any harm to you.

The DXA and pQCT tests involve exposure to some additional radiation; however we are always exposed to natural radiation from the air and soil that surround us. This is called background radiation. The scans you will receive during your visit will expose you to less than the equivalent of 3 days of background radiation.

What benefits can you expect in the study?

There are no direct benefits by participating in this study. However, your involvement will help us to see whether the diet and lifestyle associated with living in either an urban or rural environment is different, and whether this affects bone health in these two different areas of The Gambia. This study will also help to provide advice through NaNA on diet that is good for bone health.

Will you be compensated for participating in the study?

You will not get paid for participation, but you will get either transport by MRC or get the costs for the transport reimbursed.

What happens if you refuse to participate in the study or change your mind later?

You are free to participate or not in the study and you have the right to stop participating at anytime without giving a reason. This will not affect the medical care that you would normally receive.

In case you decide to withdraw your participation during the study we will not work on your samples without your permission, but any information already generated from the samples will be kept. The study doctor may also ask for tests for your safety.

Should any new information become available during the study that may affect your participation, you will be informed as soon as possible.

If you are injured in the study what compensation will be available?

We will be responsible to provide for treatment caused by procedures of the research study.

If medical treatment is required as an emergency, please refer to your health centre or clinic and contact the field worker who gave his/her telephone number to you or contact Dr Landing Jarjou on 7351849/9989957.

How will personal records remain confidential and who will have access to it?

All information that is collected about you in the course of the study will be kept strictly confidential. Your personal information will only be available to the study team members and might be seen by some rightful persons from the Ethics Committee, Government authorities and sponsor.

Who should you contact if you have questions?

If you have any queries or concerns you can contact Dr Landing Jarjou on 7351849/9989957 or Miss Sarah Dalzell on 2227975 and you can always call the personal numbers of the study staff given to you.

Please feel free to ask any question you might have about the research study.

Who has reviewed this study?

This study has been reviewed and approved by a panel of scientists at the Medical Research Council and the Gambia Government/MRC Joint Ethics Committee, which consists of scientists and lay persons to protect your rights and wellbeing.

CONSENT FORM

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|

____
(Printed name of participant)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

- confirm that my choice to participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,
- understand that I grant access to data about me to authorised persons described in the information sheet,
- have received time to consider to take part in this study,
- agree to take part in this study.

Tick as appropriate

I agree to further research on my samples as described in the information sheet	Yes ☐	No ☐
I agree to be contacted again to be asked about future studies	Yes ☐	No ☐

Participant's signature/ thumbprint* ☐

____
Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness* _______________________________________

Printed name of person obtaining consent _______________________________________

I attest that I have explained the study information accurately in _________________________ to, and was understood to the best of my knowledge by, the participant. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of person obtaining consent

____
Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

The Gambia Government/MRC Joint
ETHICS COMMITTEE

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1 August 2014

Miss Sarah Dalzell
MRC Human Nutrition Research
Elsie Widdowson Laboratory
Cambridge
United Kingdom

Dear Miss Dalzell

SCC 1389v2, Bone Health in Gambian Women: Impact and Implications of Rural-Urban Migration and the Nutrition Transition

Thank you for submitting your proposal dated 11 July 2014 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 26 July 2014.

We are pleased to approve your proposed study.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- Revised SCC application form – 11 July 2014
- Response letter – 11 July 2014
- Informed Consent Document, version 1.0 – 11 July 2014
- Eligibility form
- Questionnaire (activity/women and social health), version 1.0 – June 2014
- CV – Sarah Dalzell

APPENDIX C: ETHICAL APPROVAL FOR WMS AMENDMENT

The Gambia Government/MRC Joint
ETHICS COMMITTEE

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2 March 2015

Miss Sarah Dalzell
MRC Human Nutrition Research
Cambridge
Dear Miss Dalzell

L2015.05, Re SCC 1389: Bone Health in Gambian Women: Impact and Implications of Rural-Urban Migration and the Nutrition Transition

Thank you for submitting your letter dated 19 January 2015 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 27 February 2015.

We are pleased to approve your request, although the informed consent document is not attached but we presumed it does not require modifications given the amendments proposed. If it does, of course the informed consent document should be submitted for approval.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-
- SCC approval – 12 February 2015
- Request letter – 19 January 2015

APPENDIX D: ETHICAL APPROVAL FOR CROSS-CALIBRATION (PSC)

Scientific Coordinating Committee
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MRC The Gambia Unit

9 July 2015

Dr Landing Jarjou
Nutrition Theme
MRC Unit The Gambia
Keneba

Dear Dr Jarjou

L2015.31, Re SCC 1073, L2011.35: Permission to perform cross calibration of DXA scanners

Thank you for submitting your letter dated 16 June 2015 for consideration by the SCC at its meeting held on 6 July 2015.

We are pleased to approve your request which will be forwarded to the Ethics Committee for further consideration at its meeting on 31 July 2015.

With best wishes

Yours sincerely

Dr Anna Roca
Acting Chair, Scientific Coordinating Committee

Documents submitted for review:-
- Request letter – 22 June 2015
- Informed Consent Document (adult/child), version 1.0 – 16 June 2015

APPENDIX E: POTENTIAL MISREPORTING OF ENERGY INTAKES

In order to determine the plausibility of energy intakes for both groups, further analyses were conducted using 'Goldberg cut-offs'. 'Goldberg cut-offs' and 95% confidence intervals were calculated to enable the plausibility of reported energy intake to be estimated.

Rural and urban groups were calculated separately and together. There was no difference in the conclusion; the ratio of reported energy intake (EI) to basal metabolic rate (BMR) fell below the cut-offs for both groups.

Physical activity level was assumed to be light for both groups, which is conservative for both groups of women. A possible explanation for these implausible intakes is potential inaccuracy in composite food codes.

Analysis of contemporary recipe composition

In order to reduce the demand of a weighed dietary assessment on both field staff and participants, the Gambian Food Composition tables were designed to contain many composite codes for varieties of local sauces, with several versions of each sauce to represent the diverse ways in which they are prepared. Ratios of staple (usually rice) to sauce were also estimated based on available data at the time.

For example, the tables contain a basic version of groundnut sauce (tia durango) and also multiple versions with vegetable oil, fresh or dried fish, as well as dried leaves (e.g. baobab leaf/naa), locust bean and other ingredients which contribute important micronutrients, particularly calcium (**Figure 14:1**). The assumptions used for the proportions of ingredients in the food codes are based on data from 30 - 40 years ago; anecdotal evidence suggests that increasing sugar and oil availability may mean that some recipes require updating.

Further analyses were conducted to explore this idea; two examples are presented in the following section: 1) proportion of sugar added to porridge and 2) proportion of oil added to groundnut sauce.

FoodName
Tia durango
Tia durango and bitter tomato
Tia durango and dried challo
Tia durango and dried challo and oil
Tia durango and dried fish (unspecified)
Tia durango and dried furo
Tia durango and dried kujalo
Tia durango and fish dajiwo
Tia durango and fresh challo
Tia durango and fresh fish (unspecified)
Tia durango and fresh furo
Tia durango and fresh kujalo
Tia durango and kucha
Tia durango and mango
Tia durango and oil
Tia durango and palm oil
Tia durango and pumpkin
Tia durango and shellfish
Tia durango and tomato
Tia durango, dried challo and kucha leaves
Tia durango, dried challo and naa

Figure 14:1 A selection of Tia Durango sauces included in the Gambian Food Composition tables

Porridge and sugar

To explore more contemporary proportions of sugar added to porridge, I analysed data from a recent study (Dominguez-Salas et al., 2013), which used weighed recipes for all participants was conducted. The median percentage of sugar added to three types of porridge was 5.5%; for millet (sanyo mono, n=62), 4.9% for rice and groundnut (tiakere churo, n=94) and 5.6% for rice (mani mono, n=50). The average of the three types of porridge is 5.3%.

The current composite code for porridge and sugar in the Gambian Food Tables assumes 97.5% porridge and 2.5% sugar. This is less than half of current amount shown in the analyses above.

Table 14:1 Scenarios of energy (kcal) for mean portion of tiakere churo (groundnut porridge)

	Energy (kcal/100g)	Energy (kcal/640g)	Energy from added sugar (kcal)
Tiakere churo (No sugar)	62	397	0
Tiakere churo (2.5% Sugar)	68	435	38
Tiakere churo (5.3% Sugar)	80	509	113
Tiakere churo (8.4% Sugar)	90	575	179

Further analysis was also conducted on WMS (the present study) participant's dietary data, on occasions where sugar was added after cooking and therefore weighed separate to the porridge (n=11; five were urban). The median porridge portion was 684g and median added sugar was 57.5g, which is 8.4% of the dish, more than three times higher than the composite code, and over one and half times higher than that calculated from recipes from the study conducted by Dominguez-Salas et al. (2013). It may be that when there is opportunity to add sugar to their individual bowl and not directly into the cooking pot, participants either give themselves more, or they overestimate what portion of sugar they would normally have if it was shared among the whole pot. It may also be that those who add sugar after cooking can afford more sugar and therefore use more.

The difference in energy content of this dish is compared in **Table 14:1**. Three scenarios are included, the current code (2.5% sugar), proportion of added sugar based on contemporary recipes (5.3%), and proportion based on WMS participants (8.4%). Based on these calculations, an average 640g bowl of porridge containing 5.3% sugar, instead of 2.5%, would have an additional 74 kcal from sugar. If however, the proportion of sugar was 8.4%, the current code would underestimate the energy of the dish by 140 kcal.

Tia durango (groundnut-based sauce) and oil

A further example of oil within tia durango recipes was also analysed, results are shown in **Table 14:2.** This analysis indicates that an average portion of sauce, typically served with rice, would contain between 29 to 34kcal more than the current code estimates.

Both of these examples demonstrate that the current codes for these dishes are potentially underestimating energy intakes, with the proportion of sugar added to porridge particularly low. Work is underway to add and/or update codes in the food tables, so that they reflect contemporary recipes.

Table 14:2 Scenarios of energy (kcal) for mean portion of tia durango (groundnut sauce)

	Energy (kcal/100g)	Energy (kcal/122g portion)	Energy from added oil (kcal)
Tia Durango (No oil)	92	112	0
Tia Durango (5% Vegetable oil)	132	161	29
Tia Durango (8% Vegetable oil)	156	190	34

Goldberg Cut-off analysis: estimation of cut-offs to indicate possible misreporting of energy intake in WMS dietary data

Rural (N=74)

$$S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_t^2} = \sqrt{\frac{529}{2} + 68.89 + 225} = \sqrt{558.39} = 23.63$$

CV_{wEI} = 23%

d=2

CV_{wB} = 8.3%

CV_{tP} = 15%

$$Lower\ 95\%\ cut-off: EI_{\text{rep}}:\text{BMR} > PAL \times e^{\left[-2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

$$Upper\ 95\%\ cut-off: EI_{\text{rep}}:\text{BMR} < PAL \times e^{\left[2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

PAL = 1.55 (assuming light activity) ∴ 1.55 x e^ (±2x ((23.63/100)/√74))

Lower 95% cut-off	Upper 95% cut-off
= 1.55 x e ^ (-2 x 0.027)	= 1.55 x e ^ (2 x 0.027)
= 1.55 x e ^ -0.055	= 1.55 x e ^ 0.06
= 1.55 x 0.947	= 1.55 x 1.055
= 1.47	= 1.64

EI_{rep}: **BMR** = 1.29

This ratio is less than 1.64, but not greater than 1.47, and therefore does not fall within the plausible range and indicates underreporting.

Urban (N=58)

$$S = \sqrt{\frac{CV_{wEI}^2}{d} + CV_{wB}^2 + CV_{tP}^2} = \sqrt{\frac{529}{2} + 68.89 + 225} = \sqrt{558.39} = 23.63$$

CV_{wEI} = 23%;

d=2;

CV_{wB} = 8.3%;

CV_{tP} = 15%

$$Lower\ 95\%\ cut-off: EI_{\mathrm{rep}}:\mathrm{BMR} > PAL \times e^{\left[-2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

$$Upper\ 95\%\ cut-off: EI_{\mathrm{rep}}:\mathrm{BMR} < PAL \times e^{\left[2 \times \frac{\left(\frac{S}{100}\right)}{\sqrt{n}}\right]}$$

PAL = 1.55 (assuming light activity) ∴ 1.55 x e^ (±2x ((23.63/100)/√58)

Lower 95% cut-off	Upper 95% cut-off
= 1.55 x e ^ (-2 x 0.031)	= 1.55 x e ^ (2 x 0.031)
= 1.55 x e ^ -0.06	= 1.55 x e ^ 0.06
= 1.55 x 0.939	= 1.55 * 1.064
=1.46	= 1.64

EI_{rep}: **BMR** = 1.26

The ratio of EI to BMR does not fall between the 95% cut-offs, indicating under reporting.

Combined rural and urban groups

When groups are combined, there is little difference to the overall interpretation of the dietary data. The cut-offs are: Lower 95% cut-off = 1.49 and Upper 95% cut-off= 1.61

Mean EI:BMR (rural and urban) = 1.28

Appendix F: Peripheral quantitative computed tomography (pQCT)

Background to pQCT

Peripheral quantitative computed tomography (pQCT) provides a method to measure the peripheral skeleton, using a low level of radiation and yielding a reliable estimate of bone shape, size and strength (the ability of the whole bone to resist fracture (Bouxsein and Karasik, 2006)). Most notably, it enables the differentiation of bone mineral in trabecular and cortical compartments. It provides a true volumetric density, overcoming one of the most significant limitations of DXA. vBMD is calculated independently of bone size. Table summarises the pQCT bone mineral, geometric and muscle/fat parameters acquired in the WMS study. There are additional parameters related to bending and torsional bone strength, but these are outside the scope of this thesis. The effective dose to the participant of a set of pQCT scans (tibia and forearm) is less than one microsievert, significantly lower than DXA.

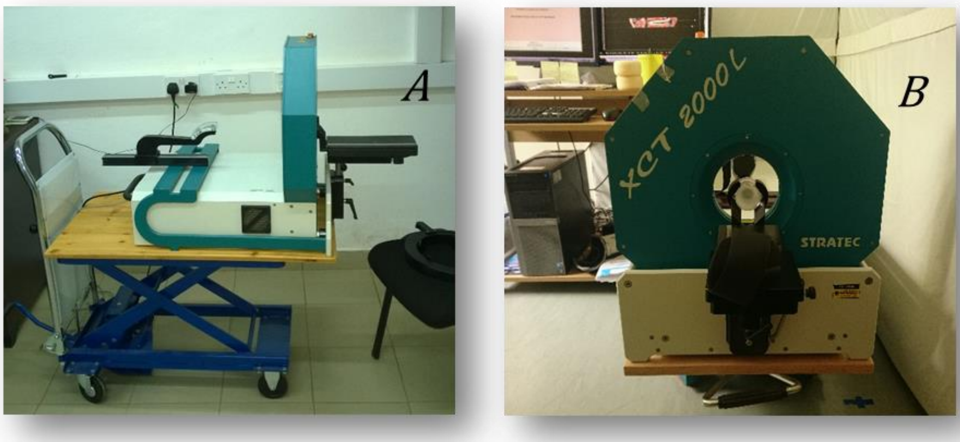
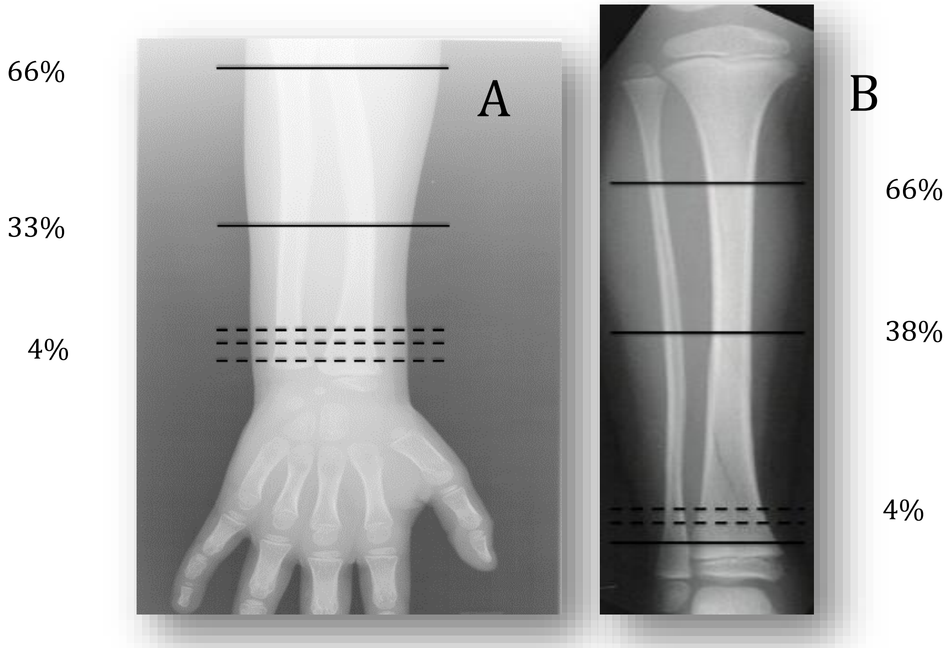


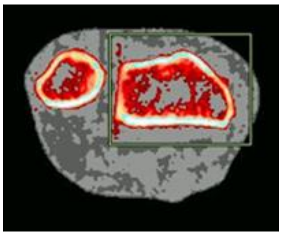
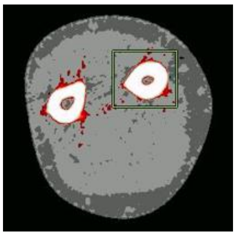
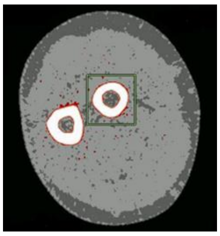
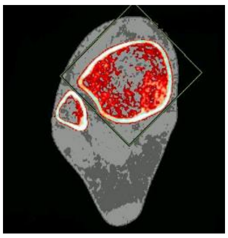
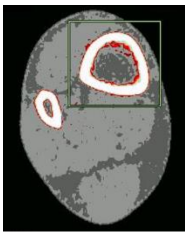
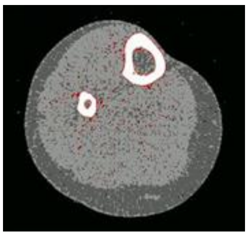
Figure 14:2 Stratec pQCT scanners: (A) XCT 2000 (B) XCT 2000L

The absorptiometric principles for pQCT are similar to DXA, using a fan beam to emit high/low energy photons. However, the X-ray source and detector are located in a circular frame, which is able to rotate around the participant's limb (forearm or leg). **Table 14:3** shows a participant having a radius measurement, with her arm placed through the gantry and hand on the hand rest. The adjustable table enables the operator to position the participant as comfortably as possible, reducing the likelihood of movement, a significant issue with pQCT.

REGION OF INTEREST (ROI)

Table 14:3 Regions of interest (ROI) at radius (A) and tibia (B); scan image parameters at each ROI



	Trabecular	**Cortical**	**Muscle/Fat**
Radius	**4%**	**33%**	**66%**
			
Tibia	**4%**	**38%**	**66%**
			

DETAILS OF pQCT BONE DENSITY MEASUREMENTS

Study equipment

Two models of pQCT scanner were used for the WMS study: a Stratec XCT 2000 and a Stratec XCT 2000L (Stratec Medizintechnik GmbH, Pforzheim, Germany). All urban participants were measured in Fajara using an XCT 2000. The majority (63%) of rural participants were measured using an XCT 2000L, with the remainder measured in Keneba, using an XCT2000.

pQCT scan acquisition

Standard operating procedures (SOPs) were adhered to for both acquisition and analysis of scans. pQCT scans were acquired by the CDBH bone imaging staff, I was present for some of these scans but not trained in the use of the equipment or positioning. Tibial and forearm length were measured according to the SOP (see **Section 6.4.1**). When a new participant was setup, these measurements along with: study ID; gender; DOB; height; weight; ethnicity and correct measurement mask were entered.

Masks

A measurement mask contains the percentage sites to be scanned. Each radius and tibia mask generally provides a series of six images, except for the muscle mask, which has three images. The XCT 2000 requires different masks, as it is a shorter model; the 4 and 33/38% sites are positioned and measured separately from the 66% site. **Table 14:4** describes the various masks used by the different pQCT scanners in WMS, indicating percentage sites and number of participants scanned with each. If the tibia length was more than 440mm a different mask was used to obtain the first two sites (4 and 38%) and then the 66% separately. The scanning procedure was explained to the participant highlighting the importance of remaining as still as possible throughout the scan. Positioning of scanner and participant was done in accordance with the manufacturers guidelines. Clothing had to be removed from the lower leg and forearm prior to scanning, along with footwear or jewellery.

Table 14:4 Description of masks used in the study

pQCT	N	Masks	Radius	% sites	Tibia	%	Muscle Mask (66%)
XCT2000	60	Urban	Radius~3S	4, 33 & 66	TIB_HBP	4, 38, 50	TIB_HBP
XCT2000	20	Rural-ENID	Radius~1	4, 33 & 66	TIBIAMRC	4,38 & 50	MUSCLE
	9	Rural- GBS	Radius~1	4, 33 & 66	TIBIAMRC	4,38 & 50	MUSCLE
XCT2000L	4	Rural-ENID	Radius~1	4, 33 & 66	TBENID	4, 38, 50 & 66	
	15	Rural-GBS	Radius~1	4, 33 & 66	Tibia~4S	4, 14, 38 & 66	
	31	Rural New	Radius~1	4, 33 & 66	Tibia~4S	4, 14, 38 & 66	

Radius Sites 4, 33 and 66%; Tibia sites: 4, 38 and 66%

Radius and tibia protocol

The software provides guidance through each step of the scanning procedure. Once the scanner was adjusted to the correct height, the arm or leg of the participant was placed through the gantry and the hand positioned on the hand rest or foot fastened to the foot holder with a Velcro strap. The limb had to be kept straight and central within the scanning field. The laser was positioned below the ulna styloid or malleolus and the scout scan performed. The scout scan can be repeated several times to make sure the end plate is visible; this is part of normal scanning procedure. By detecting the end of the bone, the scout scan then ensures that all subsequent measurement sites are appropriate and comparable between participants.

Once scanning was complete, images were visually inspected for the of presence red "streaks" which indicate significant movement and that the image may be unusable, see **Table 14:5** for description for images of varying quality. If the participant was willing these scans were repeated.

Figure 14:4 Participant undergoing pQCT scan of the radius

pQCT scan image analyses (grading)

Scans were analysed in Cambridge using Stratec software by the research assistant or myself. The first step was to check participant details were correct and matched with the measurement form. Secondly, to ensure the ROIs were labelled correctly, with radius and tibia or the whole scan selected for body composition data at the 66% site. **Table 14:6** shows an example of the correct ROI labelling of the scan image, for radius only. Scan analysis involves grading each image using standardised grading protocol from 0 to 3, 0 being a very good scan. It is also important for the scout scan to be graded, as incorrect positioning of the reference line effects the position of subsequent scan sites. Comments were recorded for the presence of artefacts that will affect results, e.g. clothing, jewellery, calcified vessels or porosity. Grading criteria are described in **Table 14:5**.

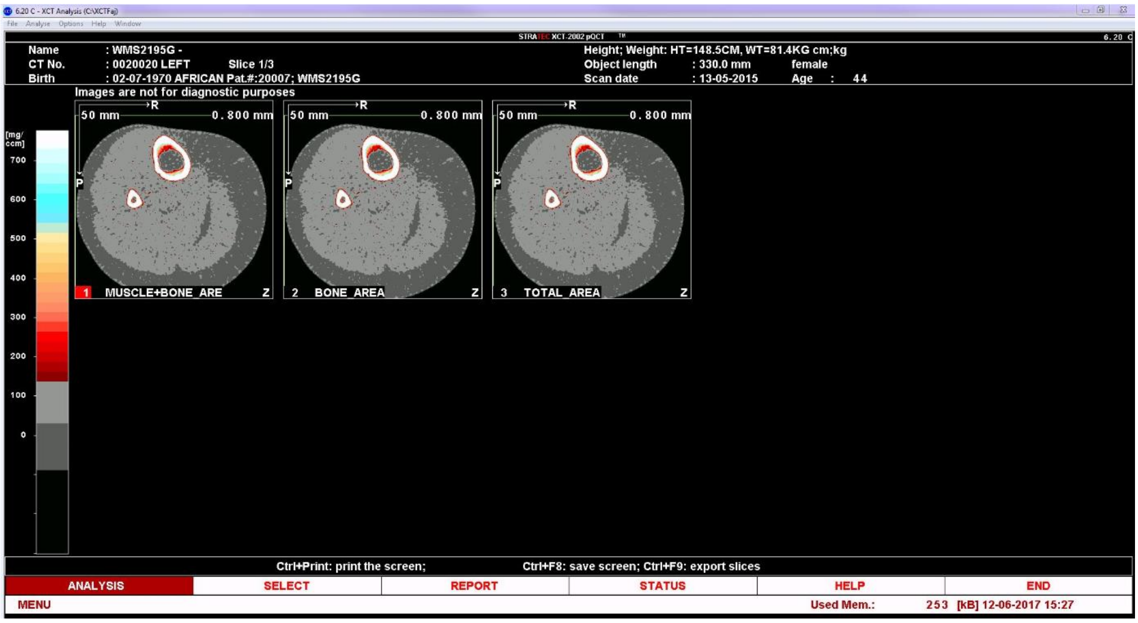
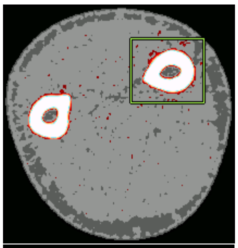
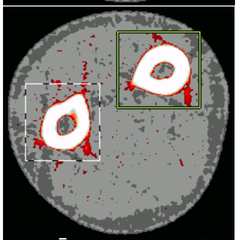
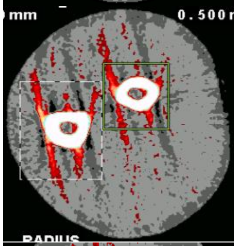
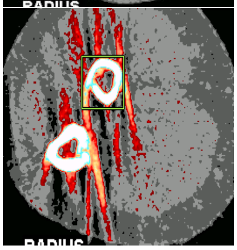


Figure 14:5 Three scan images from a 66% slice of the tibia, the whole image is included and labelled to enable calculations of muscle and fat cross sectional area at this site. The colour chart along the left side indicates the density in mg/mm; white is the most dense – cortical bone, the red trabecular and light and dark grey, muscle and fat respectively.

Figure 14:6 Six scan images of the left radius: the top three images are of the 4, 33, and 66% sites (from left to right); the radius is selected within the square ROI. All of the bottom three images are of the 66% site; these are used to calculate muscle and fat cross-sectional area.

Table 14:5 Grading criteria for pQCT scans

Grade	Scan quality	Example scan
0	Perfect scan Liittle or no movement and no abnormalities	
1	Good scan Small red patches on outside of bone	
2	Acceptable scan Medium streaks caused by movement Black regions indicating breaks in muscle Orange yellow streaks where bone shape is distorted	
3	Unusable scan A lot of streaks, covering the majority of the scan Bone no longer circular in shape Breaks in scan image and in the outline of arm or leg	

Source: MRC EWL pQCT grading protocol

pQCT basic and advanced image analysis and data preparation

In order to extract the bone and body composition data required from each image and ROI, the parameters are defined for each ROI in a ‘loop’, which involves setting the attenuation thresholds for cortical bone and choosing the appropriate modes. An example of what was used for the 4, 33, & 66% sites of the radius is shown in **Figure 14:7**. The SOP for pQCT advanced analysis was followed to determine loop settings. pQCT software provides various modes enabling separation of cortical and trabecular bone, in addition to muscle and fat. pQCT analyses were carried out under the guidance and supervision of trained staff at EWL.

This involved checking and potentially (re)naming ROIs on the various computed tomographic (CT) slices, grading the scans and following the advanced analysis protocol to create job files (a list of scans to be extracted), macros and also the cleaning of data using an in-house designed MS Access tool. This has predesigned queries, which link various raw tables of data together, enabling the correct data to be extracted from each field. Queries are based on several assumptions of where data will be located in the table.

	Upper Limb 4%	Upper Limb 33%,66%
ROIName	RADIUS	RADIUS
THBD	180	180
THBD2	180	180
THBD3	0	0
FILTERS	1	1
Trab_A_re a	45	45
Contmode	1	1
Peelmode	1	1
Hist cutth	0	0
ThCrt	710	280
ThCrt2	0	0
Cortmode	1	1
Filters2	blank	blank
Pathname	c:\loopdata\radius 09.dbf	c:\loopdata\radius 09.dbf

Figure 14:7 Example of loop settings

The use of different pQCT machines in the WMS study (2 x Keneba, 1 x Fajara) resulted in the use of different masks/templates (which determine which percentage site is measured, see **Table 14:4** for list of masks used in WMS). This meant that the slice number did not consistently correspond to the same percentage site and therefore ROI. In order to overcome this, I customised the MS Access tool to ensure that each parameter (ROI) had the correct slice specified.

Quality Assurance (QA) and Quality Control (QC)

The standard phantom scan was performed each day, to monitor the scan quality. The cone phantom was scanned weekly, to assess the precision of machine positioning, at different measurement sites and densities. A printout of the QA was filed and any issues reported.

During the study, there were a couple of visit days when the pQCT was not working properly; participants were called back for these measurements to be made at a convenient time.

APPENDIX G: CONFERENCE ABSTRACTS

Proceedings of the Nutrition Society (2017), **76 (OCE4)**, E124 doi:10.1017/S0029665117002865

Summer Meeting, 10–12 July 2017, Improving Nutrition in Metropolitan Areas

Bone health in Gambian women: impact and implications of rural-to-urban migration and the nutrition transition

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Urbanisation and the associated nutrition transition have been linked with the rapid and recent rise in osteoporotic fragility fracture incidence in many countries(1). Predictions indicate that hip fracture incidence will increase 6-fold in Africa and Asia by 2050, partially attributed to demographic transition and population ageing(2). Differences in areal bone mineral density (aBMD) between rural and urban locations indicate that urban regions of high income countries (HIC) have lower aBMD and a higher incidence of hip fracture(3). The few studies conducted in low and middle income countries (LMIC) provide inconsistent results; in contrast to HIC, most have found higher aBMD in urban populations(4).

In order to investigate the impact of migrating to an urban environment, we have conducted detailed studies of bone phenotype and factors affecting bone health in two groups of pre-menopausal Gambian women: urban migrant ($n = 58$) and rural ($n = 81$). Both groups spent their formative years in the same rural setting, urban women were known to have migrated when aged ≥16 years. Bone phenotype (bone mineral content (BMC); bone area (BA); areal bone mineral density (aBMD), and size-adjusted BMC (height, weight and BA) of the whole-body, lumbar spine and hip) was measured by dual energy x-ray absorptiometry (DXA) with further characterisation of bone phenotype by peripheral quantitative CT (pQCT). Data were also collected on anthropometry, body composition, food and nutrient intakes, physical activity, socio-demographic characteristics, vitamin D status and 24hr urinary mineral outputs (Na, K, P and Ca).

Mean age and height of rural and urban migrant groups were not significantly different ($p > 0{\cdot}05$). Urban migrant women were significantly heavier ($p < 0{\cdot}01$). Significant differences in BMC and aBMD were found between groups at all skeletal sites, with urban women having higher BMC and aBMD; BA was not significantly different. The greatest difference in BMC was found at the lumbar spine (8·5 % ± SE 3·0, $p < 0{\cdot}01$). After adjusting for size, the differences between urban and rural spine BMC remained significant (6·2 % ± SE 2·1, $p < 0{\cdot}01$). These results indicate that rural-to-urban migration is associated with higher BMC, with differences mostly attenuated by adjusting for body size, particularly weight. In this African population, higher SA-BMC may affect future fracture risk.

	Rural			Urban Migrant			
	Mean[a]	SD[b]	n	Mean[a]	SD[b]	n	p
Age (y)[ab]	43·5	41·3, 45·5	81	44·9	39·5, 47·0	58	0·3
Height (cm)	160·6	5·8	81	162·0	6·1	58	0·3
Weight (kg)[ab]	58·3	51·6, 67·3	81	67·7	55·3, 79·4	58	<0·001
LS BMC (g)	52·77	8·97	80	57·18	9·18	56	<0·01
TB BMC (g)	2116	244	81	2277	341	56	<0·01
TH BMC (g)	28·16	3·68	81	29·87	4·07	58	<0·01

LS: lumbar spine, TB: total body, TH: total hip; [a] median [b] IQR (25th and 75th)

Acknowledgements
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1. Ballane G, Cauley JA, Luckey MM *et al.* (2014) *J Bone Miner Res* **29**, 1745–1755.
2. Cooper C, Campion G, Melton LJ 3rd (1992) *Osteoporos Int* **2**, 285–289.
3. Brennan SL, Pasco JA, Urquhart DM *et al.* (2010) *J Epidemiol Community Health* **64**, 656–665.
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Summer Meeting, 10–12 July 2017, Improving Nutrition in Metropolitan Areas

Dietary intakes of Gambian women: impact of the nutrition transition

S.E. Dalzell[1], L.M.A. Jarjou[2], A. Prentice[1,2], K. Ward[1] and G.R. Goldberg[1,2]
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The Gambia is a West African country in nutritional, demographic and epidemiological transition. The climate is sub-tropical with both wet and dry seasons impacting food availability and nutritional status. The nutrition transition is associated with shifts from traditional to Western patterns of food consumption and physical activity; i.e. reduced intakes of fruit and vegetables, increased consumption of oil and sugar, and a more sedentary lifestyle.

Nutrition research in the rural district of Kiang West in The Gambia has enabled characterisation of food and nutrient intakes of a rural population. Most people are subsistence farmers, spending significant time outdoors exposed to UVB sunshine. The diet consists of cereals, predominantly rice, combined with sauces, typically composed of groundnuts or leaves, depending on the season. Due to limited consumption of animal products e.g. dairy and meat, intakes of important minerals are low, particularly calcium. Despite the habitually low calcium, reported incidence of fragility fracture is low[(1)].

The aim of this study was to investigate whether migrating to an urban environment impacted dietary intakes of bone forming minerals. This research formed part of a larger study investigating the impact of nutrition transition on bone health in Gambian women living in rural and urban areas of The Gambia. As part of this study we conducted a 2 day prospective weighed diet record. Data were collected for two groups of pre-menopausal Gambian women: urban migrant ($n = 59$) and rural ($n = 75$). Both groups spent their formative years in the same rural setting, selected urban women were known to have migrated when aged ≥16 years. Food and nutrient intakes were calculated using Gambian food composition tables and Diet in Nutrients out (DINO), a programme which enables both coding of dietary data and export of nutrient intakes[(2)].

Mean age and height of rural and urban groups were not significantly different ($p > 0{\cdot}05$). Urban migrant women were significantly heavier: urban 69·2 ± 15·6 kg and rural 60·5 ± 12·2 kg ($p < 0{\cdot}01$). There were no significant differences in energy intake or macronutrient composition of the diet; approximately 65 % of energy came from carbohydrates. Calcium intakes were low in both groups, urban migrant 294 mg/d (IQR: 235 to 385) and rural 305 mg/d (IQR: 222 to 420). Urban women had significantly lower intakes of potassium, magnesium and dietary fibre ($p < 0{\cdot}01$), related to lower consumption of fruit, green leafy vegetables and groundnuts. These differences reflect the early stages of the nutrition transition; implications for bone health and other non-communicable diseases requires further research.

	Rural*		Urban Migrant*		
	Mean[a]	SD[b]	Mean[a]	SD[b]	*p*-value
Energy kJ/d	7153	2012	7385	2199	0·5
Fat g/d	40·1	20·3	45·2	17·1	0·1
Carbohydrate g/d	307·3	83·1	305·0	94·2	0·9
Protein g/d	51·4	14·9	55·4	18·4	**0·2**
Phosphorus mg/d	707	211	697	225	0·8
Calcium mg/d[ab]	305	222, 420	294	235, 385	0·5
Potassium mg/d	2154	693	1823	518	0·002
Magnesium mg/d	432	143	364	116	0·003

* Rural: $n = 75$, Urban: $n = 59$; [a] median [b] IQR (25th and 75th)

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Salt intakes of rural and urban Gambian women

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Sodium is an essential nutrient and salt is the major source of sodium in the diet. In many high-income countries (HIC), dietary salt comes primarily from processed food rather than an addition to food in the home. Although intakes of 'processed food' are increasing in low and middle income countries (LMIC) like The Gambia, they are still low compared to many HIC, and high salt intake is not yet prioritised as a major health issue in many LMICs.

Whilst the use of local and commercially produced salt is traditional and widespread, more recent increases in availability and use of stock cubes, monosodium glutamate, and bottled and packet sauces suggest that salt intakes may be increasing. There is also evidence that these are used additively rather than singly and thus could increase sodium intakes significantly (Gambian National Nutrition Agency, *personal communication*). Locally baked bread also contains significant amounts of salt; a key characteristic of the nutrition transition is an increase in bread consumption[1]. A systematic review in sub Saharan Africa showed that most countries have salt intakes exceeding recommendations, with urban populations more likely to have higher intakes than rural[2].

Measuring sodium excretion from 24-hour urine collections is the most accurate way to assess daily salt intake. Dietary intake methods are not considered as reliable, since salt added during cooking or during a meal is not always taken into account, and because of measurement errors associated with these methods. Salt cannot be stored in the body and has to be excreted by the kidneys. As part of this physiological process, there is obligatory excretion of calcium. Hence, high sodium intakes as well as increasing risk of cardiovascular disease are adverse for bone health, associated with higher bone resorption and increased calciuria[3,4].

We have conducted a study investigating the impact of rural-to-urban migration on bone health in Gambian women [5,6]. We present here results for sodium/salt in two groups of pre-menopausal women: urban migrant ($n = 58$) and rural ($n = 71$). 24-hour urine samples were collected and aliquots analysed for sodium and for calcium using ion-specific electrodes on the Siemens Dimension® Xpand clinical chemistry system with the QuikLYTE® module.

Sodium excretion was similar in both groups, rural 98·0 (IQR: 62·6 to 133·5) mmol/24 h and urban 112·3 (IQR: 72·4 to 145·7) mmol/24 h ($p > 0{\cdot}05$). Sodium excretion expressed as salt equivalent (17·1 mmol Na = 1 g salt), showed median intakes to be, rural 5·7 (IQR: 3·7 to 7·8) g/day and urban 6·5 (IQR: 4·2 to 8·5) g/day. Intakes ranged from 2·0 to 15·4 g, and 1·8 to 12·4 g in rural and urban women respectively. As a proportion, 72 % and 78 % of rural and urban women had salt intakes above 4 g, while 42 % of rural and 55 % of urban, had intakes above 6 g. These values are consistent with the ingredients and recipe data collected as part of our dietary intake measurements in this study[6]. Urinary calcium excretion was positively associated with increasing sodium excretion ($p < 0{\cdot}01$). Further analysis is underway to investigate sodium and potassium intakes in relation to bone phenotype and markers of bone turnover.

These data indicate that salt intakes in both rural and urban Gambian women are high compared to recommendations (e.g. maximum of 5 g/day recommended by the WHO, the UK reference nutrient intake of 4 g/day, and maximum of 6 g/day). Larger studies with nationally representative samples are needed to understand the prevalence and distribution of high salt intakes in The Gambia and effects on health.

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Body composition in Gambian women: impact and implications of rural-to-urban migration and the nutrition transition.

Content

The Gambia, West Africa is experiencing various types of transition, including nutritional and epidemiological. Rapid urbanisation and the influence of globalisation are resulting in shifts in diet and lifestyle, with a related rise in NCDs. Yet, the prevalence of micronutrient deficiencies, infectious diseases, and undernutrition is still high. We have investigated whether migrating to an urban environment impacted anthropometry and body composition. This research formed part of a larger study of the impact of migration and nutrition transition on bone health in Gambian women living in rural and urban areas of The Gambia.

Data were collected for two groups of pre-menopausal women aged 35.0 to 50.9 years: urban migrant (n=58) and rural (n=81). Both groups spent their formative years in the same rural setting, and urban women were known to have migrated when aged ≥16 years. Participants had dual energy x-ray absorptiometry (DXA) and peripheral quantitative CT (pQCT) bone and body composition measurements including total lean and fat mass, and regional (android and gynoid) fat mass. Data were also collected on bone phenotype and biochemistry, food and nutrient intakes, physical activity, socio-demographic characteristics, vitamin D status, and 24hr urinary mineral outputs.

There was no significant difference in age between groups. Median age at migration for urban women was 18.5 (16.5 to 21.9) years, with an average 23.8 (18.9 to 28.6) years spent in the urban environment. The groups were of similar height ($p>0.05$). However, urban women were significantly heavier ($p<0.001$): urban 67.7 (55.3 to 79.4) kg and rural 58.3 (51.6 to 67.3) kg, with a between group difference of 13.6%. Difference in weight was attributable to significantly greater fat mass in the urban group: urban 27.0 (18.3 to 35.4) kg and rural 17.4 (13.7 to 23.2) kg, and this was primarily in the android region. Fifty-six percent of urban and 30% of rural women were overweight or obese (BMI ≥25). Of those classified as obese, 7% and 2% (urban and rural respectively) were severely or morbidly obese. Several women were underweight, 11% versus 3% of urban. Dietary data indicated that consumption of fruit and vegetables was lower in the urban group and energy from fat was higher. Differences were reflected in the urban group's higher potential renal acid load.

Overweight and underweight exist in both rural and urban regions of The Gambia, with a higher prevalence of obesity and central adiposity in urban areas. Further work is needed to understand the impacts, implications, and determinants on risk of NCDs, with the aim of developing appropriate interventions for both rural and urban contexts.

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