Life-course determinants of bone mass accrual in a transitional rural community in South India:

the Andhra Pradesh Children and Parents Study (APCAPS)

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Declaration

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

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Abstract

In urbanizing rural communities in India, bone development in children and adolescents may be affected by nutrition transition. This thesis work used a life-course approach to investigate the combined effects of early life undernutrition and urbanized lifestyles in late adolescence on bone mass accrual in a rural area in southern India that has been undergoing socioeconomic development over the past decade. The analyses used data from a prospective cohort study near Hyderabad city in India, the Andhra Pradesh Children and Parents Study (APCAPS).

Research Paper 1

The first study examined bone mass in the population of APCAPS. Over 50% of female and 30% of male participants aged 50 years and above had osteopenia or osteoporosis. Peak bone mass was low in this lean rural population (hip BMD in 20-29 year old female: 0.84±0.1; male: 0.95±0.11 g/cm^2) in comparison to the reference values from a healthy Indian population in the same age group (female: 0.90±0.11; male: 0.99±0.13). There was clearer evidence of positive association between hip BMD and lean mass than fat mass in this population.

Research Paper 2:

The second study examined longitudinal effects of early life protein-energy supplementation on bone mass in young adulthood, adjusting for current body size and lifestyles. There was no clear evidence for a long-term positive effect of prenatal and infant exposure to modest protein-energy supplementation on bone mass accrual in this rural community. Greater lean mass in young adulthood was associated with higher hip and lumbar spine BMD. Weight-bearing physical activity was positively associated with hip, LS, and whole-body BMD in males.

Research Paper 3:

The third study assessed longitudinal effects of low body mass index (BMI) during adolescence on bone mass in young adulthood. When adjusted for current BMI, there was no clear evidence for association of hip and LS BMD in young adulthood with adolescent BMI. Controlling for adolescent BMI, greater increase in BMI during adolescence was associated with higher BMD in young adulthood, although it was

still suboptimal in comparison to a healthy young Indian population.

Concluding remarks

In this transitional rural community from south India, the prevalence of osteopenia and osteoporosis was high, especially among females. Although neither modest early life supplementation nor weight gain in late adolescence was sufficient to achieve optimal peak bone mass, increase in body size in late adolescence and young adulthood was beneficial for bone mass accrual in this young population. There is a need to develop strategies to take advantage of, and also prevent any detrimental effects of, nutrition transition to improve peak bone mass in transitional rural communities in India.

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There are two types of numbering for figures. Each paper has its own independent numbering (Figure X) while the figures in all other parts of the thesis are labeled as Figure Chapter Number.X.

Abbreviations

APCAPS	Andhra Pradesh Children and Parents Study
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
DALY	Disability adjusted life years
DPA	Dual-photon absorptiometry
DXA	Dual-energy x-ray absorptiometry
FM	Fat mass
%FM	Fat mass percentage
FMI	Fat mass index
HIC	High income countries
HNT	Hyderabad Nutrition Trial
ICT	Information and Communication Technology
LM	Lean mass
LMI	Lean mass index
LMIC	Low and middle income countries
NCD	Non-communicable disease
NDBC	New Delhi Birth Cohort
NRP	Non-residential place
NSS	National Sample Survey
PA	Physical activity
PMNS	Pune Maternal Nutrition Study
PQCT	Peripheral quantitative computerized tomography
SD	standard deviation
SES	socioeconomic status
SLI	Standard of living index
SPA	Single-photon absorptiometry
SXA	Single-energy x-ray absorptiometry
USA	United States of America
U.K.	United Kingdom
wbPA	Weight-bearing physical activity

WHO	World Health Organization
W1	The first wave of data collection (2003-2005)
W2	The second wave of data collection (2009-2010)
W3	The third wave of data collection (2010-2012)

Chapter 1: Introduction

1.1 Background

Bone is commonly perceived as a dormant structure; it is, however, a dynamic organ that undergoes a cycle of remodeling – resorption of existing bone and formation of new bone - throughout its life. In healthy skeletal development, there is greater deposition of new material than resorption, resulting in bone elongation and mass accrual. After peak bone mass is attained during the third decade of life, bone resorption eventually starts to overwhelm deposition, leading to gradual loss of bone mass. This *lifecourse* of bone health is constantly affected by a number of modifiable risk factors such as diet and physical activity.

Along with socioeconomic development, many low and middle income countries (LMICs) are experiencing epidemiological transition, resulting in increased burden from non-communicable diseases (NCDs) [1,2]. Various aspects of social and physical environments change during rapid economic growth in LMICs, including development of basic infrastructure (*i.e.* water, sewer, solid waste management, electricity, transportation, health care facilities, schools), increased availability of advanced technology (*i.e.* agricultural and industrial machines, household appliance, communication technology), and systems supporting these advances in infrastructure and technology. The effects of these changes on health are complex; while infrastructural development can directly and indirectly contribute to improvement of public health, such development could also have negative effects on health. For instance, while transportation means and systems may improve people's mobility and efficiency of product distributions, which can stimulate economic growth, increased use of automobiles also leads to air pollution, which affects cardiopulmonary systems both acutely and chronically [3,4].

Nutrition is one of the key contributing factors for epidemiological transition in LMICs, partially mediating the effects of socioeconomic development on health: global and industrialized food production, marketing, and distribution systems contribute to making processed food and soft drinks more affordable and accessible in otherwise resource-limited areas [5]. Increased availability of advanced technology

and mass media may affect energy expenditures during transit (*i.e.* personal motorized vehicles), leisure, and work (*i.e.* computers) [5]. Such changes in food and activity environments have led to *nutrition transition*.

Nutrition transition can be described in terms of changes in *diet*, *physical activity*, and *body size and composition* [6]. Analyses of global economic and food availability data have shown complex association between economic growth and dietary habits. In the 1960s, higher Gross National Products were clearly associated with greater energy derived from fats; by early 1990s, there was greater availability of cheap vegetable oils and fats contributing to increased consumption of high fat diet in lower income countries as well [7]. Physical activity level has generally been declining globally largely because of the decreasing proportion of the population engaging in manual labor (*i.e.* agriculture), reduced activity load in manual labor itself due to technological development, and advancement of transportation means [8]. These changes in dietary and activity patterns have contributed to increasing prevalence of obesity in LMICs even though undernutrition is still much more common in these countries in comparison to HICs, resulting in dual burden of malnutrition. The wide range of epidemiological issues in LMICs requires multifaceted strategies to improve public health during rapid socioeconomic development. Even though certain aspects of nutrition transition may be beneficial for bone mass accrual, it is currently not well-understood how LMICs can take advantage of - but not experience detrimental effects of - nutrition transition.

India is a canonical example of an LMIC in the midst of nutrition and epidemiological transition, facing a complex epidemiological profile with dual burden of undernutrition/overnutrition and communicable/non-communicable diseases (NCD). While the Indian National Health and Family Survey has been tracking epidemiological transition of stunting (retarded growth in bone length) since early 1990s, the data on bone mass accrual in young Indians are more limited. Bone mass accrual during the growth phase, along with age-related bone loss in older adults, is a key determinant of bone health in later life. *Osteoporosis* is a condition characterized by low bone mineral density and microarchitectural deterioration, most commonly affecting the elderly. It increases risk of fragility fracture, which is associated with higher morbidity and mortality [9]. Given its aging population, India

is likely to be seeing an increased prevalence of osteoporosis and osteoporotic fracture. However, determinants of osteoporosis in the Indian population have not been examined as extensively as European countries or the United States of America (USA).

Life-course epidemiology builds and tests a theoretical model to link exposures throughout life to health outcomes in later life [10,11]. In life-course epidemiology of skeletal development and aging, studies commonly use birth weight and subsequent body size as proxy for nutritional status [12–15]. These studies have shown long-term negative effects of early life undernutrition on bone growth, suggesting a strong need for nutritional improvement in undernourished mothers and children from LMICs. On the other hand, studies from HICs have shown that urbanicity is associated with greater risk of fractures, which suggests negative effects of urbanized lifestyles on bone health [16]. However, it is currently not clear how nutrition transition in rural India, which combines early life undernutrition with urbanized lifestyles in later life, affects bone mass accrual and what types of interventions are most effective in improving peak bone mass in young Indians from urbanizing rural areas.

It is likely that the effects of nutrition transition on bone health vary depending on the timing of transition in relation to the lifecourse of bone. For instance, rural residents in LMICs who are exposed to urbanization and nutrition transition just until puberty may achieve higher peak bone mass than those who experience transition after bone development is completed. There are relatively few studies examining bone mass accrual in populations who experienced nutrition transition. Even when studies examine such populations, the information on the timing of nutrition transition in relation to the lifecourse of bone is often limited or absent even though the timing of exposure is one of the key concepts in life-course epidemiology [10].

This thesis work analyzed data from a prospective cohort study in southern India, the Andhra Pradesh Children and Parents Study (APCAPS). Anecdotally, urbanization and nutrition transition have been occurring in this study community over the past decade. Using cross-sectional and longitudinal data from APCAPS, this work evaluated evidence on the timing and impact of nutrition transition on bone

development, with a primary focus on the period before peak bone mass is achieved.

1.2 Overall aim of this thesis work

The overall objective of this thesis is to examine the combined effects of undernutrition in early life – fetal through adolescence – and nutrition transition during late adolescence on bone mass accrual in an urbanizing rural community in India.

1.3 Outline of the thesis

There are seven chapters in this thesis. Following this introduction, literature review is presented. The study setting is then described in two parts; evidence of urbanization in the study community and the overall study design for APCAPS. Three research papers based on the analyses of APCAPS data are then presented with each paper comprising one chapter. Two of these chapters also include unpublished data that were not included in the papers but are relevant to this thesis work. Three papers were published and one is under review. The thesis ends with discussion of the findings and future areas of research.

Of note, each paper was prepared as a stand-alone article and therefore there is, inevitably, some repetition of information and inconsistency in terminology and formatting due to editorial and peer review processes among the research articles. The papers are presented in the order that makes this thesis cohesive, rather than in the temporal order of publication.

Chapter 2 reviews literature on urbanization, nutrition transition, and bone health in LMICs to provide the overall context of the thesis. It includes a **systematic review paper** on urbanicity and bone mass (*published*).

Chapter 3 describes the study setting. It first reviews existing evidence on urbanization in this community and then describes the study design of APCAPS.

Chapter 4 is a **research paper** based on cross-sectional analyses of hip bone mineral density (BMD) in the overall adult population in this community (*under review*):

Main objectives:

1) To assess prevalence of osteopenia and osteoporosis in this community;

2) To examine association between hip BMD and body composition.

Chapter 5 is a **research paper** based on longitudinal analyses of early life nutritional supplementation on bone mass in young adulthood (*published*).

Main objectives:

1*)* To examine longitudinal effects of early life nutritional supplementation on hip, lumbar spine, and whole-body BMD during young adulthood;

2) To examine relative contributions of current fat and lean mass to BMD;

3) To examine association between BMD and current diet, serum vitamin D level, and physical activity.

Chapter 6 is a **research paper** based on longitudinal analyses of adolescent body size and BMD in young adulthood (*published)*. It also includes characteristics of body composition and bone outcomes in adolescents from the APCAPS community.

Main objective:

To examine longitudinal effects of thinness during adolescence on hip and lumbar spine BMD during young adulthood.

1.4 Funding

This PhD was funded by the Joint Japan/World Bank Graduate Scholarship Program. The analytical papers used data from the APCAPS. The detailed funding information for each wave of APCAPS data collection is included in Chapter 3.

1.5 Contribution of the author

The author conceptualized all research questions and conducted all analyses in the systematic review and research papers. The first, second, and third waves of APCAPS data collection had been completed by the APCAPS researchers prior to the start of the author's PhD work. The author wrote the first draft of each paper, edited the draft based on co-authors' comments, and was primarily responsible for the final draft. In 2013, as part of APCAPS' ongoing project for assessing built environment in the study villages, a pilot study for evaluating what types of non-residential places (NRPs) are available and how they are distributed in this community was conducted

(Chapter 3). The author conceptualized, designed and wrote the protocol, conducted the pilot study in 3 villages, and helped with training and management of fieldworkers who completed the mapping of NRPs in the remaining 26 villages. The author also imported all GIS data, created the first draft of the NRP categories, mapped way points, and analyzed the NRP data.

Chapter 2: Literature review

This chapter aims to provide the context of the thesis with a literature review on urbanization, nutritional transition, and bone health. The first section describes the general background on epidemiological and nutrition transition and osteoporosis. The second section discusses urbanization and health with a systematic review of articles on urban-rural comparison of bone mass. The third section reviews evidence on the effects of nutrition transition on the lifecourse of bone.

2.1 General background

2.1.1 Epidemiological and nutrition transition

Socioeconomic development has led to demographic and epidemiological transition in many parts of the world over the past century [17]. Improvements in maternal health and a decline in severe undernutrition and infectious diseases during infancy have increased childhood survival rates and life expectancy in many countries [18]. At the same time, non-communicable diseases such as coronary heart diseases (CHD), stroke, cancer, and osteoporosis have become more prevalent. NCDs now account for over 60 % of deaths globally and 80% of the global burden from NCDs are in LMICs [19,20]. Most of the NCD burden in LMICs lies in mere 23 countries, which were estimated to lose US$84 billion between 2006 and 2015 from CHD, stroke, and diabetes alone [20].

Through a series of economic reforms since the 1980s, India has been experiencing rapid socioeconomic development [21]. Such development has contributed to demographic and epidemiological transitions over the past three decades. Over 30% decrease was observed in years of life lost due to common diarrheal and lower respiratory diseases, protein-energy malnutrition during infancy, and pre-term birth complications during these decades [22]. Life expectancy at birth increased from 58 to 65 years old between 1990 and 2010 [22]. About a third of the Indian population is expected to be over 50 years old by 2050 [23]. In this aging society, NCDs now account for the majority of deaths [24]. The economic loss due to heart diseases, stroke, and diabetes alone was estimated to be nearly US$2 billion in 2015 in India [20].

Nutrition transition is one of the driving forces behind the shift in epidemiological profiles [17]. Nutrition transition is characterized by changes in diet, physical activity, and body composition [6]. In one commonly cited model of nutrition transition, the progression of nutrition transition is described in relation of technological development (agriculture and industrialization), predominant dietary and activity patterns, and nutrition-related health issues [25]. Simple put, this model suggests that, as nutrition transition progresses, changes in dietary and physical activity habits lead to shifts in body size and composition, from underweight to healthy weight, and then to obesity with high fat and low lean mass body composition. One of the key element of nutrition transition in the 20th century is reduction of famine due to improved efficiency in industrial food production and distribution [8]; however, this improvement also contributed to development of global NCD epidemics. The influence of the global food systems on obesity epidemics is widely recognized and food pricing is an important driver for dietary transition [26]. Analysis of Food and Agriculture Organization's food balance sheets suggested that the most prominent characteristics of dietary transition in the second half of the 20th century was increased consumption of cheap vegetable oils, contributing to high fat diet around the world [7].

While in reality, nutrition transition is not as simple nor linear as this model describes, HICs have generally followed this pattern over the past century. In HICs, both policy and individual attempts are now being made to introduce behavioral changes to prevent and treat overnutrition-related issues [6,27]. LMICs are currently seeing a wider range of nutritional issues than HICs and struggling with dual burden of malnutrition [28].

Nutrition transition has been occurring to varying degrees throughout India, contributing to a gradual rise in the overall NCD prevalence over the past few decades [29]. Famine was a frequent and common problem until the early 20th century in India: between 1860 and 1908, there were twenty recorded famines [30]. Famine became relatively less common during the 20th century although undernutrition-related issues still exist in India [30].

The Indian National Sample Survey[1] (NSS) has collected data on dietary trends since the 1970s [31,32]. Although much of total caloric intake (57% and 47% in rural and urban areas) still come from cereal, the consumption of cereal has been declining in both rural and urban areas. The proportion of calories from cereal in total caloric intake has declined by 10% in rural and 7% in urban areas between 1993 and 2012 [31]. Subgroup analyses show uneven patterns of cereal consumption, where the decline was more prominent in the wealthier population [33]. Potential factors contributing this decline include availability of a wider variety of food, taste change, reduction in labor work, and less time to prepare food [33].

On the other hand, fat consumption has risen by 3.5% between 1993 and 2012 [31]. Fat intake was greater in urban areas in all but the state of Punjab [31]. High socioeconomic group had greater fat intake in both urban and rural areas [31]. Sugar and honey contributed more to total caloric intake in states with higher average levels of living [31]. Patterns of protein consumption are complex and geographically more variable although urban-rural difference is small compared to fat intake [31]. The major source of protein in India is cereal [31]. Protein consumption has declined in rural India as a whole, but it has been increasing in some southern states including the former state of Andhra Pradesh, where more than 10% of calories from protein come from meat, fish, and egg [31]. Between 1993 and 2012, proportion of protein from milk and dairy products in total protein intake increased from 8.8% to 10% in India [31].

The temporal analysis of caloric intake in the NSS also shows how nutrition transition is not linear. The total caloric intake has been generally increasing but there was a dip in this increase between the 1980s and 2010s: in rural areas in the state of Andhra Pradesh, the estimated average caloric intake was 2204 kcal/day in 1983, 1995 in 2004-05, and 2365 in 2011-12 [31]. Potential explanation for this decline in caloric intake includes rural impoverishment, relative price changes, decline in calorie needs, diversification of diets, tighter food budget, and decline in subsistence consumption [34].

1. NSS examines various indicators of levels of living at national and state levels, including household consumer expenditure.

These dietary changes are accompanied by changes in body size as well. The decennial National Family Health Survey has been collecting data on nutrition, along with demographic and other health metrics since 1992, which allows analysis of secular trends in body size and composition in the Indian population over the past two decades [35]. The percentage of underweight children has decreased from 51.5% in 1992-93 to 40.4% in 2005-06; however, this proportion of underweight children is still high compared to HICs [36]. About a third of adults were underweight while more than 12% were overweight or obese in 2005-06 [36].

Both undernutrition and overnutrition are thought to have long-term effects on health through distinct mechanisms. Early life undernutrition is suggested to have long-lasting effects such as shorter adult height, lower educational attainment, reduced economic productivity, and lower offspring birth weight [37]. Even when nutritional status improves postnatally, there may be harmful effects of rapid postnatal weight gain in children born with low birth weight, including increased adulthood blood glucose concentrations and blood pressure [37]. Life-course analysis of NCD risk factors is especially important in the context of LMICs, where many experience significant lifestyle transition due to changes in environment from socioeconomic development [37].

2.1.2 Osteoporosis

Osteoporosis is a relatively silent NCD characterized by reduced bone mineral density and microarchitectural deterioration, increasing susceptibility to low-impact fracture [38]. Bone size, mass, morphology, and properties of bone materials (*i.e.* density, matrix mineralization) all contribute to bone strength [38]. Various diagnostic criteria are suggested for osteoporosis, including low bone mineral density (BMD), fracture history, bone turnover markers, family history, medication history, and menopausal status for women [38].

Low BMD is a predictor of fracture risk and has been commonly used as a criterion for diagnoses of osteoporosis. The current “gold standard” for measuring BMD is dual-energy x-ray absorptiometry (DXA), which measures differential degrees of attenuation of the x-ray beam at two energy levels as the beam goes through bone,

lean mass, and fat mass [39]. Bone mineral content (BMC in g) is derived from the measured areal BMD (g/cm^2) and bone area (BA in cm^2)[2]. BMD values <2.5 sd below peak bone mass in the population are considered to be osteoporotic in the elderly [40]. BMD between 1.0 and 2.5 sd below peak bone mass is defined to be osteopenic [40].

It is important to note that some limitations exist with the use of DXA-measured BMD as a diagnostic tool: many cases of fracture occur in individuals with normal BMDs and there are weak correlations among BMDs at different skeletal sites, and therefore a person may be osteoporotic in one skeletal site but not others [38,41]. Several studies examining South Asians living in the U.S. and U.K. to other ethnic groups have found that South Asians tend to have smaller bone sizes and lower DXA-measured bone mineral density [42–44]. Lower BMD among South Asians is partially due to smaller skeletal size, which is not automatically accounted for on DXA machines [45]. Areal BMD, a standard output of DXA measurement, does not account for depth and as a result, bones of larger width and height produce greater areal BMD and DXA-measured BMD in shorter individuals are misleadingly lower than taller individuals [46].

Despite the limitations in DXA measurement of bone density, BMD is still considered to be a good predictor for fracture risk within each ethnic group [47]. Given shorter stature among South Asians, development and use of population-specific reference values for peak bone mass are needed. In an attempt to establish Indian reference values, a national multi-center study was conducted on healthy young adults (20-29 years old) living in urban cities (Hyderabad, New Delhi, Lucknow, and Mumbai) [48]. These peak bone mass values are likely to be more appropriate for diagnosis of osteoporosis in the Indian population as they account for genetic and body size differences. However, it is also true that these reference values for peak bone mass in women were lower than South Asian women living in UK [49,50]. It may even be better to use reference values based on the Indian population who grew up in an environment that is more likely to allow optimal bone mass accrual than in places where undernutrition is common.

2 For this thesis, the term “bone mass” is used to refer to both BMC and BMD although a strict definition of bone mass is BMC.

The prevalence of osteoporosis has been steadily increasing along with demographic and nutrition transitions [51]. Although the global prevalence of osteoporosis and osteoporotic fractures is difficult to estimate due to paucity of data in LMICs, one study estimated that there were 9 million osteoporotic fractures in the world in 2000 [52]. Even though the majority of diagnosed cases of osteoporotic fractures are currently reported from HICs, much of the increase in the number of osteoporotic fractures over the next few decades is expected to occur in LMICs where remarkable demographic and nutrition transitions are occurring [51].

Osteoporosis can lead to severe clinical and economic consequences [53]. Osteoporotic fracture is associated with greater morbidity and mortality [54]. A longitudinal study based on USA Medicare records between 1986 and 2005 showed that most comorbidities associated with osteoporosis continued to increase even after mortality associated with hip fractures reached its peak in 1995 [55]. Some of the more common comorbidities reported in this study were myocardial infarction, cancer, cerebrovascular disease, chronic pulmonary disease congestive heart failure, dementia, and diabetes [55]. Globally, 5.8 million Disability Adjusted Life Years were estimated to be lost in 2000 due to osteoporotic fracture [52]. In the USA, it was estimated that annual fracture-associated costs would increase by 50% between 2005 and 2025, which would translate into a total cost of 28.5 billion dollars [53]. Although the total incidence was higher for vertebral (27%) than hip fracture (14%), the cost associated with hip fracture was estimated to be greater, accounting for 72% of the total cost [53].

The demographic transition in India suggests that the prevalence of osteoporosis and osteoporotic fracture is likely to be increasing [56]. There are few population studies that have examined prevalence of osteoporosis and osteoporotic fractures among Indians [57]. One conservative estimate from India suggested that by the end of 2015, there would be approximately 25 million people over 50 years old who suffer from osteoporosis, if we assume 10-20% of the elderly women and men to experience osteoporosis [56]. In a study from a single district near New Delhi, the incidence of hip fracture was estimated to be 159 and 105 per 100,000 for women and men above 50 years old respectively in this district [58]. A small study from Tamil Nadu has

shown greater burden of osteoporosis in rural than urban areas [59]. Despite the accumulating evidence of increasing prevalence of osteoporosis among Indians, a study in Mumbai found that the correct knowledge and awareness of osteoporosis and risk factors of osteoporosis were low among women [60].

As India continues its socioeconomic development, there is a need to assess how socioeconomic development and urbanization affect determinants of osteoporosis.

2.2 Urbanization and bone health

2.2.1 Urbanization and health

Urbanization is thought to contribute to the rising burden of NCDs [61,62]. National economic development and urbanization are often correlated [63]. Over half of the global population now resides in urban areas and by 2050, more than two-thirds of the world are expected to be urban dwellers [64]. Even though the proportion of the population living in an urban environment is currently much lower in Asia than in Europe or the Americas, the fastest growths in the urban population are seen in Asian countries [64].

In 2015, India's economic growth rate was ranked highest in the world [65]. According to the 2011 census in India, about 377 million people reside in urban areas while 833 million people live in rural areas [66]. While both the urban and rural populations have increased since 2001, the rate of growth was much higher in urban (31%) than rural (12%) areas [66]. Between 2014 and 2050, the urban population in India is expected to gain 404 million people [64].

Even though India is experiencing a steady increase in its urban population and has one of the highest number of mega-cities[3] in the world, the rate of urbanization has been slower than other middle income countries like China [67,68]. India has the highest number of rural residents in the world, whose health could potentially be heavily impacted by unplanned urbanization of their villages or migration to urban areas in near future [64]. This slow rate of urbanization in India offers an opportunity for city planning, so that the remaining rural areas can be developed into healthier cities.

For residents of transitional rural communities, the lifecourse of bone may be greatly influenced by the “lifecourse” of their urbanizing environment. The “lifecourse” of urbanizing rural areas in LMICs is distinct from HICs as rural areas in LMICs have unique issues that typically do not exist in HICs such as lack of basic infrastructure, which contributes to high prevalence of undernutrition and infectious diseases in rural areas [36]. In India in 2005-2006, there were more stunted, wasted, and

3 cities with over 10 million inhabitants.

underweight children and underweight adults in rural than urban areas [36]. 22.2% of men and 28.9% of women were overweight or obese in urban areas as compared to 7.3% and 8.6% in rural areas [36]. In contrast, the prevalence of obesity was 40% in rural areas and 33% in urban areas of the USA [45].

Cities offer both advantages and drawbacks. Urban bias is often common in LMICs, where governments focus more on economic and infrastructural development in urban than rural areas [70]. A clear example of urban bias was seen in China, where until the 1980s, urban residents in China received more benefits through a governmental rationing system for food, housing, health care, and education as well as "permanent" jobs in cities for working age groups [71,72]. On the other hand, city dwellers may also face negative consequences of urbanization such as air pollution, increased inequality, less outdoor space and opportunities to engage in physical activity, violent crimes, and poor hygiene in urban slums [73].

The process of changes in social, economic, political and physical environments during urbanization are complex, with many aspects of society developing at uneven rates and interacting with one another, often haphazardly. While it is difficult to evaluate independent, interactive, and cumulative effects of all the changes in these environments on health, the impact of physical environment on health in particular has garnered interest among public health researchers as physical environment often acts as a connection between distal (*i.e.* social and political systems) and proximal (*i.e.* individual lifestyles) determinants of health. Change in built environment, such as infrastructural development, is a crucial, and perhaps most dominant, feature of urbanization outside of population growth. Water supply, sewer, solid waste management, schools, health care facilities, transportation, electricity, designated recreational space, and telecommunication system are often lacking in rural area in LMICs. Development of each of these infrastructural and technological components could have positive and negative effects - directly or indirectly - on nutrition and health in transitional rural communities in LMICs.

For instance, power grid is one type of major public infrastructure that becomes widely available along with socioeconomic development in LMICs; in 2013, about 17% of the global population did not have access to electricity, with the majority

living in rural areas in sub-Saharan Africa and developing Asia [74]. Use of electricity may result in greater efficiency and productivity in school and at work or improve opportunities to engage in skilled work, which could lead to higher socioeconomic status; access to electricity could also encourage greater use of television, computers, or modern household appliances, which may reduce the amount of physical activity.

Transportation system is another key element in socioeconomic development that could influence health in LMICs. Faster and more efficient means to move items and people can directly strengthen health care and food distribution systems; for example, the rail system in $19^{th}/20^{th}$ century in India played a major role in the distribution of food for famine relief in India [75]. Advancement in transportation means (*i.e.* personal vehicles) and systems (*i.e.* public buses and trains, taxi) may also influence physical activity levels [76]. While HICs and some cities in LMICs have recognized the opportunity to improve transportation infrastructure in relation to health (*i.e.* safe roads for cyclists and pedestrians), such effects are rare in urbanizing rural areas in LMICs [77].

Urbanization is often associated with reduction of open space that could be used for physical activity [71]. In HICs, designated recreational areas are regularly incorporated in city planning; however, in LMICs like India, such city planning may lag behind rapid development and population growth in urban and urbanizing areas, resulting in lack of public space where urban residents can engage in leisure-time physical activity. This is particularly important in the context of LMICs, where manual labor is expected to become less common and advanced technology is rapidly replacing activities that were previously performed manually [8].

Clean water, sewage and solid waste management systems are essential to improving sanitation and reducing infectious diseases and undernutrition in LMICs [79]. On one hand, development of infrastructure for water, sewage, and solid waste management systems leads to more people having access to clean water and improved sanitation. However, excess influx of people into urban centers may overwhelm infrastructural capacity and certain parts of cities, like urban slum, may have inadequate access to such infrastructure and harbor infectious diseases. The lack of access to basic infrastructure among “urban poor” is of great concern in urbanizing areas in LMICs.

Access to primary care physicians and appropriate referral systems is key to detecting and treating NCDs in a timely manner [80]. In LMICs, access to health care is much more limited in rural areas. For instance, despite significant infrastructural improvement in both urban and rural areas, urban-rural inequalities have been widening in China – in 1998, per capita out-of-pocket health expenditure in China was nearly twice as high in rural as urban areas [71]. The Indian Infrastructure Report in 2014 states that low spending on health infrastructure (4% of Gross Domestic Product) has led to shortfall in public health care facilities; in rural areas, there was 23-40% shortage in public health care facilities [81]. Although the levels of access to health care are not sufficient in urban areas either, urban bias is evident both in terms of coverage and resource availability in India [82].

Inequality in access to, and quality of, education between urban and rural areas also exist in LMICs [71,83]. A number of studies have shown that low attainment of educational levels is associated with higher burden of diseases [84–88]. This may be because access to education results in higher socioeconomic status, greater access to resources to prevent and treat NCDs, and better health literacy, or poor health may prevent individuals from attending schools [56]. Greater investment in education may have a positive impact on health in urbanizing rural India.

Many countries have invested heavily in development of Information and Communication Technology[4], or ICT, to stimulate economic growth [90]. Although there is mixed evidence on the effects of ICT investment on economic growth in LMICs, ICT is still of significant interest to LMICs as it could potentially contribute to improved access to markets and social networks, health care, education, government services, and microfinance [89,91–93]. Mobile phones in particular have reached relatively remote and resource-limited areas in LMICs; it was estimated that mobile subscriptions increased from 738 million in 2000 to nearly 7 billion in 2015 [94]. Ubiquity and affordability of mobile phones have led to testing of its use as a new vehicle for providing health interventions or improving health systems in many

4 ICT is defined as a "set of activities that facilitates the capturing, storage, processing, transmission and display of information by electronic means" [89]. Examples of ICT includes mobile phones, radio, television, computers, network hardware, satellite systems, and any associated software.

countries with a number of studies showing promising results [95,96]. Access to information through the Internet has been steadily increasing as well – about 2 billion people from LMICs are now estimated to be using the Internet [94]. Mass media could also be used as a tool to promote health conscious behaviors (*i.e.* vaccination campaign, promotion of safe sex). At the same time, advanced communication technologies may also have negative effects on health; for example, greater body size and fat mass are positively associated with time spent on television viewing [97–99].

Even though studies have found that urbanicity is associated with higher risk of osteoporotic fracture in HICs, urbanicity may not necessarily be negatively associated with bone health in LMICs because of LMIC-specific rural issues as described above [100]. The next section presents a systematic review of urban-rural comparison of bone mass in HICs and LMICs. The aim of the next subsection is to assess existing evidence on patterns of association between urbanicity and bone mass and to highlight any difference in these patterns between HICs and LMICs.

2.2.2 Systematic review paper: Comparison of Bone Mineral Density between Urban and Rural Areas: Systematic Review and Meta-Analysis

Nutrition and epidemiological transition in urbanizing rural communities in LMICs is distinct from rural areas in HICs. This systematic review examines how urbanicity may be associated with bone mass and whether patterns of such association differ among countries at varying levels of economic development. Differences in nutritional issues in rural areas of LMICs and HICs may lead to distinct patterns of association between bone mass accrual and urbanicity. The supplemental material for this article (the PRISMA statement and summary of search strategy of the systematic review) are included in **APPENDIX A** and **APPENDIX B**.

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Conceptualized, conducted the analyses, wrote the first draft, coordinated all comments by co-authors, and was primarily responsible for the final draft.

……

NAME IN FULL (Block Capitals) Mika Matsuzaki

STUDENT ID NO: 325364

CANDIDATE'S SIGNATURE …… Date 2015-10-14

SUPERVISOR/SENIOR AUTHOR'S SIGNATURE (3 above) ……

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RESEARCH ARTICLE

Comparison of Bone Mineral Density between Urban and Rural Areas: Systematic Review and Meta-Analysis

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Abstract

Background

Studies from high income countries (HIC) have generally shown higher osteoporotic fracture rates in urban areas than rural areas. Low bone mineral density (BMD) increases susceptibility to fractures. This review aimed to assess whether urbanicity is consistently associated with lower BMD globally.

Method

Ovid MEDLINE, EMBASE, and Global Health (-April 2013) were searched for articles investigating differences in bone mineral content (BMC) or BMD between urban and rural areas. Ratio of means (RoM) of BMD were used to estimate effect sizes in meta-analysis, with an exception for one study that only presented BMC data.

Results

Fifteen articles from eleven distinct populations were included in the review; seven populations from four high income countries and four from three low and middle income countries (LMIC). Meta-analysis showed conflicting evidence for urban-rural difference in BMD; studies from high income countries generally showed higher BMD in rural areas while the results were more mixed in studies from low and middle income countries (HIC RoM = 0.05; 95% CI: 0.03 to 0.06; LMIC RoM = -0.04: 95% CI: -0.1 to 0.01).

Conclusions

Urban-rural differences of bone mineral density may be context-specific. BMD may be higher in urban areas in some lower income countries. More studies with robust designs and analytical techniques are needed to understand mechanisms underlying the effects of urbanization on bone mass accrual and loss.

Introduction

Morbidity and mortality associated with hip fracture is a major public health concern [1–4]. Suboptimal bone mineral density (BMD), muscle weakness, impaired balance and cognition can all contribute to osteoporotic hip fracture [4]. Bone mass accrual and loss are influenced by a number of modifiable risk factors throughout life, including dietary intake of calcium and protein, serum vitamin D level, and weight-bearing physical activity [5,6].

A previous systematic review showed moderate evidence for lower fracture rates in rural areas compared to urban areas [7]. Most of the studies in this review were from high income countries (HIC), as defined by the World Bank [8], due to better availability of reliable fracture records. However, the prevalence of osteoporotic fracture has been rising in low and middle income countries (LMIC), where rapid urbanization has also been taking place [9]. Lifestyles, especially dietary patterns and physical activity levels, generally vary between urban and rural areas but how they differ may be context-specific, especially in relation to stages of economic development at country level [10]. There is therefore a need to examine the effect of urbanicity on bone mass accrual and loss globally.

Bone densimetry tools like dual-energy x-ray absorptiometry (DXA) have been used for the assessment of bone mass and density in both HICs and LMICs. These data enable assessment of association between urbanicity and BMD in a global context. Bone mass data are also available from wider age groups than osteoporotic fracture records, allowing assessment of the effect of urbanicity in younger populations as well.

We assessed the evidence on comparison of bone mineral density between urban and rural areas in meta-analyses and examined any variation in patterns of urban-rural differences among countries at differing stages of economic development.

Method

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis checklist and flow diagram were referred to structure this manuscript [11].

Search Strategy

We reviewed articles investigating differences in bone mineral density or content for adults ($\geq$ 20 years) as well as children and adolescents (<20 years) living in urban or rural areas. Search terms used for bone outcomes were "bone mass", "bone mineral density", "bone mineral content", BMD, and BMC. Terms "osteoporosis" and "osteopenia" were also included in the initial search in order to be more inclusive although they were not primary outcomes of interest. In addition, we searched for studies including terms for "urban" or "rural".

Appropriate wild cards were used to account for use and non-use of space and dashes. We searched the MEDLINE, EMBASE, and GLOBAL HEALTH electronic databases for full articles published before April 2013. We excluded the following publication types: Historical Article or News or Newspaper Article or Review, Multicase or Review, Tutorial or Review of Reported Cases (OVID Medline), Review (OVID EMBASE), and Patent (OVID Global Health). The complete search strategies are listed in the Supporting Information S1 Table.

Study selection and inclusion criteria

We included studies that compared bone mineral density or content between urban and rural areas in the same country (**Fig 1**). Duplicate articles were removed (n = 104) and then two reviewers independently examined the titles and abstracts for inclusion. Articles with only abstracts available or articles written in non-English languages were excluded. Full articles

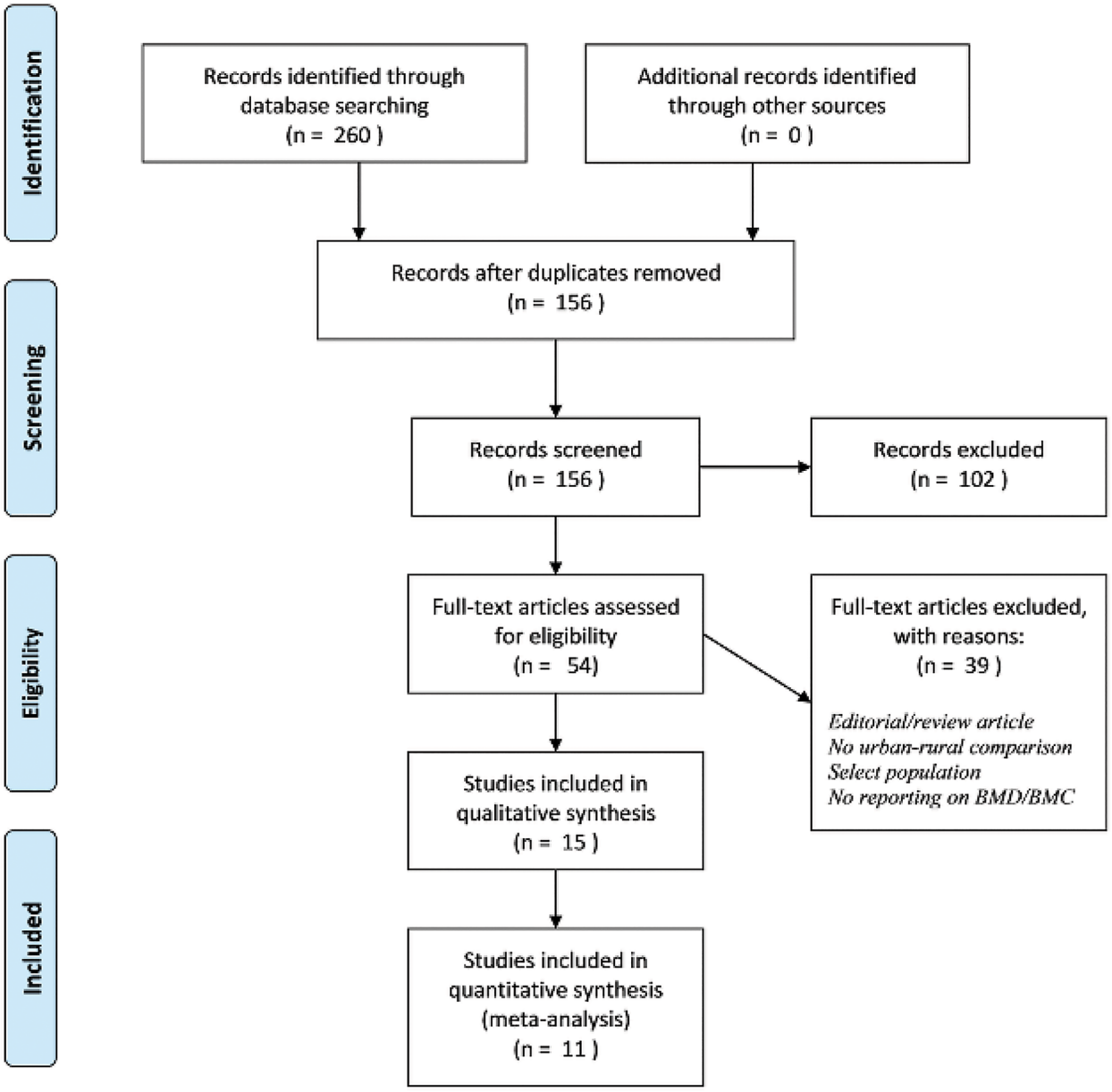

Fig 1. Flow diagram of study selection process from initial search to included studies.

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were examined when it was not clear from the titles or abstracts whether comparison of BMC or BMD in urban and rural areas was done. The discrepancies (n = 14) were resolved in a consensus meeting. Inter-rater agreement was assessed using kappa coefficients (κ) [12].

Data extraction

Data extraction was done by two independent reviewers. We extracted the following data: country, year of the study, definitions of urban or rural areas, sample size (n), age, mean and standard deviation (sd) of BMD (or BMC if BMD data were not available) in urban and rural area, and, where available, results of statistical comparison between urban and rural groups. The economic status of the countries at the time of study were identified, using the World

Bank's classification system, and categorized into either low and middle income countries or high income countries [8,13]

The Thai Epidemiological Study had three articles based on the same study population and four independent sets of data were extracted, which differed in sex (men and women) and sites of bone mass measurements (hip and lumbar spine) [14,15]. For men in the Thai Epidemiological Study, BMD data from the same study population were presented in two articles. We used the data from the 2006 article for the meta-analyses as the article had a slightly higher sample size for the rural population (duplicated data for the urban population).

Data analysis

When data from sub-groups within each sex, such as age groups and pre/postmenopausal women, were presented in articles, the data were pooled into one group to conduct statistical comparison between urban and rural areas. All hip data were based on femoral neck measurements. The analyses in this review were done separately for high income countries and low and middle income countries.

We used the ratio of means (RoM) between rural and urban populations to allow comparison of results from studies using different instruments [16,17]. RoM of BMD was calculated for all but one study, which only presented BMC data [18]. The natural logarithm of RoM and its SE were calculated for each study for the analysis. Random effects models controlling for heterogeneity in between-study variation were tested. The heterogeneity of effects across studies were estimated by Q test. Publication bias was assessed by a funnel plot and Egger's test. Q test, funnel plot, and Egger's test were only performed for HIC papers as there were too few studies from LMIC. All statistical analyses were performed in R version 3.1.1.

Results

Search results and study types

The initial search yielded 260 articles, of which 54 articles were found potentially eligible for inclusion after title and abstract search. In the full text search, 39 articles were further excluded because of one of the following reasons: they only had BMC or BMD data for either urban or rural areas; only osteoporosis prevalence data were available; only abstracts were available; or the articles were written in non-English language. A total of 15 articles met our selection criteria (**Fig 1**) [14,15,18–30]. Inter-rater agreement was high; two reviewers (MM and RP) scored 156 items and agreed on 137 (87%, $\kappa = 0.62$). No other articles were identified through hand-searching of the reference lists of these 15 articles. There were three articles published using the same population from the Thai Epidemiological Study [14,15,27]. These three articles provided four datasets (hip and lumbar spine bone mass measurements for men and for women) for our meta-analyses. There were two articles based on a study population in Malmö, Sweden, and two articles from the Norwegian Epidemiological Osteoporosis Studies (NOREPOS) [14,15,19,24–27]. A study by Gärdsell *et al* included only BMC data [18]. One HIC study did not provide sample sizes for urban and rural populations [29].

All studies had difficulty blinding researchers from urban and rural locations as the bone mass measurements were typically done within the towns where participants resided. The only cohort study included in this review was a large-scale, multi-decade study from Sweden and had low participation rates [19,24]. **Table 1** shows the characteristics of the included studies. Three studies were conducted within the last decade [14,15,22,23,27]. Six studies were from low and middle income countries (China [23,28], Thailand [14,15,27], and Sri Lanka [22]) and nine studies were from high income countries (Norway [25,26], Sweden [19,21,24,30], Poland [20], and the United States of America (USA) [29]). The age range of the study participants

Table 1. Study characteristics of the included articles.

National Income Level	Country; Study name	First author; Study year	Age range; sex	Sample size	Bone mass measurement device	Urban and rural definitions
LMIC	Thailand;	Pongchaiyakul [15];	20–84;	n = 872	DXA	U: Bangkok, a capital city, a population of 5.7 million, lifestyle similar to that in Western cities.
	Thai Epidemiological Study	2005	men and women			R: Khon Kaen, a province, a population of 1.8 million, considered one of the most typical agricultural communities in Thailand.
		Pongchaiyakul [27];	20–87; men	n = 412	DXA	(same as above)
		2005				
		Pongchaiyakul [14];	20–84;	n = 847	DXA	(same as above)
		2006	men and women			
	China;	Wanli [28];	>60;	n = 470	SPA	No definition given. All from Hongmen country of Xinxiang city.
		2005	men and women			
	China;	Gu [23];	50–70;	n = 1179	DXA	U: a city with an official urban residential (non-agricultural) registration
		2007	men and women			R: a village of a county with an agricultural residential registration according to the Chinese residential registration system
	Sri Lanka;	Ranathunga [22];	11–16;	n = 1181	DXA	U: Colombo
		2008	girls			R: Pannala
HIC	Norway;	Omsland [26];	>65;	n = 7333	SXA	Based on the population density of the election district (refers to Meyer *et al*):
	NOREPOS	2011	women			U: urban Tromsø;
						R: rural Tromsø (additionally, the rural region included Nord-Trøndelag, a rural county with a few small villages.)
		Meyer [25];	40–75;	n = 10,667	SXA	Based on the population density of the election district:
		2004	men and women			U: urban Tromsø
						R: rural Tromsø
	Sweden;	Sundberg [21];	15–16;	n = 250	DXA	U: a suburb of the city of Malmo, population size of 245,000, population density of 1595 inhabitants/km^2, the third largest city in Sweden.
		1997	boys and girls			R: Hassleholm County, population size of 50,000, 38 inhabit/km^2
	Sweden;	Ringsberg [30];	65–89;	n = 165	SPA	U: the city of Malmo, the third largest city in Sweden, population size of 240,000, a centre of trade and industry.
		2001	women			R: Sjobo, a typical agricultural community
	Sweden;	Rosengren [19];	50–80;	(1988/89) n = 437	SPA	Based on the national population records:
		2010	women	(1998/99) n = 289		U: the city of Malmo, population size of 230,383 in 1987 and 265,481 in 2002.

(*Continued*)

Table 1. (*Continued*)

National Income Level	Country; Study name	First author; Study year	Age range; sex	Sample size	Bone mass measurement device	Urban and rural definitions
						R: nine rural municipalities near the country village Sjobo, all predominantly agricultural municipalities, population size of 134,458 in 1987 and 141,989 in 2001.
		Rosengren [24];	50–80;	(1988/89) n = 323	SPA	(same as above)
		2012	men	(1998/99) n = 141		
	Sweden;	Gardsell [18];	≥40;	n = 961	SPA	Based on the Central Bureau of Statistics:
		1991	men and women			U: Malmo, the third largest city in Sweden, population size of 231,575 in 1988, a typical Swedish urban population.
						R: Sjobo, population size of 15,350 in 1988, considered one of the most typical agricultural communities in Sweden.
	Poland;	Filip [20];	30–79;	n = 503	DXA	U: Lublin urban area
		2001	women			R: Urzędów district, 40km from the nearest town, lack of industry, significant percentage of farmers.
	USA;	Specker [29];	20–66;	n = 1189	DXA	Based on the Rural-Urban Continuum Codes for South Dakota used by the U.S. Census Bureau:
	South Dakota Rural Bone Health Study	2004	men and women			Non-rural: population size of 2500 to 19,999.
						R: completely rural or population size of less than 2500.
						Hutterite: isolated communal living, agricultural-based rural lifestyle.

LMIC: low and middle income countries; HIC: high income countries; SPA: single photon absorptiometry; SXA: single-energy x-ray absorptiometry; DXA: dual-energy x-ray absorptiometry; U: Urban; R: Rural

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was from 11 to 89 years. There were two studies that examined adolescents under 20 years old [21,22]. All studies analyzed BMC and/or BMD in urban and rural areas for each sex separately. The majority of the articles were based on dual x-ray absorptiometry (DXA) data (n = 8) while five articles used single photon absorptiometry (SPA) and two articles used single x-ray absorptiometry (SXA). Bone mass measurements in all studies were done on the same type of bone densitometer within each study. There were nine studies whose main research question included urban and rural comparison of BMC/BMD [14,18,20–23,25,29].

There was a wide variation in the definition of urban and rural areas as shown in Table 1: one study gave no definition [28]; one study only gave the names of the urban and rural areas [22]; one study used rural features such as 40km from the nearest town, lack of industry, and high farming practice [20]; one study was based on the national residential registration for agricultural and non-agricultural areas [23]; all other studies used census data of population size or density, with some additionally describing the patterns in agricultural practice. One study compared physically active urban population to rural population as well as non-active urban population [30]. Another study compared two rural sub-populations, Hutterite population, who is an isolated religious community that engages in self-sufficient lifestyle through agriculture, and non-Hutterite rural population [29].

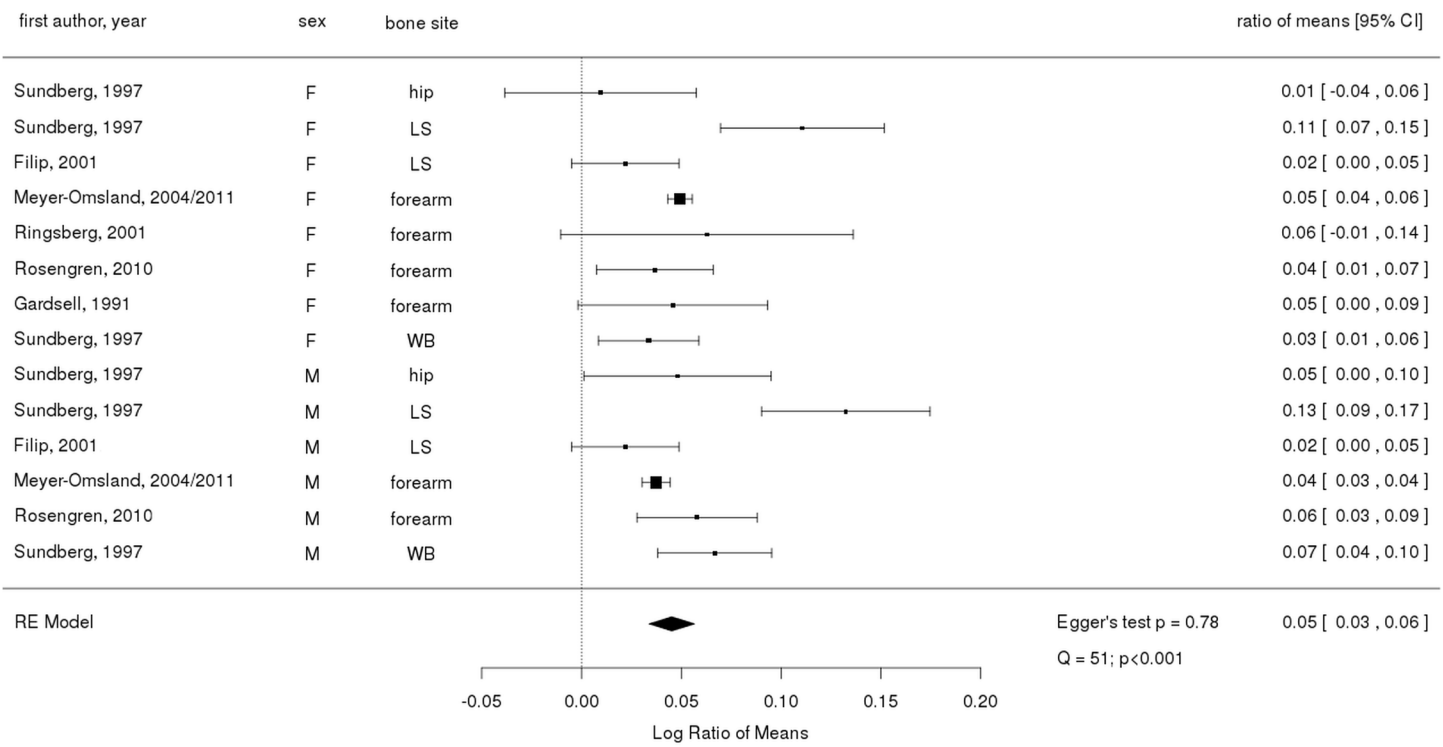

Fig 2. Ratio of means and 95% confidence interval for comparing bone mineral content or density in urban and rural populations in high income countries. Symbol sizes are proportional to sample sizes. The overall effect size was derived from a random-effects model. LS: lumbar spine. WB: whole body. F: female. M: male.

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Fig 2 (HIC) and Fig 3 (LMIC) show the meta-analysis of BMD in urban and rural populations, with the exception of BMC comparison by Gärdsell *et al* [18]. There were five articles examining hip, seven articles lumbar spine, five articles forearm, one article finger, and one article total body. Since both 1988/89 and 1998/99 data in Rosengren's cohort study showed similar patterns in BMD differences between urban and rural areas, the values from two time points were pooled for meta- analysis.

The pooled analysis showed that rural residents had a 5% higher BMD than urban residents in HIC (RoM = 0.05; 95% CI: 0.03 to 0.06). On the other hand, studies from LMICs showed mixed results (RoM = -0.04: 95% CI: -0.1 to 0.01). Publication bias for HIC studies did not indicate any systematic trend of publication bias (Egger's test p = 0.78). There was between-study heterogeneity found for HIC studies (Q = 51; p <0.001). Publication bias and heterogeneity were not tested for LMIC studies as there were too few studies.

Discussion

Three out of four studies from low and middle income countries provided evidence that bone mineral density in urban areas is higher than rural areas while there was no study from high income countries that showed higher BMD in urban areas.

Comparison with previous research

Our findings from HIC studies are generally in line with the moderate evidence found in a previous systematic review for lower risk of osteoporotic hip fracture among rural residents [7]. Mixed results in LMIC found here are in concordance with a view expressed previously on

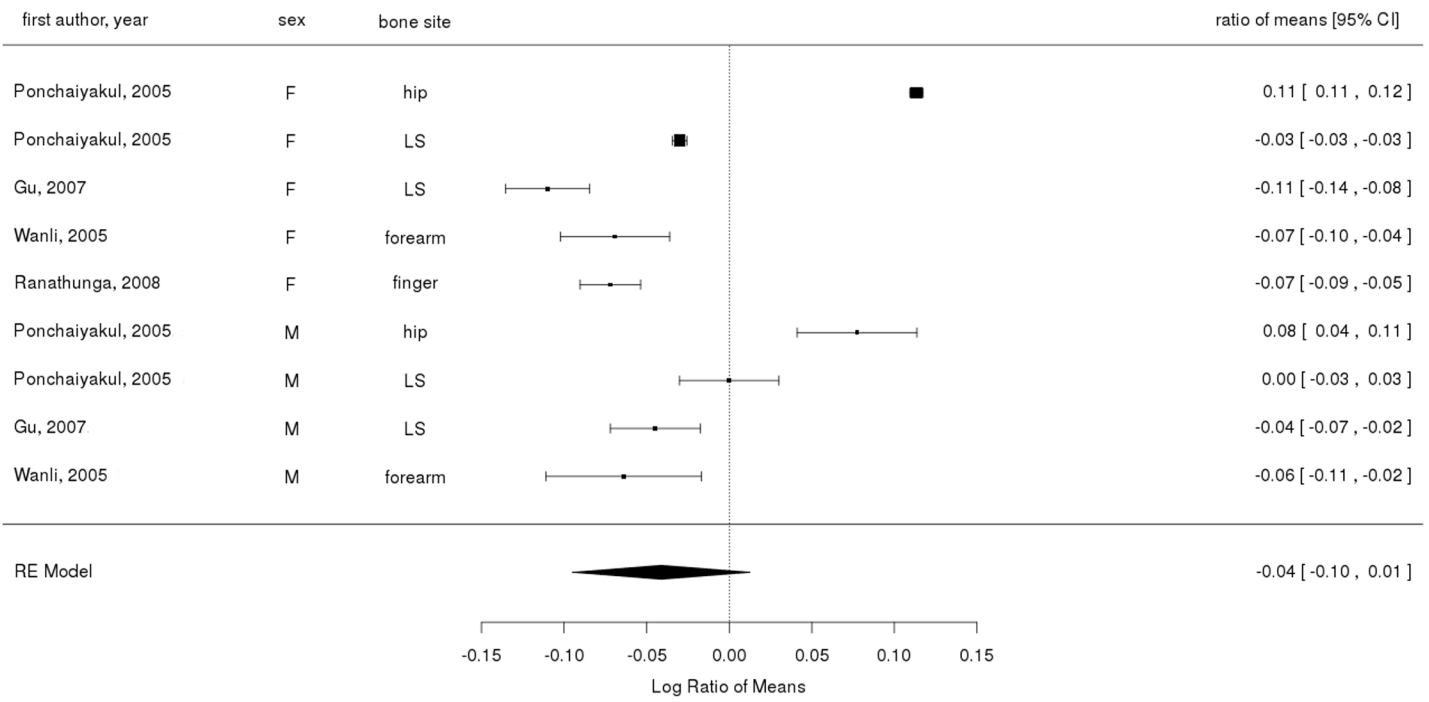

Fig 3. Ratio of means and 95% confidence interval for comparing bone mineral content or density in urban and rural populations in low and middle income countries. Symbol sizes are proportional to sample sizes. The overall effect size was derived from a random-effects model. LS: lumbar spine. WB: whole body. F: female. M: male.

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osteoporosis and urbanicity in LMIC [31]. The discrepancy in findings between HIC and LMIC shown in this review suggests that the effect of urbanization may be context-specific. There is a possibility that further economic development in LMIC may shift patterns of association between urbanicity and BMD more towards negative associations seen in HIC through environmental and individual lifestyle changes.

There are a number of potential lifestyle and environmental factors contributing to healthier bone development in rural areas in HIC, including higher physical activity level, higher serum vitamin D level, and less air pollution. A handful of studies conducted further statistical analyses to show how these risk factors may be associated with regional differences in BMD. In high income countries, longer sedentary time, lower micronutrient intake, and lower BMI are generally considered to be characteristics of urban dwellers. In Norway, urban women had lower BMI than rural women and body size adjustment attenuated the BMD differences between urban and rural areas in women [25]. Physical activity level or smoking status did not explain the regional differences. In Sweden, urban women who have engaged in regular exercise activity for twenty years had higher BMD in comparison to urban women who did not regularly exercise [30]. The difference between rural women and active urban women was less clear. In the South Dakota Rural Bone Health Study (SDRBHS), all rural residents engaged in more than 75% of their life on a farm and spent less than 1040 hours a year on non-farming work. Although there were differences in BMD between rural and non-rural populations, current physical activity level, dietary intake of calcium and vitamin D, or muscle strength did not explain these population differences. The authors speculated that the higher physical activity level during childhood and adolescence in the farming rural population may be partially responsible for the observed difference.

On the other hand, in lower income countries, urban residents may have better bone health profiles as they have better access to food, education, jobs, and social welfare that may not be available in rural areas. The studies from China showed higher BMC and BMD in urban areas [23]. Gu *et al* showed that the urban and rural difference was attenuated in men upon adjustment for body size, suggesting that higher body mass due to better nutrition may contribute to higher BMD in urban area [23]. However, for women, the urban and rural difference persisted even after adjusting for body size, income, milk consumption, calcium and vitamin D supplement intake, total physical activity, walking, and social activity. The Thai Epidemiological Study explored whether regional differences in lean and fat mass explained BMD differences [15]. Rural residents had higher lean mass and lower fat mass but did not always show higher BMD when compared to urban residents. The matched pair analysis in men showed that lean mass explained more of the variance of urban and rural difference in BMD than fat mass. Although lean mass was positively associated with BMD in women as well, the urban and rural difference in lean mass did not account for the differences in BMD as much as in men.

Strengths and limitations

There are some limitations to this review. Only full articles were reviewed while there were several conference abstracts describing urban-rural differences in BMD. Articles written in non-English languages were also excluded, which is likely to have reduced the number of articles from LMIC included in this study. For instance, Gu *et al* discusses four papers written in Chinese that showed a range of findings for urban-rural differences in BMD or the prevalence of osteoporosis in China.

The number of studies included in this review was fairly small. More studies, especially from LMIC, are needed in order to ascertain our observation on differences in urban and rural areas between HIC and LMIC. Because there were only seven countries included in this review, the interaction between national income levels and urba-rural differences could not be tested statistically formally and therefore, the conclusion should be treated with caution. Similarly to Brennan's review, the definitions of urban and rural area varied considerably among studies, which also urges careful interpretation of the results. Ten out of eleven studies were based on cross-sectional data limiting causal inference between urbanicity and bone mineral density. More cohort studies are needed in order to determine how urban and rural lifestyles and environments may influence bone mass accrual and loss throughout life.

There were also very few studies examining children and adolescents. While there were more studies examining younger adults (<50 years), most studies focused on the elderly. If lifestyles during the bone development phase are indeed important as suggested in the SDRBHS, there needs to be more studies on how lifestyle and environmental changes due to urbanization may be associated with bone development in younger populations. Suboptimal bone mineral density is a major contributing factor for osteoporotic hip fracture. Since body size is strongly associated with bone mineral density, better food availability in cities may be beneficial for bone development in lower income countries, at least during the initial phase of economic transition. Low physical activity level and excess food intake are more commonly observed among urban dwellers in higher income countries in comparison to urban areas in lower income countries. As the epidemic of osteoporosis continues to grow globally, effects of urbanization on bone health in LMICs and HICs ought to be carefully examined in order to develop appropriate interventions.

Summary Box. What is already known on this subject?

- Bone mineral density is a key determinant of osteoporotic fractures.

- Whether BMD is higher in urban than rural areas globally is not known

What does this study add?

- Bone mineral density was higher in urban areas in some low and middle income countries while no high income countries showed higher BMD in urban areas.
- There may be different underlying mechanisms of the effects of urbanization on bone mineral density in countries at various economic stages.

Supporting Information

S1 Checklist. PRISMA statement.
(DOC)

S1 Dataset. Sample size and means of bone mineral content/density for meta-analyses.
(CSV)

S1 Table. Summary of search strategy.
(DOC)

Author Contributions

Conceived and designed the experiments: MM. Performed the experiments: MM RP. Analyzed the data: MM. Wrote the paper: MM RP BK SK.

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2.3 Nutrition transition and bone development

2.3.1 Lifecourse of bone and skeletal "programming"

Bone is a dynamic organ that undergoes a cycle of resorption of existing bone and deposition of new bone throughout life. Bone size and density are both key features of bone strength. During the first three decades of life, bone grows in length, width, and mass due to a greater rate of deposition than resorption [101]. Starting with skeletal patterning in early fetal development, bone undergoes several distinct stages of growth until peak bone mass (PBM) is attained during the third decade [102–104]. About 30g of calcium is accrued prenatally, with most accretion occurring during the third trimester [105,106]. The patterns of bone growth remain similar between girls and boys until puberty. As children enter puberty, sex differences become apparent; bone tends to grow larger in and bone mass accrual lasts longer in males than females [107,108]; in Canada, average peak calcium accretion rates among healthy adolescents of 284 mg/day for females and 359mg/day for males and PBM was 0.857 ± 0.125 g/cm^2 for females and 0.910 ± 0.125 g/cm^2 for males [108,109]. Bone mass and density continue to increase after growth in bone length ceases until PBM is attained **[110]**. Peak bone size and density vary by ethnicity. Africans have greater bone size and density than Caucasians and Asians while Asians tend to have lower bone size than other ethnic groups [111–114].

Like other adult-onset diseases such as coronary heart diseases, exposure to adverse conditions during fetal development has been suggested to have long-term effects on bone health in later life [115–117]. The theory of developmental origins of osteoporotic fractures suggests that prenatal exposure to adverse conditions, such as maternal undernutrition, may result in *intrauterine programming* of bone size and shape through restricted cell division and changes in endocrine functions [106]. The biological mechanisms underlying intrauterine programming of bone development are not fully understood although epigenetic modulation of placental calcium transfer and hypothalamic–pituitary–adrenal axis have been suggested as potential contributers [118].

Birth size is commonly used in life-course studies for bone health as a measure for

pre-term birth or restricted intrauterine growth [12,13,15,119,120]. A number of studies have shown a positive association between birth weight and bone mineral content during childhood, adolescence, and even in adulthood although the evidence is more mixed for association with bone mass at younger ages [12,14,15,119–121]. Generally, studies have shown evidence for stronger association between bone mass and density and body size in adulthood than in early life [15,120,122].

2.3.2 Nutrition and bone development

Peak bone mass has been suggested as one of the key determinants of osteoporosis and osteoporotic fractures in later life [123–125]. Although genetics play a major role in determining PBM, many modifiable risk factors during early life – prenatal period, infancy, and childhood - influence whether optimal PBM may be attained [126,127]. Adequate nutrient intake is key to healthy bone development as nutrients are needed as source of bone components and metabolic fuels [107]. A number of nutrients are involved in development and maintenance of bone matrix, collagen, and cartilage, including protein, minerals (calcium, phosphorus, magnesium, zinc, copper), vitamins C, D and K. [107,127].

Several studies have examined effects of maternal nutrition during pregnancy on bone development in the offspring. In rural India, higher maternal intake of dairy products at 28 week gestation was positively associated with total body and spinal bone mass of the offspring at age 6 [128]. A small cohort study from Australia also showed a positive association between offspring BMD at age 16 and maternal intake of milk during the third trimester [129]. Calcium is one of the main nutrients that may explain the association between bone mass and intake of dairy products. An RCT from the USA showed a positive association between bone mass of neonates and daily supplementation of 2g calcium for pregnant women when these women had low (<600mg/day) calcium intake [130]. However, in a small RCT in the Gambia, daily supplementation of 1.5g calcium for mothers from gestation week 20 to delivery did not show any improvement in bone mass of the offspring during the first year of life [131]. Although some small studies suggested potential benefits of greater vitamin D level during pregnancy, a large prospective cohort study from the UK found no clear evidence between offspring bone mass at age 9 and maternal serum vitamin D level during any trimester [132,133]. Maternal intake of other

nutrients such as fat, folate, and magnesium during pregnancy may also be positively associated with bone mineral content (BMC) of the offspring during childhood and adolescence [128,129,134].

It is important to note that, although there may be association between intrauterine environment and postnatal bone development, longitudinal effects of maternal diet may be small in comparison to postnatal risk factors; for instance, in the Pune Maternal Nutrition Study, maternal consumption of milk and milk products and folate concentration at 28 week gestation explained only about 3% of the variance of total BMD in the offspring [128]. Adequate postnatal nutrition is a key determinant of PBM [127]. Severe undernutrition, such as protein-energy malnutrition, can result in retarded linear growth and low BMI is associated with lower bone mass accrual [127,135–138]. In children whose calcium and vitamin D intake are very low, supplementation of these nutrients may be beneficial in improving bone mass accrual or reducing the risk of fracture in later life [139].

In addition to diet, there is consistent evidence from both intervention and observational studies on positive effects of weight-bearing physical activity during childhood and adolescence on bone mass accrual [140–142]. Positive effects of weight-bearing physical activity may even be more important than calcium intake [143]. A mechanostat theory states that mechanical strain resulting from weight-loading stimulates alterations in bone architecture [144,145]. Certain bone sites, such as hip, experience weight-loading more than the other bone sites and may benefit more from weight-bearing physical activity over the lifecourse [146,147]. Other lifestyles such as use of tobacco, alcohol, and use of hormonal contraception may also affect peak bone mass accrual [148–150].

2.3.3 Nutrition transition and peak bone mass accrual

In urbanizing rural communities in LMICs, early life undernutrition may be combined with urbanized lifestyles in later years, which may include improved nutrient intake, excess caloric intake, unbalanced diet high in fat, carbohydrates, and salt and low in other micronutrients, sedentary lifestyle, and increased substance use. Depending on the timing, extent, and characteristics of nutrition transition and catch-up growth, nutrition transition may affect bone strength in adulthood to varying degrees. For

instance, the British Birth Cohort Study showed a positive association between diaphyseal cross-sectional area of non-dominant radius (measured by peripheral quantitative computed tomography) at age 60-64 and weight and height gain during prepubertal and postpubertal periods [120]. Such association was not found with weight gain during adulthood. In order to understand bone development in young people from urbanizing rural communities, there is a need to examine 1) whether there is a permanent effect of early life undernutrition on bone strength in populations who experience nutrition transition and 2) how the impact of nutritional transition on PBM differs at various stages of bone development.

There are several different types of studies that evaluated the impact of early life undernutrition and nutrition transition on bone development. Some studies assessed nutrition transition driven by abrupt changes in dietary environment such as international adoption, nutritional intervention, and migration from LMICs to HICs. Others examined more gradual nutrition transition driven by urbanization or historical events such as end of wars.

Compared to the number of studies on linear growth (i.e. stunting), there are fewer studies examining bone size and mass in relation to early life undernutrition and nutrition transition. For instance, a few studies have followed up on children and adolescents from LMICs who were adopted to higher income countries and observed attainment of normal height and weight for age years after adoption but detailed examination of bone size and mass was not conducted [151–154]. The following section will review intervention, famine, and immigration studies as well as cohort studies from transitional communities in LMICs with the aim of summarizing existing evidence on life-course determinants of bone mass accrual in the context of early life undernutrition and nutrition transition.

Intervention study on nutritional recovery from undernutrition

There are relatively few studies assessing association between nutrition transition due to interventions and bone size or mass in LMICs. A small study in Brazil (n = 98) examined children and adolescents (4-14 years old) who had successfully recovered from mild to severe undernutrition (weight-for-height Z-score less than –0.1645) upon nutritional treatment and support [155]. Compared to the control group who

had normal weight-for-height Z-scores, undernourished children who received nutritional interventions were able to fully catch up in both linear growth and BMC in this study. While the results are promising, this is a fairly small study and there is currently no conclusive evidence on the effects of nutritional interventions on bone mass accrual in undernourished populations in LMICs.

There exist more evidence from studies assessing the effects of weight recovery in adolescents with anorexia nervosa on subsequent bone mass accrual [156–159]. Abnormally low body weight and delay in sexual maturation seen in adolescents with anorexia nervosa are associated with reduced bone mass accrual [160–162]. A small longitudinal study from UK (n = 32) found that individuals who underwent successful treatment for anorexia nervosa (median age at disease onset = 16 years old) still had lower femur BMD in adulthood (median age = 40.2 years old) than the control subjects with no history of eating disorders [163]. Other studies have shown weight recovery and resumption of menstruation due to successful treatment resulting in catch-up accrual of bone mass in adolescents [164–166]. However, many of these studies also showed that BMD remained below the optimal levels for their ages even years after successful treatment, suggesting that severe undernutrition during this critical growth period may have permanent negative effects on bone mass.

Extrapolation of these findings for undernourished populations in LMICs requires caution as the timing of exposure to undernutrition may be more acute in an adolescent population with anorexia nervosa (*i.e.* underweight only during adolescence and normal weight during infancy) whereas undernourished adolescent populations in LMICs are more likely to have been exposed to chronic undernutrition throughout their lives. More detailed description of the timing of exposure to undernutrition and nutrition transition is needed to understand the mechanisms underlying long-term effects of undernutrition and interventions. Finally, all these studies were observational studies, which provides weaker evidence than randomized controlled trials, which would not have been ethical in these settings.

Famine Study

Elderly Chinese people in Hong Kong (n = 3832; 65 years old or older) who reported

having experienced famine (caloric restriction over 1 year) during World War II were found to have lower total hip and LS BMD than those who did not experience famine [167]. Path analyses showed that lower educational and socioeconomic status (SES) attainment as potential mediators of this association. Other potential confounders (*i.e.* weight status in adolescence and young adulthood) were not examined in this path analysis, which may have mediated the relationships examined in their models. Although the study method noted that the timing of exposure to famine was retrospectively determined through interview, the study did not present the data on the timing of famine (simply refers to exposure during "childhood") nor the process and timing of nutritional improvement.

During and shortly after World War II, Japan experienced food shortage [168]. A small study (n =88; 35-59 years old) showed that women who were infants (mean age 5 years old) during World War II had lower lumbar spine BMD in adulthood than those who were born after the war [169]. This study assumed that the study population was exposed to undernutrition before World War II and the timing of exposure to undernutrition was estimated from birth years; however, there is a possibility that those who were born after World War II were also exposed to undernutrition. The timing of exposure to nutritional improvement was not described in this study.

A small study comparing postmenopausal women from Spain and USA suggested that lower bone mass in Spanish postmenopausal women may be due to undernutrition during the Spanish Civil War, although the study did not explore the exact timing of exposure to or the nature of famine and nutrition transition [170]. Another small study (n = 133; 60 years old or older) found that Holocaust survivors who experienced severe undernutrition during childhood and adolescence showed higher prevalence of hip and lumbar spine osteoporosis than the control group who did not experience the Holocaust [171]. Among the Holocaust survivors who were 17 years or older in 1945, the prevalence of osteoporosis was lower than those who were less than 17 years old in 1945, suggesting that undernutrition before adolescence may have a greater impact on bone mass accrual [171]. The aforementioned Japanese study saw a difference in BMD between those who were under and above 5 years old during the war time, which suggests a possibility that there may be differential effects

of undernutrition even among the Holocaust survivors who were less than 17 years old (*i.e.* those who were infants vs adolescents during the Holocaust). Additionally, those who were teenagers at the end of the war may have gone through a series of nutrition transition (*i.e.* sufficient nutrition during infancy, severe undernutrition during childhood and adolescence, nutritional improvement in young adulthood). Identification of periods of nutritional insufficiency and improvement would be helpful in understanding the influence of exposure to various nutritional conditions over the lifecourse of bone.

All these study populations showed normal, or even overweight, average BMI at the time of bone mass measurement; however, these retrospective studies often lack detailed anthropometric data from earlier life, and therefore it is not clear at which stages in the lifecourse of bone these populations experienced nutritional improvement.

Migration from LMICs to HIC

Migration from LMICs to HICs can result in significant dietary transition [172]. Several studies on migrants from LMICs to HICs have suggested that migration at younger age may be more beneficial for bone mass accrual. A study examining recent migrants from Sudan to the USA (n = 143; mean age = 30.4 years old) showed a positive association between whole-body and hip BMD – but not spinal BMD - and length of stay in the USA (median = 3.4 years) [173]. The study showed lower spinal BMD than the reference values for African Americans. The association between whole-body and hip BMD and length of stay in the USA was attenuated by body weight as well as milk consumption for hip BMD, suggesting that dietary change and weight gain after moving to the USA may have contributed to this improvement in whole-body and hip bone mass accrual; however, this was a small cross-sectional study and there is no prospective data on when and how nutrition transition occurred because of the migration [173]. There was also no description of BMD at the time of immigration and change in BMD after immigration could only be speculated.

In studies examining Chinese migrants to the USA and Denmark, older ages at migration were negatively associated with BMD [174–176]. Among Southeast Asian women who migrated to the USA too, older age at migration as well as late

menarche, which was assumed to be due to early life undernutrition, were associated with lower BMD [177]. Based on the hypothesis that coastal residents were less affected by drought-related famine because of the access to seafood, the authors also examined association with bone mass and coastal birth and found a positive association. These results suggest that Southeast Asian women who did not experience early life undernutrition or experienced transition from undernutrition at a younger age had higher BMD during adulthood. Farming at age 18 was also associated with increased BMD, which supports the importance of physical activity during the growth phase even in an undernourished population.

Although all the reviewed articles suggested that dietary changes (*i.e.* greater consumption of dairy products after migration) may have contributed to differences in BMD, like the famine studies, there was no prospective data on anthropometry, diet, or physical activity prior to or at the time of migration and thus the effects of nutrition transition could only be speculated. Additionally, these studies examined age at the time of migration in older individuals, who may benefit from the transition through distinct mechanisms from those for younger populations who are in developmental phases.

Cohort studies on bone development

While analysis of early life nutrition and linear growth is fairly common in cohort studies, there are fewer studies that examined lifecourse determinants of bone mass accrual in young populations who experienced both early life undernutrition and nutrition transition before attaining peak bone mass. LMIC-specific cohort studies on nutrition transition are important as early life undernutrition is not as common in HICs. Several large-scale cohort studies from LMICs, including five studies from the Consortium of Health-Orientated Research in Transitioning Societies (COHORTS), have examined the longitudinal effects of early life environment and health on growth and adult-onset NCD in Brazil, Guatemala, India, the Philippines, and South Africa [178–181]. The COHORTS suggest that both higher birth weight and faster growth during the first 2 years are positively associated with height in adulthood [182]. The studies from Brazil and India examined association of adulthood bone mass and early life body size [13,183]. The 1982 Pelotas Birth Cohort Study found a positive association between BMC and BMD at age 18 and adolescent BMI at age 11

[183]. This association was largely mediated by BMI and fat free mass at age 18. The New Delhi Birth Cohort (NDBC) found that BMI gain at different stages of bone development was associated with different bone outcomes in adulthood [13]. Peak bone mineral content (BMC) was positively associated with BMI gain during infancy while higher areal BMD and bone apparent mineral density were associated with greater BMI gain in childhood and adolescence. Adulthood height and BMI mediated these associations respectively.

There are relatively few large-scale cohort studies in India that assessed long-term effects of early life nutrition on bone mass using dual-energy x-ray absorptiometry. The Pune Maternal Nutrition Study (PMNS) and NDBC are two cohort studies from India that examined the longitudinal effects of early life nutrition on bone mass [13,128]. **Table 2.1** shows a summary of the characteristics of each study based on publication to date.

The differences in study settings for PMNS, NDBC, and our study (APCAPS) reflect uneven rates of socioeconomic development in India. The study population in the PMNS is more similar to the APCAPS population than NDBC as they live in a rural area near a large city (Pune). Undernutrition is common in these study villages in PMNS. Maternal pre-pregnancy BMI was low (18.1 ± 1.9 kg/m^2) and mothers had inadequate protein intake during pregnancy [184]. In contrast, the NDBC is undertaken within the city of New Delhi and the study population is relatively well-off by national standards although over 40% of the families lived in one-room tenements [185]. As described in detail in Chapter 3, the APCAPS population resides in 29 villages located near a large city (Hyderabad) and the community has been experiencing urbanization at uneven rates over the past decade.

Dual-energy x-ray absorptiometry (DXA) measurements were conducted at different points in the lifecourse of bone in these three cohort studies. PMNS examined long-term effects of early life nutrition on bone mass in children (6 years old). The NDBC took bone measurements in adults in their 30s. APCAPS examined bone mass in young adults (18-23 years old).

In PMNS, higher total and spinal BMC and BMD were observed in children (6 years

old) whose mothers who had a higher frequency of intake of calcium-rich foods during pregnancy (milk, milk products, pulses, non-vegetarian foods, green leafy vegetables, fruit). Children whose mothers had higher folate levels at 28 week gestation had higher total and spine BMD, independent of parental size and DXA measurements.

Although all three studies took a lifecourse approach in assessing determinants of bone mass accrual, the study settings and designs are distinct. APCAPS aims to strengthen evidence on effects of early life undernutrition and nutrition transition during late adolescence on bone mass accrual. The next four chapters will include description of the study setting of APCAPS and three research papers based on the cross-sectional and longitudinal analyses of the data from the first three waves of data collection.

Table 2.1: Comparison of study designs of DXA bone cohort studies in India.

	PMNS	NDBC	APCAPS
Study characteristics			
Year of initial recruitment	1994-1996	1969-1972	1987-1990
Place of study	6 villages near Pune	A defined area (12km^2) in South Delhi	29 villages near Hyderabad
Urbanicity	rural	urban	rural/urbanizing
Initial recruitment	All married women of reproductive age from the study villages (n=2,675) were recruited. 1102 women became pregnant. 762 live births.	20755 never-married women from the defined area were followed bimonthly. Pregnancies were identified and the newborns were enrolled (n = 8181).	Hyderabad Nutrition Trial: All women (aged 13-45) considered exposed to the risk of pregnancy were monitored for last menstrual period monthly to identify pregnancies early in the antenatal period. 1826 mothers gave 2601 births during the trial period (1987-1990).
Participant SES	low	middle	low-middle
Follow-up studies (sample size)	annually in 1994-2009 [186]; detailed study at age 6 (n = 653)[187] and 12 (n = 690) [188].	every 6 months in 1973-1992 (except for 1980-1982)[13]	2003-2005 (n = 1165)[189]
		1998-2002 (n = 1526)[190]	2009-2010 (n = 1446)
		2006-2009 (n = 565*)[13]	2010-2012 (n = 6944)
Exposure measures			
Prenatal	maternal data (18 and 28 week gestation):	NA	Pregnant/lactating women and children under 6 years old

	anthropometry dietary intakes physical workload circulating micronutrients (red cell folate and plasma ferritin, vitamin B12, and vitamin C) [187]		(Hyderabad Nutrition Trial): protein-energy supplementation provided along with early childhood education, health, hygiene and nutrition education for the mothers, and delivery of other national programmes (immunization, anemia control and basic health care) in the Integrated Child Development Services (ICDS) scheme. neonates birth weight (only a small subset could be linked).
Infancy and childhood† (age)	(Parental total body and LS BMC and BMD when children were six years old.)	SD scores for height and BMI for each subject at age six months and at birthdays from age 1 to 11 years. Changes in height and BMI during infancy (birth to 2 years) and childhood (2-11 years)	NA
Adolescence† (age)	NA	SD scores for height and BMI at birthdays from age 12 to 18 years. Changes in height and BMI during adolescence (11 years to adulthood)	anthropometric data, physical activity‡, diet‡, tobacco/alcohol use, reproductive data from age 13 to 18.

Adulthood† (age)	NA	SD scores for height and BMI at birthdays from age 18 to 21 years. Lifestyle variables (exercise scores, tobacco use, alcohol consumption) Socio-economic status (occupation, education, material possessions) (for women) reproductive history (parity and age at menarche) Body size (height and BMI)	(Parental BMC and BMD when index children were at least 20 years old.)
Outcome measures (bones)			
Pre-adulthood (age)	Total body and total spine BMC and BMD (6 years old; n = 698) [128]	NA	NA
Adulthood (age)	NA	Bone mineral area, content, and areal density at femoral neck (FN), lumbar spine (LS; L1-4) and forearm and volumetric bone mineral density at FN and LS (n = 565).[13]	Bone mineral area, content, and areal density for total hip, lumbar spine (L1-4), whole-body (n = 1504 index children at W2/3).
DXA machine type	Lunar DPX-IQ 240 pencil beam machine	Hologic QDR 4500A fan beam DXA machine	Hologic Discovery A and 4500W.
Main research questions for	To examine the relationship	To examine association between	To examine association of

longitudinal analyses of bones	between maternal nutritional status (body size, body composition, dietary intakes, micronutrient in blood and physical activity) and bone health in the offspring	early life body size and growth and adulthood bone mass.	exposure to early life nutritional supplementation and to adolescent body size and bone mass during young adulthood.
Main findings from longitudinal analyses	Children (6 years) of mothers who had a higher frequency of intake of calcium-rich foods during pregnancy (milk, milk products, pulses, non-vegetarian foods, green leafy vegetables, fruit) had higher total and spine bone mineral content and BMD, and children of mothers with higher folate status at 28 wk gestation had higher total and spine BMD, independent of parental body size and DXA measurements.	The associations between peak bone mass and infancy/childhood/adolescent BMI and skeletal growth are mediated by the attainment of adult height and BMI.	Chapter 7 in this thesis

PMNS: Pune Maternal Nutrition Study; NDBC: New Delhi Birth Cohort; APCAPS: Andhra Pradesh Children and Parents Study; BMI: body mass index; BMD: bone mineral density; DXA = dual-energy x-ray absorptiometry; SD = standard deviation.

* bone measurements were taken in these participants.

† infancy/childhood <12 years old; adolescence 12 to 18 years old; adulthood here is defined as ≥18 years old.

‡ dietary and physical activity data were collected during adolescence but the W1 questionnaire was different and shorter than the W2/3 questionnaire, which was tested for validity and reliability.

Chapter 3: Study setting

This chapter describes the study setting and method of the Andhra Pradesh Children and Parents Study. The first section aims to describe urbanization in the APCAPS community using three different approaches: census data (2001, 2011), night time light intensity (1992, 2002, 2012), and built environment (2013). The second section describes the APCAPS study design and data availability. Research papers included in Chapter 4, 5 and 6 all analyzed data from APCAPS; therefore only the methodological information pertaining to all research papers are described in Chapter 3 and any additional methodological information specific to the research papers are included in respective chapters.

3.1 Urbanization in a transitional rural community in India

The APCAPS villages (n = 29) are located in the Ranga Reddy district in the state of Telangana **(FIGURE 3.1).** This thesis uses data from three waves of data collection in APCAPS, which were all conducted before the state of Andhra Pradesh was split into two states, Telangana and Andhra Pradesh in June, 2014 [191].

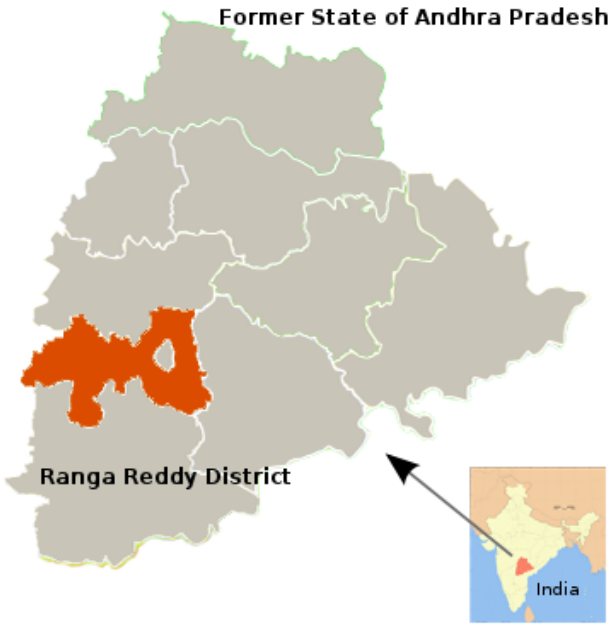


FIGURE 3.1: Maps of the Ranga Reddy district in former Andhra Pradesh state in India[5].

5. "Rangareddy district in Telangana" by Adityamadhav83 - Own work. Licensed under CC BY-SA 3.0 via Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Rangareddy_district_in_Telangana.png#/media/File:Rangareddy_district_in_T

The study villages are located about 50-100km away from Hyderabad, one of six mega-cities[6] in India. Since 1990s, the economy in Hyderabad has grown significantly due to its strong information technology, financial, and pharmaceutical sectors [192]. The proximity of the Ranga Reddy district to Hyderabad led to an increase in both the total and urban populations in the past decade, where over 70% of the population in this district resided in urban areas in 2011, compared to 54% in 2001 [193].

Anecdotal evidence points to uneven rates of urbanization of the APCAPS villages in the last decade **(Figure 3.2).**

FIGURE 3.2: Photos from two study villages showing uneven rates of urbanization in the APCAPS community: Thummalur (left) and Ibrahimpatnam (right).

According to the national decennial census, the APCAPS community saw an overall modest increase in its population size between 2001 and 2011 (**Table 3.1)[194]**. The population sizes among the APCAPS villages varied greatly in 2011, from about 600 to 17000.

Table 3.1: The population size and density in 2001 and 2011 in the APCAPS villages and the city of Hyderabad.

Village names	2001 population size	2011 population size‡

elangana.png

6 . Mega-cities are cities with over one million people.

	(population density)	
Ibrahimpatnam*	Khalsa: 6839 (271.4) Bagath: 17692 (702.1)	Khalsa: 17345 Bagath: 12349
Sheriguda*	NA	NA
Patelguda*	NA	NA
Mangalpalli	4134 (180.1)	4812
Uppariguda*	NA	NA
Pocharam	930 (144.0)	1598
Ramireddyguda†	1386 (990)	728
Seetharampet†	NA	NA
Nomula	1788 (152.0)	1932
Lingampalli	1592 (302.7)	1459
Engalgda	568 (546.2)	605
Polkampalli	2835 (203.5)	3055
Nainampalli	1741 (183.6)	1877
Raipole	4980 (501.0)	5106
Dandumylaram	5140 (402.5)	5296
Rachaloor	3777 (183.5)	4036
Lemur	4769 (139.8)	4828
Mankhal	10587 (343.4)	11916
Thummalur	2850 (123.8)	2859
Maheshwaram	6892 (325.6)	8795
Mansanpalli	1756 (168.0)	2125
Gudur	1926 (160.0)	2264
Kandurkur	4413 (135.7)	4999
Gummadivalli	2483 (101.9)	2728
Thimmapur	3390 (215.5)	3466
Meerkhanpet	4033 (211.3)	4377
Sardarnagar	1484 (551.7)	1600
Aakulamylaram§	NA	NA
Nedunur	5148 (195.1)	4652

* Sheigua, Patelguda, and Uppairguda have been counted as part of Ibrahimpatnam for census since 1991. Ibrahimpatnam has been split into two village since 2001.
† Ramireddyguda and Seetarampet have been combined for census since 2001.
‡ Population density could not be estimated for 2011 as the area sizes were not yet available for 2011 census as of May 2015.
§ Aakulamylaram has been counted as part of Kandukur for census since 1991.

Urbanization is often associated with greater convenience and employment and education opportunities, attracting more people to migrate to urbanized areas and leading to a rapid increase in population size [195]. However, population size, especially in transitional rural settings, may not always reflect degrees of urbanization and, more importantly, do not describe the process of urbanization that may influence people's choices on modifiable risk factors for non-communicable diseases. Another limitation of census data is that changes in boundaries of villages as decided by the government make it difficult to make longitudinal comparison of urbanicity of the same areas.

The need to develop an urbanicity scale based on other environmental factors has been increasingly recognized [196]. Assessment of environmental measures such as night-time light intensity and built environment may provide clearer understanding of the process of urbanization in transitional rural communities in low and middle income countries.

Night time light intensity (NTLI)

Night-time light intensity has been used to estimate urbanicity in LMICs as it reflect improvement in access to electricity, a key piece of infrastructural development in LMICs [197,198]. Availability of longitudinal NTLI data allows assessment of urbanization in the APCAPS community over the past few decades.

A detailed description of NTLI estimation in APCAPS is included in **APPENDIX C.** Briefly, the NTLI data (1992 to 2012) were obtained from the USA. National Oceanographic Atmospheric Administration (NOAA). These data were derived from repeated measurements of visible-near infrared emissions from the earth's surface by weather satellites on clouds-free nights. After data cleanup to remove artifacts (i.e. fires and lightening) and calibration, "stable" datasets are published on NOAA's website, which are updated periodically. NTLI values are on a scale of relative intensity of light ranging from 0 (no light) to 63 (high intensity). In years where data from multiple satellites were available, maximum values were taken rather than means as it is not uncommon to see 0 due to artifacts.

In an external sub-study, this NTLI-based urbanicity scale was validated against a

scale based on fieldworkers' perception of urbanicity of the study villages. Eight fieldworkers on the APCAPS team were asked to rank all villages by their perception of relative levels of development in this community. The average ranking among eight fieldworkers for each village was used to create an urbanicity scale based on fieldworkers' perception. Both the fieldworkers' ranking and NTLI-based scores were then categorized into tertiles (high, medium, and low urbanicity groups). The tertiles based on NTLI scores were found to be in good agreement with the fieldworkers' perception. Additionally, the agreement with fieldworkers' perception was improved when 14 indicators of urbanicity taken from household and village-level data were added to NTLI scores to form a single urbanicity scale(NTLI+)[7] (unpublished).

The NTLI values confirm anecdotal stories about rapid urbanization in the APCAPS community over the past decade **(Table 3.2)**. The APCAPS community has overall become more urbanized. The variation in night-time intensity has also increased over the past twenty years, suggesting the presence of uneven rates of urbanization.

TABLE 3.2: Mean night-time light intensity for APCAPS villages and the city of Hyderabad in 2012.

Village names	1992	2002	2012
Ibrahimpatnam	10.4	15.1	29.1
Sheriguda	8.7	12.4	27.2
Patelguda	10.0	10.0	27.0
Mangalpalli	6.8	10.0	21.2
Uppariguda	6.0	10.0	19.0
Pocharam/ Ramireddyguda*	6.0	7.0	13.0
Seetharampet	4.6	7.0	10.4
Nomula	4.6	7.0	10.0
Lingampalli	5.1	8.0	12.0
Engalgda	7.0	11.0	19.0

7 Based on literature review on potential indicators of urbanicity, 14 questions were selected to be part of this urbanicity scale, of which 7 were at the household-level (motor vehicle ownership, phone ownership, television ownership, in-residence water source, toilet, construction material, household size) an 7 at the village level (education, occupation, post-office, bank and credit society, college, health facility, population size). Using principal component analyses, those 14 items and NTLI values were weighted and summed to assign a single urbanicity score for each village.

Polkampalli	6.0	12.0	20.0
Nainampalli	5.1	10.1	17.0
Raipole	5.8	9.8	16.0
Dandumylaram	3.7	7.0	10.4
Rachaloor	0.9	7.0	12.0
Lemur	5.0	7.0	14.7
Mankhal	11.0	15.1	36.9
Thummalur	5.8	9.2	14.3
Maheshwaram	6.0	12.1	22.2
Mansanpalli	4.0	8.1	20.7
Gudur	0.0	6.2	11.2
Kandurkur	4.6	8.5	18.0
Gummadivalli	4.0	5.7	10.0
Thimmapur	2.4	6.0	14.6
Meerkhanpet	4.0	5.0	10.8
Sardarnagar	11.8	25.3	42.2
Aakulamylaram	0.0	5.0	12.0
Nedunur	4.9	6.1	13.1
Mean (sd)	**5.5 (2.9)**	**9.4 (4.1)**	**18.0 (8.1)**

* Pocharam and Ramireddyguda are counted as one village.

Built environment (BE)

As part of the preliminary work for the development of a built environment survey, all non-residential places (NRPs) were mapped in these villages in 2013 in order to better understand available components of built environment in this community. The data from this mapping project were also used to develop an alternative urbanicity scale. This section describes the method of the mapping project and compares its validity against three other urbanicity scales: fieldworkers' perception of urbanicity in the study villages, NTLI, and NTLI+.

Author contribution: Conceptualized the mapping project, wrote the protocol, tested the protocol in three villages, and analyzed the results.

Background

Urbanization brings significant changes in built environment [199]. Over the past

decade, an increasing number of studies have examined the impact of built environment on health [200–202]. There have been few BE surveys developed for use in rural settings in India [203,204]. Types and geospatial distribution of non-residential places specific to rural India have not been well-documented. The lack of such data presents a challenge in deciding which components to assess in a built environment survey. Mapping and categorization of non-residential places can serve both as preliminary work for the development of a built environment survey and an alternative urbanicity scale for use in transitional rural communities. The NRP-based urbanicity scale was compared and validated against other urbanicity scales.

Methods

In a pilot study in August 2013, the APCAPS team mapped all NRPs in three villages of distinct degrees of urbanization (Ibrahimpatnum, Aakulamailaram, Engalgua) to test feasibility of complete mapping of NRPs in this community. These three villages were selected based on populations sizes and advice by local experts. The fieldworkers walked or drove on every street in each village and recorded the description of main services and/or items and global positioning system (GPS) data at each NRP. Based on the description of NRPs in this pilot study, 24 domains and 112 NRP categories were developed (**APPENDIX D**). Upon confirmation of feasibility of complete mapping of NRPs in the pilot study, NRPs in the remaining villages were mapped, using these newly defined NRP categories. The measurements were taken once per NRP. The categorization of NRPs in Akulamailaram was done by two coders and their inter-coder agreement was fair [$\kappa = 0.77$]. The detailed protocol of the mapping in the pilot study is included in **APPENDIX E**.

The overall relationship between NRP and NTLI-based scores was first examined in a scatterplot to identify any outliers. If outliers were found to be due to artifacts (i.e. external light contamination), these villages were removed from the comparative analysis of urbanicity scales. The urbanicity scores were then categorized into tertiles (high, medium, low urbanicity groups). Percent agreement (% agreement) and κ coefficients were then assessed among urbanicity scales based on fieldworkers' perception of urbanicity, NTLI, NTLI+, numbers of NRPs, and numbers of types of NRPs.

Results

There was a wide variation in the numbers and types of NRPs in these villages **(Table 3.3);** Ibrahimpatnam had over 1200 NRPs while Engalguda only had 16 NRPs. On average, 19% of NRPs were food-related. Eateries and chicken/egg stores were the most common food-related NRP type, followed by dairy products and vegetable stalls. Most villages had governmental ration centers. General store was the most populous type NRP in this community.

Table 3.3: Characteristics of non-residential places in 29 study villages of the Andhra Pradesh Children and Parents Study (2013)

Village names	Total number of NRPs	Number of NRP types (total 112 types)	Number of processed food vendors	Number of food ingredient vendors
Ibrahimpatnam	1264	98	112	92
Sheriguda	147	52	15	2
Patelguda	156	43	5	9
Mangalpalli	153	49	18	3
Uppariguda	53	24	1	6
Pocharam/ Ramireddyguda*	105	33	3	4
Seetharampet	33	20	0	5
Nomula	47	20	2	1
Lingampalli	86	31	4	2
Engalgda	16	9	0	0
Polkampalli	119	39	2	4
Nainampalli	64	29	1	4
Raipole	211	65	22	18
Dandumylaram	176	49	7	10
Rachaloor	103	39	4	5
Lemur	140	42	10	3
Mankhal	106	41	6	6
Thummalur	110	44	5	4

Maheshwaram	558	94	41	20
Mansanpalli	123	49	6	9
Gudur	84	29	2	3
Kandurkur	402	97	21	22
Gummadivalli	76	38	6	22
Thimmapur	114	41	5	5
Meerkhanpet	135	50	4	17
Sardarnagar	54	21	1	0
Aakulamylaram	54	30	4	9
Nedunur	159	41	6	9
Mean (sd)	**173 (241)**	**43 (22)**	**11 (22)**	**11 (17)**

* Pocharam and Ramireddyguda are counted as one village.

Comparison to other urbanicity scales

Two villages (Sardar nagar and Mankhal) showed very high NTLI scores but ranked low on all other urbanicity scales including total number of NRPs (**FIGURE 3.1.3)**. This is likely due to their proximity to an airport, which adds extra light intensity to the surrounding area. For the comparison of NRP and NTLI based scales, Sardar nagar and Mankhal were removed.

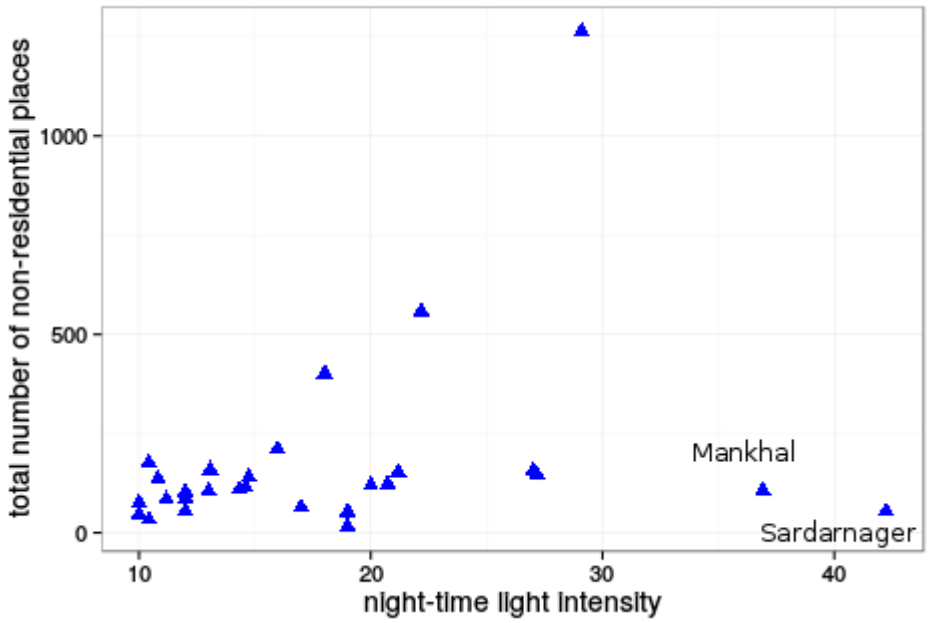


Figure 3.3: Relationship between night-time light intensity scores and the total number of non-residential places.

The rankings of village urbanicity varied among the urbanicity scales **(APPENDIX F)**. The urbanicity scales based on the total number NRPs showed fair to moderate agreement with other scales **(TABLE 3.4)**.

Table 3.4: Percent agreement and κ statistics for inter-rater agreement of tertiles of urbanicity (high, medium, and low) among the urbanicity scales in 26 villages.

	Number of NRP	NRP types

	% agreement	κ (95%CI)	% agreement	κ (95%CI)
Fieldworker	50.0	0.44 (0.14,0.75)	50.0	0.44 (0.14,0.75)
NTLI	69.2	0.41 (0.01,0.81)	65.4	0.47 (0.1,0.83)
NTLI+	53.8	0.12 (-0.32,0.55)	46.2	0.29 (-0.1,0.68)
NRP types	78.6	0.5 (0.12,0.88)		

2 villages (Mankhal and Sardarnagar) were removed from the NTLI and NTLI+ analyses as their NTLI scores were enhanced by the nearby airport. The scales are based on: Number of NRP = number of available non-residential places; NRP types = number of types of available non-residential places; Fieldworker = fieldworkers' perception of urbanicity of the study villages; NTLI = night-time light intensity; NTLI+ = night-time light intensity plus 14 indicators of urbanicity.

There is no consensus on what constitutes as the "gold standard" in defining urbanicity [196]. The census data may be useful for comparing overall patterns of urbanization with other studies since populations size and density are common indicators used in defining urbanicity. In terms of understanding the urbanization over the past two decades in the study community, NTLI is likely to offer better evidence as it reflect infrastructural development in these villages. Data on non-residential places offer direct measures of changes in environment due to urbanization, which may be useful for understanding the relationship between health and built environment. Although the NRP-based urbanicity scales were in good agreement with other urbanicity scales, they were not used for analyzing association between osteoporosis and urbanicity in this thesis as urbanicity in this community was highly skewed and there were too few data points from more urbanized villages to draw any sound conclusions from statistical analyses.

There are some limitations to using NRPs for an urbanicity scale as well. We currently only have cross-sectional data from 2013, so urbanization over time cannot be described. In addition, some less developed villages are located near more developed villages, and residents in some villages may be affected by urbanization of these nearby villages. The bus system as well as "autos" (motorized rickshaw) and personal ownership of scooters are also fairly common in these villages. The residents in the study villages are therefore not completely limited to only what is available in these villages.

Summary

- Night-time light intensity data suggest that urbanization has been occurring in the APCAPS community for the past decade at uneven rates.
- The urbanicity scale based on the total number of non-residential places is in good agreement with other urbanicity scales.
- Data on non-residential places can offer more specific information on the process of urbanization and its impact on lifestyles than urbanicity scales based on census, night-time light intensity, or fieldworkers' perception.

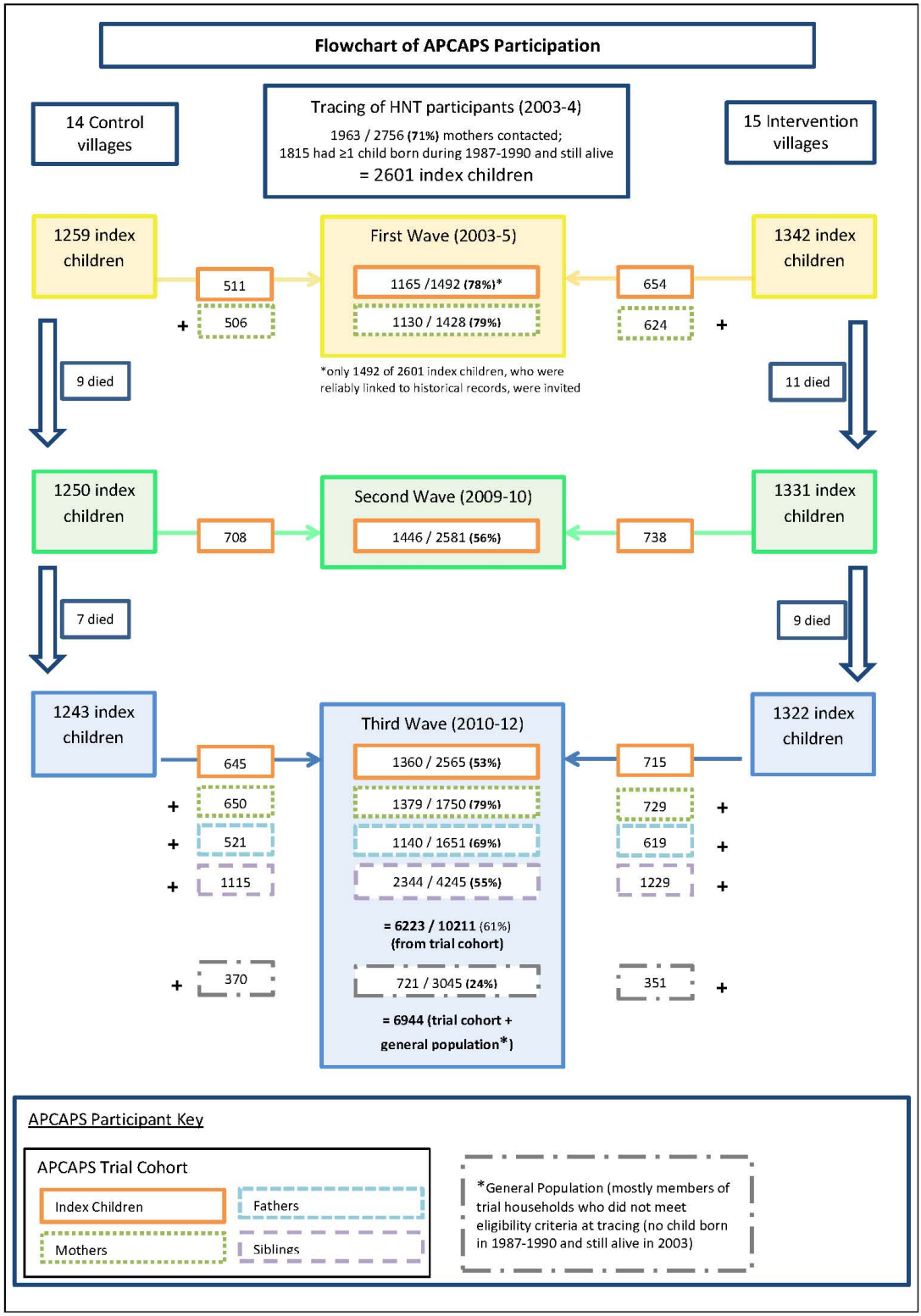
3.2 The Andhra Pradesh Children and Parents Study (APCAPS)

The thesis analyzed data from three waves of data collection in the Andhra Pradesh Children and Parents Study. APCAPS is a prospective cohort study established through long-term follow-up of the Hyderabad Nutrition Trial (HNT: 1987-1990). A detailed description of the HNT and three waves of APCAPS data collection has been previously published [205]. The following section will describe the study design, availability of relevant data, and the method of DXA artifact coding. Any additional methodological information that are specific to the research papers are described in the respective chapters. All bone mineral density (BMD) examined in this thesis was areal BMD and all hip data are based on total hip.

3.2.1 Study Design

Figure 3.4 summarizes the numbers participating in each phase of the study.

FIGURE 3.4: Flow chart of participants in the Andhra Pradesh Children and Parents Study (APCAPS). Adapted with the permission from the authors [205].



Hyderabad Nutrition Trial (1987-1990)

HNT assessed the Integrated Child Development Services (ICDS) scheme in a controlled stepped wedge design trial in 1987-90. ICDS is a national community outreach program initiated in 1975 that provides food supplementation to pregnant and lactating women and children under the age of six years along with early childhood education, health, hygiene and nutrition education for the mothers, and delivery of other national programmes (immunization, anemia control and basic health care) from the ICDS centers [205,206]. The children who took part in the HNT are termed **index children** in this thesis.

A total of 29 villages in two adjacent administrative areas near Hyderabad city (50-100km) in Ranga Reddy district were selected, one with the full ICDS programme already in place (15 intervention villages) and the other awaiting implementation of the nutritional supplementation part of the programme (14 control villages) **(FIGURE 3.5)**. Both groups had the other parts of the programme implemented by the start of the HNT.

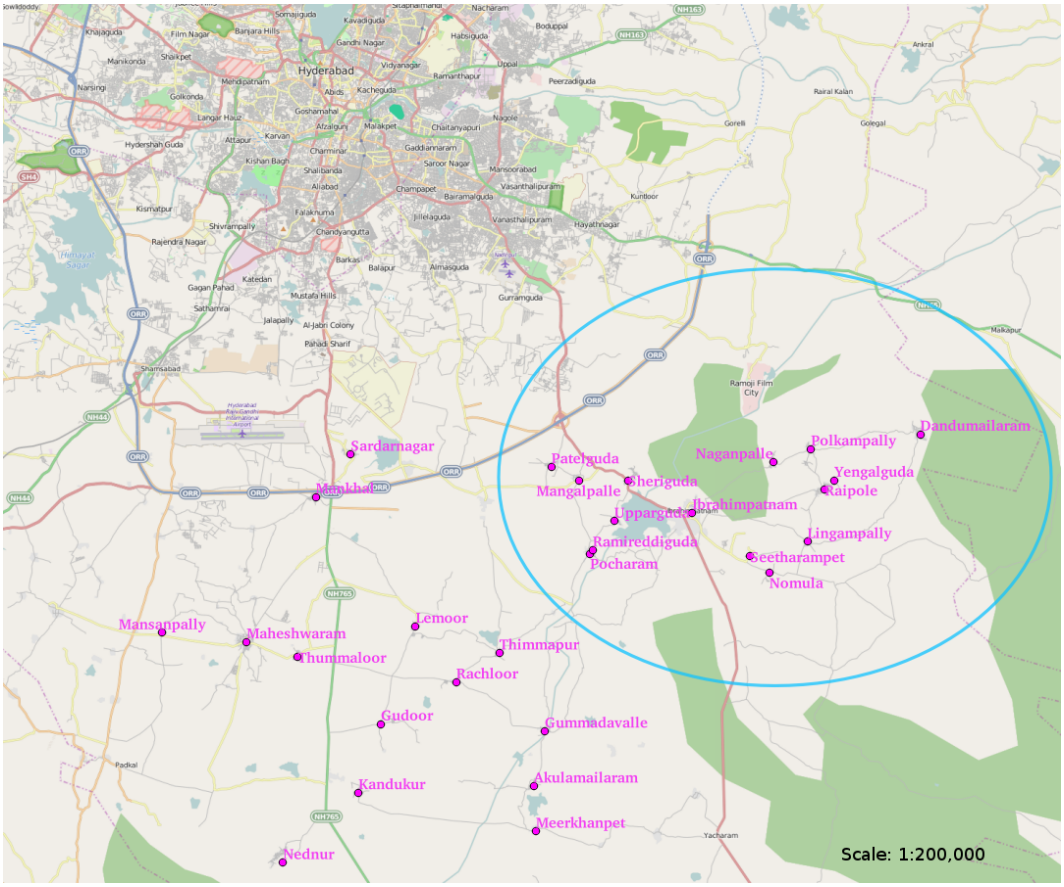


Figure 3.5: Map of the APCAPS villages.

The villages in the blue circle were intervention villages during the Hyderabad Nutrition Trial.

The full ICDS programme was eventually implemented in all of the control villages as well, after the HNT trial period ended in 1990 (**Table 3.5**).

Table 3.5: First year of ICDS implementation in APCAPS villages.

Intervention villages	ICDS start year	Control villages	ICDS start year
Ibrahimpatnam	1984	Rachaloor	2001
Sheriguda	1984	Lemur	2001
Patelguda	1984	Mankhal	2002
Mangalpalli	1984	Thummalur	2001
Uppariguda	1982	Maheshwaram	2001
Pocharam	1984	Mansanpalli	2002
Ramireddyguda	1985	Gudur	2002
Seetharampet	1984	Kandurkur	2001
Nomula	1984	Gummadivalli	2001
Lingampalli	1984	Thimmapur	2002
Engalguda	1984	Meerkhanpet	2001
Polkampalli	1984	Sardarnagar	2002
Nainampalli	1984	Aakulamylaram	2000
Raipole	1984	Nedunur	2001
Dandumylaram	1984		

In ICDS, a nutritional supplement made of corn-soya blend and soybean oil was made available daily to all pregnant and lactating women and children under 6 years. The meal (upma) contained, on average, 2.09 MJ (478kcal) and 20-25g protein for pregnant and lactating women (40-50% for a pregnant woman weighing 50kg, based on the Recommended Daily Allowance for protein intake in the USA. [207]) and about 1.25 MJ (299 kcal) and 8-10g protein for children under 6 years old. There were no supplemental nutrients added to upma. The meal was to be collected daily by the mothers or their children from the anganwadi[8] but they were not required to consume the supplement at the facility. Anganwadi workers also provided nutrition and health education to the women, and encouraged them to attend antenatal care and seek timely health advice.

8. Child day-care centre, delivering pre-school education to children between the ages of three to six years, and monitoring their nutritional status, and providing referrals when deemed necessary.

During the trial period, women 'at risk' of pregnancy (13-45 years old) were monitored and those who became pregnant were followed closely during pregnancy until childbirth. There were 4338 live births during the trial period, of which 2601 children (1815 households had more than one children in 1987-1990) were still alive when the APCAPS birth cohort was established in 2003-2005. Since the ICDS programme was introduced to all intervention villages by 1985 (Table 3.5), all index children in the intervention villages, who were born between 1987 and 1990, were exposed to the nutritional supplementation since the fetal period, as long as their mothers took the opportunity to consume it during pregnancy and feed it to their children. Index children from the control villages were not exposed to the nutritional supplementation as the nutritional supplementation was not made available in these villages until 2000 or later.

First wave of data collection (W1: 2003-2005)

Thirteen years after the end of HNT, the first wave of data collection was conducted. A probability algorithm matching on family name pairs and child information was used to retrospectively link the index children in W1 to the historical records from HNT. Only the index children who could be reliably linked to their historical records (n = 1492) and their mothers who were still alive at W1 (n = 1090) were invited in this study.

1165 adolescents (78% response) underwent an interviewer-administered questionnaire and clinical examination (intervention group, n = 654; control group, n = 511); 1064 mothers (97% response) underwent a brief questionnaire and examination. Although a clinical examination of mothers during pregnancy and index children for the first year of life was conducted during HNT, the data were recorded on separate files without linkable identifiers, and therefore these data could not be easily linked to W1 data. Linkable birth weight data were available only for 603(40%) of the 1492 index children. Of note, unlinked birth weight data (n = 2964) showed a difference in means between the intervention and control areas (intervention: 2655g (SD 424); control 2594g (430)) [208]. Those mean values are fairly low and close to the low birth weight range (<2500g) [209].

Second wave of data collection (W2: 2009-2010)

2581 index children who were still alive at W2, irrespective of historical record linkage, were invited for W2 (2009-2010). The index children were now young adults aged 18 to 23 years old. The main aim of W2 was to assess body composition measured by DXA and the propensity to develop type 2 diabetes and coronary heart disease. The DXA measurement in W2 also allowed assessment of skeletal health in these young adults.

Third wave of data collection (APCAPS family study, 2010–12)

In 2010, a family cohort study was established by extending the data collection to the parents and siblings of the index children. A total of 6223 (61%) out of 10211 eligible individuals participated during this phase. In addition, 721 family members from trial families with no living index children were also recruited, yielding data on a provisional total of 6944 participants in this wave. The DXA measurement in W3 allowed assessment of skeletal health in the total population in this community.

Some index children participated in both W2 and W3. As a result, 780 index children have data from both W2 and W3, with a mean follow-up time of 2 years. Since the second and third waves of data collection were conducted within a relatively short period of time, the analyses in Chapter 4 and 6 combined data from these two waves of data collection (W2/3) for index children. In cases where participants attended both waves of data collection, the data from W3 were used, unless there were artifacts in DXA scans from W3, which prompted the use of data from W2.

Research Paper 3 (Chapter 5) was written in 2012, using only W2 data on young adults who were HNT participants. After W3 data became available for analyses in late 2013, W2/3 data on all adult participants (18 years and older) were analyzed for Research Paper 2 (Chapter 4) and W2/3 data on index children were used in Research Paper 4 (Chapter 6).

3.2.2 Measurements

The data were collected either within the villages or at the National Institute of Nutrition: W1 at clinics in the villages where the participants resided; W2 at a single clinic at NIN; W3 at clinics at NIN for DXA scans and vascular measures and at clinics

in the villages for other measures.

QUESTIONNAIRE DATA

A semi-structured questionnaire was administered to all participants by a trained interviewer.

Socio-demographic data (W1/2/3)

A subset of questions (14/29) from the Standard of Living Index (SLI), a summary measure of household level asset-based scale devised for Indian surveys, was used to estimate socioeconomic position as joint family structures are common in rural India[210]. This subset was selected as they were believed to be most informative for this study population [210]. The following information was collected in the APCAPS questionnaire: the quality of house, toilet facilities, source of lighting and drinking water, ownership of clock, radio, television, bicycle, motorcycle, car, refrigerator, telephone, and agricultural land. These items were weighted to give a maximum score of 34, using weights developed by the International Institute of Population Science in India (**Table 3.6**) [35].

Table 3.6: Standard of living index scores in the Andhra Pradesh Children and Parents Study.

Category	Item score
House type	Pucca = 4; Semi-pucca = 2; Kutcha = 0
Toilet facility	Own flush toilet = 4; Public or shared flush toilet, or own pit toilet = 2; No facility = 0
Source of lighting	Electricity = 2; Kerosene, gas or oil = 1; Other source of lighting = 0
Main fuel for cooking	Electricity, liquid petroleum gas, or biogas = 2; Coal, charcoal or kerosene = 1; Other fuel = 0
Source of drinking water	Pipe, handpump or well in residence/yard/plot = 2; Public tap, handpump, or well = 1; Other water source = 0
Separate room for cooking	Yes = 1; No = 0
Ownership of house	Yes = 2; No = 0

Ownership of agricultural land	5 acres or more = 4; 2.0 to 4.9 acres = 3; Less than 2 acres or acreage not known = 2; No agricultural land = 0
Ownership of irrigated land	At least some irrigated land = 2; No irrigated land = 0
Ownership of livestock	Yes = 2; No = 0
Ownership of durable goods	Car or tractor = 4 (each); Moped/scooter/motorcycle, telephone, refrigerator, or colour television = 3 (each); Bicycle, electric fan, radio/transistor, sewing machine, black and white television, water pump, bullock cart, or thresher = 2 (each); Mattress, pressure cooker, chair, cot/bed, table, or clock/watch = 1.

Education was classified into four levels: no formal education; primary (1 to 4 years); secondary (5 to 12 years); and beyond secondary. Occupation was classified into four categories: students, manual employment, professional employment, and unemployment.

Substance use (W1/2/3)

Current tobacco use (smoking, chewing, or snuffing tobacco) and alcohol use (yes or no) were defined as use within the last 6 months. For tobacco use, although participants were categorized into three groups (current, former, and never), there were few former users (*i.e.* W3: 0.7%), therefore those who had previously or never used tobacco were combined.

Diet and physical activity (W1/2/3)

For W1, a questionnaire with only a few dietary and physical activity questions was administered and these questions had not been validated; therefore, the data on diet and physical activity from W1 were not used in this thesis. The semi-quantitative questionnaires from W2/3 had been adapted and evaluated for use in this setting previously and their performance was found to be satisfactory [211,212].

Dietary intake over the past year was estimated with a semi-quantitative food frequency questionnaire collecting information on frequency of intake (daily, weekly, monthly, yearly/never) of 98 commonly consumed food items. The nutrient content of a single portion of each food item on the list was estimated based on the Indian food composition tables [213]. Physical activity in the previous week was assessed

across the following domains: work, travel, leisure (sports/games/exercise), household, and sedentary. For each activity, the average amount of time spent on the activity and the frequency of the activity were documented. Weight-bearing physical activity (wbPA) was defined as activities involving standing, walking, running, and extraneous weights. The average hours per day spent on wbPA at work, during commute, and for leisure were calculated from the weekly frequency and duration per day.

Reproductive history (W1/W2/W3)

For W1, four puberty stages (corresponding to Tanner's early, middle, and late puberty and post-puberty) were set based on time since the onset of menstruation (girls) and testicular volume (boys). The boys assessed testicular volume in private, using Prader's orchidometer with volumes ranging from 1 ml to 25 ml. This self-assessment method has previously been validated against measurement by clinicians in an external sub-study [208]. For W2/3, menopausal status was set as a binary variable based on current menstruation status (yes or no).

CLINICAL DATA

Anthropometric data (W1/2/3)

Standing height was measured to the nearest 1 mm with a plastic stadiometer (W1/2/3: Leicester height measure, Chasmors, London). Weight was measured to the nearest 0.1kg on a digital weighing machine (W1: HD 305; Tanita, Tokyo, Japan; W2/3: SECA 899). Measurements were taken twice and the average of two values was used in the analysis (% coefficient of variation (%CV) for W2 height: 0.55%; weight 0.07%; W3 height: 0.67%; weight 0.09%). Body mass index (BMI) was calculated as weight (kg) / height (m2). Cutoff points of BMI $\leq$ 17.0 and $\leq$ 18.5 were used for underweight in adolescence and adulthood respectively [214,215].

Serum Vitamin D (W2)

Assays were conducted on fasting venous samples at the National Institute of Nutrition. Serum $25(OH)D_3$ and $25(OH)D_2$ were extracted in quantitative high-performance liquid chromatography assays and detected at 265 nm using an ultraviolet detector (%CV: 7%). Of note, even though blood samples from W3 have been stored in -80C freezer, these samples were not stored in tubes with tightly

sealed cryovials (i.e. O-ring lids), and therefore, it was not possible to retrospectively assess serum vitamin D levels for W3 due to concerns for slow evaporation of the samples over time.

Dual x-ray absorptiometry scanning (W2/3)

Bone mass measurements were assessed using DXA machines (W2: 91% Hologic Discovery A model; 9% Hologic 4500W). Whole body scan was performed with the participant supine on the scanning bed with their arms resting by their sides. Women suspected of pregnancy were excluded from DXA scanning. Standard Hologic software options were used to define regions of the body (head, arms, trunk, and legs). Bone mineral density (BMD in g/cm^2) was calculated from bone mineral content (BMC in g) and bone area (BA in cm^2) for total hip and lumbar spine (L1-L4). Fat and lean mass indexes (FMI and LMI) were based on fat and lean mass (kg) from whole-body scans / height (m^2). %CV were determined to be 0.7% for hip bone mineral density (BMD), 1.3% for LS BMD, and 0.9% for whole-body BMD (n = 30).

DXA artifact coding

Scanned images of hip, lumbar spine, and whole body were examined for artifacts by Dr. Heli Viljakainen (W2: hip and lumbar spine), Dr Amy Taylor (W2: whole-body), and the author of this thesis (W3: hip, LS, WB). For the third wave of data collection, the artifact coding used a protocol based on the protocols developed by Dr Taylor and Dr Viljakainen at University of Bristol and Ms. Jennifer Thompson at University of Cambridge. The protocol for DXA artifact coding for W3 is included in the **APPENDIX G**.

All DXA images were first printed off the Hologic machine by the DXA technician. The printed copies of the standard Hologic DXA report were then digitized by the APCAPS team in Hyderabad. The aforementioned researchers examined the digitized copies of the DXA images for artifacts. All large foreign objects, major movements and major cutoff (i.e. more than 1 finger) were excluded from bone data analyses. Scans with minor movements (i.e. blurred toes and movement in head only) and with small foreign objects (i.e. a ring, toerings, or earrings)were included. For lumbar spine scans in W2, pathological changes such as osteoarthritis affecting two or more vertebrae were excluded; if only one vertebra was affected, the scan was re-analyzed

after the affected part was excluded [216]. For body composition analyses, we excluded images with all the same criteria as bone data analyses with the exception of inclusion of images with foreign objects.

Unclear digital copies of hip or whole-body images were re-digitized by the APCAPS team at the National Institute of Nutrition in Hyderabad and re-assessed by the artifact coders. All the lumbar spine images from W3 for older siblings, mothers, and fathers were too blurry for artifact coding, requiring raw images to be examined. The protocol for W3 raw data transfer out of the National Institute of Nutrition in Hyderabad has not been established; therefore, for research paper 2, in which W3 data on all adults were included, only hip and whole-body body composition data were analyzed.

Quality control

We produced detailed protocols and regularly checked compliance to standardize the work of the fieldwork team. The anthropometric equipment was calibrated at the start of every clinic. Although fieldworkers could not be "blinded" for the group assignments, DXA estimates of BMD were automated to reduce the possibility of bias. DXA scans were analyzed by a single trained technician. For quality assurance of DXA scans, a spine phantom was scanned every day to check for acceptable ranges.

3.2.3 Summary of data

Table 3.7 summarizes relevant exposure and outcome variables used for the lifecourse analyses of bone mass of index children [126,127,205,217].

Table 3.7: Summary of exposure and outcome measures for index children in the Andhra Pradesh Children and Parents Study.

Domain	Parameters	Measures	W1	W2	W3
		Total number of participants	**1165**	**1446**	**1358***
Exposure					
Early life nutritional intervention	ICDS nutritional supplementation	villages (intervention vs control)	1165	1446	1358
Socio-demographics	Birth details	birth dates	1165	1446	1358
		birth weight (24 hours)	162	161	111
		birth weight (7 days)	493	317	291
		birth weight (1 year)	574	367	345
	Socioeconomic status	education	1110	1445	1357
		occupation	1105	1445	1357
		household asset-based scores	1109	1435	1352
Anthropometry	Body size	height	1113	1445	1356
		weight	1115	1446	1356
	Body composition	DXA fat mass	NA	1401	635
		DXA lean mass	NA	1401	635
Lifestyle	Alcohol	consumption pattern	1093	1444	1357
	Tobacco	consumption pattern	1101	1444	1357
	Diet	consumption pattern of 98 food items	NA	1443	1354
	Physical activity	activity patterns in work, travel,	NA	1444	1354

		leisure, household, and sedentary domains			
Reproductive health	Reproductive history	number of pregnancies (girls)	NA	135	286
		number of live birth (girls)	NA	135	285
		number of live children (girls)	505	NA	403
	Puberty	age at menarch (girls)	471	172	202
		testicular volume (boys)	588	485	202
	Period	menstrual status	NA	465	519
Biomarker	Nutrients	serum vitamin D	NA	1041	NA
		serum calcium	NA	1404	1309
Outcome					
Skeletal health	Bone mass	DXA hip bone mass[†]	NA	1386	633
		DXA lumbar spine bone mass[†]	NA	1387	633
		DXA whole body bone mass[†]	NA	1401	635

[*] One index children were removed from the analyses for having unrealistic values for DXA body composition.
[†] Numbers for DXA measurements are total numbers of DXA scans regardless of presence of artifacts. The numbers after the removal of artifacts are shown in research paper chapters.

Ethics Statement

The study received approvals from the ethics committees of the NIN (Hyderabad, India), the Indian Council of Medical Research (ICMR), Centre for Chronic Disease Control, and London School of Hygiene and Tropical Medicine (London, UK). Approval was also sought from the village heads and their panchayats in each of the 29 villages. Written informed consent or witnessed thumbprint if illiterate was obtained from the participants prior to their inclusion in the study.

Funding

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PhD studentships: W2 (Amy Taylor, Wellcome Trust Award to the University of Bristol; Ruth Sullivan, Economic & Social Research Council UK Award to the London School of Hygiene & Tropical Medicine; Bharati Kulkarni., Queensland University of Technology, Australia); W3 (Poornima Prabhakaran, Wellcome Trust Capacity Strengthening Strategic Award to the Public Health Foundation of India and a consortium of UK universities, grant 084754; Mika Matsuzaki, Joint Japan/World Bank Graduate Scholarship Program).

Chapter 4: Research Paper 1

4.1 Research Paper 1: Association of Hip Bone Mineral Density and Body Composition in a Rural Indian Population: The Andhra Pradesh Children and Parents Study (APCAPS)

This chapter describes hip bone mineral density in the adult population to provide the overall context of the population in the study community. The analyses also include assessment of the association between hip bone mineral density and fat and lean mass. The first section takes a publication style with a brief introduction followed by the methodological information specific to this chapter and the findings. The chapter ends with a summary of main findings.

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Conceptualized, wrote the first draft, coordinated all comments by co-authors to revise the draft, and was primarily responsible for the final draft

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SUPERVISOR/SENIOR AUTHOR'S SIGNATURE (3 above) .....

Association of Hip Bone Mineral Density and Body Composition in a Rural Indian Population: The Andhra Pradesh Children and Parents Study (APCAPS)

Short title: Hip BMD and Body Composition in a Rural Indian Population

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Abstract

Background: Fat mass is variably associated with bone mass, possibly due to differential mechanical and biological effects of fat mass. We examined the association of fat mass with bone mass in a lean population.

Objective: To investigate association between hip bone mineral density and fat mass in a cross-sectional study from southern India.

Design: The Andhra Pradesh Children and Parents Study is a prospective cohort study in Hyderabad, India. In 2009-2012, the study collected data on anthropometric measures, bone area, bone mineral content, bone mineral density (BMD), fat mass, and lean mass measured by dual-energy x-ray absorptiometry, and socioeconomic data of the adult participants.

Results: The median BMI (kg/m^2) was 20.1 kg/m^2. females had relatively higher fat mass as compared to males. In models adjusted for lean mass, there was an association between hip bone mineral density and fat mass in females (β (95% confidence interval): premenopausal 0.025 (0.006 to 0.045); postmenopausal 0.045 (0.014 to 0.076) g/cm^2) but not in males (0.001 (-0.012 to 0.0014)). The association between hip BMD and fat mass was stronger in postmenopausal than premenopausal females. Hip BMD was consistently associated with lean mass, in both males and females.

Conclusions: In this relatively lean population, lean mass was more consistently associated with hip BMD than fat mass. Weight gain through lean mass improvement may be a more reliable public health strategy for strengthening bone health in transitional rural communities in low and middle income countries.

Introduction

Osteoporotic fracture is associated with morbidity and mortality [1]. A study estimated that the number of hip fractures will rise to 6.3 million globally by 2050 [2]. Hip bone mineral density is a predictor of overall risk of fractures in later life [3]. Body mass is one of the key determinants of hip bone mineral density [4].

Fat mass is one of the main components of body mass. On one hand, sheer mechanical loading of fat mass stimulates bone formation [5,6]. However, *in vivo* and *in vitro* studies have suggested negative effects of fat on bone mass accrual through several biological mechanisms [5–8]. Leptin, an adipocyte hormone, was shown to have an anti-osteogenic property in mice [9]. Shared precursor stromal cells suggest competitive cell lineages between osteogenesis and adipogenesis [10]. Peroxisome proliferator-activated receptor (PPAR) γ pathway is a key regulator of adipogenesis and also an inhibitor for osteoblastogenesis [7].

Epidemiological studies have shown inconsistent results on association between bone and fat mass [11–14]. Many studies examined this association in the context of obesity and osteoporosis partially because of concerns for higher fracture rates among obese individuals [15]. However, it is possible that the balance between mechanical and biological mechanisms may vary depending on the combination of body size and composition. Asians have been shown to have higher proportion of fat mass at lower body mass index (BMI) [16]. The patterns of association between fat and bone mass in the Indian population may therefore be distinct from the European and American populations. There have been few large-scale studies examining this association in the Indian population.

The Andhra Pradesh Children and Parents Study (APCAPS) is a prospective cohort study from southern India. The study population has been undergoing a nutritional and epidemiological transition due to urbanization over the past decade; as a result, this population has a wide variation in body sizes and compositions. The current study assessed how fat and bone mass may be associated in this transitional rural population.

Methods

Ethics Statement

The study received approvals from the ethics committees of the NIN (Hyderabad, India), the Indian Council of Medical Research (ICMR), Centre for Chronic Disease Control, and London School of Hygiene and Tropical Medicine (London, UK). Approval was also sought from the village heads and their panchayats in each of the 29 villages. Written informed consent or witnessed thumbprint if illiterate was obtained from the participants prior to their inclusion in the study.

Study design

The analyses in this study used data from two waves of data collection (2009-2010; 2010-2012) of the APCAPS study, a prospective cohort study established through long-term follow up of the participants of the Hyderabad Nutrition Trial (HNT: 1987-1990). The HNT studied impact of the Integrated Child Development Services scheme, a national community outreach programme providing food supplementation along with health, hygiene, and nutrition education, immunization, anemia control, and basic health care to pregnant and lactating females and children under the age of six years [17]. A detailed description of the initial trial (HNT) and the first wave of data collection in APCAPS (i.e. the first follow-up of the HNT, 2003-2005) has previously been published [17,18].

Since the second and third waves of data collection (W2/3) were conducted within a relatively short period of time (2009-2012), the analyses in this manuscript used combined data from these two waves of data collection. W2/3 examined markers for chronic diseases affecting cardiovascular, musculoskeletal, and mental health. All consenting participants underwent dual-energy x-ray absorptiometry (DXA) measurements at the National Institute of Nutrition (NIN) in Hyderabad and physical measurements at NIN (W2) or the village clinics (W3). In cases where participants attended both waves of data collection, the data from the third wave were used, unless there were major artifacts in the DXA scans from W3, which prompted the use of data from W2. The current manuscript analyzed data on the adult participants only (18 years old and above).

Measurements

Questionnaire data

A semi-structured questionnaire was administered to all participants by a trained interviewer. A subset of questions (14/29) from the Standard of Living Index (SLI) in the National Health Family Survey-2, a summary measure of household level asset-based scale devised for Indian surveys, was used to estimate socioeconomic position as joint family structures are common in rural India [19]. We collected information on the quality of house, toilet facilities, source of lighting and drinking water, ownership of clock, radio, television, bicycle, motorcycle, car, refrigerator, telephone, and agricultural land. These items were weighted to give a maximum score of 34, using weights developed by the International Institute of Population Science in India [19]. Occupation was classified into four categories: students, manual employment, professional employment, and unemployment. Current tobacco use was defined as smoking, chewing, or snuffing tobacco in the last 6 months. Menopausal status was set as a binary variable (yes or no).

Anthropometric data

Weight was measured to the nearest 0.1kg with a digital SECA balance and standing height was measured to the nearest 1 mm with a plastic stadiometer (Leicester height measure). Measurements were taken twice and the average of two values was used in the analysis. Body mass index (BMI) was calculated as weight (kg) / height (m^2).

DXA scanning

Bone mass measurements were assessed by DXA (Hologic Discovery A model). Whole body scan was performed with the participant supine on the scanning bed with their arms resting by their sides. females suspected of pregnancy were excluded from DXA scanning and the scans were taken only after confirming the negative pregnancy by conducting urine pregnancy test. Standard Hologic software options were used to define regions of the body (head, arms, trunk, and legs). The coefficients of variation were determined to be 0.7% for hip bone mineral density (BMD), 1.3% for LS BMD, and 0.9% for whole-body BMD (n = 30). Scans were coded for artifacts by a visual inspection and those with major movement and foreign objects as well as incomplete scans were excluded from the analyses of hip bone mineral density (BMD; g/cm^2). Major movements and incomplete scans were counted as artifacts and removed from analysis with fat and lean mass. BMD (g/cm^2) was calculated from bone mineral content (g) and bone area (cm^2) for total hip. Fat to lean mass ratio (FLR: fat mass /

lean mass^{x}) was calculated using the allometric coefficients from sex-stratified models regressing log-transformed fat mass upon log-transformed lean mass as x (x = 1.57 for females; 1.66 for males). FLR was multiplied by 100 to improve clarity. Total fat and lean mass (kg) were estimated from whole-body scans. Osteopenia and osteoporosis were defined based on the BMD values in healthy Indian young adults (Hip BMD mean (sd): females = 0.901 (0.111); males = 0.988 (0.131)) [20].

Statistical analysis

Descriptive statistics were calculated for premenopausal females, postmenopausal females, and males separately.

The associations between hip BMD and fat mass, lean mass, and FLR were examined in multilevel regression models (Model 1, 2, 3, and 4) that accounted for household-level clusters (Table 3 and Supplemental Material Table S1). Three-level nested multilevel models to adjust for both village and household-level clusters were considered but the small intraclass correlations for the village level suggested that adjustment for the household-level clustering was sufficient. All models were stratified by sex. There was evidence of an interaction between fat mass and menopausal status; therefore, regression models for females were stratified by menopausal status. Fat mass (kg) was log-transformed as its distribution was positively skewed. Hat, PRESS, and Cook statistics were examined to remove outliers for regression models.

Model 1 examined the association between hip BMD and fat mass (kg) or lean mass (kg), adjusting for age (years), height (cm), and SLI. Model 2 assessed association between hip BMD and fat mass, adjusting for lean mass, age, height, SLI. Model 3 examined association between hip BMD and FLR, adjusting for age, height, and SLI. Model 4 further adjusted Model 3 for weight. Further adjustment for other potential confounders (vegetarianism and current tobacco use) did not materially change the results and therefore parsimonious models are presented.

All analyses were conducted using R, version 3.1.1 and multilevel modeling was done with nlme version 3.1-118.

Results

Of the 7375 participants of the second/third wave of data collection whose age, sex, height, and weight information were available, 4251 participants (58%) underwent DXA scans during W2/3. Of those, scans without major artifacts were available in 4243 (99.8%) participants for hip BMD. 97.5% of the DXA participants also had scans without major artifacts for whole-body estimation of fat and lean mass. Information on the other descriptive variables were available for ≥99% of the DXA participants.

Table 1 summarizes the key characteristics of the participants. Although the average BMIs were similar between females and males, females had higher fat to lean mass ratio than males. Current tobacco use was more common among males. There were few vegetarians in this community. Young females had much lower hip BMD in comparison to healthy young Indians (**Table 2**) [20].

Table 1: Characteristics of the participants of the Andhra Pradesh Parents and Children Study (2009-2012).

	Premenopausal female		Postmenopausal female		Male	
	n	mean(sd)	n	mean(sd)	n	mean (sd)
age (year)	1271	29.7(10.1)	643	46.9(7.3)	2320	33.1(15.2)
height (cm)	1270	152(5.7)	642	150.2(5.7)	2319	164.9(6.7)
weight (kg)	1270	47.9(9.8)	643	48.9(9.8)	2320	55.4(10.3)
BMI (kg/m^2)	1270	20.6(3.9)	642	21.6(3.9)	2319	20.3(3.4)
Fat mass (kg)	1267	14.9(5.6)	643	16.1(5.7)	2314	10.2(4.9)
Lean mass (kg)	1267	31.8(4.9)	643	31.9(4.7)	2314	43.5(6.4)
FLR	1203	6.19(1.49)	560	6.66(1.56)	2255	1.9(0.73)
wbPA (hours)	1271	93.2(122.3)	643	153(145)	2320	143.2(119.3)
vegetarian (n)	1270		643		2318	
yes		56		12		43
no		1214		631		2275
tobacco use (n)	1270		643		2319	
current		70		178		767
never/former		1200		465		1552
SLI	1268	17.7(4.9)	643	16.6(4.7)	2313	18.2(4.6)
occupation (n)	1271		643		2319	
student		178		4		471
employed: manual		680		525		1697
employed:		20		0		47

professional			
unemployed	393	114	104

BMI = body mass index; FLR = fat to lean mass ratio; SLI = Standard of Living Index; wbPA = weight-bearing physical activity.
All values are mean (standard deviation) except for occupation (%), vegetarian (n), menopause status (n), and tobacco use (%).
FLR for women: fat mass / lean mass$^{1.57}$ x 100; for men: fat mass / lean mass $^{1.66}$ x 100
Current tobacco use included smoking, chewing, or snuffing tobacco in the last 6 months; former users stopped using tobacco products 6 months ago or more.

Table 2: Description of mean hip bone mineral density, osteopenia (%), and osteoporosis (%) by sex and age groups for the participants of the Andhra Pradesh Parents and Children Study (2009-2012).

	Female				Male			
age	n	BMD (g/cm^2)	osteopenia (%)	osteoporosis (%)	n	BMD (g/cm^2)	osteopenia (%)	osteoporosis (%)
20-29	665	0.84(0.1)	28.4	0.9	1163	0.95(0.11)	14.6	0.3
30-39	204	0.86(0.1)	24.5	0.5	108	0.93(0.11)	25	0
40-49	647	0.84(0.11)	30.9	1.1	253	0.92(0.12)	29.2	1.6
50≤	248	0.75(0.12)	51.2	14.9	516	0.89(0.12)	39.7	3.3

BMD = bone mineral density (g/cm^2).
Osteopenia is defined as 1 to 2.5 standard deviations (sd) and osteoporosis as more than 2.5 sd below peak bone mass in a healthy Indian population for each sex.

In the multilevel models adjusting for age, height, and SLI, fat mass and lean mass were each positively associated with hip BMD (**Table 3**). The positive associations between hip BMD and fat mass remained robust in females upon further adjustment for lean mass in Model 2, although the effect sizes were attenuated from 0.001 (premenopausal and postmenopausal) in Model 1 to 0.0002 (premenopausal) and 0.0004 (postmenopausal) g/cm^2 increase in hip BMD for one percent increase in fat mass. There was no clear evidence of association between hip BMD and fat mass in males after adjusting for lean mass. There was clear and consistent evidence for a positive association between hip BMD and lean mass. In models examining association between hip BMD and fat to lean mass ratio (**Supplemental Material S1 Table**), FLR was negatively associated with hip BMD upon adjustment for total weight. Adjusting models for other potential confounders such as vegetarianism, tobacco use, and parity (for females only) did not materially change the results on association between hip BMD and fat mass.

Table 3: Association of hip bone mineral density (BMD) and fat and lean mass in the participants of the Andhra Pradesh Parents and Children Study (2009-2012).

		Model 1		Model 2	
		β	p	β	p
		95% CI		95% CI	
Pre-menopausal female	Fat mass (kg)	0.1	<0.001	0.025	0.01
		(0.085 to 0.116)		(0.006 to 0.045)	
	Lean mass (kg)	0.011	<0.001	0.009	<0.001
		(0.009 to 0.012)		(0.008 to 0.011)	
Post-menopausal female	Fat mass (kg)	0.13	<0.001	0.045	0.008
		(0.108 to 0.153)		(0.014 to 0.076)	
	Lean mass (kg)	0.012	<0.001	0.01	<0.001
		(0.011 to 0.014)		(0.007 to 0.012)	
Male	Fat mass (kg)	0.082	<0.001	0.001	0.92
		(0.072 to 0.093)		(-0.012 to 0.014)	
	Lean mass (kg)	0.01	<0.001	0.01	<0.001
		(0.009 to 0.01)		(0.009 to 0.011)	

Sample size: pre-menopausal female (n = 1200); post-menopausal female (n = 560); male (n = 2130).
CI = confidence interval.
All models are multilevel models adjusting for household level clustering. ε_{ij} and υ_j are errors terms for multilevel regression models accounting for individual and household level differences:
Model 1: HIP BMD $\sim \beta_0 + \beta_1$ FAT MASS or LEAN MASS $+ \beta_2$ AGE $+ \beta_3$ HEIGHT $+ \varepsilon_{ij} + \upsilon_j$
Model 2: HIP BMD $\sim \beta_0 + \beta_1$ FAT MASS $+ \beta_2$ LEANMASS $+ \beta_3$ AGE $+ \beta_4$ HEIGHT $+ \varepsilon_{ij} + \upsilon_j$
Age (years); Height (cm); Fat and lean mass (kg)
Fat mass (kg) has been log-transformed.

Discussion

Hip bone mineral density was low in this relatively lean population in rural India. There was a positive association between hip BMD and fat mass in females (who also had relatively higher fat to lean mas ratio, but not in males. Hip BMD was consistently associated with lean mass in this population.

Comparison to previous studies

A number of epidemiological studies have examined the association between bone and fat mass: some showed no clear evidence of association while both positive and negative associations were also suggested [12,13,21–26]. As with many of the previous studies, we saw more consistent evidence for a positive effect of lean mass than fat mass [24,27]. Previous studies on Asian populations have shown attenuation of the association between BMD and fat mass upon adjustment for lean mass, similar to our findings [24,25,27–29]. Our study also showed that for a given body size, lower fat to lean mass ratio may be associated with higher hip BMD, which suggests that both body mass and composition may contribute to healthy bone mass accrual. A large DXA study from China examined association between bone mass and body composition in a similarly lean population [12]. In unadjusted models, fat mass was positively associated with hip BMD, but on adjustment for body weight, fat mass became negatively associated with hip BMD, similar to the findings from our study.

Fat may have opposing effects on bone mass accrual through mechanical and biological mechanisms. Positive association between mechanical loading and bone mass accrual have been shown consistently in studies examining the effects of body mass and weight-bearing physical activity [4,6,30–33]. The mechanostat theory suggests that weight causes structural adaptation through local mechanical strain, which was supported by direct measurement of mechanical strain in live animals and humans [5,33]. On the other hand, *in vitro* and *in vivo* studies have suggested several biological mechanisms underlying the association between fat and bone mass. Osteoblasts and adipocytes originate in common stromal cells in bone marrow, suggesting plasticity between these two cell lineages [34]. Insufficiency in PPAR $\boldsymbol{\gamma}$, a key regulator for adipocyte differentiation, increased osteoblastogenesis *in vitro* and bone mass *in vivo* [7]. Leptin is a hormone produced by adipocytes, regulating both fat distribution and bone turnover through the hypothalamic neural networks [26].

The combined effect of mechanical and biological properties of fat on bone mass accrual is not well-established in epidemiological studies [35] and may differ depending on sex, menopausal status, and ethnicity. [36–38]. In models adjusted for lean mass, hip BMD was positively associated with fat mass in females, but not in males. Certain fat hormones are suggested to be beneficial for bone mass accrual may be more strongly associated with bone mass accrual in females [23]. Another potential explanation may be that the amount of fat mass in males in this lean population was too low to detect its contribution to hip BMD in the presence of lean mass. However, a study from the United States, where the males had greater fat mass than the males in this study population, still showed no strong evidence for a positive association between BMD and fat mass in males, suggesting that sexual dimorphism may contribute to the varying degrees of association between fat and bone mass between females and males. [23].

The association between hip BMD and fat mass was also slightly greater in postmenopausal than premenopausal females. Several studies have shown greater positive effects of fat mass in postmenopausal females although underlying biological mechanisms are not well-understood [38,39].

It is important to note that this population had low hip BMD when compared to the values in a healthier Indian population [20]. One potential explanation for this finding is that although this rural community has become more developed and has been experiencing a nutritional transition over the past decade, gain in weight and lean mass during mid to late adulthood may not be able to fully mitigate adverse effects of undernutrition at younger ages when the majority of bone mass accrual occurs. Our previous study showed that gain in weight and lean mass in late adolescence and young adulthood was positively associated with bone mass but it is currently unknown how long the window of opportunity to improve bone mass lasts [218].

Strengths and limitations

This study is one of the few large-scale DXA studies examining the Indian population. The study subjects reside in an urbanizing rural community where there is a wide

range of combinations in body sizes and compositions, allowing assessment of the association between hip BMD and fat mass in both underweight and overweight populations. The use of DXA provides more accurate estimation of fat and lean mass than anthropometric measurements.

There are some limitations in this study as well. The cross-sectional nature of the study does not allow causal inference. This community has been experiencing a nutritional transition due to urbanization over the past decade. There may be risk factors from the past that are contributing to current bone mass, although, in young adults, our previous studies did not find strong evidence for longitudinal effects of early life [40] and adolescent undernutrition[218] on bone mass during adulthood after controlling for current body mass. The study population was also lean compared to higher income countries [41]; therefore, our findings may not be generalizable to other populations but the findings may be of interest to other lean populations whose body compositions are more similar to this study population in India. Another limitation is the lack of data on fractures and fat hormones, which would be of clinical and biomedical importance.

Conclusions

In this relatively lean population, hip BMD was associated with lean mass in both males and females, but hip BMD was associated with fat mass in females only. Weight gain through lean mass improvement may be a more reliable public health strategy for strengthening bone health in transitional rural areas in low and middle income countries.

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Supplemental Material S1 Table. Multilevel regression models examining association between hip bone mineral density and fat to lean mass ratio in the participants of the Andhra Pradesh Parents and Children Study (2009-2012).

		Model 3		Model 4	
		β 95% CI	p	β 95% CI	p
Premenopausal female	FLR	0.008 (0.004 to 0.011)	<0.001	-0.01 (-0.014 to -0.006)	<0.001
Postmenopausal female	FLR	0.014 (0.009 to 0.019)	<0.001	-0.009 (-0.014 to -0.003)	0.005
Male	FLR	0.01 (0.004 to 0.017)	0.003	-0.046 (-0.054 to -0.039)	<0.001

Sample size: premenopausal female (n = 1200); postmenopausal female (n = 560); male (n = 2248).
CI = confidence interval; FLR = fat to lean mass ratio
FLR for women: fat mass / lean mass$^{1.57}$ x 100; for men: fat mass / lean mass $^{1.66}$ x 100
All models are multilevel models adjusting for household level clustering. ε ij and υ j are errors terms for multilevel regression models accounting for individual and household level differences.
Model 3: HIP BMD ~ β 0 + β 1 FLR + β 2 AGE + β 3 HEIGHT + ε ij + υ j
Model 4: HIP BMD ~ β 0 +β 1 FLR+β 2 AGE+β 3 HEIGHT+β 4 WEIGHT+ ε ij +υ j
Age (years); Height (cm); Fat and lean mass (kg)

Summary

- The adult population in the APCAPS community generally showed low hip bone mineral density when compared to the Indian reference values.
- There was a notable difference in body composition as well as the associations between bone mass and body composition between females an males in this study population.
- There was clearer evidence of positive association between bone mass and lean mass than fat mass.

Chapter 5: Research Paper 2

5.1 Research Paper 2: Life-course determinants of bone mass in young adults from a transitional rural community in India: the Andhra Pradesh Children and Parents Study (APCAPS)

This chapter describes lifecourse analysis of the effects of exposure to early life protein-energy supplementation on total hip, lumbar spine, and whole-body bone mineral density in young adulthood. The chapter takes a publication style with a brief introduction followed by the methodological information specific to this chapter and the findings on the combined effect of early life nutritional supplementation and current body size and composition. The second section in this chapter includes unpublished data on the effects of early life nutritional supplementation on bone area.

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Conceptualized, conducted the analyses, wrote the first draft, coordinated all comments by co-authors, and was primarily responsible for the final draft.

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Life-course determinants of bone mass in young adults from a transitional rural community in India: the Andhra Pradesh Children and Parents Study (APCAPS)[1–3]

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ABSTRACT

Background: Undernutrition and physical inactivity are both associated with lower bone mass.

Objective: This study aimed to investigate the combined effects of early-life undernutrition and urbanized lifestyles in later life on bone mass accrual in young adults from a rural community in India that is undergoing rapid socioeconomic development.

Design: This was a prospective cohort study of participants of the Hyderabad Nutrition Trial (1987–1990), which offered balanced protein-calorie supplementation to pregnant women and preschool children younger than 6 y in the intervention villages. The 2009–2010 follow-up study collected data on current anthropometric measures, bone mineral density (BMD) measured by dual-energy X-ray absorptiometry, blood samples, diet, physical activity, and living standards of the trial participants (n = 1446, aged 18–23 y).

Results: Participants were generally lean and had low BMD [mean hip BMD: 0.83 (women), 0.95 (men) g/cm^2; lumbar spine: 0.86 (women), 0.93 (men) g/cm^2]. In models adjusted for current risk factors, no strong evidence of a positive association was found between BMD and early-life supplementation. On the other hand, current lean mass and weight-bearing physical activity were positively associated with BMD. No strong evidence of an association was found between BMD and current serum 25-hydroxyvitamin D or dietary intake of calcium, protein, or calories.

Conclusions: Current lean mass and weight-bearing physical activity were more important determinants of bone mass than was early-life undernutrition in this population. In transitional rural communities from low-income countries, promotion of physical activity may help to mitigate any potential adverse effects of early nutritional disadvantage. *Am J Clin Nutr* doi: 10.3945/ajcn.113.068791.

INTRODUCTION

Urbanization has been linked to the rise of many noncommunicable diseases (NCDs)[4], including osteoporosis (1). A conservative estimate has suggested that ~25 million Indian adults will have osteoporosis by 2015 (2). In rural India, rapid socioeconomic development is resulting in drastic changes in diet and activity patterns, which are key determinants of bone mass in young adults. Whereas the exact mechanisms underlying the association between urbanization and bone health are unclear, several potential risk factors have been identified, including a lower physical activity level, serum 25-hydroxyvitamin D [25(OH)D] concentration, and fruit and vegetable intake (3, 4).

The possibility of a developmental origin of osteoporosis has been suggested (5). A meta-analysis of observational studies found a weak positive association between adult bone mass and birth weight—a proxy measure for early-life nutrition (6). However, birth weight may be influenced by factors other than maternal nutrition, and direct evidence from nutritional trials is lacking (7).

There are many potential risk factors for bone mass accrual in adults. Previous studies have generally shown a positive association between bone mass accrual and weight-bearing physical activity (wbPA), whereas evidence on the effects of nutrition on bone mass is inconsistent (8–12). BMI, and also its main components of fat mass and lean mass, have been independently associated with bone mass accrual (13). Low serum 25(OH)D

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[2] The initial trial was funded jointly by the Indian Council of Medical Research and the US Agency for International Development. The first follow-up study (2003–2005) was funded by a personal fellowship to SK (Eden Fellowship in Paediatrics, granted by the Royal College of Physicians, United Kingdom), and the second follow-up study (2009–2010) was funded by the Wellcome Trust, United Kingdom.

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[4] Abbreviations used: BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; LS, lumbar spine; NCD, noncommunicable disease; SLI, Standard of Living Index; wbPA, weight-bearing physical activity; 25(OH)D, 25-hydroxyvitamin D.

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concentration and dietary intake of calcium, protein, and calories may contribute to poor bone health and osteoporosis (2, 14–18). How these adult risk factors play a role in bone mass accrual in young adults who have been experiencing significant nutritional and lifestyle transitions since their childhood is not well understood.

The Andhra Pradesh Children and Parents Study was established to understand societal and individual risk factors for NCDs in a transitional rural community. The study population experienced early-life undernutrition; two decades later, other lifestyle risk factors associated with rapid economic development are emerging (19, 20). This transition provided an opportunity to examine bone development in young adults whose bone mass may be influenced by both previous undernutrition and current urbanized lifestyles (21). In this manuscript, we present the analyses of life-course determinants of bone mass of young adults in this transitional rural community.

SUBJECTS AND METHODS

Study design

The analyses in this study used data from the second follow-up of the Andhra Pradesh Children and Parents Study, established through long-term follow up of the Hyderabad Nutrition Trial. The Hyderabad Nutrition Trial evaluated the Integrated Child Development Services scheme—a national community outreach program providing food supplementation along with health, hygiene, and nutrition education, immunization, anemia control, and basic health care to pregnant and lactating women and children younger than 6 y (22, 23).

The study received approvals from the ethics committees of the National Institute of Nutrition (Hyderabad, India), the Indian Council of Medical Research, and London School of Hygiene and Tropical Medicine (London, United Kingdom). Approval was also sought from the village heads and their committees in each of the 29 villages. The participants provided written informed consent, or a witnessed thumbprint if illiterate, before their inclusion in the study.

Initial trial (1987–1990) and first follow-up study (2003–20005)

A detailed description of the initial trial and the first follow-up study of the Hyderabad Nutrition Trial was previously published (24). Briefly, a controlled "stepped wedge design" trial was conducted in 1987–1990, using the opportunity afforded by the incremental expansion of Integrated Child Development Services program. A total of 29 villages in 2 adjacent administrative areas near Hyderabad City in India were selected: one with the Integrated Child Development Services program already in place (15 intervention villages) and the other awaiting implementation (14 control villages) (**Figure 1**). In the intervention villages, a nutritional supplement made of corn-soya blend and soybean oil was available daily to all pregnant and lactating women and children younger than 6 y. The meal (*upma*) contained, on average, 2.09 MJ and 20–25 g protein for pregnant and lactating women and ~1.25 MJ and 8–10 g protein for children younger than 6 y. Supplementation was associated with a small but statistically robust (61 g; 95% CI: 18, 104 g; $P = 0.007$) increase in the birth weight of the offspring (24). The first follow-up study in 2003–2005 examined 1165 adolescents aged 13–18 y who were still resident in these villages. The adolescents in the intervention villages were 14 mm (95% CI: 4, 23 mm; $P = 0.007$) taller and had more favorable measures of insulin resistance and arterial stiffness, as evidenced by a 20% (95% CI: 3%, 39%; $P = 0.02$) lower HOMA score and 3.3% (95% CI: 1%, 5.7%; $P = 0.008$) lower augmentation index.

Second follow-up study (2009–2010)

The second follow-up study examined markers for chronic diseases affecting cardiovascular, musculoskeletal, and mental health in the cohort of young adults who took part in the initial trial. All consenting participants underwent clinical examinations at the National Institute of Nutrition, Hyderabad.

Measurements

Questionnaire data

A semistructured questionnaire was administered to all participants by a trained interviewer. A subset of questions (14/29) from the Standard of Living Index (SLI), a summary measure of household level asset-based scale devised for Indian surveys, was used to estimate socioeconomic position as joint family structures are common in rural India (22). We collected information on the quality of house, toilet facilities, source of lighting and drinking water, ownership of clock, radio, television, bicycle, motorcycle, car, refrigerator, telephone, and agricultural land. These items were weighted to give a maximum score of 34 by using weights developed by the International Institute of Population Science in India (22). Education was classified in 4 levels: no formal education, primary (1–4 y), secondary (5–12 y), and beyond secondary.

The food and physical activity questionnaires were developed and evaluated previously in this setting, and their performance was found to be satisfactory (25, 26). Dietary intake over the past year was estimated with a semiquantitative food-frequency questionnaire that collected information on the frequency of intake (daily, weekly, monthly, or yearly/never) of 98 commonly consumed food items. The nutrient content of a single portion of each food item on the list was estimated based on the Indian food-composition tables (27). Physical activity in the previous week was assessed across the following domains: work, travel, leisure (sports, games, exercise), household, and sedentary. For each activity, the average amount of time spent on the activity and the frequency of the activity were documented; wbPA was defined as activities involving standing, walking, running, and extraneous weights. The average hours per day doing wbPA at work, during commute, and for leisure were calculated from the weekly frequency and duration per day.

Anthropometric data

Weight was measured to the nearest 0.1 kg with a digital SECA balance, and standing height was measured to the nearest 1 mm with a plastic stadiometer (Leicester height measure; Chasmors Ltd). Measurements were taken twice, and the average of 2 values was used in the analysis. BMI was calculated as weight (kg)/height $(m)^2$.

Serum 25(OH)D

Assays were conducted on fasting venous samples at the National Institute of Nutrition. Serum 25-hydroxyvitamin D_2 and

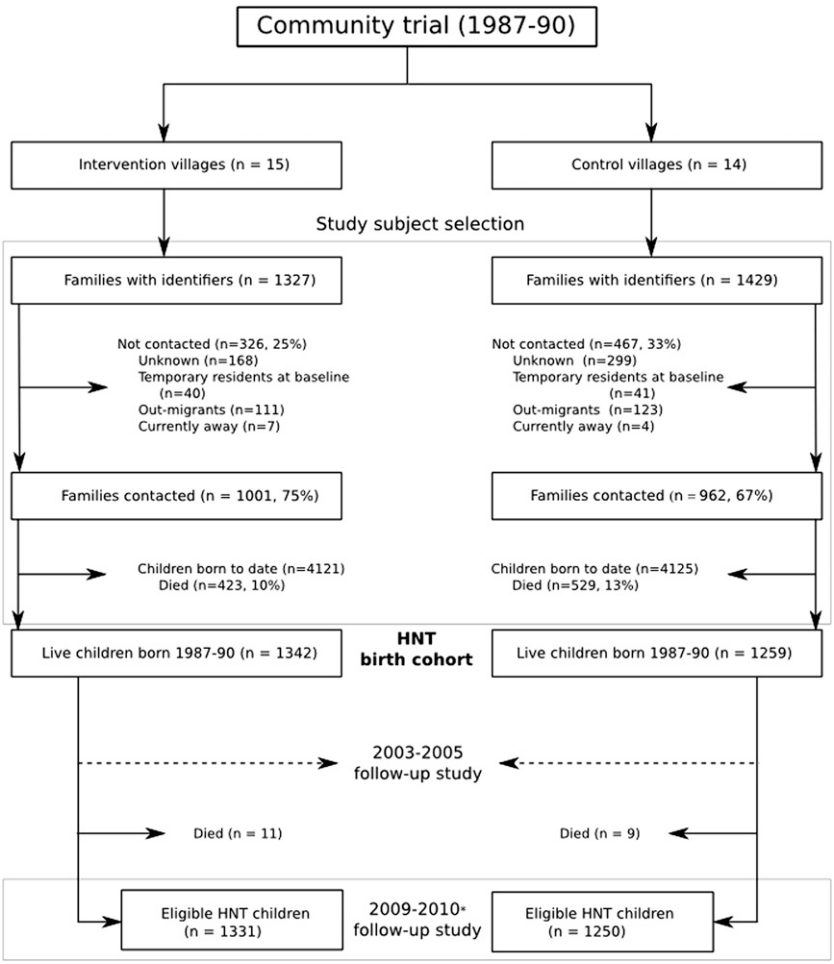

FIGURE 1. Flowchart of participant recruitment at follow-up in the Andhra Pradesh Children and Parents Study. *Includes people who migrated out of villages: intervention (n = 326), control (n = 366). HNT, Hyderabad Nutrition Trial.

25-hydroxyvitamin D_3 were extracted in quantitative HPLC assays and detected at 265 nm by using an ultraviolet detector (CV: 7%).

Dual-energy X-ray absorptiometry

Bone mass, lean mass, and fat mass measurements were assessed with dual-energy X-ray absorptiometry (DXA): 91% of scans were performed with a Hologic Discovery A model and 9% with a Hologic 4500W. The same software version was used on both machines. On the basis of repeated measurements for 30 participants with Hologic Discovery A, the CVs were determined to be 0.7% for hip bone mineral density (BMD), 1.3% for LS BMD, and 0.9% for whole-body BMD. The whole-body scan was performed while the participant lay supine on the scanning bed with their arms resting by their sides. Women suspected of pregnancy (n = 26) were excluded from DXA scanning. Standard Hologic software options were used to define regions of the body (head, arms, trunk, and legs). Scans were coded for artifacts by a visual inspection, and those with major movement and incomplete scans were excluded. For LS scans, pathological changes such as osteoarthritis affecting $\geq$2 vertebrae were excluded; if only one vertebra was affected, the scan was reanalyzed after the affected part was excluded (28). BMD (g/cm^2) was calculated for total hip, LS (L1–L4). Whole-body BMD was included for analyses in men only, because most women wore bangles and other jewelry, which were counted as major artifacts. Whole-body lean mass (kg) and fat mass (kg) were used for the analyses.

Quality control

We produced detailed protocols and regularly checked compliance to standardize the work of the fieldwork team. The anthropometric equipment was calibrated at the start of every clinic. The BMD estimation process was automated in software, which reduces the potential for bias arising from the DXA technician, who knew the intervention group assignment. Hip and LS DXA scans were analyzed by a single trained technician. For quality assurance of DXA scans, a spine phantom was scanned every day to check for acceptable ranges.

Statistical analysis

Descriptive statistics were calculated for each sex. The associations of hip and LS BMD with early-life supplementation and current risk factors were modeled in multilevel regression models that accounted for sibling pairs and village clusters (284 sibling pairs from 29 villages). All multilevel models also adjusted for DXA scanner types. Variables with positively skewed

distributions were logarithmically transformed. The analysis was done on an intent-to-treat basis by using the area of birth as proxy for intervention and control villages. List-wise deletion was used to handle the missing data.

Current risk factors included in the multilevel regression models were age, sex, height, fat mass, lean mass, household SLI, serum 25(OH)D, wbPA, and dietary intake of fruit and vegetables, calcium, protein, and total calories. Three models were fitted for each of the 2 outcome variables (hip and LS BMD) to incrementally adjust for various risk factors: model 1 (age and sex), model 2 (age, sex, anthropometric, and socioeconomic factors, eg, height, fat mass, lean mass, and SLI), and model 3: age, sex, anthropometric and socioeconomic factors, serum 25(OH)D, dietary factors (fruit and vegetable, calcium, protein, and energy intakes), and wbPA.

Interaction terms for sex and supplementation and for sex and wbPA were examined, and a robust interaction term between sex and supplementation was found for hip BMD (P = 0.03 for hip, 0.61 for LS). Sex-stratified multilevel regression analysis was performed for all models. The intraclass correlation analysis for model 3 showed that between-village variability in the outcome was quite small compared with between-sibling variability (data not shown). All analyses were conducted by using R, version 2.15.2, and multilevel modeling was done with nlme version 3.1–105.

RESULTS

Of the original Hyderabad Nutrition Trial cohort, all 2601 participants born between 1987 and 1990 were invited to the clinic, 1446 (56%) of whom accepted the invitation and attended. The participants in the study were more likely to be men than were the eligible nonparticipants (*see* Supplemental Table 1 under "Supplemental data" in the online issue), partially attributable to the emigration of women because of marriage. DXA scans without major artifacts were available in 1351 (93%) participants for hip and 1360 (94%) for LS. Serum 25(OH)D data were available for 1037 (71%) participants. Information on the other variables including SLI, dietary intake estimates, and physical activity were available for ≥98% of the participants.

The key characteristics of the participants are shown in **Table 1**. These young adults were generally lean (BMI: 19.5). The median total energy intake was 2735 kcal/d. Although Indian standards were unavailable and caution should be exercised in applying standards across populations, 57% of the participants had low serum 25(OH)D concentrations (<20 ng/mL) (29) and 70% of the participants had a low fruit and vegetable intake (<400 g/d) (30) according to international standards. Women were more likely to be unemployed than were men, with most engaging in household work (n = 166), whereas men were more likely to be students or skilled manual laborers. Women spent fewer hours on wbPA at work, during commuting, or for leisure.

TABLE 1
Participant characteristics of the Andhra Pradesh Children and Parents Study cohort in 2009–2010[1]

	Women	Men
Total subjects [*n* (%)]	465 (32.2)	981 (67.8)
Age (y)	20.44 ± 1.2[2]	20.2 ± 1.2
Height (cm)	152.64 ± 5.3	166.67 ± 6.2
Weight (kg)	44.61 ± 7.5	54.81 ± 8.7
BMI (kg/m^2)	19.12 ± 2.9	19.71 ± 2.8
Lean mass (kg)	29.93 ± 3.9	43.42 ± 5.5
Fat mass (kg)	12.2 (11.8, 12.6)[3]	9.1 (8.9, 9.3)
SLI	17.62 ± 4.5	18.65 ± 4.2
Education [*n* (%)]		
No/primary only	131 (28.2)	171 (17.5)
Secondary	305 (65.6)	751 (76.6)
Beyond secondary	29 (6.2)	58 (5.9)
Occupation [*n* (%)]		
Unemployed	170 (36.6)	28 (2.8)
Student	143 (30.8)	399 (40.3)
Manual work	90 (19.4)	188 (19)
Skilled manual work	48 (10.3)	303 (30.6)
Professional	14 (3)	72 (7.3)
Serum vitamin D (ng/mL)	18.52 (17.8, 19.3)	23.12 (22.3, 24)
Dietary intake		
Calcium (mg/d)	423.8 (405, 443.5)	618.7 (600.3, 637.6)
Protein (g/d)	47.5 (46.1, 49)	75.2 (73.5, 76.9)
Energy (kcal/d)	1987.4 (1930.9, 2045.6)	3107.9 (3042.8, 3174.5)
Fruit and vegetables (g/d)	210 (200, 220.5)	341.1 (329, 353.8)
Dairy products (g/d)	118.8 (106.6, 132.4)	159.1 (149.4, 169.5)
Lifestyle		
Tobacco use [*n* (%)]	0 (0)	188 (19.2)
Alcohol use [*n* (%)]	8 (1.7)	217 (22.1)
wbPA (h)	0.71 (0.63, 0.79)	1.9 (1.8, 2)

[1] SLI, Standard of Living Index; vitamin D, serum 25-hydroxyvitamin D; wbPA, weight-bearing physical activity.
[2] Mean ± SD (all such values).
[3] Geometric mean; geometric 95% CI in parentheses (all such values; for variables with a skewed distribution).

Tobacco and alcohol use were rare among women, whereas approximately one-fifth of male participants reported current tobacco or alcohol use.

Hip and LS BMD values in this population were generally low when compared with the reference values for the Indian population (31) (**Table 2**). In multivariable models adjusted for current risk factors, early-life supplementation was not positively associated with BMD (Table 2). For men, the negative association between early-life supplementation and BMD remained robust in the fully adjusted model for hip BMD, whereas this association was attenuated when the serum 25(OH)D concentration was added to the model for LS BMD. Of the current risk factors assessed in model 3, height and lean mass were positively associated with hip, LS, and whole-body BMDs (**Table 3**, **Table 4**, and **Table 5**). Fat mass was positively associated with LS BMD in women and whole-body BMD in men. The positive associations between hip BMD and wbPA remained robust when fat mass was added to the models but became attenuated with the addition of lean mass (*see* Supplemental Table 2 under "Supplemental data" in the online issue). The serum 25(OH)D concentration was not strongly associated with BMD in the multivariable models. The positive associations of dietary intake of calcium, protein, and total calories with BMD were attenuated in the fully adjusted models, except for calcium and hip BMD in women.

DISCUSSION

Young adults from this transitional rural community were generally lean, and their BMD was low. No strong evidence of an association was found between BMD and early-life supplementation. Current height, BMI, and wbPA were associated with bone mass, whereas no clear evidence of an association with bone mass was found for other current risk factors, including social position and dietary intakes of calcium, protein, and calories. Strong evidence of an association was found between BMD and lean mass.

Comparison with previous research

The Guatemala trial randomized 4 villages within pairs to offer either low-energy (1.38 MJ) or high-energy (3.76 MJ, proteins, and micronutrients) supplements to pregnant women and children until the age of 7 y (7). Follow-up in adolescence (mean age: 16.7 y) showed a positive association between bone mass and protein-calorie supplementation, but this association was not statistically robust when the models were adjusted for current weight and stature. In an urban cohort of participants from India (the New Delhi Birth Cohort study), bone mass at age 33–39 y was positively associated with birth weight (proxy measure for early-life undernutrition), but this association was also attenuated to null on adjustment for current anthropometric and lifestyle risk factors (32). In our study, no strong evidence of a positive association was found between bone mass and early-life nutritional supplementation; if anything, there was weak evidence of an inverse association between the two. Higher osteoporotic fracture rates are observed in urban areas in high-income countries, which may be partially because of the more sedentary lifestyles among city dwellers (3). Anecdotal evidence suggests that urbanization of the study area over the past decade may

TABLE 2
Multilevel models examining the association between supplemental nutrition and BMD in the Andhra Pradesh Children and Parents Study cohort in 2009–2010[1]

	BMD[2]		Early-life supplementation effect[3,4]		
	Intervention	Control	Model 1	Model 2	Model 3
	g/cm²	*g/cm²*			
Hip					
Women	0.83 ± 0.09	0.84 ± 0.1	−0.005 (−0.025, 0.015)	0.003 (−0.014, 0.021)	0.004 (−0.015, 0.022)
P			0.62	0.7	0.7
Men	0.93 ± 0.11	0.96 ± 0.12	−0.03 (−0.046, −0.015)	−0.02 (−0.033, 0.006)	−0.02 (−0.041, −0.007)
P			<0.001	0.006	0.009
LS					
Women	0.86 ± 0.1	0.86 ± 0.1	−0.003 (−0.025, 0.018)	−0.002 (−0.021, 0.017)	−0.005 (−0.025, 0.016)
P			0.76	0.85	0.65
Men	0.92 ± 0.1	0.94 ± 0.11	−0.02 (−0.037, −0.003)	−0.01 (−0.032, 0.004)	−0.005 (−0.03, 0.02)
P			0.02	0.12	0.68
WB					
Men	1.06 ± 0.08	1.07 ± 0.08	−0.02 (−0.029, −0.002)	−0.007 (−0.021, 0.006)	−0.012 (−0.034, 0.009)
P			0.03	0.27	0.25

[1] Sample sizes: women (hip BMD intervention, n = 413; control, n = 196; LS BMD intervention, n = 412; control, n = 412) and men (hip BMD intervention, n = 480; control, n = 466; LS BMD intervention, n = 486; control, n = 471; WB BMD intervention, n = 458; control, n = 444). BMD, bone mineral density; LS, lumbar spine; SLI, Standard of Living Index (tertiles: low, 0–17; middle, 17–20; high, 21–32); WB, whole body.

[2] Values are means ± SDs.

[3] Values are β coefficients; 95% CIs in parentheses.

[4] Model 1: hip BMD (n = 368 women, n = 838 men); LS (n = 366 women, n = 850 men); and WB (n = 808 men). Model 2: hip BMD (n = 366 women, n = 833 men); LS (n = 364 women, n = 845 men); and WB (n = 804 men). Model 3: hip BMD (n = 329 women, n = 535 men); LS (n = 326 women, n = 538 men); and WB (n = 515 men). Models 1–3 are multilevel regression models accounting for villages and sibling effects and adjusted for types of dual-energy X-ray absorptiometry machines in addition to the following variables: model 1 [adjusted for age in (y)], model 2 [adjusted for age, height (cm), lean mass (kg), fat mass (kg), and SLI (tertiles)], and model 3 [adjusted for age, height, lean mass, fat mass, SLI, serum 25-hydroxyvitamin D (ng/mL), calcium intake (mg/d), protein intake (g/d), energy intake (kcal/d), and weight-bearing physical activity (h/d)].

TABLE 3
Women: univariable and multivariable models examining current risk factors of hip and lumbar spine BMD in the Andhra Pradesh Children and Parents Study cohort in 2009–2010[1]

	Hip BMD (n = 329)				Lumbar spine BMD (n = 326)			
	Univariable		Multivariable[2]		Univariable		Multivariable[2]	
	β coefficient (95% CI)	P	β coefficient (95% CI)	P	β coefficient (95% CI)	P	β coefficient (95% CI)	P
Age (y)	0.005 (−0.003, 0.012)	0.25	0.001 (−0.007, 0.008)	0.89	−0.001 (−0.008, 0.007)	0.89	−0.005 (−0.013, 0.004)	0.28
SLI low[3]	−0.029 (−0.054, −0.005)	0.022	−0.017 (−0.043, 0.009)	0.19	−0.044 (−0.07, −0.019)	0.001	−0.034 (−0.062, −0.007)	0.019
SLI mid[3]	−0.029 (−0.054, −0.005)	0.007	−0.019 (−0.044, 0.006)	0.13	−0.044 (−0.07, −0.019)	0.004	−0.031 (−0.057, −0.004)	0.028
Height (cm)	0.003 (0.001, 0.005)	0.001	−0.002 (−0.004, 0)	0.094	0.005 (0.003, 0.006)	<0.001	0.002 (0, 0.004)	0.077
Fat mass (kg)	0.082 (0.053, 0.111)	<0.001	0.01 (−0.027, 0.047)	0.6	0.088 (0.058, 0.118)	<0.001	0.053 (0.014, 0.093)	0.012
Lean mass (kg)	0.011 (0.009, 0.013)	<0.001	0.011 (0.008, 0.014)	<0.001	0.008 (0.006, 0.011)	<0.001	0.004 (0, 0.008)	0.04
Vitamin D (ng/mL)	0.023 (−0.002, 0.048)	0.067	0.02 (−0.002, 0.043)	0.085	0.004 (−0.022, 0.03)	0.74	0.003 (−0.022, 0.027)	0.84
Fruit and vegetables (g)	0.009 (−0.007, 0.025)	0.25	−0.004 (−0.026, 0.018)	0.72	0.014 (−0.002, 0.031)	0.089	0.001 (−0.022, 0.025)	0.9
Calcium (mg/d)	0.017 (0, 0.033)	0.052	0.036 (0.003, 0.069)	0.037	0.016 (–0.001, 0.033)	0.074	0.01 (−0.026, 0.046)	0.57
Protein (g/d)	0.012 (−0.014, 0.037)	0.37	−0.087 (−0.22, 0.044)	0.19	0.021 (−0.005, 0.047)	0.11	−0.04 (−0.181, 0.1)	0.57
Energy (kcal)	0.009 (−0.01, 0.029)	0.35	0.042 (−0.079, 0.16)	0.486	0.016 (−0.004, 0.036)	0.12	0.031 (−0.097, 0.16)	0.63
wbPA (h)	0.02 (0.001, 0.039)	0.042	0.014 (−0.006, 0.033)	0.161	0.012 (−0.008, 0.031)	0.23	0.016 (−0.005, 0.037)	0.13

[1] All models were adjusted for types of dual-energy X-ray absorptiometry machine in multilevel regression models accounting for village clusters and sibling pairs. Multivariable models were additionally adjusted for age, SLI, height, fat mass, lean mass, serum 25-hydroxyvitamin D, wbPA, and dietary intake of fruit and vegetables, calcium, protein, and energy. Serum 25-hydroxyvitamin D, fruit and vegetable intake, calcium intake, protein intake, energy intake, and wbPA were log transformed to account for skewed distributions. BMD, bone mineral density (g/cm^2); SLI, Standard of Living Index; wbPA, weight-bearing physical activity.

[2] Based on model 3.

[3] SLI tertiles: low, 0–17; middle, 17–20; high, 21–32.

TABLE 4
Men: univariable and multivariable models examining current risk factors of hip and lumbar spine BMD in the Andhra Pradesh Children and Parents Study cohort in 2009–2010[1]

	Hip BMD (n = 535)				Lumbar spine BMD (n = 538)			
	Univariable		Multivariable[2]		Univariable		Multivariable[2]	
	β coefficient (95% CI)	P	β coefficient (95% CI)	P	β coefficient (95% CI)	P	β coefficient (95% CI)	P
Age (y)	0.006 (0, 0.013)	0.049	−0.002 (−0.008, 0.005)	0.66	0.006 (0, 0.012)	0.036	0.002 (−0.005, 0.009)	0.62
SLI low[3]	−0.01 (−0.028–0.008)	0.29	−0.00005 (−0.021, 0.021)	0.996	0.005 (−0.012, 0.022)	0.54	0.013 (−0.008, 0.034)	0.23
SLI mid[3]	−0.01 (−0.028, 0.008)	0.067	−0.003 (−0.023, 0.017)	0.76	0.005 (−0.012, 0.022)	0.99	0.013 (−0.007, 0.033)	0.2
Height (cm)	0.003 (0.002, 0.004)	<0.001	−0.004 (−0.005, −0.002)	<0.001	0.002 (0.001, 0.003)	<0.001	−0.002 (−0.003, 0)	0.037
Fat mass (kg)	0.074 (0.056, 0.091)	<0.001	−0.027 (−0.053, −0.002)	0.041	0.052 (0.035, 0.068)	<0.001	−0.01 (−0.036, 0.016)	0.47
Lean mass (kg)	0.01 (0.009, 0.011)	<0.001	0.012 (0.01, 0.015)	<0.001	0.007 (0.006, 0.008)	<0.001	0.008 (0.005, 0.01)	<0.001
Vitamin D (ng/mL)	0.024 (0.003, 0.046)	0.029	0.016 (−0.003, 0.035)	0.096	0.023 (0.002, 0.043)	0.029	0.015 (−0.005, 0.034)	0.14
Fruit and vegetables (g)	0.027 (0.015, 0.039)	<0.001	0.001 (−0.018, 0.02)	0.94	0.018 (0.008, 0.029)	0.001	0.012 (−0.006, 0.031)	0.2
Calcium (mg/d)	0.025 (0.012, 0.039)	<0.001	−0.021 (−0.053, 0.01)	0.18	0.015 (0.003, 0.027)	0.018	−0.011 (−0.042, 0.02)	0.5
Protein (g/d)	0.058 (0.039, 0.076)	<0.001	0.037 (−0.068, 0.143)	0.49	0.036 (0.019, 0.052)	<0.001	0.029 (−0.081, 0.138)	0.6
Energy (kcal)	0.042 (0.025, 0.058)	<0.001	0.005 (−0.093, 0.103)	0.92	0.022 (0.009, 0.036)	0.001	−0.02 (−0.121, 0.082)	0.7
wbPA (h)	0.04 (0.025, 0.054)	<0.001	0.017 (0, 0.033)	0.051	0.031 (0.017, 0.045)	<0.001	0.02 (0.004, 0.037)	0.02

[1] All models were adjusted for types of dual-energy X-ray absorptiometry machine in multilevel regression models accounting for village clusters and sibling pairs. Multivariable models were additionally adjusted for age, SLI, height, fat mass, lean mass, serum 25-hydroxyvitamin D, wbPA, and dietary intake of fruit and vegetables, calcium, protein, and energy. Serum 25-hydroxyvitamin D, fruit and vegetable intake, calcium intake, protein intake, energy intake, and wbPA were log transformed to account for skewed distributions. BMD, bone mineral density (g/cm^2); SLI, Standard of Living Index; wbPA, weight-bearing physical activity.

[2] Based on model 3.

[3] SLI tertiles: low, 0–17; middle, 17–20; high, 21–32.

TABLE 5
Men: univariable and multivariable models examining current risk factors of whole-body BMD in the Andhra Pradesh Children and Parents Study cohort in 2009–2010[1]

	Whole-body BMD (n = 562)			
	Univariable		Multivariable[2]	
	β coefficient (95% CI)	P	β coefficient (95% CI)	P
Age (y)	0.011 (0.007, 0.016)	<0.001	0.007 (0.002, 0.012)	0.012
SLI low[3]	0.0003 (−0.013, 0.013)	0.97	0.011 (−0.005, 0.026)	0.17
SLI mid[3]	−0.0008 (−0.013, 0.013)	0.9	0.009 (−0.005, 0.023)	0.22
Height (cm)	0.002 (0.001, 0.003)	<0.001	−0.002 (−0.003, −0.001)	0.001
Fat mass (kg)	0.026 (0.014, 0.039)	<0.001	−0.043 (−0.062, −0.024)	<0.001
Lean mass (kg)	0.006 (0.005, 0.007)	<0.001	0.008 (0.007, 0.01)	<0.001
Vitamin D (ng/mL)	0.024 (0.008, 0.039)	0.004	0.014 (0, 0.029)	0.057
Fruit and vegetables (g)	0.017 (0.008, 0.025)	<0.001	0.002 (−0.012, 0.016)	0.76
Calcium (mg/d)	0.02 (0.01, 0.03)	<0.001	-0.0003 (0.023, 0.023)	0.98
Protein (g/d)	0.035 (0.022, 0.049)	<0.001	0.047 (−0.032, 0.127)	0.24
Energy (kcal)	0.024 (0.013, 0.036)	<0.001	−0.039 (−0.112, 0.035)	0.3
wbPA (h)	0.022 (0.012, 0.033)	<0.001	0.013 (0.001, 0.025)	0.043

[1] All models were adjusted for types of dual-energy X-ray absorptiometry machine in multilevel regression models accounting for village clusters and sibling pairs. Multivariable were models additionally adjusted for age, SLI, height, fat mass, lean mass, serum 25-hydroxyvitamin D, wbPA, and dietary intake of fruit and vegetables, calcium, protein, and energy. Serum 25-hydroxyvitamin D, fruit and vegetable intake, calcium intake, protein intake, energy intake, and wbPA were log transformed to account for skewed distributions. BMD, bone mineral density (g/cm^2); SLI, Standard of Living Index; wbPA, weight-bearing physical activity.
[2] Based on model 3.
[3] SLI tertiles: low, 0–17; middle, 17–20; high, 21–32.

have proceeded more rapidly in the intervention villages than in the control villages. In the first follow-up study in 2003–2005, we observed taller statures in children from the intervention villages, raising the possibility of a positive effect of early-life supplementation on skeletal growth during early adolescence. Any positive effects of early-life supplementation during early adolescence may have been negated as a result of changes in lifestyles during late adolescence and young adulthood in more urbanized villages. Higher rates of physical activity may be associated with more rural lifestyles (eg, agricultural occupations) in the control villages, which may partially explain the inverse association between early-life supplementation and BMD; however, our study did not have appropriate measures to examine this possibility definitively. Inclusion of population size as a marker of urbanization did not materially change our results.

Current wbPA and height and lean mass were strong determinants of bone mass in this study, consistent with the findings of previous studies that examined bone mass in individuals in the growth phase (11, 32–35). Fat and lean mass, 2 main components of body mass, have been suggested, although not consistently, as independent determinants of BMD (13, 36, 37). Our analyses of relative contributions of fat and lean mass to BMD showed clear evidence of a positive association between BMD and lean mass, but not fat mass. A similar analysis from Thailand suggested that uniformly low body fat, a common characteristic observed in rural areas of low- and middle-income countries, may obscure the association between BMD and fat mass because of a lack of sufficient variability (ie, low study power) (13). The addition of lean mass to the models attenuated the association between BMD and wbPA, which suggests that this association may be partially mediated through lean mass accumulation. On the other hand, lean mass as a surrogate for physical activity may be measured with relatively less error as compared with physical activity assessed by questionnaire, which further supports the role of physical activity in bone mass accrual. Our finding on physical activity is particularly important for this transitional rural community because physical inactivity has been suggested to partially explain the association between lower bone mass and urbanization (1, 4, 38, 39).

The magnitudes of the effects of dietary calcium intake have varied in previous studies (40–42), and it is possible that calcium intake may only be positively associated with bone mass in individuals with very low vitamin D concentrations (12). In this study population, no evidence of an association was found between BMD and dietary calcium, even though the serum 25(OH)D concentration was fairly low (43, 44). Studies have shown a positive association between bone mass and protein intake, although a high protein intake has been suggested to be both detrimental and protective for bones (17, 45). The extremely low energy intake of adolescents may be associated with low BMD, but the effect of a high calorie intake in healthy young adults is unclear (46, 47). Our results showed no evidence of an association between BMD and protein or energy intake. Fruit and vegetable (Tables 3, 4, and 5) and dairy product (data not shown) intakes have also been suggested as potential determinants of bone mass, but no associations were observed in this study (4, 48).

Strengths and limitations

The main strength of this study was the combined assessment of effects of both early-life and current risk factors on BMD. Compared with observational studies using birth weight as a proxy measure for early-life nutrition, the initial supplementation trial with a controlled design in our study should have reduced the chances of confounding by other factors and more

directly assessed the effects of early-life nutrition on bone mass accrual. The rapid but uneven socioeconomic development of the area over the past decade has also introduced remarkable changes in the current lifestyles of the study population, providing sufficient variation in these exposures for their effects to be robustly examined. The study population size was large compared with other DXA bone studies in young adults from low- and middle-income countries, which added further strength to the study.

The study also had some limitations. The DXA facility location was not convenient or feasible to reach for some participants, and the response rate of participants invited to clinic was 56% of the original study sample. The response rates were similar between intervention and control villages, but the possibility of selection bias could not be ruled out. Women were overrepresented among the nonparticipants as a result of migration due to marriage. We did not have direct data on nutritional supplementation; mothers in the intervention area were presumed to have taken the supplement, which could also have been shared with other family members. As a result, the dose of nutritional supplement may have been too modest for persistent effects on bone development. On the other hand, our results mimicked real-life circumstances, which provided plausible estimates of the long-term effects of nutritional supplementation programs. Although we collected detailed information on types and amounts of physical activity, we did not have information on the amounts of loads that each type of physical activity applied to bones. Finally, the cross-sectional assessment of bone mass did not allow us to examine the causality between these exposures and outcomes.

Implications

Bone remodeling is influenced by many factors throughout life. In emerging-economy countries, many rural communities are experiencing the effects of rapid economic development superimposed on early disadvantages. Whereas improved nutrition may be able to mitigate some of the effects of early-life undernutrition, more urbanized lifestyles may also have negative effects on bone mass accrual in the future. Physical activity may be incorporated into daily lives relatively cheaply and easily and is also important for the prevention of other NCDs, such as cardiovascular diseases (49). Our findings provide yet another reason for promoting physical activity in rural communities in low- and middle-income countries that are undergoing rapid urbanization.

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Online Supplemental Tables

Table 1: Characteristics of young adults who attended and those who did not attend clinics at the 2009-2010 study of the Andhra Pradesh Children and Parents Study. Values are numbers (percentages) unless stated otherwise.

	Intervention area (n = 1342)			Control area (n = 1259)		
Characteristics	**Participants (n=738)**	**Non-participants (n=604)**	**p***	**Participants (n = 708)**	**Non-participants (n = 551)**	**p***
Mean(SD) age (years)†	20.7(1.1)	20.7 (1.1)	0.12	20.7 (1.1)	20.7 (1.1)	0.43
Women	236 (32)	423 (70)	<0.001	223 (31.5)	399 (72.4)	<0.001
Occupation‡	(n = 737)	(n = 598)	<0.001	(n = 695)	(n = 532)	<0.001
Full time student	608 (82.5)	415 (69.4)		534 (76.8)	328 (60.3)	
Full time employment	90 (12.2)	119 (19.9)		124 (17.8)	155 (28.5)	
Other (neither, both)	39 (5.3)	64 (10.7)		37 (5.3)	49 (9)	
Birth weight (g)	(n=198)	(n=136)	0.74	(n=273)	(n=165)	0.26
	2715.6(416.5)	2730.3(381.4)		2639.6(432.4)	2592.4(426.7)	

* These p-values are based on unpaired t tests or X^2 tests for heterogeneity with appropriate degrees of freedom.
† As of January 1 2009.
‡ Based on 2003 data.

Table 2: Models with or without lean mass examining associations of weight-bearing physical activity with hip and lumbar spine BMD of the Andhra Pradesh Children and Parents Study cohort in 2009-2010. Values are β coefficient (95% CI) and p-values.

	Hip BMD				Lumbar spine BMD			
	Model 3 without lean mass		Model 3		Model 3 without lean mass		Model 3	
	β coefficient	p	β coefficient	p	β coefficient	p	β coefficient	p
Women								
Fat mass (kg)	0.078 (0.047 to 0.11)	<0.001	0.009 (-0.027 , 0.047)	0.59	0.077 (0.045, 0.11)	<0.001	0.053 (0.014 , 0.093)	0.01
Lean mass (kg)			0.011 (0.008 , 0.014)	<0.001			0.004 (0 , 0.008)	0.04
wbPA (hour)	0.024 (0.004, 0.045)	0.021	0.014 (-0.006 , 0.033)	0.16	0.02 (-0.001, 0.04)	0.06	0.016 (-0.004 , 0.037)	0.12
Men								
Fat mass (kg)	0.053 (0.047, 0.11)	<0.001	-0.028 (-0.053 , -0.002)	0.04	0.042 (0.02, 0.063)	<0.001	-0.009 (-0.035 , 0.017)	0.49
Lean mass (kg)			0.012 (0.01 , 0.015)	<0.001			0.008 (0.005 , 0.01)	<0.001
wbPA	0.028	0.003	0.016	0.06	0.028	0.002	0.02	0.02

(hour)	(0.004, 0.045)	(0 , 0.033)	(0.011, 0.045)	(0.003 , 0.036)

Sample size: Hip BMD Women n=329, Men n=535; Lumbar spine BMD Women n=326, Men n=538.
BMD: Bone mineral density (g/cm^2); LS: Lumbar spine; wbPA: Weight bearing physical activity
All models adjusted for DXA machine types in multilevel models accounting for village clusters and sibling pairs. Multivariable models additionally adjusted for early life supplementation, age, height, SLI, dietary intake (fruit and vegetable, calcium, protein, calories), and serum vitamin D.

5.2 Unpublished data from Research Paper 2

Was early life protein-energy supplementation associated with hip and lumbar spine bone area and mineral content?

Early life nutrition may be a more important determinant of bone size than density. This section shows the results of same regression analyses from Research Paper 2 applied to hip and LS bone area and mineral content to augment the findings in this chapter.

There was no clear evidence of differences in mean values for hip and lumbar spine BA and BMC in young adults from the intervention and control villages in the APCAPS community (**Table 5.1**). There was some evidence for a small positive association between hip BA and BMC and early life nutrition supplementation in females (**Table 5.2**). In males, there was a negative association between BMC and the intervention area. Overall, the effect sizes were fairly small even when there was evidence for association and, for most outcomes, there was no clear evidence of association. It is therefore unlikely that the early life intervention had a strong beneficial effect on bone size in this young population.

Table 5.1: Mean comparison of bone area and bone mineral content of hip and lumbar spine in the intervention and control areas of the Andhra Pradesh Children and Parents Study (2009-2012).

		Bone area (cm^2)		Bone mineral content (g)	
		intervention	**control**	**intervention**	**control**
Hip	**female**	28.38 (2.39)	28.23 (2.78)	23.86 (3.7)	23.84 (3.89)
	male	35.66 (3.49)	35.87 (3.34)	33.48 (5.17)	34.8 (5.59)
Lumbar spine	**female**	48.61 (4.36)	48.75 (4.91)	42.22 (7.38)	42.51 (7.88)
	male	57.25 (5.05)	57.43 (5.66)	53.38 (8.65)	54.63 (9.21)

LS: lumbar spine.
All values are mean (standard deviation).
Hip: female n = 277 (intervention); 239 (control): male n = 519 (intervention); 482 (control).
Lumber Spine: female n = 276 (intervention); 239 (control): male n = 521 (intervention); 487 (control)

Table 5.2: Multivariable models for association between bone area in hip and lumbar spine in young adulthood (18-25 years) and early life nutritional supplementation in the Andhra Pradesh Children and Parents Study (2009-2012).

		BA				BMC			
		Model 1		Model 2		Model 1		Model 2	
		β	p	β	p	β	p	β	p
		95% CI		95% CI		95% CI		95% CI	
Hip	**female**	0.17	0.46	0.4	0.03	0.04	0.91	0.57	0.04
		(-0.3 to 0.65)		(0.04 to 0.76)		(-0.67 to 0.75)		(0.04 to 1.11)	
	male	-0.32	0.27	0.00	0.99	-1.43	0.00	-0.81	0.01
		(-0.9 to 0.26)		(-0.43 to 0.43)		(-2.36 to -0.5)		(-1.44 to -0.18)	
LS	**female**	-0.05	0.91	0.00	0.99	-0.19	0.79	0.08	0.89
		(-0.91 to0.82)		(-0.61 to 0.62)		(-1.62 to 1.23)		(-1.09 to 1.25)	
	male	-0.31	0.45	-0.04	0.90	-1.27	0.12	-0.74	0.29
		(-1.15 to 0.52)		(-0.64 to 0.57)		(-2.86 to 0.33)		(-2.12 to 0.65)	

LS: lumbar spine; BA: bone area (cm^2); BMC: bone mineral content (g); CI: confidence interval
Model 1: bone outcomes (BA/BMC in hip/LS) ~ age + early life nutrition supplementation
Model 2: bone outcomes ~ age (years) + height (cm) + lean mass (kg) + fat mass (kg; log-transformed) + standard of living index + early life nutrition supplementation

Summary

- There was no clear evidence of positive association between bone mass in young adulthood and early life protein-energy supplementation.
- Current body mass, especially lean mass, was positively associated with bone mass in young adults.
- Weight-bearing physical activity may be an important determinant of bone mass in this lean population.

Chapter 6: Research Paper 3

6.1 Research Paper 3: Adolescent undernutrition and early adulthood bone mass in an urbanizing rural community in India

This chapter shows results from lifecourse analyses of adolescent underweight on bone outcomes in young adulthood. The first section takes a publication style with a brief introduction followed by the methodological information specific to this chapter and the findings on the effect of adolescent body size on bone mass in young adulthood. The second section includes unpublished data in five subsections: the first three subsections extend upon the analyses from Research Paper 3 to include missing data analyses and analysis of bone area; the last two subsections focus on body size and composition and bone health in adolescents in this community.

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Conceptualized, conducted the analyses, wrote the first draft, coordinated all comments by co-authors, and was primarily responsible for the final draft.

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ORIGINAL ARTICLE

Adolescent undernutrition and early adulthood bone mass in an urbanizing rural community in India

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Abstract

Summary The long-term effects on bone health of nutritional status in adolescence are unclear. The impact of adolescent and current body mass on bone mass in young adulthood in rural India was assessed. Current lean mass was a more important determinant of bone mass than thinness during adolescence in this population.

Purpose/introduction Adolescence is a crucial period for skeletal growth. However, the long-term effects on bone health of nutritional status in adolescence, particularly in the context of nutritional transition, are unclear. The current manuscript assessed the impact of adolescent and current body size on bone mass in young adulthood in an Indian rural community that is undergoing rapid socioeconomic changes.

Methods The Andhra Pradesh Children and Parents Study is a prospective cohort study in Hyderabad, India. In 2003–2005, the study collected anthropometric and cardiovascular data on adolescents (mean age=16 years old). The second and third waves of the study in 2009–2012 collected data on current anthropometric measures, areal bone mineral density (aBMD) in hip and lumbar spine (L1–L4) measured by dual-energy X-ray absorptiometry, and living standards of the trial participants who were now young adults (mean age=22 years old).

Results The median body mass index (BMI) of the 722 participants included in this analysis was 16.8 kg/m^2 during adolescence, while the median BMI as young adults was 19.3 kg/m^2. Lower aBMD during adulthood was associated with lower adolescent BMI (β (95 % confidence interval) for hip aBMD 0.017 (0.013 to 0.022) and LS aBMD 0.012 (0.008 to 0.016)). This association was attenuated upon adjustment for current fat and lean mass (β (95 % CI) for hip aBMD 0.00 (−0.005 to 0.005) and LS aBMD 0.005 (0.000 to 0.01)). There was clear evidence for positive associations between aBMDs and current lean mass.

Conclusions Current lean mass was a more important determinant of bone mass than thinness during adolescence in this population. Weight gain during late adolescence and young adulthood coupled with improvement in lean mass may help to mitigate any adverse effects that pre-adulthood undernutrition may have on bone mass accrual.

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Introduction

Suboptimal peak bone mass is associated with higher risk of osteoporotic fractures in later life [1, 2]. Studies from high income countries have shown that 90 % of peak bone mass is accrued before age 18 in healthy individuals [1, 3, 4]. Skeletal growth during adolescence is therefore an important determinant of peak bone mass. Large body size, high level of weight-bearing physical activity, and adequate micronutrient intake are some of the key determinants of bone mass accrual [1].

Undernutrition is commonly observed in low and middle income countries (LMICs). In India, the prevalence of undernutrition remains high although it has been slowly declining over the last 2 decades [5, 6]. As a result, some young adults who experienced undernutrition during childhood and adolescence have attained at least normal body mass index as adults [7].

A number of studies have suggested positive associations between adult bone mass and birthweight as well as weight during infancy [8–10]. On the other hand, association between peak bone mass and thinness during adolescence has not been adequately studied in lean populations from LMICs. Some studies from high income countries examined longitudinal effects of anorexia nervosa during adolescence and showed that successful recovery from anorexia nervosa may mitigate some of the negative effects of low body weight during adolescence [11, 12]. Since adolescence is a crucial period for skeletal growth, it is important to understand whether undernutrition during adolescence has any long-term effects on bone mass.

While studies have generally found a positive association between body mass and bone mass, fat and lean mass may be differently associated with bone mass [13–15]. Lean mass is influenced by both diet and physical activity level. Weight-bearing physical activity during adolescence is associated with higher bone mass [4, 16]. It is therefore important to understand how gains in overall weight, fat mass, and lean mass may contribute to skeletal development in young adults who experienced nutritional transition during adolescence.

The Andhra Pradesh Children and Parents Study (APCAPS) is a prospective cohort study from southern India. The study community has been experiencing nutritional transition due to urbanization over the past decade. The current manuscript assessed whether being underweight during adolescence is associated with lower peak bone mass in young adults, some of whom have experienced improvements in nutritional status since adolescence.

Methods

Study design

The analyses in this study used data from three waves of data collection (2003–2005, 2009–2010, and 2010–2012) of the APCAPS study, established through long-term follow up of the Hyderabad Nutrition Trial (HNT). The HNT studied impact of the Integrated Child Development Services (ICDS) scheme, a national community outreach program providing food supplementation along with health, hygiene, and nutrition education, immunization, anemia control, and basic health care to pregnant and lactating women and children under the age of 6 years [17].

Initial trial (1987–90) and the first wave of data collection (W1: 2003–5)

A detailed description of the initial trial (HNT) and the first wave of data collection (the first follow-up of the HNT) have previously been published [18]. Briefly, a controlled "stepped wedge design" study was conducted in 1987–1990, using the opportunity afforded by the incremental expansion of ICDS program. A total of 29 villages in two adjacent administrative areas near Hyderabad city in India were selected, one with ICDS program already in place (15 intervention villages) and the other awaiting implementation (14 control villages). In the intervention villages, a nutritional supplement made of corn-soya blend and soybean oil was available daily to all pregnant and lactating women and children under 6 years. The meal (upma) contained, on average, 2.09 MJ and 20–25 g protein for pregnant and lactating women and about 1.25 MJ and 8–10 g protein for children under 6 years old. The supplementation was associated with a small but statistically robust (61 g; 95 % CI 18 to 104 g; p=0.007) increase in the birth weight of the offspring [18]. During the first wave of data collection in 2003–2005, 1165 adolescents aged 13–18 years who were still resident in these villages were reexamined [18]. The adolescents in the intervention villages were 14 mm (95 % CI 4 to 23 mm; p=0.007) taller and had more favorable measures of insulin resistance and arterial stiffness as shown by a 20 % (95 % CI 3 to 39 %; p=0.02) lower homoeostasis model assessment score, which describes levels of insulin resistance, and 3.3 % (95 % CI 1 to 5.7 %; p=0.008) lower augmentation index.

The second and third waves of data collection (W2/3: 2009–2012)

Since the second and third waves of data collection were conducted within a relatively short period of time (2009–2012), the analyses in this manuscript combined data from these two waves of data collection (W2/3). W2/3 examined markers for

chronic diseases affecting cardiovascular, musculoskeletal, and mental health. All consenting participants underwent DXA measurements at the National Institute of Nutrition (NIN), Hyderabad, and physical measurements at NIN (W2) or the village clinics (W3). In cases where participants attended both waves of data collection, the data from the third wave were used, unless there were artifacts in DXA scans from the W3, which prompted the use of data from W2.

The present analyses were restricted to participants from the first wave of data collection who also underwent DXA scans during W2/3.

Measurements

Questionnaire data (W1/2/3)

A semi-structured questionnaire was administered to all participants by a trained interviewer. A subset of questions (14/29) from the Standard of Living Index (SLI) in the National Health Family Survey-2, a summary measure of household level asset-based scale devised for Indian surveys, was used to estimate socioeconomic position, as joint family structures are common in rural India [19]. We collected information on the quality of house, toilet facilities, source of lighting and drinking water, ownership of clock, radio, television, bicycle, motorcycle, car, refrigerator, telephone, and agricultural land. These items were weighted to give a maximum score of 34, using weights developed by the International Institute of Population Science in India [19]. Education was classified in four levels: no formal education, primary (1 to 4 standard), secondary (5 to 12 standard), and beyond secondary level education. Current tobacco use was defined as smoking, chewing, or snuffing tobacco in the last 6 months.

Puberty (W1)

Four puberty stages were set based on sexual maturation on the basis of time since the onset of menstruation (girls) and testicular volume (boys) [20]. The boys assessed testicular volume in private, using Prader's orchidometer with volumes ranging from 1 to 25 ml. This self-assessment was validated against measurements by clinicians in an external sub-study [20].

Anthropometric data (W1/2/3)

Weight was measured to the nearest 0.1 kg with a digital SECA balance, and standing height was measured to the nearest 1 mm with a plastic stadiometer (Leicester height measure). Measurements were taken twice, and the average of two values was used in the analysis (coefficients of variation for height 0.67 %; weight 0.09 %). Body mass index (BMI) was calculated as weight (kg)/height (m^2). Cutoff points of BMI $\leq$17.0 and $\leq$18.5 were used for underweight in adolescence and adulthood respectively [21, 22].

DXA scanning (W2/3)

Bone mass measurements were assessed with DXA on a Hologic Discovery A model. The whole body scan was performed with the participant supine on the scanning bed with their arms resting by their sides. On the basis of repeated measurements for 30 participants with Hologic Discovery A, the coefficients of variation were determined to be 0.7 % for hip bone mineral density (BMD), 1.3 % for LS aBMD, and 0.9 % for whole-body aBMD. Women suspected of pregnancy were excluded from DXA scanning, and the scans were taken only after confirming the negative pregnancy by conducting urine pregnancy test. Standard Hologic software options were used to define regions of the body (head, arms, trunk, and legs). Scans were coded for artifacts by a visual inspection, and those with major movement as well as incomplete scans were excluded from the present analyses. For lumbar spine (LS) scans, pathological changes such as osteoarthritis affecting two or more vertebrae were excluded; if only one vertebra was affected, the scan was reanalyzed after the affected part was excluded [23]. Areal bone mineral density (aBMD in g/cm^2) was calculated from bone mineral content (BMC in g) and bone area (BA in cm^2) for total hip and lumbar spine (L1–L4). Fat and lean mass indexes (FMI and LMI) were based on fat and lean mass (kg) from whole-body scans/height (m^2). Major movements were counted as artifacts and removed from analysis with fat and lean mass.

Statistical analysis

Descriptive statistics were calculated for each sex. The associations of hip and lumbar spine BA, BMC, and aBMD with adolescent body size were modeled in multilevel regression models that accounted for village clusters (29 villages). FMI was log-transformed because it had a positively skewed distribution. Complete-case analysis was used.

Four models were fitted for each of the two outcome variables (hip and LS aBMD): Model 1 assessed each of the explanatory variables (adolescent BMI, current (young adulthood) BMI, current FMI, current LMI), adjusting for sex, age at W1, age at W2/3, height at W1 (cm), and height at W2/3 (cm). Model 2 examined the association between aBMD and adolescent BMI, adjusting for current BMI, age at W1, age at W2/3, sex, height at W1, and height at W2/3. In model 3, current BMI in model 2 was replaced with current FMI and LMI. Model 4 replaced current BMI in model 2 with conditional BMI to examine the effect of change in BMI, by using residuals from a regression model in which current BMI was regressed on adolescent BMI. We also adjusted model 3 for other potential confounders such as puberty stages at W1,

adolescent height, current height, adolescent SLI, current SLI, and current tobacco use. Interaction terms between sex and adolescent BMI, current FMI, current LMI, and change in BMI were examined in models 3 and 4. There was weak evidence for an interaction with current FMI for LS aBMD, where the effect of FMI was slightly higher in female (the interaction term β: 0.08; p=0.04). Residuals from the multilevel models were checked for normality (Shapiro-Wilk test) and heteroscedasticity and found reasonably normally distributed and homoscedastic at each level.

All analyses were conducted using *R*, version 3.0.0, and multilevel modeling was done with nlme version 3.1-109.

Quality control

We produced detailed protocols and regularly checked compliance to standardize the work of the fieldwork team. The anthropometric equipment was calibrated at the start of every clinic. The aBMD estimation process was automated in software, which reduces the potential for bias arising from the DXA technician. Hip and LS DXA scans were analyzed by a single trained technician. For quality assurance of DXA scans, a spine phantom was scanned every day to check for acceptable ranges.

Ethics statement

The study received approvals from the ethics committees of the NIN (Hyderabad, India), the Indian Council of Medical Research (ICMR), Centre for Chronic Disease Control, and London School of Hygiene and Tropical Medicine (London, UK). Approval was also sought from the village heads and their panchayats in each of the 29 villages. Written informed consent or witnessed thumbprint if illiterate was obtained from the participants prior to their inclusion in the study.

Result

Of the 1165 participants of the first wave of data collection (W1), 722 participants (62 %) had their height and weight measured both as adolescents and as young adults and also underwent DXA scans during the second and third waves of data collection (W2/3). Of those, scans without major artifacts were available in 710 (98 %) participants for hip aBMD and 715 (99 %) for lumbar spine aBMD. Eighty-seven percent of the participants also had scans without major artifacts for whole-body estimation of fat and lean mass. Information on

Table 1 Characteristics of the subjects who participated in the Andhra Pradesh Parents and Children Study both in 2003–2005 (W1) and in 2009–2012 (W2/3)

	Women			Men		
	n[c]	W1	W2/3	n[c]	W1	W2/3
Age (year)	220	15.8 (1)	22 (1.3)	502	15.9 (0.9)	21.8 (1.3)
Height (cm)	220	151.2 (5.9)	153.1 (5.6)	502	158.5 (8.7)	167 (6.2)
Weight (kg)	220	40.6 (6)	45.9 (8.2)	502	41.9 (7.5)	56.0 (9.4)
Body mass index (kg/m^2)	220	17.7 (2.2)	19.6 (3.2)	502	16.6 (1.9)	20.1 (3)
Fat mass index (kg/m^2)	216	*n/a*	5.8 (2)	499	*n/a*	3.4 (1.6)
Lean mass index (kg/m^2)	216	*n/a*	13.1 (1.5)	499	*n/a*	15.9 (1.8)
SLI	217	12.9 (5)	18.1 (4.7)	499	10.6 (4.3)	18.7 (4.3)
Occupation (%)	220			502		
Student[b]		84.2	27.3		85.5	34.3
Employed		15.8	32.3		12	61.2
Neither		11.7	40.5		2.6	4.6
Tobacco use (n)[a]	215			500		
Current		0	2		1	85
Former		0	0		0	3
Never		215	220		499	415

All values are mean (sd) unless otherwise noted

W1 the first wave of data collection (2003–2005), *W2/3* the second and third waves of data collection (2009–2012), *SLI* standard of living index, *n/a* not available

[a] Current tobacco use included smoking, chewing, or snuffing tobacco in the last 6 months; former users stopped using tobacco products 6 months ago or more

[b] Student for W1 included one man who worked and studied at the same

[c] All n were same for W1 and W2/3 except that SLI was available for 219 women in W2/3. Tobacco use information was available for 222 women in W2/3

Table 2 Mean hip and lumbar spine bone mass of participants of the Andhra Pradesh parents and children study in 2009–2012 (W2/3)

Total hip	Women		Men	
	n	Mean (sd)	*n*	Mean (sd)
BA (cm^2)	217	28.43 (2.59)	493	35.83 (3.44)
BMC (g)	217	23.92 (3.98)	493	34.25 (5.36)
BMD (g/cm^2)	217	0.837 (0.096)	493	0.952 (0.115)
Lumbar spine				
BA (cm^2)	216	48.81 (4.89)	499	57.56 (5.63)
BMC (g)	216	42.76 (8.02)	499	54.68 (8.99)
BMD (g/cm^2)	216	0.869 (0.104)	499	0.945 (0.105)

BA bone area, *BMC* bone mineral content, *BMD* bone mineral density

the other variables including SLI and occupation was available for ≥99 % of the participants.

Table 1 summarizes the key characteristics of the participants. Fifty-six percent of the participants were underweight (BMI<17) during adolescence. The participants were still lean as young adults although the percentage of underweight individuals (BMI≤18.5) decreased to 38 %. A majority of the participants were students during W1, but only a third of the participants were students during W2/3. Women were more likely to be unemployed and engaged in household work as young adults. Both hip and lumbar spine aBMD values were generally lower than the reference values for the Indian population (Table 2) [24]. Underweight adults (BMI≤18.5) showed lower aBMD than others with normal BMI (data not shown).

Positive association between current aBMD and adolescent BMI was attenuated upon adjustment for current BMI in hip, but the association remained for lumbar spine (Tables 3 and 4). There was no strong evidence of interactions between the change in BMI between W1 and W2/W3 and adolescent BMI (data not shown). In model 3, current lean mass index was strongly associated with current aBMD, whereas there was no strong evidence for association between current aBMD and adolescent BMI or current fat mass index. Model 4 showed positive associations between current aBMDs and conditional BMI. Adjustment for other potential confounders (puberty stages at W1, adolescent height, current height, adolescent SLI, current SLI, and current tobacco use) did not materially change the results.

Table 3 Multivariable models examining associations between body mass index during adolescence (2003–2005) and current bone mineral density (2009–2012) in hip in young adults of the Andhra Pradesh children and parents study (2003–2012)

	Hip BMD							
	Model 1		Model 2		Model 3		Model 4	
	β		*β*		*β*		*β*	
	(95 % CI)	*p*	(95 % CI)	*p*	(95 % CI)	*p*	(95 % CI)	*p*
Adolescent BMI	0.017 (0.013 to 0.022)	<0.001	0.003 (−0.003 to 0.008)	0.32	0.00 (−0.005 to 0.005)	0.97	0.015 (0.011 to 0.019)	<0.001
Current BMI	0.015 (0.012 to 0.017)	<0.001	0.014 (0.01 to 0.017)	<0.001				
Current FMI	0.087 (0.062 to 0.113)	<0.001			−0.018 (−0.047 to 0.011)	0.23		
Current LMI	0.03 (0.026 to 0.034)	<0.001			0.032 (0.026 to 0.038)	<0.001		
Conditional BMI[a]	0.015 (0.012 to 0.019)	<0.001					0.014 (0.01 to 0.017)	<0.001

Conditional BMI was estimated from current BMI regressed on adolescent BMI

Model 1 is a base model examining association between BMD and each of four explanatory variables (adolescent BMI, adulthood BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003–2005 (adolescence), age at the second and third waves (W2/3) in 2009–2012 (current/adulthood), height at W1 (cm), and height at W2/3 (cm)

Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BMD (g/cm^2) adjusting for current BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/W3

Model 3 examined association between adolescent BMI and adulthood BMD, adjusting for current FMI (kg/m^2), current LMI (kg/m^2), sex, age at W1, and age at W2/W3, height at W1, and height at W2/W3

Model 4 examined association between adolescent BMI and adulthood BMD, adjusting for conditional BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/W3

BMI body mass index (kg/m^2), *FMI* fat mass index (kg/m^2, log-transformed), *LMI* lean mass index (kg/m^2)

Table 4 Multivariable models examining associations between body mass index during adolescence (2003–2005) and current bone mineral density (2009–2012) in the lumbar spine in young adults of the Andhra Pradesh children and parents study (2003–2012)

	LS BMD							
	Model 1		Model 2		Model 3		Model 4	
	β		*β*		*β*		*β*	
	(95 % CI)	*p*	(95 % CI)	*p*	(95 % CI)	*p*	(95 % CI)	*p*
Adolescent BMI	0.012	<0.001	0.006	0.03	0.005	0.06	0.011	<0.001
	(0.008 to 0.016)		(0.001 to 0.011)		(0.00 to 0.01)		(0.007 to 0.015)	
Current BMI	0.008	<0.001	0.006	<0.001				
	(0.006 to 0.011)		(0.003 to 0.009)					
Current FMI	0.048	<0.001			−0.013	0.41		
	(0.024 to 0.072)				(−0.043 to 0.017)			
Current LMI	0.016	<0.001			0.014	<0.001		
	(0.012 to 0.02)				(0.008 to 0.02)			
Conditional BMI[a]	0.007	<0.001					0.006	<0.001
	(0.004 to 0.01)						(0.003 to 0.009)	

Conditional BMI was estimated from current BMI regressed on adolescent BMI

Model 1 is a base model examining association between BMD and each of four explanatory variables (adolescent BMI, adulthood BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003–2005 (adolescence), age at the second and third waves (W2/3) in 2009–2012 (current/adulthood), height at W1 (cm), and height at W2/W3 (cm)

Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BMD (g/cm^2) adjusting for current BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/W3

Model 3 examined association between adolescent BNU and adulthood BMD, adjusting for current FMI (kg/m^2), current LMI (kg/m^2), sex, age at W1, and age at W2/3, height at W1, and height at W2/W3

Model 4 examined association between adolescent BMI and adulthood BMD, adjusting for conditional BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/W3

LS lumbar spine, *BMI* body mass index (kg/m^2), *FMI* fat mass index (kg/m^2 , log-transformed), *LMI* lean mass index (kg/m^2)

Discussion

There were less underweight young adults from this transitional rural community compared to during adolescence. Although these young adults on average had low bone mass, there was no clear evidence for an association between bone mass during young adulthood and thinness during adolescence when adjusted for current fat and lean mass. There was stronger evidence for a positive association between bone mass and lean mass than fat mass in young adulthood.

Comparison with previous research

While there are a number of studies examining association between bone mass in later life and birthweight, there are relatively few studies focusing on the long-term effects of nutritional status during adolescence on adult bone mass [8]. The New Delhi Birth Cohort examined the associations between bone mass during adulthood (age 33–39) and early life height and weight [25]. This cohort also had low BMI (15.1 kg/m^2 for boys and 15.4 kg/m^2 for girls) at age 11, although by age 33–39, the average BMI had increased to above 25. The study found positive associations between femoral neck and lumbar spine BMC and aBMD during adulthood and BMI at age 11, but similarly to our findings, these associations were attenuated upon adjustment for adult BMI. They also assessed changes in BMI in infancy, childhood, and adolescence and found that the change in BMI during adolescence was most strongly associated with adulthood bone mass. In our previous analyses, we found no strong evidence for a positive association between areal bone mineral density as young adults and early life nutritional supplementation [26].

The study subjects in the APCAPS gained weight between late adolescence and young adulthood. The Penn State Young Women's Health Study compared healthy women who gained weight in late adolescence (17–22 years) to those who had stable weight [27]. Those who gained weight had higher aBMD and greater bone cross sectional area in proximal femur shaft. This result is in line with our findings where larger gain in BMI during young adulthood is associated with higher aBMDs. Of note, their study also found that the bone strength index decreased in women who became overweight during late adolescence and suggested a potential negative effect of excess weight gain during adolescence on bone strength.

Studies have suggested different patterns of associations between aBMD and fat and lean mass [14, 28–30]. In our study, there was no strong evidence for positive associations between aBMDs and fat mass. On the other hand, there was more consistent evidence for a positive association between aBMD and lean mass than fat mass, similarly to previous studies assessing relative contributions of fat and lean mass to bone mass accrual [28, 29].

It is important to note that aBMD in this study population was generally lower than values for bone mass values for young adults reported in a national DXA study in India [25]. The individuals with current BMI in the normal range had aBMD values closer to the national reference values than those with lower BMI values [25]. The current study found stronger evidence for an association between bone mass in young adulthood and current BMI than adolescent BMI. The weight gain during late adolescence may not have been sufficient for some of the study participants to achieve full catch-up growth. It is also possible that weight-bearing physical activity level was not high enough during late adolescence and young adulthood in this population.

Strengths and limitations

The main strength of this study is the availability of longitudinal data on height and weight, allowing the assessment of long-term effects of undernutrition during adolescence on bone mass in young adulthood. The study subjects experienced a unique circumstance where nutritional status of the study subjects improved greatly toward the end of the skeletal growth phase due to socioeconomic development in their villages. This setting allowed for an assessment of potential mitigation of the effects of undernutrition in early adolescence through improved nutritional status in late adolescence and young adulthood. Another strength of the study was the use of fat and lean mass from DXA scans to understand how different types of body mass may be distinctly associated with bone mass.

The study also had some limitations. The DXA measurements were not performed during adolescence, making it less clear whether and how bone mass improved, as weight, fat mass, and lean mass increased during adolescence. However, the association between body size and bone mass has been shown repeatedly in previous studies [14, 27], and therefore, it is reasonable to assume that the study subjects who were mostly underweight during adolescence also had lower z-scores for bone mass for their age than healthier adolescents. Finally, due to a lack of detailed nutritional and activity data from W1, we could not explore long-term effects of lifestyle risk factors during adolescence that may have been important for skeletal growth.

Conclusions

In healthy individuals, much of bone mass accrual occurs during adolescence. As socioeconomic development continues in low- and middle-income countries, many children and adolescents are experiencing the effects of nutritional transition. Our findings suggest that weight gain combined with improvement in lean mass in young adulthood may be able to help mitigate adverse effects of undernutrition during adolescence on bone mass in young adulthood.

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Conflicts of interests None.

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6.2 Unpublished data from Research Paper 3

This section shows analyses that were not included in Research Paper 3 but are relevant to this thesis work. The first three subsections extended upon the analyses from Research Paper 3. First, W1 characteristics of index children who did and did not participate W2/3 are compared (6.2.1). This comparison is followed by missing data analyses using a dataset with imputed values to update the results of regression models in Table 3 and 4 in Research Paper 3 (6.2.2). These regression models were then applied to bone area in hip and lumbar spine as well to assess association between adolescent thinness and bone size in adulthood (6.2.3). The last two subsections focused on body composition and bone outcomes in adolescents. In the fourth subsection, BMI values in adolescents at W1 (2003-2005) and W3 (2010-2012) were compared to Indian reference values to assess whether there have been any obvious shift in body size in adolescents in this community. Lastly, using cross-sectional data from a small group of adolescents who participated in W3, association between hip bone size and mass and body size and composition were analyzed to assess how thinness may associated with bone outcomes in adolescents.

6.2.1 W1 characteristics of W2/3 participants and non-participants

How did index children who participated in W2/3 differ from non-participants?

Table 6.1 compared W1 characteristics of index children who did and did not participate W2/3. The baseline population in this analysis is defined as those who had at least height measurement taken at W1 (n = 1111).

Table 6.1: Comparison of characteristics of index children who did and did not participate in the second or third wave of data collection (2009-2012) in the Andhra Pradesh Children and Parents Study.

	Participants		Non-Participants		
	n	mean(sd) or %	n	mean(sd) or %	p
age (years)	722	15.9(0.9)	385	15.9(0.9)	0.64
sex	220	30%	298	77%	<0.001
BMI (kg/m^2)	722	16.9(2.1)	389	17.9 (2.6)	<0.001

occupation	722		389		<0.001
student	590	82%	274	70%	
employed	95	13%	71	18%	
neither	37	5%	44	11%	

All children included in this analysis attended the first wave of data collection (2003-2005).
All data are based on W1 data.
Age was determined at the time of clinic visit in the first wave of data collection from W1. There were four people who had their anthropometric data taken but their clinic visit dates were not available and therefore their ages could not be determined.
p-values are based on unpaired t tests or 2 tests for heterogeneity with appropriate degrees of freedom.
There were two people who responded to be both studying and working. These people were included as students.

About 65% of index children who participated in W1 also underwent DXA measurement during W2/3. There were more female non-participants than participants in W2/3. Mean BMI was lower in W2/3 participants than non-participants, which may have biased the association between adulthood bone outcomes and adolescent body size. In the next subsection, the analyses from Research Paper 3 were updated with a dataset with imputed values for missing data.

6.2.2 Missing data

Did missing data affect the findings in Research Paper 3?

There were 390 index children who attended W1 but did not have any DXA scans without artifacts from W2/3. The regression analyses in Research Paper 3 were updated using a dataset with imputed values for missing data. The multivariable analyses in Table 6.3 and 6.4 used the same models as ones from Table 3 and 4 from Research Paper 3.

Method

Table 6.2 shows variables used in Research Paper 3 and counts of missing data. The baseline population is defined as those who had at least their height measurement taken during W1 (n = 1111). All variables with missing values were imputed through multiple imputation using R package *mice* (version 2.22) [219,220].

Table 6.2: Availability of variables used in multivariable regression models for association between bone outcomes (bone area, mineral content, mineral density) in adulthood and adolescent body size.

wave of data collection	variable	available	missing
All	sex	1111	0
	village	1111	0
W1 (2003-2005)	age*	1107	4
	height	1111	0
	weight	1111	0
	BMI	1111	0
W2/3(2009-2012)	age	745	366
	height	745	366
	weight	745	366
	BMI	745	366
	hip BA	713	398
	hip BMC	713	398
	hip BMD	713	398
	LS BA	718	393
	LS BMC	718	393
	LS BMD	718	393
W1 to W2/3	conditional BMI	745	366

W1: first wave of data collection; W2/3: second and third waves of data collection; BMI: body mass index; BA: bone area (cm2); BMC: bone mineral content; BMD: bone mineral density; LS: lumbar spine.
All numbers are counts of available and missing data.
BA, BMC, and BMD counts include only scans without artifacts. Scans with artifacts were counted as missing.
* There were four individuals whose W1 clinical visit dates were not available but their anthropometric data were available.

All missing data were imputed using multiple imputation (number of iteration = 5) with predictive mean matching (pmm) under the assumption of missing at random. For derived variables (BMI, FMI, LMI, conditional BMI), passive imputation and handling as 'just another variable' were considered. A previous study has shown that pmm generally improved upon bias and coverage in comparison to passive imputation and therefore, the current analysis used pmm to impute both observed and derived variables [221]. DXA values that were rejected for analyses due to major artifacts in Research Paper 3 were assigned NA and imputed in this dataset. After multiple imputation, all imputed data were checked for plausibility (*i.e.* no negative BMD values) and patterns of distributions in diagnostic graphs comparing observed

and imputed values. **Figure 6.1** shows example diagnostic graphs with actual and imputed values for hip and LS BMD, which suggested that all values were plausible and the general distributions of imputed values are similar to the observed values.

Results

The results with the imputed dataset were similar to the findings in Research Paper 3 (**Table 6.3 and 6.4**). Although a small positive association between LS BMD and adolescent BMI persisted after adjusting for FMI and LMI, the overall evidence on longitudinal effects of adolescent BMI on BMD remained weak in this analysis. Current body mass, especially lean mass, was again an important determinant of BMD and greater increase in BMI during late adolescence, adjusting for adolescent BMI, was associated with higher BMD in young adulthood.

Comments

Multiple imputation is generally considered to be a better method of handling missing data than listwise deletion. The current analysis updated the analyses in Research Paper 3 with a dataset with imputed values for missing data. The findings were materially unchanged from Research Paper 3 and confirmed the importance of current lean mass over adolescent thinness.

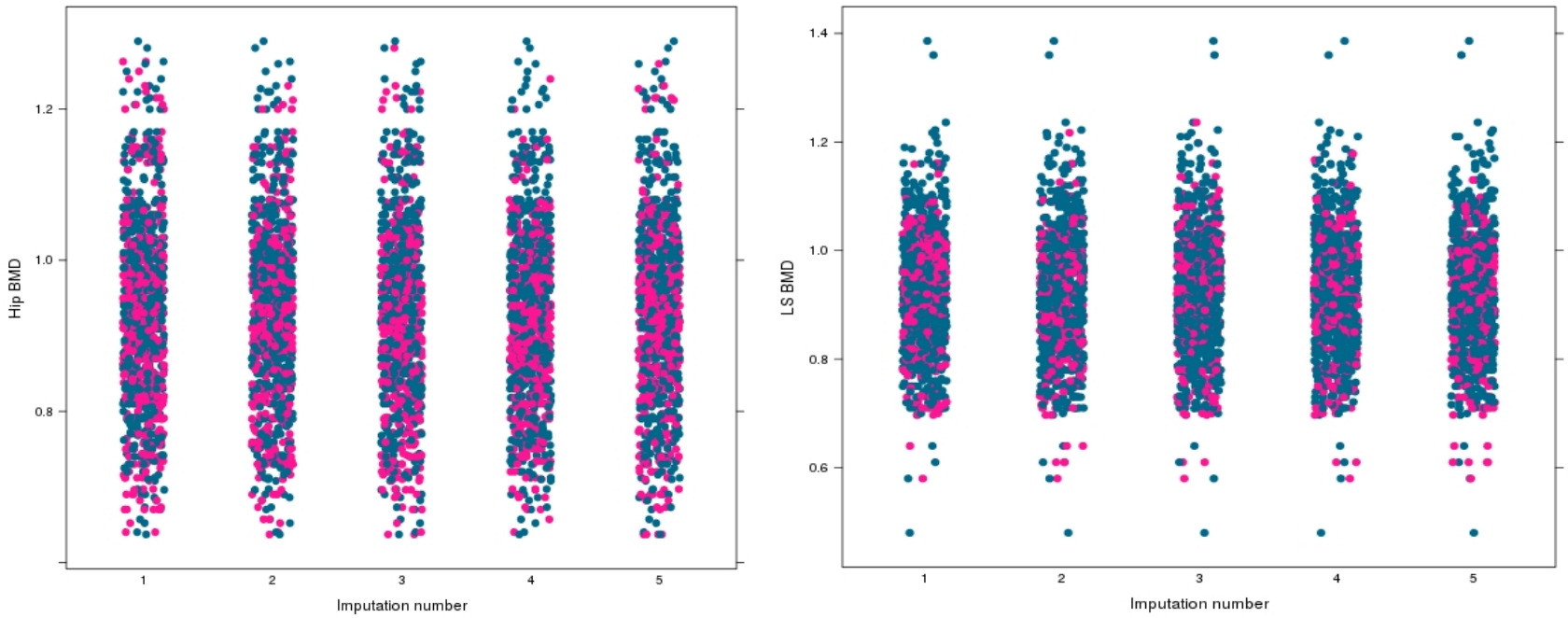


Figure 6.1: Diagnostic graphs of observed and imputed values for hip and lumbar spine bone mineral density in adulthood.

BMD: bone mineral density; LS: lumbar spine.
Observed values are in blue and imputed in pink.

Table 6.3: Multivariable models examining associations between current bone mineral density in hip and body mass index during adolescence in young adults of the Andhra Pradesh Children and Parents Study (2003-2012) using a dataset with imputed values for missing data.

	Model 1		Model 2		Model 3		Model 4	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
adolescent BMI	0.009 (0.004 to 0.013)	<0.001	0.001 (-0.003 to 0.005)	0.51	0.001 (-0.004 to 0.005)	0.79	0.009 (0.004 to 0.014)	0.002
current BMI	0.009 (0.005 to 0.014)	0.002	0.009 (0.004 to 0.013)	0.001				
current FMI	0.044 (0.014 to 0.073)	0.006			0.018 (-0.014 to 0.051)	0.25		
current LMI	0.019 (0.013 to 0.024)	<0.001			0.018 (0.012 to 0.023)	<0.001		
conditional BMI	0.009 (0.005 to 0.013)	<0.001					0.009 (0.004 to 0.013)	0.001

BMI = body mass index (kg/m^2); FMI = fat mass index (kg/m^2); LMI = lean mass index (kg/m^2).
Conditional BMI (change in BMI) uses residuals from a model where current BMI is regressed on adolescent BMI.
Model 1 is a base model examining association between hip BMD and each of four explanatory variables (adolescent BMI, adulthood BMI, change in BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003-2005 (adolescence), age at the second wave (W2) in 2009-2012 (current/adulthood).
Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BMD (g/cm^2) adjusting for sex, age at W1, age at W2, adulthood BMI, adolescent height, and adulthood height.
Model 3 examined association between adolescent BMI and adulthood BMD, adjusting for sex, age at W1, and age at W2, change in BMI, adolescent height, and adulthood height.
Model 4 examined association between adolescent BMI and adulthood BMD, adjusting for age at W1, age at W2, sex, current fat mass (kg, log-transformed), current lean mass (kg), adolescent height, and adulthood height.

Table 6.4: Multivariable models examining associations between current bone mineral density in lumbar spine and body mass index during adolescence in young adults of the Andhra Pradesh Children and Parents Study (2003-2012) using a dataset with imputed values for missing data.

	Model 1		Model 2		Model 3		Model 4	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
adolescent BMI	0.009 (0.005 to 0.011)	<0.001	0.003 (-0.001 to 0.007)	0.09	0.005 (0.001 to 0.009)	0.01	0.009 (0.006 to 0.012)	<0.001
current BMI	0.008 (0.006 to 0.01)	<0.001	0.007 (0.004 to 0.009)	<0.001				
current FMI	0.028 (0.005 to 0.052)	0.017			0.002 (-0.025 to 0.03)	0.87		
current LMI	0.012 (0.007 to 0.016)	<0.001			0.009 (0.004 to 0.014)	0.001		
conditional BMI	0.006 (0.004 to 0.009)	<0.001					0.007 (0.004 to 0.009)	<0.001

BMI = body mass index (kg/m^2); FMI = fat mass index (kg/m^2); LMI = lean mass index (kg/m^2).
Conditional BMI (change in BMI) uses residuals from a model where current BMI is regressed on adolescent BMI.
Model 1 is a base model examining association between LS BMD and each of four explanatory variables (adolescent BMI, adulthood BMI, change in BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003-2005 (adolescence), age at the second wave (W2) in 2009-2012 (current/adulthood).
Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BMD (g/cm^2) adjusting for sex, age at W1, age at W2, adulthood BMI, adolescent height, and adulthood height.
Model 3 examined association between adolescent BMI and adulthood BMD, adjusting for sex, age at W1, and age at W2, change in BMI, adolescent height, and adulthood height.
Model 4 examined association between adolescent BMI and adulthood BMD, adjusting for age at W1, age at W2, sex, current fat mass (kg, log-

transformed), current lean mass (kg), adolescent height, and adulthood height.

6.2.3 Adolescent thinness and adulthood bone size

Is thinness during adolescence associated with lower bone size?

In Research Paper 3, stronger evidence was found for positive association between hip and LS bone mass and current lean mass than adolescent BMI. Bone density is a key determinant of bone strength and risk of fracture; however, bone size also contributes to bone strength. This subsection extends upon the analyses from Research Paper 3 and section 6.2.2 and apply the same regression models to bone area of hip and lumbar spine. The analyses in this subsection used the same imputed dataset to account for missing data as described in the previous subsection (section 6.2.2*)*.

Results

There was no clear evidence of association between adulthood BA and adolescent BMI in this analysis (**Table 6.5 and 6.6**). Increase in BMI during late adolescence and young adulthood (conditional BMI) was positively associated with adulthood LS BA after adjusting for adolescent BMI. There was some evidence for a negative association between BA and current FMI while current LMI was positively associated with both hip and LS BA.

Comments

Adolescent thinness was not associated with hip and lumbar spine bone area in this young adult population in rural India. There was stronger evidence for association between LS BA and change in body mass during late adolescence than hip BA, which may be due to regional differences in speed and timing of bone growth [222]. Overall, there was stronger evidence for association between BMD and body size than BA.

Table 6.5: Multivariable models for association between bone area in hip and adolescent body mass index in the Andhra Pradesh Children and Parents Study (2009-2012) using a dataset with imputed values for missing data.

	Model 1		Model 2		Model 3		Model 4	
	β (95% CI)	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**
adolescent BMI	0.02 (-0.17 to 0.22)	0.81	-0.04 (-0.22 to 0.14)	0.66	-0.09 (-0.31 to 0.13)	0.41	0.02 (-0.18 to 0.22)	0.82
current BMI	0.06 (-0.11 to 0.22)	0.47	0.07 (-0.09 to 0.24)	<0.001				
current FMI	-0.85 (-2.27 to 0.58)	0.22			-1.17 (-2.46 to 0.19)	0.07		
current LMI	0.36 (0.2 to 0.51)	<0.001			0.44 (0.23 to 0.65)	<0.001		
conditional BMI	0.07 (-0.09 to 0.24)	0.36					0.07 (-0.09 to 0.24)	0.36

BMI: body mass index (kg/m^2); FMI: fat mass index (kg/m^2, log-transformed); LMI = lean mass index (kg/m^2)
Conditional BMI was estimated from current BMI regressed on adolescent BMI
Model 1 is a base model examining association between BA (cm^2) in hip and each of four explanatory variables (adolescent BMI, adulthood BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003-2005 (adolescence), age at the second and third waves (W2/3) in 2009-2012 (current/adulthood), height at W1 (cm), and height at W2/3 (cm).
Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BA adjusting for current BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/3.
Model 3 examined association between adolescent BMI and adulthood BA, adjusting for current FMI (kg/m^2), current LMI (kg/m^2), sex, age at W1, and age at W2/3, height at W1, and height at W2/3.
Model 4 examined association between adolescent BMI and adulthood BA, adjusting for conditional BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/3.

Table 6.6: Multivariable models for association between bone area in lumbar spine and adolescent body mass index in the Andhra Pradesh Children and Parents Study (2009-2012) using a dataset with imputed values for missing data.

	Model 1		Model 2		Model 3		Model 4	
	β (95% CI)	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**	**β (95% CI)**	**p**
adolescent BMI	0.23 (-0.05 to 0.5)	0.01	0.052 (-0.28 to 0.38)	0.74	0.15 (0.25 to 0.55)	0.42	0.23 (-0.04 to 0.5)	0.009
current BMI	0.23 (0.1 to 0.36)	0.001	0.21 (0.04 to 0.37)	0.014				
current FMI	-1.77 (-3.05 to -0.5)	0.007			-3.0 (-4.23 to -1.79)	<0.001		
current LMI	0.59 (0.33 to 0.85)	<0.001			0.48 (0.28 to 0.96)	0.002		
conditional BMI	0.20 (0.04 to 0.37)	0.013					0.21 (0.04 to 0.37)	0.014

BMI: body mass index (kg/m^2); FMI: fat mass index (kg/m^2, log-transformed); LMI = lean mass index (kg/m^2)
Conditional BMI was estimated from current BMI regressed on adolescent BMI
Model 1 is a base model examining association between BA (cm^2) in lumbar spine and each of four explanatory variables (adolescent BMI, adulthood BMI, adulthood fat mass, and adulthood lean mass), adjusting for sex, age at the first wave of data collection (W1) in 2003-2005 (adolescence), age at the second and third waves (W2/3) in 2009-2012 (current/adulthood), height at W1 (cm), and height at W2/3 (cm).
Model 2 examined association between adolescent BMI (kg/cm^2) and adulthood BA adjusting for current BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/3.
Model 3 examined association between adolescent BMI and adulthood BA, adjusting for current FMI (kg/m^2), current LMI (kg/m^2), sex, age at W1, and age at W2/3, height at W1, and height at W2/3.
Model 4 examined association between adolescent BMI and adulthood BA, adjusting for conditional BMI, sex, age at W1, age at W2/3, height at W1, and height at W2/3.

6.2.4 Body mass index in adolescents in 2003-2005 and in 2010-2012

Has nutrition transition been occurring in this community over the past decade?

One way to describe nutrition transition is to examine population-level shift in body size [6]. In this subsection, adolescent BMI from the W3 cohort (2010-2012) were compared to adolescent BMI in the W1 cohort (2003-2005) and BMI reference values estimated from healthy Indian children and adolescents in order to examine longitudinal shift in body size at the community level. This descriptive analysis was intended to assess any obvious temporal trend in adolescent body size in this community as the number of adolescents in W3 was small - the small sample size does not allow for reliable statistical comparison as we would need over 120 adolescents in each group to detect a BMI difference of 0.5 with 90% confidence and 80% power.

Results

The BMI values were similarly low in 2003-2005 and 2010-2012 in comparison to the reference values for Indian adolescents (**Table 6.7**) [223]. There may be a trend for a slight increase in median BMI values in adolescents males between W1 and W3. Due to small sample sizes, the interpretation of these values is limited.

Comments

Although longitudinal analyses of index children in Research Paper 3 showed that prevalence of underweight decreased between adolescence and young adulthood, underweight is still prevalent among adolescents in this community. Larger sample size at future data collection can help detect nutrition transition in adolescents from this community.

Table 6.7: Median height, weight, and body mass index by age groups for adolescents from the APCAPS community in 2003-2005 and 2010-2012 in comparison to the Indian reference values.

		2003-2005				2010-2012				Reference		
	age	n	height	weight	BMI	n	height	weight	BMI	height	weight	BMI
female	14	47	150.4	38.2	16.7	28	148.1	37.0	16.8	154.7	46.4	18.7
	15	114	151.6	40.2	17.2	38	151.2	41.6	17.7	156.1	48.4	19.3
	16	222	151.3	40.5	17.6	57	150.6	39.3	17.8	156.9	49.7	19.9
	17	117	152.8	42.0	18.1	72	152.3	40.3	17.4	157.4	50.9	20.5
male	14	40	150.4	35.6	15.1	30	150.7	35.7	15.5	159.9	48.2	19.4
	15	129	154.7	38.9	16.0	46	159.3	39.1	16.4	164.5	53.1	19.9
	16	248	160.8	43.2	16.4	58	162.6	45.4	16.9	168.1	56.8	20.3
	17	162	161.9	45.6	17.1	92	165.4	47.6	17.4	171.0	59.5	20.6

W1: first wave of data collection (2003-2005); W3: third wave of data collection (2010-2012); BMI: body mass index (kg/m^2)
The reference values for adolescent height, weight, and BMI are based on population-level data in India [223].

6.2.5 Association between hip BA and BMD and BMI in adolescents

How is body size associated with bone size and bone mass in adolescents?

There was no DXA measurement during W1. As a result, we can only speculate how bone size and mass in index children changed during nutrition transition in late adolescence and young adulthood. 282 adolescent siblings (12.5 to 17.4 years old) of the index children underwent DXA measurement in W3. This section analyzed these cross-sectional data to examine association between hip bone size and mass and body mass and composition in adolescents in order to understand how thinness during adolescence in this community may be associated with bone outcomes.

Table 6.8 shows body composition characteristics of adolescents who attended W3. Female and male adolescents had similar mean BMIs but fat mass index was much higher in females than males. **Table 6.9** shows mean hip BA, BMC, and BMD in adolescents. Normative reference data for bone mass in total hip are not available for Indian adolescents; however, the means for total hip BMD for adolescents in APCAPS were consistently lower than the reference values for femoral neck (which constitutes part of total hip), after standardizing the APCAPS and reference values to account for differences between Hologic and Lunar DXA machines [224–226]. This suggests that these adolescents are likely to have lower hip bone mass for age than healthy Indian adolescents. Larger sample size is needed for statistical comparison.

Table 6.10 shows association between bone outcomes and body mass and composition in adolescents. Low BMI was associated with lower hip BA, BMC, and BMD. Fat mass may be negatively associated with BA and BMC while greater lean mass was consistently associated with higher bone size and mass. Although this result does not confirm how BMI was associated with BA and BMD in adolescents in W1, it provides some support to the assumption that thinness during adolescence was associated with lower bone size and mass in the index children in 2003-2005.

Table 6.8: Characteristics of body composition in adolescent participants (13-17 years old) of the third wave of data collection (2010-2012) in the Andhra Pradesh Children and Parents Study.

	Female (n = 120)	Male (n = 162)
Body mass index (kg/m^2)	17.73(2.65)	17.22(2.57)
Fat mass (kg)	11.19(3.79)	7.36(3.27)
Fat mass index (kg/m^2)	4.94(1.57)	2.86(1.22)
Lean mass (kg)	28.18(4.26)	37.14(7.47)
Lean mass index (kg/m^2)	12.44(1.34)	14.36(1.83)

All values are mean (standard deviation).
Fat (or lean) mass index = fat (or lean) mass (kg) / height^2 (m^2).

Table 6.9: Mean hip bone area, bone mineral content, and bone mineral density of adolescent participants (13-17 years old) of the third wave of data collection in the Andhra Pradesh Children and Parents Study (APCAPS).

Age (n)	n	BA (cm^2)	BMC (g)	BMD (g/ cm^2)
Female				
13	8	23.52 (2.53)	18.06 (3.81)	0.761 (0.104)
14	19	25.35 (3.14)	19.3 (3.79)	0.759 (0.094)
15	23	26.8 (2.21)	21.31 (3.08)	0.796 (0.103)
16	30	26.43 (2.89)	21.23 (4.03)	0.804 (0.117)
17	40	27.17 (2.46)	22.56 (2.89)	0.831 (0.086)
All	120	26.38 (2.78)	21.17(3.65)	0.802 (0.102)
Male				
13	12	26.14 (5.05)	20.63 (5.55)	0.78 (0.088)
14	20	28.41 (3.99)	22.43 (4.7)	0.786 (0.087)
15	30	30.85 (3.6)	24.81 (6.42)	0.795 (0.145)
16	41	33.34 (3.08)	28.28 (4.63)	0.846 (0.102)
17	59	34.73 (3.67)	31.25 (5.99)	0.898 (0.134)
All	162	32.24 (4.55)	27.43(6.61)	0.843 (0.13)

All values are mean (standard deviation)
BA: bone area; BMC: bone mineral content; BMD: bone mineral density; FN: femoral neck.

Table 6.10: Multivariable models examining association between bone area, mineral content, and mineral density in hip and body mass index in adolescents (aged 13-17 years old) in the third wave (W3: 2010-2012) of data collection in the Andhra Pradesh Children and Parents Study.

	BA				BMC				BMD			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Height	0.31 (0.26 to 0.35)	<0.001	0.13 (0.07 to 0.2)	<0.001	0.35 (0.29 to 0.42)	<0.001	-0.00 (-0.09 to 0.08)	0.94	0.00 (0 to 0.01)	<0.001	-0.00 (-0.01 to 0)	0.02
BMI	0.18 (0.06 to 0.31)	0.005			0.76 (0.59 to 0.93)	<0.001			0.019 (0.02 to 0.02)	<0.001		
FM			-2.74 (-3.83 to -1.65)	<0.001			-2.71 (-4.19 to -1.23)	<0.001			-0.008 (-0.05 to 0.04)	0.69
LM			0.36 (0.27 to 0.45)	<0.001			0.75 (0.63 to 0.87)	<0.001			0.013 (0.01 to 0.02)	<0.001

Sample size = 282 (female = 120; male = 162)
BA: bone area (cm^2); BMC: bone mineral content (g); BMD: bone mineral density (g/cm^2); CI: confidence interval; BMI: body mass index (kg/m^2); FM: fat mass (kg, log-transformed); LM: lean mass (kg)
All models adjusted for household-level clustering in multilevel models.
Model 1: BA or BMC or BMD in hip ~ age (years) + sex + height (cm) + BMI
Model 2: BA or BMC or BMD in hip ~ age + sex + height + FM (log-transformed) + LM

Chapter 7: Discussion

The current work aimed to study lifecourse determinants of bone mass accrual in a rural Indian population which has been experiencing urbanization and nutritional transition over the past decade. The first research paper examined the prevalence of osteopenia and osteoporosis as well as the association between hip BMD and body composition in the overall adult population in the study community (Chapter 4). Two longitudinal analyses were then conducted to assess long-term effects of early life and adolescent undernutrition and body composition on bone mass in young adulthood (Chapter 5 and 6). This chapter briefly summarizes the key findings from each analysis and discusses the overall findings, strengths, and limitations of the study beyond what has been discussed in the previous chapters. The chapter ends with potential areas of future research and public health implications for bone health in transitional rural communities in low and middle countries.

7.1. Summary of findings from research papers

7.1.1 Bone mass in a rural Indian population

What was previously known:

In HICs, bone mineral density may be higher in rural areas while association between BMD and urbanicity may be more mixed in LMICs, possibly due to high prevalence of undernutrition in some rural areas of LMIC. Although the prevalence of undernutrition has been declining through nutrition transition, over a third of rural Indians are still underweight [227]. While there is clear evidence of negative association between hip BMD and low body mass, previous studies have shown variable results on association between bone mass and body composition (lean and fat mass) [136,228–230]. In lean Indian populations, both fat and lean mass may be equally important determinants of bone mass.

What this study adds:

Over 50% of female and 30% of male participants aged above 50 years old had osteopenia or osteoporosis in this community. Peak bone mass was low in this lean rural population (hip BMD in 20-29 year old female: 0.84±0.1; male: 0.95±0.11 g/cm^2) in comparison to the reference values from a healthy Indian population in the

same age group (female: 0.90±0.11; male: 0.99±0.13) [48]. There was clearer evidence of association between hip BMD and lean mass than fat mass in this lean population. A small positive association between hip BMD and fat mass remained only in females after adjustment for lean mass.

7.1.2 Early life protein-energy supplementation

What was previously known:

Early life undernutrition is associated with poor skeletal growth [107,231,232]. A theory of developmental origins of osteoporosis suggests that fetal undernutrition may cause intrauterine programming of skeletal size and shape, which may increase risk of osteoporosis in later life [233]. Postnatal protein energy malnutrition also leads to poor skeletal growth and is a major health issue in India [234]. Data from the first wave of data collection in APCAPS (2003-2005) showed that modest protein-energy supplementation in early life was associated with slightly greater height during early adolescence [189]. It is unknown whether this modest protein-energy supplementation in early life was associated with greater bone mass in young adulthood.

What this study adds:

There was no clear evidence for a long-term positive effect of prenatal and infant exposure to modest protein-energy supplementation on bone mass accrual in this rural community. Greater lean mass in young adulthood was associated with higher hip and LS BMD. Hours spent on weight-bearing physical activity was positively associated with hip, LS, and whole-body BMD in males but not in females. These findings suggest that overall nutritional improvement in late adolescence and young adulthood may be a more important determinant of bone mass in adulthood than modest protein-energy supplementation in early life.

7.1.3 Undernutrition and nutrition transition in adolescence

What was previously known:

90% of peak bone mass is thought to accrue by age 20 in healthy populations [127,235,236]. Early adolescence is a period of peak bone mass accrual [222]. Several studies on adolescents with anorexia nervosa disorders showed partial catch-up accrual of bone mass in adolescents who successfully completed treatment

[158,162,163]. It is not clear whether nutrition transition in late adolescence and young adulthood leads to significant catch-up accrual of bone mass in a population who has experienced undernutrition until mid adolescence.

What this study adds:

When adjusted for current BMI, there was no clear evidence for association of hip and LS BMD in young adulthood with adolescent BMI. Controlling for adolescent BMI, greater increase in BMI during late adolescence was associated with higher BMD in young adulthood. These findings suggest that nutrition transition in late adolescence was beneficial for bone mass accrual in this formerly undernourished population. However, the suboptimal bone mass in these young adults in comparison to a healthy young Indian population suggests that weight gain during adolescence was not sufficient to achieve optimal bone mass.

7.2 Overall review of the study

7.2.1 Overall findings

This thesis work used a lifecourse approach to provide evidence on the impact of nutrition transition on bone development in an LMIC setting. The findings from this thesis suggest that neither modest protein-energy supplementation in early life nor the recent nutrition transition was sufficient to achieve optimal bone mass accrual in these young Indians. The strong association between bone mass in young adulthood and increase in BMI during adolescence suggests that nutrition transition in adolescence and young adulthood was beneficial for bone mass accrual even if optimal bone mass was not attained. Clear evidence of association between bone and lean mass and weight-bearing physical activity points to the importance of physical activity even for lean populations.

Comparison to previous cohort studies in India

In comparison to the other two DXA cohort studies in India, APCAPS differed in two main aspects. First, we assessed bone mass in young adults while children and adults in mid 30s were examined in PMNS and NDBC respectively. Bone mass in these young adults in APCAPS is likely to be near the peak values but not yet at the stage of age-related loss. Secondly, index children in APCAPS live in an urbanizing rural

community and have experienced a combination of early life undernutrition and improved nutrition (estimated by change in BMI) in late adolescence and young adulthood. The study population in NDBC was urban dwellers who were relatively well-off although interestingly, they were short and thin for their ages during early adolescence like the adolescents in the APCAPS community [13,121]. At the time of bone mass measurements, the participants of NDBC had high average BMI (mean age 36 years old; BMI >25 kg/m^2). Young adults in APCAPS were still lean (mean age 21 years old; BMI = 20 kg/m^2) but the prevalence of underweight decreased from 56% to 38% between adolescence (mean age 15 years old) and young adulthood. Bone mass in these young adults should be close to their peak but their LS BMD (20-29 year old female 0.84±0.1; male: 0.95±0.11 g/cm^2) were lower, especially in females, than the NDBC population (female: 0.99±0.12; male: 0.98±0.12 g/cm^2) [13].

This comparison raises two questions. First, although the participants in NDBC were short and thin during early adolescence like index children in APCAPS, their LS BMD in adulthood were comparable to the reference BMD values in a healthy Indian population. Since the environment and SES of NDBC participants were different from APCAPS, there may be factors other than body size in adolescence that contributed to better peak bone mass attainment in the NDBC population. It may also be possible that index children in APCAPS will continue accruing bone mass until their mid 30s although if we assume that 90% of peak bone mass is accrued by the third decade as in healthy populations [126], it seems unlikely that females in the APCAPS community would be able to catch up to the same level as the NDBC population. There may also have been an increase in BMI at younger ages in the NDBC population, allowing an earlier start to catch-up accrual of bone mass, or pre-adolescent nutritional status in the NDBC population may have been better than in the index children in APCAPS. It is also unclear why BMD values are better in male participants in APCAPS despite similar average BMIs in females and males in adulthood. One potential reason for this difference is greater lean mass and physical activity level during adolescence in males. This is plausible as, during a focus group discussion on the built environment, adolescents in the APCAPS community mentioned limited space for adolescent females to engage in physical activity.

7.2.2 Strengths and limitations

Anthropometric data from 2003-2005 and 2009-2010 allowed analyses of the timing and extent of nutrition transition in the young population, which generally corresponded to the overall temporal change in urbanicity in the APCAPS study site. While there are numerous studies examining the effects of early life nutrition on linear growth or rickets in LMICs, there are fewer studies evaluating the effects on bone size and mass. There are also more studies examining bone mass in older populations as clinical manifestation of osteoporosis appear more commonly among the elderly. However, PBM is one of the key determinants of bone health in later life [127]. This study was able to provide insights into bone mass accrual in a young population.

However, APCAPS started as a cohort study to examine cardiovascular health and therefore, questionnaires in W1/2/3 did not include questions related to potential burden of poor skeletal growth such as osteoporotic fracture and economic impact on household-level income. We were also unable to ascertain longitudinal change in bone mass during adolescence in index children as there was no DXA measurement in W1 although previous studies have consistently shown a negative association between bone mass and low BMI [126,127,231,237]. Additionally, analysis on a small sample of adolescents whose bone mass was measured during W3 showed a positive association between bone mass and body size (Table 6.10), which suggests that it is likely that underweight during adolescence was also associated with lower bone mass in W1.

Secondly, while data from W1/2/3 were collected prospectively, information before adolescence was obtained retrospectively. Matching of birth weight records to participants in W1 was previously attempted but only a small portion of the participants could be reliably linked and therefore, association between bone mass in adulthood and birth weight could not be examined [208]. The effects of maternal diet and health during pregnancy (beyond ICDS nutritional supplementation status) could not be assessed in this work due to lack of these data, which would be helpful for understanding what other nutritional interventions may be most effective in improving bone mass accrual in early life.

Although W2/3 used the same validated FFQ, the dietary data were very limited in W1 and therefore dietary transition in this community could not be described in this thesis. While weight gain may be achieved from intakes of various macronutrients, healthy skeletal growth requires many other nutrients including calcium and vitamin D. Since the index children in this study still exhibited suboptimal bone mass in young adulthood, there is a need to assess whether nutrition transition occurs unevenly among different nutrients (*i.e.* increase in fat intake, little change in calcium intake) and whether supplementation of micronutrients during adolescence can augment the benefit of nutrition transition in urbanizing rural areas in LMICs.

Potential bias

Selection bias and measurement bias may have influenced the association between bone mass in adulthood and early life undernutrition. For the longitudinal analyses, participants in W2/3 were compared to non-participants in terms of W1 characteristics. This comparison showed that these two groups differed in some of these characteristics, most notably in low participation by females. This may be due to emigration for marriage among females, which is common in this rural area. There is a possibility that females who emigrated for marriage may be from households with different lifestyles or income levels, which could potentially affect the levels of bone mass. However, degrees of differences between participants and non-participants were similar between the intervention and control areas, which reduces the impact of selection bias on association between bone mass in adulthood and early life supplementation.

Two main exposure variables in this thesis work were HNT intervention status and adolescent BMI. Classification of index children who received intervention or control was not biased as it was based on places of residence. There is a possibility that some index children in the intervention area received only partial or no food supplementation because the mothers shared the supplementation food with other family members, did not consume it during pregnancy, or did not give it to children. This may have attenuated the effect of early life supplementation. However, this analysis allows for a more realistic evaluation of the outcomes of this intervention programme in rural India as these behaviors are likely to exist in real-world settings.

In terms of adolescent BMI, anthropometric measurements were done on well-calibrated machines and it is unlikely that the systemic measurement error in adolescent BMI grossly distorted the true association between adolescent undernutrition and adulthood bone mass [208]. Coefficients of variation for height and weight in W1 could not be determined to estimate the level of random measurement errors as only one measurement was taken.

In terms of the outcome measurement, DXA is currently considered to be the "gold standard" for diagnosis of osteoporosis [238]. However, there are a number of factors that could influence the values including obesity and scan positioning [238,239]. Obesity was still fairly rare in this population, especially among young individuals (1.3% for 20-29 year old participants) and therefore, it is unlikely that this error had a large impact on the findings. Careful examination of each DXA scan was conducted and scans with poor positioning were removed from analyses.

7.3 Future areas of research

As the APCAPS community continues to become urbanized, this thesis work can serve as a baseline study to describe how nutrition transition in rural India affects bone health. Currently, increase in body size from nutrition transition seems to be improving bone mass accrual in the young population in this community. However, as the systematic review in Chapter 2 showed, urbanicity may be negatively associated with bone mass in higher income countries. In LMICs, nutrition transition to the fourth stage of Popkin's model (increase in NCD risk factors: Figure 1.1) seems to be occurring at an earlier stage of socioeconomic development [240]. There is a need to develop strategies to simultaneously take advantage of and prevent detrimental effects of nutrition transition in order to change the current trajectory of epidemiological transition in LMIC.

In understanding how nutrition transition affects bone health in rural communities in LMICs, there are three domains of research questions to explore: epidemiological transition of bone health, dietary and nutrition transition, and environmental change due to urbanization. **Figure 7.1** summarizes available data from APCAPS to highlight which additional data are needed to understand the effects and underlying mechanisms of nutrition transition in LMICs.

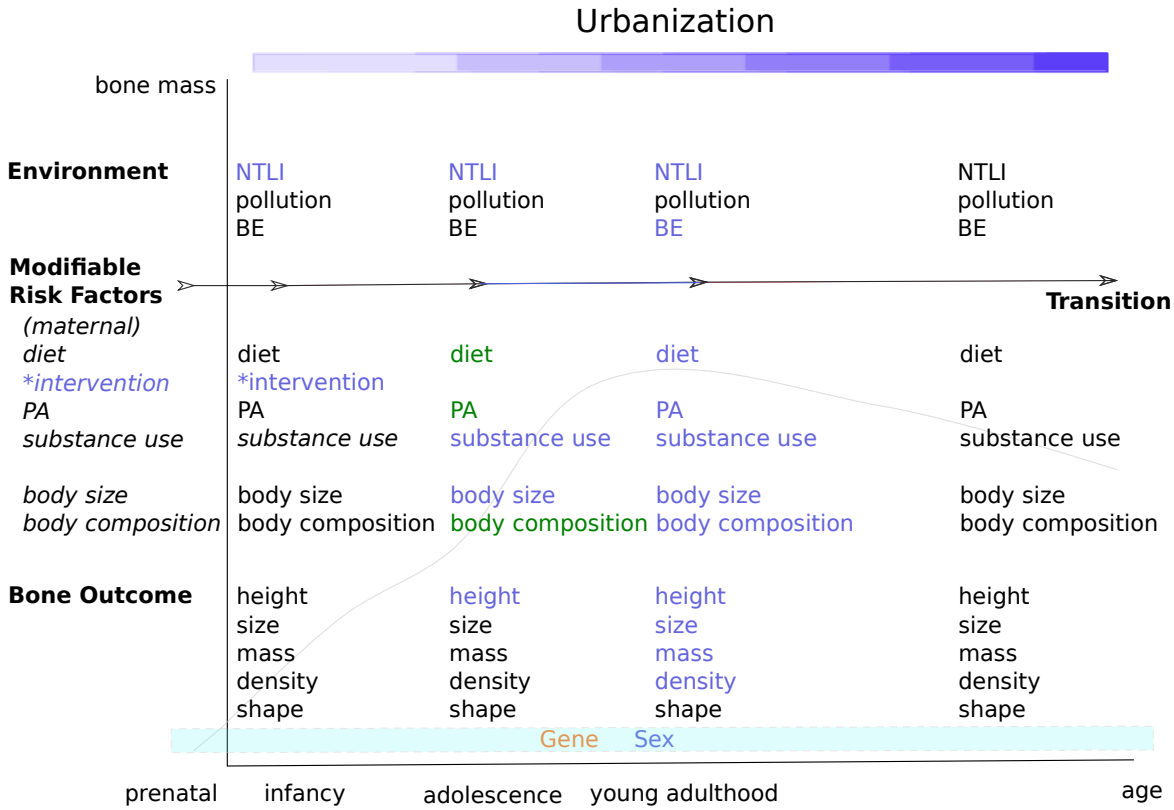


Figure 7.1: Parameters for studying the effects of nutrition transition on bone development and osteoporosis

NTLI: night-time light intensity; BE: built environment; PA: physical activity
The parameters in blue are available in APCAPS. The parameters in green are available but not measured on same scales as the following data collection. Genes can be studied using W3 blood samples but they have not been examined yet. The parameters in italics represent risk factors that are all or predominantly secondary exposure.

Longitudinal data on bone outcomes, modifiable risk factors, and environment can not only describe how transition occurs as rural areas become urbanized but also allow analyses of how these three transitions are associated with one another. Currently, APCAPS has data on overall environmental transition (urbanization) and nutrition transition in terms of body size. Building upon longitudinal and cross-sectional findings from this study, several topics may be further explored in future studies, with the goal of developing interventions at individual, familial, and community levels in urbanizing rural India:

Follow-up study on bone size and mass

Follow-up measurement of bone mass will allow analyses of within-person change in bone mass accrual in this rural population (**FIGURE 7.2**) as well as population-level

changes in bone mass **(Figure 7.3)** in this transitional rural community.

Figure 7.2: Nutrition transition and potential within-person change in peak bone mass accrual.

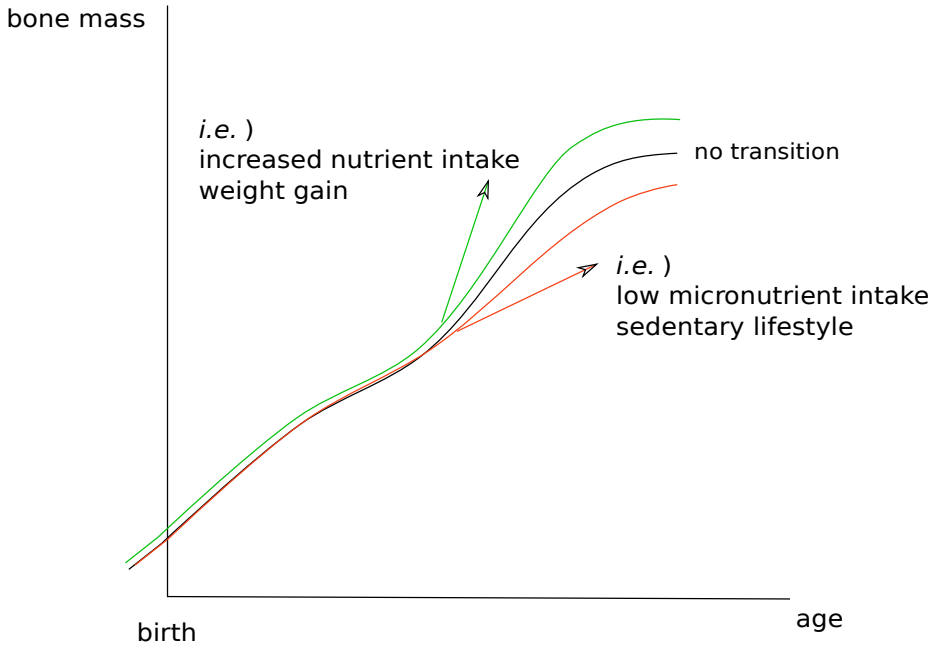
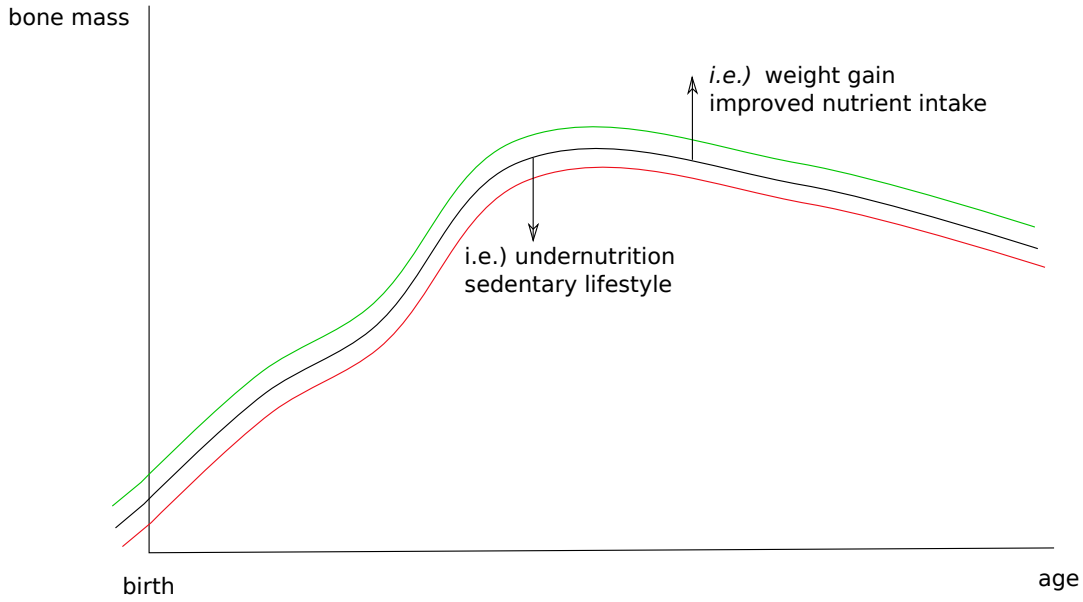


Figure 7.3: Nutrition transition and potential population-level shift in bone mass accrual.



The majority of the participants in W2/3 were adults but there were some children and adolescents who underwent bone mass measurements in 2009-2012. Though we lack DXA measurements from W1, these data from W2/3 could serve as baseline data to examine longitudinal change in bone mass accrual in adolescence and young adulthood (Figure 7.2). Additionally, DXA data can estimate longitudinal change in body composition in adolescents during nutrition transition.

Follow-up study on diet and nutrition

While the analysis of body size addresses one aspect of nutrition transition, diet and physical activity data from the next follow-up study can provide further insights into how people's lifestyles are changing and what types of interventions may be effective in transitional rural communities in India. Along with follow-up data on bone mass, association among dietary transition, nutrition transition, and bone mass accrual can also be explored.

Assessment of burden of osteoporosis in this community.

It is currently not well-understood how osteoporosis affects rural Indian populations. For instance, the prevalence of osteopenia is fairly high among young adults but it is unclear if this leads to osteoporotic fracture at younger ages. The clinical and economic burden of osteoporosis may be assessed in future studies by collecting data on prevalence of osteoporotic fracture, premature death, disability, comorbidity, and medical costs related to osteoporosis, and impact on productivity and household income.

Survey of built environment in the APCAPS community.

Collaboration between city planners and public health specialists can help build healthier cities that can tackle complex profiles of malnutrition and diseases. Development of a new survey on the built environment for the APCAPS community is underway. Longitudinal analyses of the built environment in urbanizing rural India can provide better understanding of the process of urbanization, environmental determinants of bone mass accrual, impact on dietary and nutrition transition, and potential environmental interventions.

7.4 Concluding remarks

In many low and middle income countries, the problem of undernutrition is being replaced by NCD risk factors as the countries undergo socioeconomic development. While evidence on causes, process, and impact of urbanization and nutrition transition has been accumulating in HICs, there is much more work to be done in LMICs. The lifecourse framework for the impact of nutrition transition on bone health is especially important in LMIC settings, where significant transition may occur at various points in the lifecourse of bone. The current study provides insights into the impact of the combined effect of early life undernutrition and nutrition transition during late adolescence and young adulthood on bone mass accrual. Depending on the timing of nutrition transition, we may need to implement distinct strategies to mitigate negative effects of early life exposure to undernutrition and urbanized lifestyles in later life in order to promote healthy aging in transitional rural communities in LMICs. Continuation of prospective data collection on determinants of bone health in this community can augment efforts to promote healthy growth and development in younger populations from transitional rural communities.

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APPENDIX A: PRISMA statement for the systematic review (Chapter 2)

PRISMA 2009 Checklist

Supporting Information Checklist S3: PRISMA Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4/OSM
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4/OSM
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4/5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7-12
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figure 2-3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	13
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

APPENDIX B: Summary of search strategy of the systematic review (Chapter 2)

Supporting Information Table S1: Summary of search strategy

Medline	EMBASE	Global Health
1. exp Bone Density/ or exp "Bone and Bones"/ or exp "Osteoporosis"/ 2. (osteoporosis or osteopenia or bone mass or bone mineral density or bone mineral content or BMD or BMC).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] 3. 1 or 2 4. exp Urban Health/ or exp Urban Population/ 5. exp Urbanization/ 6. (urban$ or city or non-rural or nonrural).mp. [mp=title, abstract, original title, name of substance word,	1. exp bone density/ 2. exp bone/ or exp bone development/ or exp osteoporosis/ 3. (osteoporosis or osteopenia or bone mass or bone mineral density or bone mineral content or BMD or BMC or bone density).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword] 4. exp urban rural difference/ or exp urban population/ 5. exp urbanization/ 6. (urban$ or city or non-rural or nonrural).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device	1. exp bones/ or exp osteoporosis/ 2. exp bone density/ 3. (osteoporosis or osteopenia or bone mass or bone mineral density or bone mineral content or BMD or BMC).mp. [mp=abstract, title, original title, broad terms, heading words] 4. 1 or 2 or 3 5. exp rural urban relations/ or exp rural urban migration/ or exp urban population/ 6. urbanization.sh. 7. (urban$ or city or non?rural).mp. [mp=abstract, title, original title, broad terms, heading words] 8. 5 or 6 or 7 9. exp rural health/ or exp urban rural migration/ or exp rural depopulation/

subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
7. 4 or 5 or 6
8. exp Rural Health/ or exp Rural Population/
9. rural$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
10. 8 or 9
11. (urban-rural or rural-urban).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
12. bone.mp. [mp=title, abstract, subject headings, heading word, drug manufacturer, drug manufacturer, device trade name, keyword]
7. 4 or 5 or 6
8. exp rural population/
9. rural$.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
10. 8 or 9
11. (urban-rural or rural-urban).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
12. 7 and 10
13. 11 or 12
14. 1 or 2 or 3
15. 13 and 14
16. bone.mp. [mp=title, abstract, subject headings, heading word, drug manufacturer, drug manufacturer, device trade name, keyword]
or rural communities/ or exp rural population/
10. rural$.mp. [mp=abstract, title, original title, broad terms, heading words]
11. 9 or 10
12. 8 and 11
13. (rural-urban or urban-rural).mp. [mp=abstract, title, original title, broad terms, heading words]
14. 12 or 13
15. bone.mp. [mp=abstract, title, original title, broad terms, heading words]
16. limit 15 to abstracts
17. 4 and 14 and 16
18. limit 17 to english language
19. 8 or 11 or 13
20. 4 and 16 and 19
21. limit 20 to english language

original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
13. limit 12 to abstracts
14. 7 and 10
15. 11 or 14
16. 3 and 13 and 15
17. limit 16 to (english language and humans)
18. 7 or 10 or 11
19. 3 and 13 and 18
20. limit 19 to (english language and humans)
21. (Historical Article or News or Newspaper Article or Review or Review, Multicase or Review, Tutorial or Review of Reported Cases).pt.
22. (17 not (Historical Article or News or Newspaper Article or Review or Review, Multicase or Review, Tutorial

trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
17. limit 16 to abstracts
18. 15 and 17
19. limit 18 to (human and english language)
20. 7 or 10 or 11
21. 14 and 17 and 20
22. limit 21 to (human and english language)
23. Review.pt.
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25. 19 not 23

or Review of Reported Cases)).pt.

APPENDIX C: Protocol for data processing for night-time light intensity (NTLI) scores.

Credit: This protocol was originally developed by Robin Wilson (Southampton University) and Chris Baker (London School of Hygiene and Tropical Medicine) and adapted for this thesis by Mika Matsuzaki. Areal tracing of the study villages were done by Chris Baker and APCAPS NTLI data processing was done by Robin Wilson. Original image and data processing are credited to USA. National Oceanographic Atmospheric Administration National Geophysical Data Center and Defence Meteorological Satellite Programme data collected by US Air Force Weather Agency.

Background

The dataset for night-time light intensity values for analyzing urbanicity in the APCAPS community was taken from the USA National Oceanographic Atmospheric Administration (NOAA). Since 1992, the satellites operated by the Defence Meteorological Satellite Programme - Operational Linescan System (DMSP-OLS) have been capturing visible-near infrared emissions from the earth's surface on cloud-free nights. The values provided by the DMSP-OLS satellites undergo data cleanup and manipulation to remove artifacts (i.e. fire and lightening) before they are published as 'stable' datasets. This thesis uses Version 4.0 NTLI data [241]. NTLI is not measured with any pre-existing physical unit. Instead the values are "digital numbers" that relate to relative intensity of light ranging from 0 (no light) to 63 (high intensity).

APCAPS data processing

Boundaries for all APCAPS villages were developed via aerial tracing of Bing Sat imagery, using Java OpenStreetMap Editor (JOSM) **(Figure C1)**.

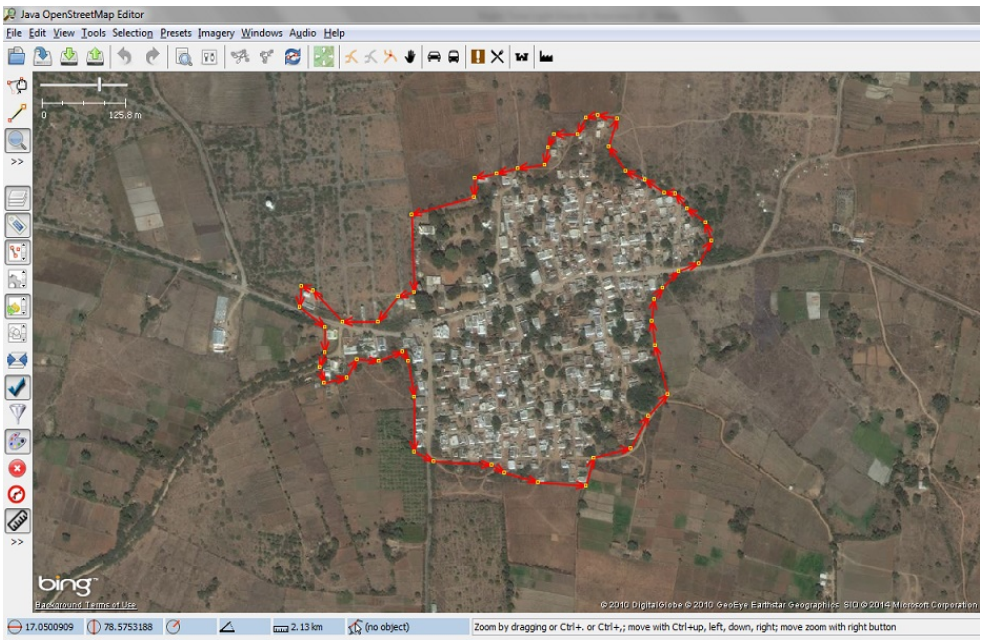

Figure C1: Aerial traced boundary around Meerkhanpet village. Image was created in Java OpenStreetMap Editor. Image credit: Chris Baker (London School of Hygiene and Tropical Medicine).

This created 28 files that demarcate the built up area of each village. The villages of Pocharam and Ramreddiguda have merged over the past 10 years and represent only one village. If it was not clear where a particular village ended, the field team took GPS coordinates on the ground to identify the 'true' boundary point. **Figure C2** shows the 28 APCAPS 'villages' as red polygons along with the 1km x 1km satellite grids which intersect with any part of the village. Some grids are included where only a very small section of the village boundary intersects. Four villages are contained within one grid cell, all others require further statistical reduction to achieve one 'village' value. To avoid the problem of one grid cell's high value inflating the village's total NTLI value, a 'super-resolution' methodology was adopted.

In Figure 3.1, the village falls mostly into a grid whose NTLI value is 1. A small section of the village lies in a grid whose NTLI value is 10. Calculating the mean value for the village by simply averaging the two cells ((1+10)/2 = 5.5) would overestimate the NTLI value for this village. The 'super-resolution' method splits each cell into 100 smaller squares (100m x 100m), and takes values only from those smaller cells that cover part of the village. The mean comes out very differently; ((65 * 1) + (4 * 10)) / 69 = 1.52; a far better estimate. The concept is demonstrated in a

digram in **Figure C3**.

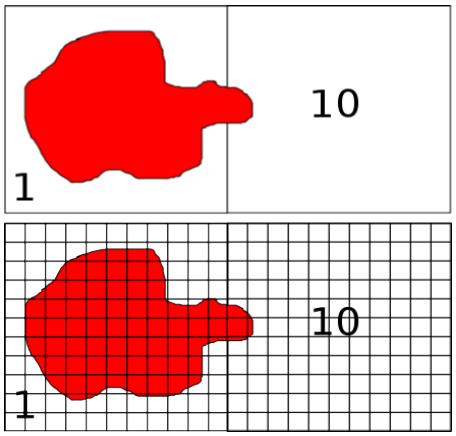

Figure C3: An example diagram demonstrating a super-resolution technique to better estimate average night-time light intensity. Credit: Robin Wilson and Chris Baker.

NTLI data are sometimes provided by multiple satellites as a satellite is only in service for a few years and its replacement usually launches before its predecessor is brought out of service. When two or more satellites provide readings for the same geographical location it is preferable to take the maximum of these values rather than the mean since it is not uncommon for artefacts and technical problems with the satellite to return “0” values which would underestimate the true value if included in a mean calculation.

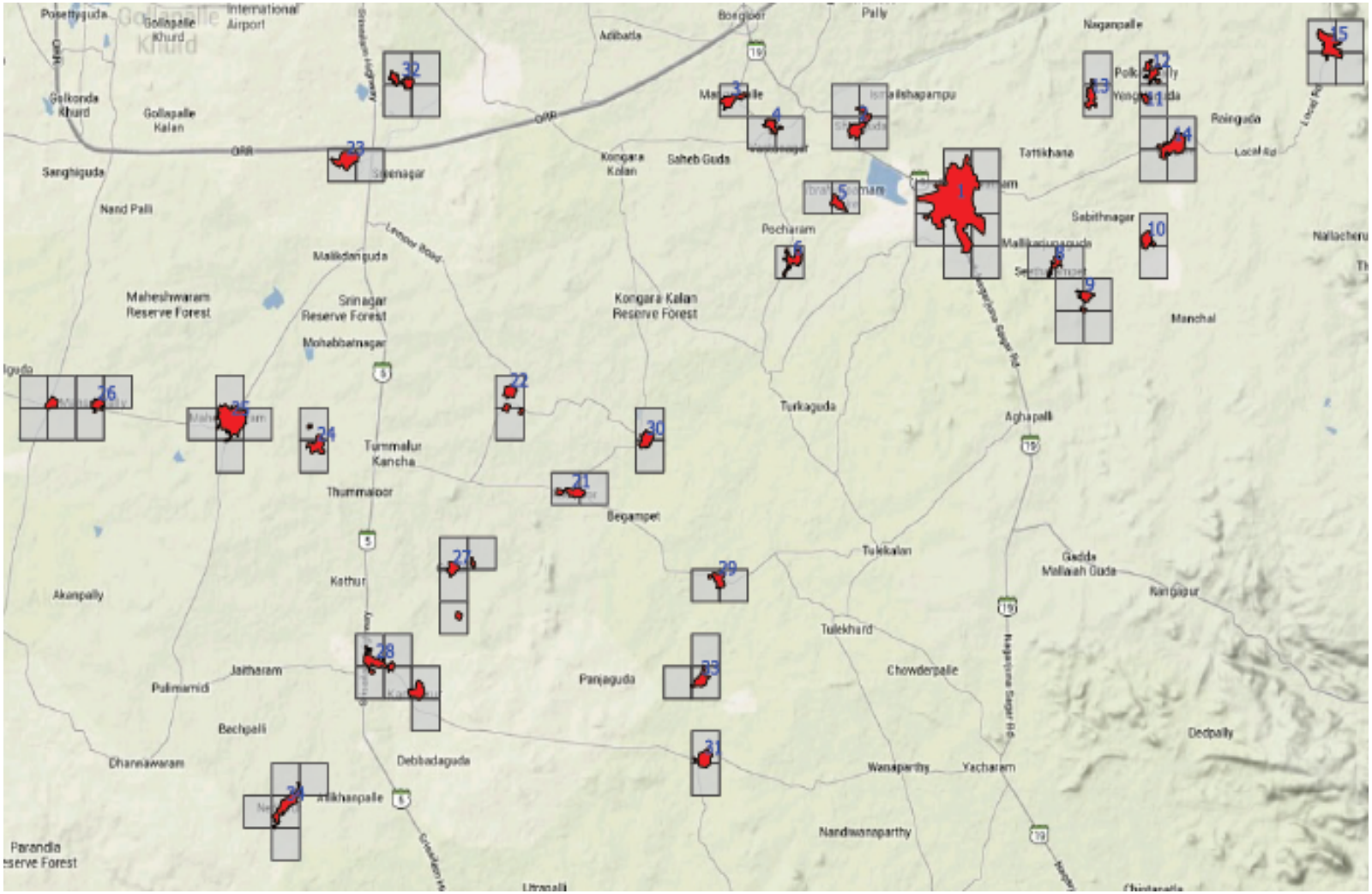

Figure C2: APCAPS village boundaries and intersecting 1km x 1km satellite grids. Credit: Robin Wilson and Chris Baker.

Limitations

The satellite sensors can only measure light which is emitted and visible from the atmosphere. Therefore, lights inside buildings are not captured. The values therefore, do not reflect the 'true' amount of light being used in a particular settlement. Several DMSP-OLS satellites have been used with each one being in use for a few years before replacement. They have not undergone precise inter-satellite or inter-annual calibration although the current dataset is still sufficient to demonstrate overall changes in NTLI over the past two decades.

APPENDIX D: Numbers of non-residential places by categories in Ibrahimpatnum, Aakulamailaram, Engalguda

Domain	NRP categories	Ibrahimpatnum	Aakulamailaram	Engalguda
Agricultural & Allied	Agricultural supplies & products	7	1	0
	Animal husbandry & veterinary services	2	2	0
Communications	Internet Café	6	0	0
	Mail/Courier, inc. post office	3	0	0
	Media / Press	2	0	0
	Mobile Phone Shop and Services	35	1	0
	Public Phone	1	0	0
Construction	Cable TV services	1	0	0
	Borewell Servicing	7	0	0
	Cement/Brick Services and Shop	15	0	0
	Construction Agency	6	0	0
	Hardware	31	0	0
	Metal & welding services	36	0	0
	Stone Service and Shop	7	0	0
	Timber & woodwork	33	0	0
Education	College- above X	15	0	0
	High school-X	2	1	0
	Middle – VII	2	0	0
	Primary-IV	9	1	1

	Vocational	8	0	0
	Special needs education	0	0	0
Energy Supplies	Cooking gas/wood	3	0	0
	Petrol station	2	0	0
	Electricity supply, sub stations	1	0	0
Environment	Recycling/Scrap Dealer	5	0	0
	Commercial & Govt. Bank	8	0	0
	Individual ATM	3	0	0
Finance	Insurance	2	0	0
	Loans	9	0	0
	Non-commercial bank, credit societies, co-operatives (inc. agricultural co-operatives), DWARCA	1	1	0
Food processed	Only fried snacks or fast food	25	0	0
	Sweets, ice cream	7	0	0
	Tea/ coffee point, limited food items	22	1	0
	Bakery	8	0	0
	Fruit juice	2	0	0
Food raw	Haleem/meat	4	0	0
	Soft drink	8	0	0
	Tiffin centre/restaurant	36	3	0
	Fruit	31	0	0
	Grains	10	1	0

	Meat	11	4	0
	Chicken	14	2	0
	Dairy	6	2	0
	Market	1	0	0
	Spice	1	0	0
	Vegetable	18	0	0
Food services	Government Ration Center	5	1	0
	Flour/rice/spice mill	8	1	0
	Water plant; including filtering/bottling units	4	1	0
	Water points; taps/pumps	0	0	0
	Warehouse, godowns	1	0	0
	Water tanks/storage	0	0	2
Government	Police/Fire	1	0	0
	Documentation Assistance	11	0	0
	Office & govt services e.g. gram panchayat, mee seva, BSNL/telephone exchange	8	1	0
	Registration Office, e.g. land registry, marriage registry	2	0	0
	Political Party Office	2	0	1
	Welfare Provider	2	0	0
Medical	AYUSH - ayurveda, yoga, unani, siddhi,	0	0	0

	homeopathy			
	Dentist	0	0	0
	Diagnostic center	5	0	0
	Government Aanganwadi	2	2	2
	Hospital- inpatient	10	0	0
	Out patient clinic	24	2	0
	Pharmacy	16	0	0
Miscellaneous	Miscellaneous (please provide description)	4	0	0
Products Electronics	Electronic items	13	0	0
	Watches and Clocks	4	0	0
Products Fashion	Bangle Store & Accessories, “emporium”	24	4	0
	Clothes- ready made	50	0	0
	Fabric store	3	1	0
	Gold/ Silver Jewelery	25	1	0
	Shoes	15	0	0
Products Misc	Bookstore & Stationary	3	0	0
	Flowers	6	2	0
	General Household Store	168	0	2
	Gifts	3	0	0
	Bags & boxes	0	0	0
Recreation	Event organizers; catering, tents, music band	21	2	0

	Function Hall, Community Hall, meeting space	8	0	1
	Gym & Fitness center, swimming pool	2	0	0
	Music/Video/Arts	8	0	0
	Open Space for physical activity, inc. playgrounds	1	0	0
	Theater/Cinema	1	0	0
	Library	0	1	0
Religious	Religious Center	16	6	5
	Burial place/Cremation	0	0	1
Services Professional	Employment Consultancy	1	0	0
	Law Firm / Advocate	3	0	0
	Real Estate	5	0	0
Services Semi-skilled	Ironing	15	1	0
	Xerox & Printing	24	0	0
Services- Skilled	Electronic servicing	19	2	0
	Hair Salon/Beauty Parlor	37	3	0
	Hostel / Guesthouse / Resort	1	0	0
	motorized machinery servicing	15	0	0
	Photo studio/ videographer	21	2	0
	Tailor & upholstery	56	1	0
	Cobbler	0	0	0
Substance	Alcohol branded shop	6	0	0

	Alcohol- homebrew shop	3	0	0
	Alcohol drinking joint	0	0	0
	Tobacco products	11	0	0
Transport	Bicycle Servicing	5	0	0
	Bus stop/station. Auto-rickshaw point	2	0	1
	Goods transport company	1	0	0
	Travel Agency	0	0	0
	Vehicle Parts, Supplies & Servicing	86	0	0
	Vehicle Rental and Sales	11	0	0
Manufacturing	Handicrafts; baskets, pottery, rugs, books	0	0	0
	Large scale craftsmanship e.g. boats	0	0	0
	Factory	0	0	0

NRP: non-residential places. NRPs were defined as buildings that provide retail or services, including but not limited to: shops, governmental offices, medical facilities, religious facilities, event places, and open space used for physical activity.

APPENDIX E: Protocol for mapping non-residential places in rural India

Built Environment Survey Development: Mapping of Non-Residential Places

Contributors: Chris Baker, Mika Matsuzaki, Chitra Sharma

Version: 2013 October 18

RATIONALE

* Anecdotal evidence shows that rural villages in India are becoming rapidly urbanized. Census data on population size and density may not accurately reflect these changes.
* Availability and accessibility of shops, restaurants, and public services and space are key elements of environmental profiles.
* Types of services and products available in rural Indian villages have not been well-documented.
* Sampling one street in rural settings may not adequately capture representative built environment of the villages.
* APCAPS villages are small enough that it is likely that we can map information on all non-residential places.
* The same information collected in this exercise may be used for development of urbanicity scales as well.

OBJECTIVES

1. Assess feasibility of mapping all non-residential places (NRP).
2. Collect data on types and locations of of non-residential places as a preparatory step for developing built environment survey as well as an alternative urbanicity scale.

PREPARATION

TEAM

* 1 Telugu speaker/GPS recorder
* 1 person to note types of NRPs

INSTRUMENT

* GPS device
* Pen
* Paper to document types of shops
* Map of the village
* Writing board
* An extra set of AA batteries
* Cables for connecting GPS device to a laptop
* Laptop to download waypoints when the GPS device becomes full

DEFINITION

Non-residential places (NRP): Buildings that provide retail or services, including but not limited to: shops, governmental offices, medical facilities, religious facilities, event places, and open space used for physical activity.

Village boundaries: Defined by the last building (houses, NRPs, other landmarks) on the outer streets that cross into the neighboring villages or space.

Road types: Paved (concrete) or unpaved (mud).

Approach road: Defined by whether buses from other villages ride on this road into the village of interest.

Temporary or permanent places are defined by whether the NRPs move from one place to another, whether daily or monthly or seasonally, or remain open only for certain seasons.

SECTION 1: FIELDWORK

A: Before fieldwork

1. Consult with fieldworkers and/or village heads where they think are streets with high densities of non-residential places as you will walk down these streets first.

(This step is for CB and CS)

2. Prearrange tables with columns for: GPS waypoint number, raw descriptions, categories, temporary or permanent or not clear, road types, primary fuel types. Categories can be added post field site visit. Be sure to add the team members initials and coder's names.

3. Erase all existing waypoints on the device after copying them onto a computer, so that the numbering starts from 1 for each day of the visit.

(This step is for CB and CS)

4. In Open Street Map (OSM), perform aerial tracing of the roads in each village, so that we have maps for all the villages. Print a map of the field site before visiting, so that you can check which streets you've surveyed and which streets you have not been on.

B: Field site

* If you need to take a break while mapping, please note the two waypoints, between which you took a break. We will be calculating how long it takes to complete this survey to show feasibility of this mapping project to other researchers and we would like to remove time that was not spent on mapping activities from this calculation.

5. Walk down the streets with high NRP densities. Record GPS coordinates and types of all NRPs. Mark on your map which streets you are surveying. Download waypoints if the GPS device becomes full before the end of the visit.

GPS device keeper (Telugu speaker):

* Turn on the GPS device. Wait until the estimation range is within 15m.
* Stand within 3m from the front of each non-residential place and mark the location. Tell the recording person the number.
* If the places are not open, ask neighbors what types of places they are.

* If there are multiple NRPs in the same building (i.e. 3 story building), make a new waypoint for each NRP at the same place (i.e. If there are 3 NRPs in a building, take 3 separate GPS coordinates).

Recording person:

* Note the number from the GPS device.

* Write down the description of non-residential places. If not sure about the exact type, document items sold in the place.

The following items should all be noted:

- Shops, restaurants, and other services in permanent locations
    - fuel types for prepared food places
- Street venders and other temporary places (mark as temp)
- Governmental offices and services
- Bus and other transportation hubs
    - Take a photo of the timetables if available
- Schools of all levels
- Religious facilities (including unmanned places)
- Function (event) places
- Approach road types (mud/paved/mixed) – Mark on the map where the first NRP stands on the approach roads. Note types of roads under that waypoint.
- Last building (NRP or house) on outer roads on the edges of the villages (This information will help define village boundaries.)
- Open space that is used for physical activity – if not clear, ask passerbys.

6. Walk (or drive if necessary) on all other streets. Stop at each NRP you find and record the GPS coordinates within 3 m from the front of the NRP as described in Step 5.

C: After fieldwork

7. Download Garmin GPSmap's waypoints. Check that the waypoints have been recorded and downloaded successfully and back up the data before erasing waypoints. There is a CD for downloading waypoints on a Windows machine. For Linux, the following command downloads waypoints:

sudo gpsbabel -i garmin -f /dev/ttyUSB5 -o gpx -F waypoint.gpx
(Change /dev/ttyUSB5 accordingly to the result of lsusb or dmesg)

8. Record raw data into a spreadsheet.

SECTION 2: Data Cleanup and Analyses

1. Categorize types of non-residential areas. The category list is attached to the end of this protocol. If there are multiple categories that apply to one place, note them all in separate columns. If there is no category that matches the type of NRP, add a new category. If it is not possible to categorize and a second coder is required, add under XX: Unclear. Please note the coder's name.

(The following steps to be done by CB, MM, or CS)

2. Map both waypoints and descriptions of waypoints on Open Street Map (OSM) or Google Physical Map in Quantum GIS (QGIS).

3. Calculate the number of hours it took to record NRPs, using GPS records.

4. Count the total number of NRPs as well as numbers in each category.

5. Estimate the densities of NRPs to determine the center(s) of the village.

APPENDIX F: Comparison of village ranking of urbanicity defined by population size (2011), night-time light intensity (2012), and numbers of non-residential places (2013).

Village names (APCAPS village ID)	Census ranking	NTLI ranking	NRP total number ranking	NRP type ranking	Fieldworkers ranking
Ibrahimpatnam (1)	1	1	1	1	1
Mangalpalli (4)	8	14	7	7	6
Pocharam (6) /Ramireddyguda (7)	16	15	15	17	20
Nomula (9)	19	22	22	22	10
Lingampalli (10)	22	20	17	18	19
Engalgda (11)	23	23	23	23	23
Polkampalli (12)	13	12	11	14.5	8
Nainampalli (13)	20	13	20	19.5	13
Raipole (14)	5	6	4	4	5
Dandumylaram (15)	4	8	5	7	7
Rachaloor (21)	11	17	16	14.5	9
Lemur (22)	7	10	8	10	14
Mankhal (23)	2	3	14	12	12
Thummalur (24)	14	11	13	9	16
Maheshwaram (25)	3	2	2	3	2
Mansanpalli (26)	18	7	10	7	4
Gudur (27)	17	18	18	19.5	11

Kandurkur (28)	6	4	3	2	3
Gummadivalli (29)	15	21	19	16	15
Thimapur (30)	12	16	12	12	17
Meerkhanpet (31)	10	19	9	5	22
Sardarnagar (32)	21	5	21	21	21
Nedunur (34)	9	9	6	12	18

Five villages were excluded in this ranking comparison as the national census data were not available (Sheriguda, Patelguda, Uppariguda, Seethakampet,Aakulamylaram).

APPENDIX G: Protocol for DXA artifact coding

DXA artifact coding

2014-06-16

Contributor: Mika Matsuzaki

OBJECTIVE

The goal of this exercise is to find out which scans cannot be used for data analysis. Foreign objects, movements, cut-out regions, and skeletal diseases like sclerosis are all artifacts that affect the accuracy of DXA values. At the end of this exercise, all DXA values accompanied with scan or analysis grade 4 will be removed from data analysis. DXA scan grade 3 will be revisited for 2nd opinions. DXA values with analysis grade 3 will be edited, so that the values are usable. For all participants, check whether hip BMD, LS BMD, whole-body BMD, lean mass, and fat mass values are correct since they were manually entered.

A. Scan grade

Scan grade refers to how well participants' bodies were scanned as well as how clear digitized DXA reports are. Describe the quality of scanning by checking:

a. body alignment (straight) and inclusion (all parts inside the scan)
b. any foreign objects (bright/large/numerous = exclude (4); small/only earrings OR only 1 small ring OR only faint and small toe rings = include (2))

c. any movement (large movement showing >2 limbs, 2 heads etc or zigzag bones that look like fractures = exclude (4); small movement showing slightly wavy bone lines or slight blur in head = include (2))

d. any cut-off region (only part of 1 finger or part of head = include (2); everything else = exclude (4))

e. paper scan availability and quality: is the scan available? Is the available scan clean enough to judge artifacts on?

Table G1: Artifact grading criteria for DXA scanning quality.

Grade	Note
1 = include	Perfect
2 = include but minor artifacts: Make a comment on why it's 2 under variable *comment*	Slight movement, small foreign objects, not positioned perfectly but mostly straight, small cut-off region
3 = unsure and need 2nd opinion: Make a comment on why it's 3 under variable *comment*	If you aren't sure whether to do 2 or 4, mark 3.
4 = exclude because of major artifacts: Make a comment on why it's 4 under variable *comment*	Major movement, major foreign objects, body severely bent, large cut-off regions
9: no paper scan available	No high-quality paper scan available. Need to request Santhi to resend it.

B. Analysis grade

Analysis grades define whether the scans are ready for analyses based on scan grade and any other relevant information. Describe whether DXA values are ready for analysis as is by checking:

a. earrings or faint ring or toerings only OR only slightly bent body OR only small cut-out region OR only slight movement = 2

b. only 1 LS region contains a foreign body = 3: in comment, say which region needs to be excluded

c. more than 1 LS regions contain a foreign body = 4: in comment, say which regions contain foreign objects

d. if scan grade is 4, analysis grade is 4 too.

Table G2: Artifact grading criteria for DXA bone analysis.

Grade	Note
1 = include	Perfect
2 = include but minor artifacts:	Leave minor artifacts as they are since they are unlikely to influence the values significantly.
3 = unsure and need 2nd opinion OR the scan or values need to be manually edited.	If you aren't sure whether to do 2 or 4, mark 3. OR if the scan or values are usable if manually edited, mark so.
4 = exclude because of unfixable major artifacts:	Unfixable artifacts.

C. Body composition grade

For fat and lean mass analyses, foreign objects were permitted. Describe whether DXA values are ready for body composition analysis as is by checking:

a. earrings or faint ring or toerings only = 1

b. Only slightly bent body OR only small cut-out region OR only slight movement OR foreign metal objects only= 2

c. need 2nd opinions = 3

d. severe deformity OR major cutoff OR missing body parts OR major movement = 4; in comment, describe issues.

Table G3: Artifact grading criteria for DXA body composition

Grade	Note
1 = include	Perfect
2 = include but minor artifacts:	Leave minor artifacts as they are since they are unlikely to influence the values significantly.
3 = unsure and need 2nd opinion OR the scan or values need to be manually edited.	If you aren't sure whether to do 2 or 4, mark 3. OR if the scan or values are usable if manually edited, mark so.
4 = exclude because of unfixable major artifacts:	Unfixable artifacts.

Example coding is showed in Table A3.5.3. For both scan and analysis grades, note any issues with the scans in the comment section. If any numbers are not entered, please add rows to the spreadsheet and enter hip BMD, LS BMD, whole-body BMD, lean mass, and fat mass values as well as any artifact observations.

Table G4: Examples of DXA artifact coding.

hip.scan.grade	hip.analysis.grade	hip.comments	ls.scan.grade	ls.analysis.grade	ls.comments	wb.scan.grade	wb.analysis.grade	wb.fo	wb.mv	wb.bc	m.wb.comments
1	1		1	1		4	4	2	0	1	earrings, bracelets, toerings
1	1		1	1		2	2	1	1	1	slight

1	1	1	1	2	2	1	0	1

movement in feet and small fo ring, buttons toerrings

ls = lumbar spine; wb = whole-body; fo = foreign object; mv = movement; bc = body composition grades