DETERMINANTS AND MEASUREMENT OF LEAN BODY MASS IN INDIAN ADULTS

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KEYWORDS

Anthropometry, Appendicular skeletal muscle mass, Body composition, Body fat percent, Developmental origins of health and disease, dual energy X-ray absorptiometry, Fat free mass, Fat mass, Grip strength, Indian, Isotope dilution technique, Lean body mass, Lifestyle, Muscle mass, Nutrition supplementation, ^{18}O, Prediction equations, Programming, Validation.

ABSTRACT

A high prevalence of adiposity related chronic diseases like diabetes and cardiovascular disease is a significant public health challenge in developing countries like India. Double burden of childhood under nutrition and adult onset adiposity in these settings is particularly difficult to tackle. Substantial evidence demonstrates that the elevated risk of chronic diseases in a chronically under nourished population such as India is related to the peculiar body composition with a low lean body mass (LBM) and high fat mass in Indians. Lean body mass, including muscle mass, has an independent association with insulin sensitivity and is important for the prevention of chronic diseases. In addition, muscle strength, an indicator of functional competence of muscle mass, is a strong predictor of health-related quality of life. Information on the determinants of LBM, muscle mass and muscle strength in Indian adults, although critical for developing interventions for improving muscle mass and strength, is not available.

It has been argued that this high fat-low muscle mass phenotype is "programmed" in early life as a result of under nutrition during critical periods of development. In addition, nutrition and physical activity throughout the life course are known to have important influence on the body composition of an individual. The majority of the studies have, however, assessed the role of early nutrition or later lifestyle determinants on adult lean and muscle mass in isolation. Studies using life course approach with comprehensive assessment of early as well as later life factors that impact adult lean and muscle mass are particularly important, but lacking.

One of the reasons for the lack of information on the lean and muscle mass of Indians is related to the difficulties involved in accurate and precise estimation of these body compartments. The present thesis therefore includes three studies which focus on the determinants as well as the measurement of lean and muscle mass in Indians. These studies provide population specific evidence which is essential due to known ethnic differences in the body composition patterns.

The first study examined the importance of early nutrition and current lifestyle as determinants of LBM and muscle strength in participants of a birth cohort (Andhra Pradesh Children and Parents Study, APCAPS). This cohort, which was established to assess the long-term impact of balanced protein-energy supplementation (2.51 MJ, 20 g protein) during pregnancy and childhood, provided a unique opportunity to examine both the early and current life factors as determinants of LBM. The study participants (n= 1446, 32% females) were born in 29 villages (15 intervention, 14 control) near Hyderabad, India. Their LBM and appendicular skeletal muscle mass (ASM) were measured by DXA along with grip strength and lifestyle indicators including diet and physical activity. Participant characteristics (mean) including age (20.3 y) and BMI (19.5 kg/m^2) were similar in the intervention and control group; but current dietary energy intake was higher in the intervention group. Unadjusted LBM and grip strength estimates were also similar in the two groups. However, after adjusting for potential confounders, intervention group participants had lower LBM (β = -0.75 kg; 95% CI: -1.41 to -0.09 kg; p = 0.03), ASM and grip strength than controls although the magnitude of these differences was small (<0.1 SD). Multivariable regression analyses showed that current factors including socio-economic position, dietary energy intake and physical activity were

important determinants of LBM and muscle strength. The study thus showed that in this transitioning community, modest protein calorie supplementation in early life did not have a lasting positive effect on the LBM and muscle strength of the rural young adults. On the other hand, adult socio-economic position, diet and physical activity were important determinants of lean mass indices. The study does not support the role of "programming" of lean and muscle mass by nutrition supplementation provided through a government funded programme in India and has important policy implications.

Estimates of LBM in the first study, however, could be influenced by the method used for its estimation because substantial differences in the estimates of body composition using different methods are possible. The second study in this thesis, therefore, compared the estimates of lean body mass and fat mass measured by DXA with those using isotope dilution technique. The study included participants who were healthy volunteers (n=152; 48% males) aged19-70 y, representing a wide range of body mass index (14-40 kg/m^2). Their body composition was assessed by 2 methods: DXA and isotope (^{18}O) dilution technique on the same day. Agreement between the estimates of lean mass, fat mass (FM) and body fat percentage (% BF) by the two techniques was assessed using the Bland Altman method. Analyses indicated that the LBM estimates were higher by about 7% (95% Confidence Interval (CI): 6 to 9%), FM estimates were lower by about 21% (95% CI: -18 to -23%) and %BF estimates were lower by about 7.4% (95% CI: - 8.2 to - 6.6%) using DXA compared to the isotope dilution technique. Bland Altman analyses showed wide limits of agreement indicating poor agreement between the methods. The bias in the LBM estimates was higher at a higher BMI whereas bias in the %BF estimates was

higher at lower values of the %BF. The study thus showed that the two commonly used reference methods for body composition assessment may show substantial differences in body composition estimates with wide limits of agreement. These values were, however, highly correlated indicating that the differences in the absolute values at individual level may not affect the results of the studies exploring the relationship of body composition using either of these methods with health outcomes. The results of the first study assessing the relationship of early and current life determinants with the lean body mass of young adults (estimated using DXA) may therefore be robust to the method used for body composition assessment.

Further studies are, however, required for enhanced our understanding about the determinants of lean and muscle mass in Indians. This information, although critical for developing intervention strategies focused on improvement of the muscle and lean mass, is, however, scarce. The paucity of information is mainly due to difficulties involved in quantifying these body compartments in resource-poor settings as inexpensive methods suitable for large scale epidemiological studies are not available. The third study in this thesis, therefore, developed anthropometric prediction equations to estimate the LBM and appendicular lean soft tissue (ALST, also known as appendicular skeletal muscle mass or ASM: an indicator of skeletal muscle mass) using DXA as a reference method.The study participants were healthy volunteers (n= 2220; 36% females; age 18-79 y) representing a wide range of body mass index (14-44 kg/m^2). Their LBM including ALST was assessed by DXA along with anthropometric measurements. The sample was divided into prediction (60%) and validation (40%) sets. In the prediction set, a number of prediction models were constructed using DXA measured LBM and ALST estimates as dependent variables

and a combination of anthropometric indices as independent variables. These equations were cross-validated in the validation set. Simple equations using age, height and weight explained > 90% variation in the LBM and ALST in both men and women. Additional variables (hip and limb circumferences and sum of SFTs) increased the explained variation by 5-8% in the fully adjusted models predicting LBM and ALST. More complex equations using all the above anthropometric variables could predict the DXA measured LBM and ALST accurately as indicated by low standard error of the estimate (LBM: 1.47 kg and 1.63 kg for men and women, respectively) as well as good agreement by Bland Altman analyses. These equations could be a valuable tool in large epidemiological studies assessing these body compartments in Indians and other population groups with similar body composition.

The present thesis thus examined important determinants (both early nutrition as well as current life factors) of the lean body mass operating during life course in rural young adults residing in a transitioning community. This information would help development of intervention strategies targeting at improvement of lean body mass in Indian adults. The validation study comparing the body composition estimates by DXA with those by isotope dilution technique provides population specific evidence on the differences in the body composition estimates by the two methods. Moreover, to facilitate future research studies on the lean and muscle mass in other resource poor settings with limited access to advanced techniques of body composition assessment, the third study developed population specific prediction equations for estimation of lean and muscle mass.

Structure of the thesis

The thesis includes six chapters. The first chapter (Introduction) provides background information on the subject while the second chapter provides review of relevant literature. The above described three studies are presented in chapters 3, 4 and 5 in the form of scholarly publications in international peer-reviewed journals. The sixth chapter presents general discussion and conclusions of the thesis.

Contribution of the candidate to the project

Three studies included in this thesis were parts of a large study entitled "Nutritional challenges, abdominal adiposity and type 2 diabetes in Indians" funded by the Wellcome trust, UK. The planning and conduct of the study was a team work and the candidate gratefully acknowledges support from all the co-investigators of the study.

The candidate was a collaborator on this project and performed the following specific roles:

1. Provided input for - a. planning the study
   b. field work organization for data collection
   c. training the field staff
   d. design and administration of study questionnaire
   d. enrolment of the participants
2. Ensured quality control of DXA scans and analyses
3. The candidate completed a short training course on ' stable isotopes in nutrition' organised by the International Atomic Energy Agency at the St. John's Research Institute, Bangalore and supervised the isotope measurement and dosing. The candidate co-ordinated the isotope analyses at St. John's Research Institute, Bangalore
4. Data management and cleaning
5. Data analyses
6. Drafting and communicating the manuscripts for publication in high impact international journals.

List of publications

Peer reviewed International journal articles

1. **Bharati Kulkarni,** Andrew P Hills, Nuala M Byrne. Nutritional influences during the life course on the lean body mass of individuals in developing countries. Nutrition Reviews 2014;72:190-204

2. **Bharati Kulkarni**, Hannah Kuper, KV Radhakrishna, Andrew P Hills, Nuala M Byrne, Amy Taylor, Ruth Sullivan, Liza Bowen, Jonathan C Wells, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Sanjay Kinra. The Association of Early Life Supplemental Nutrition With Lean Body Mass and Grip Strength in Adulthood: Evidence From APCAPS. American Journal of Epidemiology 2014;179: 700-9.

3. Sanjay Kinra, KV Radha Krishna, Hannah Kuper, KV Rameshwar Sarma, Poornima Prabhakaran, Vipin Gupta, Gagandeep Kaur Walia, Santhi Bhogadi, **Bharati Kulkarni**, Aniket Kumar, Aastha Aggarwal, Ruby Gupta, D Prabhakaran, K Srinath Reddy, George Davey Smith, Yoav Ben-Shlomo, Shah Ebrahim. Cohort Profile: Andhra Pradesh Children and Parents Study (APCAPS). Int J Epidemiol2013 Sep 9. [Epub ahead of print]

4. **Bharati Kulkarni**, Hannah Kuper, Amy Taylor, Jonathan C Wells, KV Radhakrishna, Sanjay Kinra, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, AV Kurpad, Nuala M Byrne, Andrew P Hills. Comparison of body composition estimation by Dual-energy X-ray absorptiometry and isotope dilution technique in Indian men and women. British Journal of Nutrition [In press]

5. **Bharati Kulkarni**, Hannah Kuper, Amy Taylor, Jonathan C Wells, KV Radhakrishna, Sanjay Kinra, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Nuala M Byrne, Andrew P Hills. Development and validation of anthropometric prediction equations for estimation of lean body mass and

appendicular lean soft tissue in Indian men and women.J Appl Physiol 2013; 115: 1156–1162

Conference presentations: Oral

Bharati Kulkarni, Hannah Kuper, KV Radhakrishna, Andrew P Hills, Nuala M Byrne, Amy Taylor, Ruth Sullivan, Liza Bowen, Jonathan C Wells, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Sanjay Kinra. "Determinants of lean body mass in young indian adults – evidence from Hyderabad nutrition trial". The sixteenth Annual Meeting of SNEHA / MRC International Workshop on Developmental Origins of Health and Adult Disease, Hyderabad, India. February 3-5, 2012.

Conference presentations: Poster

Bharati Kulkarni, Hannah Kuper, KV Radhakrishna, Andrew P Hills, Nuala M Byrne, Amy Taylor, Ruth Sullivan, Liza Bowen, Jonathan C Wells, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Sanjay Kinra. "Early life Nutrition Intervention and lean body mass in young Indian adults". International Conference on Nutrition and Growth at Paris, France. March 1-3, 2012.

Bharati Kulkarni, Hannah Kuper, Amy Taylor, Jonathan C Wells, KV Radhakrishna, Sanjay Kinra, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, AV Kurpad, Nuala M Byrne, Andrew P Hills. Comparison of body composition estimation by Dual-energy X-ray absorptiometry and isotope dilution technique in Indian men and women. The seventeenth Annual Meeting of SNEHA / MRC International Workshop on Developmental Origins of Health and Adult Disease, Mysore, India. February 1-3, 2013.

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LIST OF ABBREVIATIONS

BMI	Body mass index
%BF	Percent body fat
2 C	Two compartment model (of body composition)
3 C	Three compartment model (of body composition)
4 C	Four compartment model (of body composition)
ADP	Air displacement plethysmography
AIC	Akaike's information criteria
ALST	Appendicular lean soft tissue
ASM	Appendicular skeletal muscle mass
BIA	Bioelectrical impedance analysis
CAMA	Corrected arm muscle area
CT	Computerised tomography
CV	Coefficient of variation
CVD	Cardiovascular disease
DOHaD	Developmental origins of health and disease
DPA	Dual photon absorptiometry
DXA	Dual energy X ray absorptiometry
EAA	Essential amino acids
FFM	Fat free mass
FFMI	Fat free mass index
FFQ	Food frequency questionnaire
FM	Fat mass
FMI	Fat mass index
FTIR	Fourier transform infrared spectroscopy
IAEA	International atomic energy agency
IGF-1	Insulin like growth factor 1
IRMS	Isotope ratio mass spectrometry
ISAK	International Society for Advancement of Kinanthropometry
IUGR	Intrauterine growth retardation
LBM	Lean body mass

LBW	Low birth weight
MAC	Mid arm circumference
MET	Metabolic equivalent of task
MRI	Magnetic resonance imaging
PE	Pure error
PUFA	Polyunsaturated fatty acids
REE	Resting energy expenditure
RET	Resistance exercise training
SEE	Standard error of the estimate
SLI	Standard of living index
SM	Skeletal muscle mass
SPA	Single photon absorptiometry
TBK	Total body potassium
TBW	Total body water
TM	Total mass
TNF-alpha	Tumour necrosis factor - alpha
TSF	Triceps skinfold thickness
UWW	Under water weighing
WC	Waist circumference
WHO	World Health Organisation

STATEMENT OF ORIGINAL AUTHORSHIP

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

QUT Verified Signature

Signature:

Date: 23 JULY 2014

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Chapter1: Introduction

1.1 BACKGROUND

Rising prevalence of adiposity-related chronic diseases in India

South Asian countries including India are experiencing a rapid increase in non-communicable diseases including type 2 diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease (CVD) that are known to be associated with obesity (Reddy and Yusuf 1998, Ramachandran, Snehalatha et al. 2001, Misra and Khurana 2009). Recent estimates indicate that currently India has the highest global number of patients with diabetes (61.3 million), which is estimated to increase to 101.2 million in 2030 (Whiting, Guariguata et al. 2011). Moreover, insulin resistance and clustering of cardiovascular risk factors (the metabolic syndrome) are frequently seen in South Asians at a young age (Chan, Malik et al. 2009). For example, a study from Delhi reported a high prevalence of insulin resistance in post-pubertal children which was associated with excess body fat and abdominal adiposity (Misra, Vikram et al. 2004, Ranjani, Sonya et al. 2013). The social and economic costs of this high burden of chronic diseases in the young productive age group are enormous.

The high burden of adiposity-related chronic disorders in the Indian population is surprising considering the low prevalence of obesity using body mass index (BMI) criteria in this population. Although the main drivers of the increase in chronic diseases may be high energy intakes and low physical activity (similar to other settings), a peculiar body composition of Indians is considered to be an important determinant of the elevated risk of metabolic syndrome in this population.

A number of studies have demonstrated that even at BMI and waist circumference levels considered 'normal' using internationally accepted standards, South Asians including Indians, have greater adiposity and a higher risk of metabolic perturbations than individuals belonging to other ethnic groups (Deurenberg Yap, Chew et al. 2002, Mohan, Sandeep et al. 2007, Kulkarni, Shatrugna et al. 2010). The higher adiposity is associated with a lower lean and muscle mass which independently contribute to the reduced insulin sensitivity in this ethnic group (Unni, Ramakrishnan et al. 2009).

Importance of lean body mass and muscle mass

Majority of the studies assessing the relationship between body composition and the risk of chronic diseases have focussed on the fat mass with less attention to the lean body mass, muscle mass or functional competence of muscle mass. Skeletal muscle, the largest component of LBM, plays a central role in a number of physiologic processes and energy metabolism. Muscle mass is known as the primary metabolic "sink" for glucose and triglyceride disposal and a number of studies have shown that a lower LBM is associated with increased risk of insulin resistance (Braith and Stewart 2006, Atlantis, Martin et al. 2009). In addition, muscle mass is important for a number of physiologic processes such as recovery from acute stress, physical work capacity as well as for the prevention of chronic diseases like osteoporosis (Wolfe 2006). Moreover, muscle mass is an important determinant of resting metabolic rate and a higher muscle mass may contribute to the prevention of obesity by influencing energy balance (Wolfe 2006).

Muscle strength: functional competence of muscle mass

A number of studies have demonstrated that muscle strength, a commonly used indicator of functional competence of muscle mass, is a strong predictor of health-related quality of life and is associated with decreased risk of chronic diseases and premature mortality(Rantanen, Masaki et al. 2012). Muscle strength has also been associated with duration of hospital stay indicating that muscle strength is an important indicator of general health status (Kerr, Syddall et al. 2006). Lower hand grip strength is also associated with impaired glucose tolerance and diabetes (Sayer, Dennison et al. 2005). Moreover, LBM and muscle strength are important measures of human capital and understanding their determinants is important.

Determinants of lean body mass and muscle mass

Evidence suggests that the lean body mass during adulthood is influenced by nutritional status in early childhood years as well as the diet and physical activity patterns during later life.

Early life influences on the lean body mass

It has been suggested that the adult Indian phenotype of low lean body mass and excess body fat in relatively thin individuals may be programmed *in utero.* This hypothesis is supported by studies that have shown that the 'low lean mass and high fat mass' phenotype is observed even at birth. For example, a study in Pune, India

that compared body composition of Indian newborns with that of Caucasian babies in the UK, demonstrated that the Indian babies had a lower muscle mass but preserved body fat (Yajnik, Fall et al. 2003). Another study from Mysore confirmed these findings and showed that although the Indian newborns were small and thin compared with the UK neonates, they had low lean mass but relatively preserved truncal body fat. The study also showed that this phenotype persisted at the age of 4 years (Krishnaveni, Hill et al. 2005).

In addition, a large volume of evidence (mainly based on observational studies in humans and animal experiments) has emerged during the past two decades indicating that the nutrition and growth patterns during early life may be associated with subsequent risk of adiposity and metabolic syndrome (Bavdekar, Yajnik et al. 1999, Barker, Eriksson et al. 2002, Brawley, Itoh et al. 2003, Gluckman, Hanson et al. 2008). This is commonly referred to as 'programming', i.e. during the critical periods in early stages of development, environmental factors such as maternal under nutrition have long-lasting impact on the phenotype and may 'program' the metabolism of an individual. The foetus probably adapts to the inadequate nutrition by sacrificing tissues that require high-quality building blocks, like muscle and bone, and instead lays down less demanding tissue like fat (Fall 2009). The high fat mass may also confer survival benefit to the offspring if the nutritional adversity continues in later life. As these changes occur during critical periods of early development, the resultant changes tend to be long lasting.

If the foetal programming hypothesis is correct, maternal nutritional status would be expected to influence the body composition and disease risk in the

offspring because of its association with the foetal nutrition. However, only a few studies have assessed the relationship between maternal nutritional indicators and the health outcomes in the offspring. For example, the Pune Maternal Nutrition Study from India showed that maternal vitamin B12 deficiency was associated with increased body fat and insulin resistance in the children (Yajnik, Deshpande et al. 2008). Other studies investigating the relationship of maternal dietary intakes and the lean body mass in the offspring, however, did not find such a relationship (Hawkesworth, Prentice et al. 2008).

Using nutrition supplementation trials to investigate the early life influences on the adult lean body mass

The evidence in support of intrauterine programming of adult lean body mass and chronic diseases is largely circumstantial based on observational studies in humans and animal experiments. Moreover, the majority of these studies have used birth weight as a proxy measure of foetal nutrition. A major criticism of these studies is that birth weight is a poor measure of intrauterine nutrition because it is multi-factorial in origin and a more direct assessment of exposure is desirable (Paneth and Susser 1995, Kuzawa 2004). However, the direct evidence of a relationship between maternal nutrition during pregnancy and later health outcomes is scarce due to the difficulties in conducting longitudinal studies with many years of follow-up. Previously conducted nutrition supplementation trials in pregnant women provide an opportunity for the direct assessment of the long-term impact of the nutrition intervention on the health of the offspring (Gupta, Ray et al. 2007, Stewart, Christian et al. 2009). For example, a study from Guatemala which assessed the effect of

supplemental nutrition in early life on body composition at a later age, indicated that intervention was associated with a taller height and a higher lean body mass, especially in females during adolescence (Rivera, Martorell et al. 1995). This evidence, however, is not supported by some of the other follow up studies of nutrition trials (Hawkesworth, Prentice et al. 2008).

Early nutrition and muscle strength

Apart from age and sex, a number of factors including diet and physical activity are known to influence muscle strength but these do not fully account for the variation in muscle strength between individuals (Robinson, Jameson et al. 2008). Several epidemiological studies have indicated that poor intrauterine growth (indicated by low birth weight) is associated with lower handgrip strength, even after adjustment for potential confounding factors (Kuh, Bassey et al. 2002, Inskip, Godfrey et al. 2007, Yliharsila, Kajantie et al. 2007). In addition, growth during infancy and early childhood could also influence the muscle mass and strength in later life (Kuzawa, McDade et al. 2010). However, evidence on the long term impact of early nutrition interventions on the muscle strength in later life is not available.

Life-course influences on the LBM & muscle strength

Apart from early life influences, nutrition and physical activity in later years are major modifiable determinants of LBM (Wackerhage and Rennie 2006, Morris and Jacques 2012). Higher intake of protein is associated with higher LBM and studies have demonstrated a beneficial effect of protein and amino acid

supplementation on muscle protein synthesis and LBM (Houston, Nicklas et al. 2008, Tang, Moore et al. 2009). Physical activity, particularly resistance exercise, is known to have a strong positive effect on LBM especially when complemented with higher intake of protein (Yang, Breen et al. , Pennings, Koopman et al. 2011).

Lean body mass, muscle mass and muscle strength are thus influenced by nutritional and other influences operating throughout the lifecourse. Comprehensive assessment of both early and later life influences on the LBM and muscle strength is therefore essential. Majority of the previous studies have, however, examined "programming" of the LBM by early nutrition or the role of lifestyle determinants in isolation. In order to address this knowledge gap, the first study of the present thesis examined the role of early nutrition supplementation as well as current life determinants on the LBM, muscle mass and muscle strength in young adults from a birth cohort (Andhra Pradesh Children and Parents study) established to examine the long term impact of protein energy supplementation during pregnancy and early childhood. The study also provides valuable evidence on the long term impact of early supplemental nutrition provided through a government programme on the lean mass and muscle strength of young adults. The participants' lean and muscle mass were examined at age 18-21 y using DXA and grip strength was assessed as an indicator of functional competence of muscle mass.

Methods for the assessment of LBM and muscle mass

It is evident that precise and accurate estimation of LBM is critical to examine the relationship between early nutrition and later LBM. Difficulties in the

accurate assessment of this important body compartment could be one of the major reasons for relatively small number of studies in this area (for example, in comparison to consideration of body fat content). In addition, the estimates of LBM in the first study could be influenced by the method used (DXA) for its estimation as substantial differences in the estimates of body composition using different methods are possible. The second study in this thesis therefore compared the estimates of lean body mass and fat mass measured by DXA with the estimates using isotope dilution technique

DXA is increasingly used as the method of choice for body composition assessment because of its precision and low dose of radiation exposure. Moreover, DXA scans are quick, non-invasive, operator-independent, require little subject compliance and are relatively cheap. In addition to the estimation of total lean body mass (and fat mass), DXA enables the estimation of regional body composition and appendicular skeletal muscle mass which are important for a number of health outcomes (Szulc, Munoz et al. 2010, Wijnhoven and Snijder 2011).

DXA, however, is not without limitations. Although studies have shown that DXA estimates of body composition are highly correlated with those using criterion techniques, modest variations between the estimates have been reported. Compared to the 4 compartment model, both over-estimation (Schoeller, Tylavsky et al. 2005) and underestimation (Visser, Fuerst et al. 1999) of LBM by DXA has been observed. Validation studies comparing DXA with other criterion techniques tend to be population-specific due to ethnic variations in body composition (Liu, Byrne et al.

2011). Studies comparing DXA estimates of body composition with other precise techniques have not been reported from India.

The second study of the present thesis, therefore, compared the body composition estimates using DXA with another precise technique, isotope dilution, which is commonly used as a criterion technique to validate other simpler methods of body composition assessment. The study provides information on the possible influence of the measurement technique on the relationship of various determinants with the LBM of the young adults examined in the first study.

Anthropometry for estimation of LBM and muscle mass

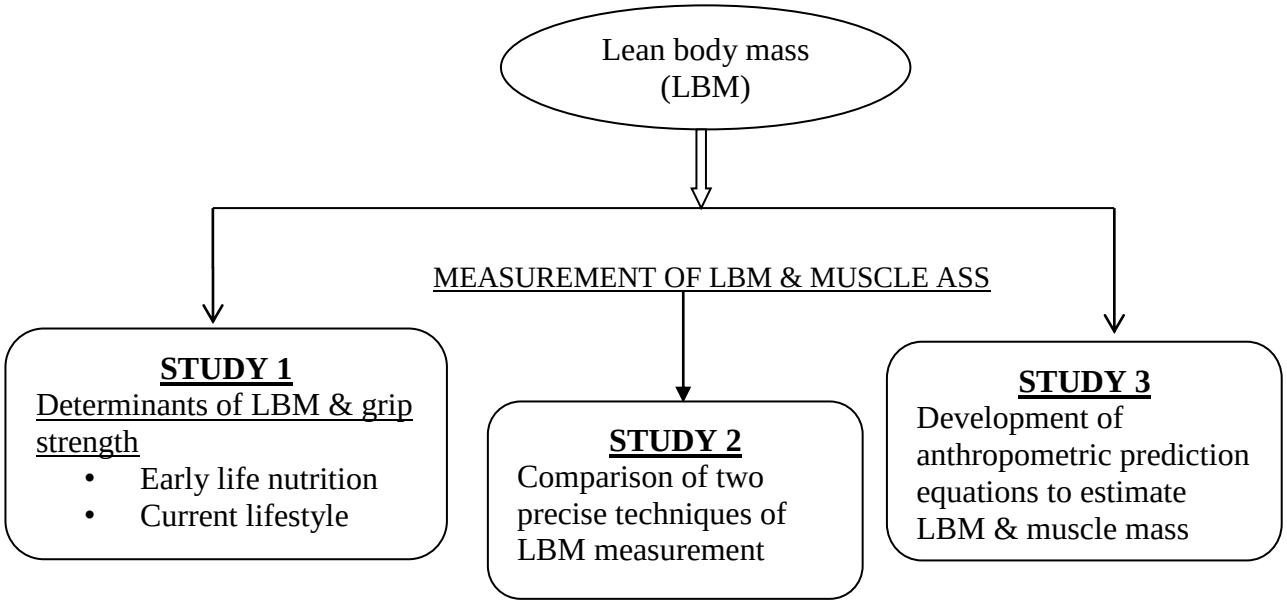
The advanced body composition assessment techniques such as DXA, computerized tomography (CT), magnetic resonance imaging (MRI), air displacement plethysmography (ADP) as well as a 4 compartment model are expensive and largely restricted to laboratory settings. Large epidemiological studies require simple, inexpensive and portable methods for assessment of body composition suitable for field settings. Anthropometric assessment of body composition fulfils these criteria and therefore anthropometry continues to be a practical tool in epidemiological studies carried out in resource poor settings.

Anthropometric methods depend on prediction equations to convert anthropometric measurements to the estimates of body composition. Commonly used prediction equations for calculating body composition estimates using precise techniques of body composition as reference have been developed in Caucasian

populations (Durnin and Womersley 1974, Jackson and Pollock 2004). Population-specific prediction equations are, however, desirable due to ethnic differences in body composition. Only one study has been reported on anthropometric equations to estimate LBM and muscle mass compartments developed in a small sample of Indian adults using 24 h creatinine excretion as a reference method (Kuriyan and Kurpad 2004). Large scale studies using advanced and precise technique of body composition assessment as a reference method are necessary to develop anthropometric equations of high predictive quality in Indians.

The third study included in this thesis therefore developed anthropometry based prediction equations to estimate LBM and appendicular skeletal muscle mass in a large sample of Indian men and women using DXA as a reference technique.

Thus the central theme of this thesis is focused on the LBM and muscle mass of Indian adults and the three studies included in the thesis contribute to improved understanding about the determinants and measurement of these important body compartments.



1.2 MAIN OBJECTIVES AND HYPOTHESES

The main objectives and hypotheses for these three studies are as follows

STUDY 1 (CHAPTER 3)

Main Objective

To examine the role of nutrition supplementation in early life as well as current lifestyle as determinants of LBM, muscle mass and muscle strength in adulthood.

Hypothesis

Nutrition supplementation in early life as well as current lifestyle including diet and physical activity are positively associated with LBM, muscle mass and muscle strength in adulthood.

STUDY 2 (CHAPTER 4)

Main Objective

To examine the agreement between the estimates of LBM and fat mass by two precise techniques: DXA and isotope dilution technique

Hypothesis

There is a good agreement between LBM and fat mass measurements using DXA and the isotope dilution technique.

STUDY 3 (CHAPTER 5)

Main Objective

To develop anthropometric prediction equations to estimate LBM and muscle mass in Indian men and women.

Hypothesis

Sex-specific anthropometric prediction equations can be developed for estimation of lean and muscle mass in Indians using DXA as a reference technique.

Chapter 2: Literature Review

2.1 Rising prevalence of adiposity-related chronic diseases

A rapid increase in obesity-related chronic diseases including type 2 diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease (CVD) has been observed in most of the countries and it is estimated that 347 million individuals are currently affected globally. The number has increased substantially since 2007 (Unwin, Gan et al. 2010) and it is predicted that the number will increase to 380 million in 2025. (Danaei, Finucane et al. 2011). Furthermore, more than 80% of diabetes deaths occur in low- and middle-income countries (Mathers and Loncar 2006). The number of people with diabetes in developing nations is expected to rise by 69 % by the year 2030 (Shaw, Sicree et al. 2010).

Asia is the world's most populated region and it is estimated that >60% of the world's population with diabetes will come from this region in 2025 (Chan, Malik et al. 2009). Unlike in the West, where older populations are most affected, the burden of diabetes in Asian countries is disproportionately high in young to middle-aged adults (Chan, Malik et al. 2009). India tops this list with the highest number of people affected with diabetes (International Diabetes Federation 2009). The prevalence of diabetes has risen rapidly in India, especially among the urban slums and among the rural poor, and the age at diagnosis of type 2 diabetes has fallen by many years (Mohan, Deepa et al. 2006). A recent large nationwide risk factor surveillance study among >10000 Indians (aged 20–69 years) indicated that two in three men and one in two women had high lifetime predicted risks for cardiovascular disease (Jeemon, Prabhakaran et al. 2011). Another multicenter study in the three regions of India - India Health Study - that assessed cardio-metabolic risk factors in

about 4000 adults in the age group of 35-69 years, indicated that across the regions, more than 80% of the participants met the criteria for abdominal adiposity and 10-28% were considered diabetic (Daniel, Prabhakaran et al. 2011). Moreover, socio-economic gradient in the disease risk in India has been changing. A higher prevalence of cardiovascular risk factors has been observed in individuals with low educational, occupational and socioeconomic status in recent studies (Gupta, Deedwania et al. 2012) in contrast to earlier reports that showed a higher risk in affluent groups(Sarvotham and Berry 1968).

In addition, Indian and other south Asian migrants to other countries are also known to have an elevated risk of diabetes and cardiovascular disease when compared to the indigenous populations of the host country (Barnett, Dixon et al. 2006, Forouhi and Sattar 2006). For example, the Southall study in London which is one of the largest studies on South Asian immigrants comparing 1711 South Asians with 1761 Europeans, indicated that age standardized prevalence of metabolic syndrome was much higher in South Asians (46.3% *vs*. 18.8%) than the Europeans (Tillin, Forouhi et al. 2005). Another study which compared the change in metabolic parameters in South Asian and European patients attending a diabetes clinic in the UK indicated that South Asians developed type 2 diabetes almost 11 years earlier than Europeans (46 years *vs*. 57 years) and at a BMI lower than their European counterparts (28.7 kg/m^2 versus 29.9 kg/m^2) (Mukhopadhyay, Forouhi et al. 2006). In addition, South Asians were found to be more predisposed to develop microvascular and macrovascular complications of diabetes than European whites (Misra and Khurana 2011).

The above evidence emphasizes the daunting challenges for prevention and treatment of obesity-related chronic diseases including diabetes in India as the costs associated with the treatment of these chronic diseases are enormous. The data from studies on Indian

immigrants indicate that certain innate factors specific to Indian and other Asian populations play a role in their higher susceptibility to chronic diseases. An understanding of the mechanisms and factors responsible for this increased susceptibility is important for planning strategies for effective prevention and treatment of these diseases.

2.2 Causes of increasing prevalence of diabetes and metabolic syndrome in India

Demographic shift, particularly population ageing, is known to be the major driver of emergence of chronic diseases like diabetes and cardiovascular disease. Diabetes is strongly associated with factors characterising less successful ageing including morbidities like ischemic heart disease, stroke, cancer and impaired physical performance. (Hodge, Flicker et al. 2013). Urbanization has been invoked as a major determinant of diabetes especially in low income countries including India as urban residents tend to have a more sedentary lifestyle than people living in rural agricultural zones (Ramachandran, Mary et al. 2008). Migration from rural to urban areas was also seen to be associated with increases in obesity, which drives changes in other risk factors (Ebrahim, Kinra et al. 2010). Rapid nutrition transition as a result of globalization and urbanization in many developing countries, including India, is associated with marked changes in the diet and physical activity patterns that promote obesity. Studies in India have also demonstrated that physical activity and dietary intake patterns influence the risk of cardio-metabolic disorders (Mohan, Gokulakrishnan et al. 2005, Radhika, Van Dam et al. 2009, Daniel, Prabhakaran et al. 2011). A population based epidemiological study from North India which assessed the cardiovascular risk factors in more than 2000 participants showed that although the prevalence of cardiovascular risk factors (smoking, hypertension, dyslipidemias, diabetes and metabolic syndrome) was low in adolescents, rapid escalation of these risk factors by age of 30-39 years was noted (Gupta,

Misra et al. 2009). These findings suggest need for behavior change interventions focused at young adults.

It has been suggested that higher susceptibility of Indians to diabetes and cardiovascular disease can be partly explained by genetic factors (Radha, Vimaleswaran et al. 2006) in addition to the environmental factors described above. However, genetic factors cannot explain the rapid increase in the prevalence of these diseases in the past three decades which strongly suggests the role of environmental factors.

2.3 Relationship between Body mass index (BMI) and risk of chronic diseases

Overweight and obesity are simply defined as excessive or abnormal accumulation of body fat. At the population level, the BMI is commonly used as the measure of relative body fatness because the BMI is simple and easy to measure. Early evaluation of weight-height indices as measures of adiposity (Keys, Fidanza et al. 1972), found that the BMI had the highest correlation with percent body fat as measured by skin-fold thickness and hydrodensitometry. BMI has been widely used for a number of decades to monitor both forms of malnutrition (undernutrition and overnutrition), and WHO has identified cut-off points to classify underweight, healthy weight, overweight and obesity based on BMI criteria (WHO 2000). WHO has also identified separate public health action points of BMI indicating moderate, high and very high health risks for Asians as consistent evidence has demonstrated that Asians tend to have an increased risk of chronic diseases at a given BMI compared to other ethnic groups (WHO 2004).

Despite the fact that BMI cannot distinguish between fat mass and LBM, it is often used in epidemiological studies as an indicator of adiposity because more precise techniques of body composition assessment may not be feasible in such settings. Many epidemiological studies have analysed the complex association between obesity, chronic diseases and survival using BMI as an indicator of obesity. For example, a meta-analysis that analysed data on about 900,000 participants from prospective studies, confirmed that obesity, as measured by higher BMI, was associated with increased mortality mainly due to cardiovascular disease, diabetes, stroke and liver disease, in both men and women (Lewington, MacMahon et al. 2009). The relationship between BMI and cardiovascular disease mortality in populations with lower prevalence of obesity, however, appears to be J-shaped. A 10-year prospective study in 220,000 Chinese men indicated that above BMI of 20 kg/m^2, there was a positive association between BMI and the risk of cardiovascular disease mortality but below this BMI range, the association appeared to be reversed (Chen, Yang et al. 2006).

2.4 Ethnic differences in chronic disease risk

A number of studies from industrialized countries have reported major differences in the patterns of chronic disease prevalence in different ethnic groups sharing similar environment. For instance, South Asians in the United Kingdom are known to have an increased risk of type 2 diabetes and cardiovascular disease (Forouhi, Sattar et al. 2006, Narasimhan, McKay et al. 2012). Although it has been postulated that higher rates of infection may contribute to the higher risk of coronary artery disease in the South Asians, current evidence for this hypothesis is not convincing (Stefler, Bhopal et al. 2012).

Ethnic differences are likely to be of very complex aetiology as ethnicity incorporates a wide range of attributes including genetic, phenotypic, physiological, behavioural and lifestyle differences. Ethnic differences in body composition have been considered as one of the factors contributing to ethnic differences in chronic disease risk. Focusing on body composition, therefore, represents a valuable way to integrate the multiple levels of biological factors affecting cardio-metabolic risk. (Wells 2012). In addition, body composition variability is strongly associated with the cardio-metabolic risk across ethnic groups. Although studies assessing the ethnic variations in body composition have largely focussed on the fat mass, variability in lean mass and its components may be an important determinant of disease risk. A number of studies have shown that South Asians have reduced muscle mass compared with Europeans (Rush, Goedecke et al. 2007,Lear, Kohli et al. 2009, Stanfield, Wells et al. 2012).

2.5 Ethnic differences in BMI and body composition relationship

Though simple to measure, BMI is an imperfect measure of obesity because it does not differentiate between fat mass and fat-free mass. A large number of studies have shown that major ethnic differences exist between the BMI-body fat relationship and BMI systematically underestimates percent body fat in South Asians, including Indians (Deurenberg, Deurenberg Yap et al. 2002, WHO 2004, Kagawa, Binns et al. 2007, Kulkarni, Shatrugna et al. 2010). For example, Deurenberg et al. assessed the BMI-body fat relationship in 291 adults from three different ethnic groups in Singapore (Chinese, Malays and Indians). The results indicated that for a given BMI, Asians had significantly higher body fat percent (%BF) than Caucasians. Among the three Asian groups, for the same BMI,

Indians had the highest and Chinese had the lowest %BF (Deurenberg-Yap, Schmidt et al. 2000).

A study from Delhi, India assessed the body composition of 123 healthy volunteers (86 males aged 18–75 years and 37 females aged 20–69 years) using skinfold thickness measurements showed that BMI did not accurately predict overweight in this group. BMI cut-offs of 21.5 and 19.0 were associated with a %BF of 25 and 30, in males and females, respectively (Dudeja, Misra et al. 2001). Another study in low-income women from Hyderabad, India indicated that at a BMI of 20 and 21, the participants had %BF of 30 and 35, respectively (Kulkarni, Shatrugna et al. 2010). In Caucasian populations, the BMI of 30 corresponds with a %BF of over 25% in young adult males and 35% in young adult females (Romero-Corral, Somers et al. 2008).Moreover, a study from Canada that estimated the abdominal adipose tissue (subcutaneous and visceral) in 822 participants - Aboriginal, Chinese, European, and South Asian - aged between 30 and 65 years, showed that BMI significantly underestimated visceral abdominal fat in South Asian and Chinese participants than the Europeans (Lear, Humphries et al. 2007).

Recognising the fact that Asians have a different relationship between BMI, body fat and health risks than Europeans, a WHO expert consultation identified potential public health action points indicating moderate, high and very high health risks, respectively (23·0, 27·5 and 32·5) along the BMI continuum (WHO 2004). However, the consultation did not redefine the cut-off points of BMI for specific Asian sub-populations because the evidence was thought to be inadequate. The ethnic differences in body composition are considered to be one of the important reasons for the higher susceptibility to the chronic diseases among Indians and other Asians.

2.5.1Partitioning of BMI into fat mass index and fat-free mass index

As BMI cannot differentiate between fat and fat-free mass, Van Itallie et al. proposed that BMI can be partitioned into two subcomponents namely fat mass index (FMI) and fat-free mass index (FFMI) (VanItallie, Yang et al. 1990). FFMI and FMI are the ratios of fat-free mass and fat mass with a common denominator of height (in m^2).

Thus,

$$BMI\ (kg/m^2) = FFMI\ (kg/m^2) + FMI\ (kg/m^2)$$

Therefore,

$$FFMI = (BMI\text{-}FMI) \text{ and } FMI = (BMI - FFMI)$$

A perfect inverse relationship thus exists between the values of FMI and FFMI at a constant BMI. Both indices are useful for comparison of body composition of individuals who differ in height and allow differentiation of overweight due to higher FM, FFM or both combined.

FFMI and FMI percentiles have been developed in European Caucasians (Schutz, Kyle et al. 2002) but data on other population groups are not available. Racial and ethnic differences in the FFMI, however, have been documented. A recent study investigating body composition of 1339 healthy adults using DXA indicated that FFMI differed among the four ethnic groups (Caucasians, Asians, Hispanics, African-Americans) with the highest values in African Americans and the least in Asians (Hull, Thornton et al. 2011). Further studies are needed to identify the interracial differences in FFMI in relation to disease risk.

Although partitioning of the BMI into FMI and FFMI improves the understanding of the BMI-disease risk relationship, it does not adequately characterize these body compartments because both fat and fat-free mass have a heterogeneous composition. Fat mass distribution at different anatomical sites and composition of fat-free mass have important influences on the disease risk though these relationships are not well understood at present.

2.5.2 Difference between lean body mass and fat free mass

Fat free mass represent body mass devoid of all extractable fat whereas lean body mass includes essential fat (located chiefly within the central nervous system, bone marrow and internal organs) in addition to fat free mass (McArdle, Katch et al. 2010). FFM is considered to be an in vitro entity appropriate for carcass analysis whereas LBM is considered as an in vivo entity relatively constant in water, organic matter and mineral content (Behnke 1963). In normally hydrated healthy adults, FFM and LBM differ only in the essential fat component. The most widely used body composition assessment methods using two compartment models partition the body into fat mass and fat-free mass.

2.5.3 Components of lean body mass

Over the past few years, imaging techniques like CT and MRI scanning have enabled mapping and quantification of various tissues and organs that constitute LBM. LBM is the metabolically active compartment of the body and the principal contributor to the resting energy expenditure (REE). However, it is a heterogeneous compartment containing organs / tissues with a wide range of specific metabolic rates. For example, skeletal muscle which

constitutes 40-50% of total body weight, accounts for only 20-30% of REE whereas organs like the brain, liver, heart and kidneys collectively contribute to <6% of total body weight, but account for about 60-70% of REE in adults (Gallagher, Belmonte et al. 1998). Small differences in the organ masses and activities can have a significant impact on the inter-individual variability in energy expenditure and thus may be relevant for metabolic predisposition to leanness or fatness(Heymsfield, Thomas et al. 2012). Ethnic differences in the organ masses have also been noted. For example, African Americans were found to have a significantly smaller proportion of LBM contributed by high metabolic rate organs than do whites (Gallagher, Albu et al. 2006). The authors suggested that this helps explain findings of lower REE, adjusted for LBM in African Americans than in Whites. Studies in other ethnic groups have not been reported yet and future studies in this area will add considerably to the understanding of metabolic susceptibility to obesity in certain ethnic groups.

2.6 Early life influences on adult health and body composition

Early life environment, in addition to the adult lifestyle, is believed to have a profound and permanent effect on body composition and disease risk. The concept that experiences in early life may have a lasting influence on health and well being in adult life has been suspected for a long time, the work of Barker and colleagues rekindled interest in the area of the 'foetal origins of disease' (FOAD) hypothesis (Barker 1990). The term FOAD was subsequently replaced by 'developmental origins of health and disease' (DOHaD) because later research showed that apart from foetal life, events in infancy also influence the later disease because the 'plastic' phase of development continues beyond foetal life. Another reason for this change was that the DOHaD terminology emphasizes that the developmental

period has implications not only for disease and its prevention, but also for health promotion (Gluckman and Hanson 2006).

A number of studies carried out by Professor Barker's group showed an inverse association between birth weight and risk factors such as adiposity, blood pressure, insulin resistance, metabolic syndrome, as well as cardiovascular mortality (Barker, Hales et al. 1993, Barker 2002). Initially, these findings were received with considerable scepticism (Wilson 1999), however a number of studies in different settings later confirmed that impaired growth in early life is associated with higher risk of later cardiovascular disease and diabetes or impaired glucose tolerance (Bavdekar, Yajnik et al. 1999, Eriksson and Forsén 2002).

An important observation that has been consistently reported by the majority of these studies is that the association between low birth weight and disease risk in adulthood emerges most strongly after adjustment for the current weight or BMI. Some researchers have, therefore, argued that the change in size between birth and adulthood may be the key predictor of disease (Lucas, Fewtrell et al. 1999) rather than the low birth weight. Studies have shown that catch up growth in low birth weight babies is associated with higher fat mass during adulthood (Walker, Gaskin et al. 2002, Dulloo, Jacquet et al. 2006). It has been postulated that the LBM represents 'metabolic capacity' and fat mass represents 'metabolic load'. The relative proportions of these two tissue compartments across population groups could, therefore, be a more important determinant of chronic disease risk (Wells 2007) (Figure 2.1).

As adult BMI is a composite index of fat and lean mass, it is difficult to interpret the role of adult BMI in the causation of disease. Recent studies that have assessed body composition are therefore important to improve the understanding of early life programming of the metabolic syndrome.

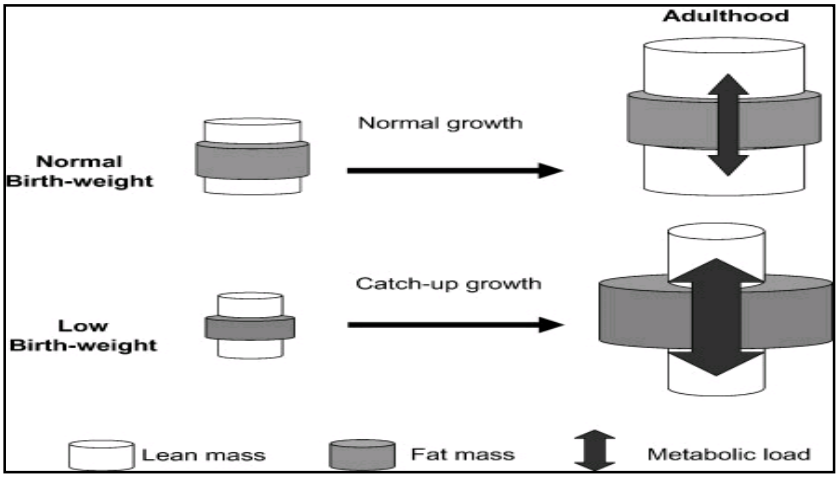


Figure 2.1.Schematic diagram showing pathways in which child growth can influence metabolic risk.

(adapted from (Wells 2007)

2.6.1 Relationship between birth weight and adult body composition

2.6.1.1 Evidence from the developed countries

A number of studies have assessed the relationship of early life influences on the adult BMI and body composition using birth weight as a proxy for foetal nutritional status. The evidence is dominated by studies from developed nations as most of these studies have used historical records of body size measurements at birth and during infancy and such records are extremely rare in developing countries. A number of anthropometric measures including

weight, BMI, waist circumference, waist: hip ratio, skin-fold thickness etc have been reported along with body composition measures.

For example, a study assessing the relationship of early life nutritional status with the body composition in older men in the Hertfordshire cohort indicated that birth weight was significantly and consistently positively associated with adult BMI and LBM but not with adult fat mass (Aihie Sayer, Syddall et al. 2004). Another UK study corroborated these findings and examined the relationship between birth weight and muscle mass (estimated from creatinine excretion in a timed overnight urine collection) in 217 men and women born in Preston in 1935-43. Birth weight category was positively associated with muscle mass but not with non-muscle mass (Phillips 1995). A Finnish study which assessed the relationship of birth weight with adult body composition in 928 men and 1075 women born in 1934–1944, with measurements at birth recorded, indicated that low birth weight was associated with lower LBM and grip strength (Yliharsila, Kajantie et al. 2007). Abdominal obesity, however, was not predicted by birth weight in this study. Another study from the USA assessed the birth weight and body composition relationship in 192 men from the military service by measuring their thigh circumference and anterior skin-fold thickness at the mid-thigh to estimate thigh muscle plus bone area and subcutaneous fat area (Kahn, Narayan et al. 2000). Birth weight had a significant positive association with thigh muscle plus bone area, but not the thigh subcutaneous fat area.

In general, most studies have indicated a significant positive association between birth weight and LBM. In contrast, the findings have been less consistent for body fat and fat distribution and negative, positive and non-significant associations with birth weight have been reported. For example, a case-control study in men born in Hertfordshire compared the

body composition and fat distribution of men (n=32; age 64-72 years) with a low (mean 2.76 kg) or high (mean 4.23 kg) birth weight (Kensara, Wootton et al. 2005). After adjustment for weight and height, the low-birth-weight group had a higher percentage body fat (29.3% compared with 25.3%; P = 0.029) and fat mass (24.49 kg compared with 21.67 kg; P = 0.039) but a lower fat-free soft tissue (56.32 kg compared with 59.22 kg; P = 0.024), muscle mass (27.25 compared with 29.22 kg; P = 0.022), and muscle-to-fat ratio. On the other hand, a study in 91 adults in the age group 34-56 y from the USA did not find any association between birth weight and visceral fat (measured using CT) after adjustment for age, sex, ethnicity, BMI, or smoking status (McNeely, Fujimoto et al. 2007).Similarly, a study in 587 women participating in the Michigan Bone Health and Metabolism Study which examined the relationship of birth weight to longitudinal changes in adult body composition, did not find any association (Rillamas-Sun, Sowers et al. 2012).

One of the major criticisms of the DOHaD hypothesis is that a number of factors related to adult lifestyle as well as genetic factors confound the relationship between birth weight and later body composition and statistical models cannot completely control for these confounding factors. Evidence based on twin studies is therefore valuable as twins share genetic and environmental exposures and confounding by these factors is largely taken care of. A Belgian study examined the association between birth weight and adult body composition in 447 female and 229 male twin pairs in the age group of 18-34 y (Loos, Beunen et al. 2001, Loos, Beunen et al. 2002). The results indicated that twins with higher birth weight were taller and slightly heavier as adults than the twins with lower birth weight. The heavier twins also had more lean body mass and less subcutaneous and abdominal fat. Similarly, a large study from New Zealand which investigated the relation between birth weight and body composition using DXA in 2228 dizygotic and 842 monozygotic

female twins aged between 18 and 80 y showed that a higher birth weight was associated with a higher proportion of lean to fat mass as adults (Skidmore, Cassidy et al. 2009). However, this association was determined by shared common environment of the twins rather than by fetal nutrition.

Most of the earlier studies used anthropometry for the assessment of body composition. Later studies using advanced techniques such as DXA and the 4-compartment model for the measurement of body composition, have confirmed the findings of the above mentioned studies. For example, a study from the UK assessing the relationship of birth weight with the LBM using DXA in adolescents, supported the hypothesis that foetal growth measured by birth weight, programs LBM in later life. The authors further speculated that these observations partially explain the paradox that though the low birth weight is associated with a lower risk of obesity as measured by BMI, it increases the susceptibility to the cardiovascular disease (Singhal, Wells et al. 2003). Another prospective study from the UK confirmed the association between birth weight and LBM in children and adolescents aged 4-20 y (Chomtho, Wells et al. 2008). The study assessed the body composition using the 4-compartment model in 391 healthy participants and indicated that each 1 SD increase in birth weight was associated with 0.18 SD increase in LBM in boys. These associations were independent of puberty, physical activity, social class, ethnicity, and parental BMI. Birth weight, however, was not significantly related to fat mass in both boys and girls.

2.6.1.2 Evidence from the developing countries including India

The investigation of the developmental origins hypothesis is highly relevant for India and other developing countries due to the high prevalence of under nutrition in young

children and rapid nutrition transition resulting in increasing rates of obesity in adults. However, a few studies exploring the role of early life influences on body composition in adulthood have been reported to date because of the prolonged follow-up required to investigate these associations. The studies, in general, support the DOHaD hypothesis. Table 2.1 presents the studies reported from the developing countries.

A study by Yajnik et al. carried out detailed anthropometric measurements in 631 term babies from Pune, India and compared them with 338 term babies from Southampton, UK. The Indian babies had significantly lower birth weight compared with the British babies (mean: 2.7 *vs*. 3.5 kg) and were small in all the other body measurements including length, head, mid-upper-arm and abdominal circumferences, subscapular and triceps skin-fold thicknesses, as well as placental weight. The largest deficit was observed in abdominal and mid-arm circumferences while the smallest deficit was in subscapular skin-fold thickness, indicating low muscle and visceral mass and relatively high adiposity. They speculated that the poor muscle and visceral mass and higher adiposity of the Indian babies may persist in later life and predispose them to an insulin-resistant state (Yajnik, Fall et al. 2003). Another study from the same centre, which assessed the body composition and cardiovascular risk in relation to their birth size, in 698 children at 6 years, indicated that larger size at birth and faster growth in all body measurements from birth to 6 years predicted higher LBM and fat mass at 6 years and these associations were stronger with LBM than with fat mass (Joglekar, Fall et al. 2007).

Another study from Mysore, India, assessed body composition of 663 children at birth, 1year and 4years and compared it with equal number of British children from Southampton. The study confirmed the ‘thin-fat’ phenotype in the Indian newborns. At four

years of age, the sub-scapular skin-fold thickness in Indian children was larger than the UK and Dutch standards indicating higher central adiposity in Indian children despite all other body measurements remaining smaller (Krishnaveni, Hill et al. 2005). The high fat phenotype, however, could not be confirmed in a study from Bangalore, India, that assessed body composition of 408 newborns using anthropometry (Muthayya, Dwarkanath et al. 2006). The study indicated that skin-fold thickness at various sites in Indian babies was similar to those reported in a Western population with comparable birth weights. Although the reasons for the discrepancy of the findings of the above studies are not clear, the authors argued that the fact that the earlier studies made no adjustments for the gestational age which may provide a partial explanation.

A population-based cohort study from Delhi, which examined the anthropometric indices of adult body composition in relation to birth size and BMI during childhood in 1526 men and women aged 26–32 years, indicated that the birth weight and BMI gain in early childhood were correlated positively with adult LBM whereas higher BMI and greater BMI gain in late childhood and adolescence were associated with increased central adiposity in adulthood (Sachdev, Fall et al. 2005).

Table 2.1 Studies from developing countries assessing the relationship of birth and childhood growth measurements with body composition at a later age

No.	Country (city)	Birth Measurements	Frequency of growth measurement	Body composition assessment method	N	Age	Relation between birth wt, childhood growth & body composition	Reference
1	India (Pune)	Birth weight & length	Every 6 mo	DXA, Anthropometry	698	6 y	LBM++ FM +	Joglekar CV, et al. (2007)
2	India (New Delhi)	Birth weight & length	To 1 y: every 3 mo 1-6 y : every 6 mo	Anthropometry	1526 men & women	26-32 y	BMI gain in infancy: LBM+ BMI gain in later childhood: FM+	Sachdev HS, et al. (2005)
3	India (Mysore)	Birth weight, length, arm muscle area (AMA)	Growth annually to 5 y 6 monthly to 9.5 y	Anthropometry Muscle strength by dynamometer	275 boys 299 girls	9.5 y	Birth size: related to AMA at 9.5 y + Birth size and AMA at birth: related to grip strength at 9.5 y +	Krishnaveni GV, et al. (2010) Barr JG, et al. (2010)
4	India (Vellore)	Birth weight	--	DXA	61 LBW & 56 NBW men	20 y	LBW: related to LBM -	Thomas N, et al. (2012)
5	The Philippines (Cebu)	Birth weight	Weight velocities measured every 6 mo till 24 mo	Anthropometry Muscle strength by dynamometer	770 men	About 21 y	Birth weight and early weight gain: related to LBM+ , muscle area +, muscle strength+	Kuzawa CW,, et al. (2010).

6	Brazil (Pelotas)	Birth weight & length	Weight and length at 6 mo, 1 and 4 y	BIA	172 boys	9 y	Birth wt: related to LBM+, Growth after 1 yr: related to LBM+ and FM+	Wells JC, et al. (2005).
7	Brazil (Pelotas) (5 y follow up of cohort no. 6)	Birth weight	Growth during 0-6, 6-12 and 12-48 months	Isotope dilution technique	222 males and 203 females	14 y	Weight gain in infancy: related to LBM+ in males and FM+ in females. After 1 yr: weight gain related to FM+ and LBM+ in both sexes	Wells JC, et al. (2012)
8	Brazil (Pelotas)	Birth weight	HAZ, WAZ, WHZ at 2 & 4 y of age	BIA	132 LBW, 2119 NBW men	About 18 y	HAZ, WAZ, WHZ at 2 and 4 y: related to lean mass index + and fat mass index + at 18 y	Gigante DP, et al. (2007)
9	Korea (Seoul)	SGA	Growth in childhood	Anthropometry	46 boys and 53 girls	Pubertal	Catch up in height :related to skeletal muscle mass + ; Catch up in weight- related to FM+	Ko JM, Park HK, Yang S, Hwang IT (2012)
10	Cameroon (Yaounde)	Birth weight from records	Cross sectional anthropometry in childhood	Anthropometry BIA	162 boys and girls	4 y	Birth weight: related to LBM +(indirect) through association with REE	Said-Mohamed R (2012)
11	South Africa	Birth weight	Weight at 12, 24 & 60 mo	DXA	160 boys and 142 girls	15.5 y	Early growth: related to LBM + (weak)	Kuzawa CW, et al. (2012)

12	Guatemala	Birth weight and length	Weight and height at 1,3, 5 and 7 y in sub-samples	Anthropometry	358 women and 352 men	32.7 y	Weight and length at birth: related to LBM+; BMI gain in later childhood – related to FM+	Corvalan C, et al. (2007)

LBM: Lean body mass; FM: Fat mass; DXA: Dual energy X-ray absorptiometry; BIA: Bioelectrical impedance; LBW: low birth weight; NBW: normal birth weight; SGA: small for gestational age
+ indicates positive relationship; - indicates negative relationship

Studies from other developing countries also support these findings. For example, a study from the Philippines which assessed the relation between birth weight and growth in the first 2 years of life with body composition in 770 young men, showed that birth weight as well as early weight gain were positively related to LBM, muscle mass and muscle strength at the time of follow up (Kuzawa, McDade et al. 2010). Similarly, another study from Brazil showed a positive relationship between birth weight and LBM assessed using BIA in 9 year old boys (n = 172) (Wells, Hallal et al. 2005). A similar study from South Africa assessing these relationships in 15 year old boys and girls, however, showed a weak association of birth weight and early childhood growth with LBM estimated using DXA (Corvalán, Gregory et al. 2007).

2.6.2 Relationship between birth weight and muscle strength

A number of factors such as age, sex, body size, physical activity etc. influence muscle strength but these do not fully account for the variation in muscle strength between individuals. Evidence based on a number of observational epidemiological studies suggests a positive association between birth weight and handgrip strength, even after adjustment for potential modifying and confounding factors mentioned above (Kuh, Hardy et al. 2006, Inskip, Godfrey et al. 2007, Ylihärsilä, Kajantie et al. 2007, Sayer, Syddall et al. 2008). A recent meta-analysis which included 20,481 participants from 13 studies (mean ages 9.3 to 67.5 y) showed a 0.86 kg (95% CI: 0.58, 1.15) increase in muscle strength per additional kilogram of birth weight, after adjustment for age, sex and height (Dodds, Denison et al. 2012). Table 2.2 shows studies reported on the relationship between birth weight and muscle strength in later life.

A study from the US which included participants from the East Flanders Prospective Twin Survey used a twin pair approach, using both 'individual' data and 'within-pair' differences, to investigate the influence of birth weight on hand grip strength. The study also examined whether this association may be mediated through LBM. Analyses showed that the birth weight was positively associated with hand grip strength (β = 2.60 kg, 95% CI 1.52, 3.67, $p<0.001$) and LBM (β = 4.2, 95% CI 3.16, 5.24, $p<0.001$), adjusted for gestational age, sex and adult age. However, the association between birth weight and grip strength was attenuated following adjustment for LBM indicating that the association was mediated by LBM (Ridgway, Sharp et al. 2011).

Table 2.2 Studies assessing the relationship between birth weight and muscle strength in later life

No	Study	Location	No. of participants	Age	Relation between birth weight and grip strength
1	(Sayer, Cooper et al. 1998)	UK	411 M , 306 F	67.5 (63-73)	M: 2.03 (0.69,3.36), p<0.01 F: 1.16 (-0.07,2.38), p=0.06
2	(Sayer, Syddall et al. 2004)	UK	730 M, 673 F	65.0 (59-71)	M: 2.42 (1.49,3.35), p<0.001 F: 1.73 (0.90,2.56), p<0.001
3	(Yliharsila, Kajantie et al. 2007)	Finland	928 M, 1075 F	61.5 (56-69)	M: 1.84 (0.62,3.06), p<0.01 F: 1.79 (0.94,2.64), p<0.001
4	(Kuh, Bassey et al. 2002)	UK	1371 M, 1404 F	53	M: 3.05 (1.90,4.21),p<0.001 F: 2.00 (1.18,2.82), p<0.001
5	(Inskip, Godfrey et al. 2007)	UK	1562 F	30.6 (20-40)	2.16 (1.62,2.70), p<0.001
6	(Saigal, Stoskopf et al. 2007)	Canada	104 ELBW (45M,59F) 125 NBW (59M,66F)	23	Mean difference (pooled estimate) ELBW – NBW: -6.4kg (95%CI: -9.1,-3.7), p<0.001
7	(Rogers, Fay et al. 2005)	Canada	53 ELBW (17M,36F) 31 controls (17M,14F)	17(16-19)	ELBW *vs* controls: 72.5 vs 93.2 for males 51.6 *vs* 54.8 for females, p<0.001
8	(Martorell, Ramakrishnan et al. 1998)	Guatemala	39 IUGR (20M,19F) 292 controls (149M,143F)	15	Mean differences for IUGR *vs* controls, adjusted for age and gestational age: M: -2.65(SE: 1.56), p=0.09 F : -4.24 (SE: 1.32), p=0.02

9	(Barr, Veena et al. 2010)	India	M/F 574	9.3	0.40 (95%CI: 0.02, 0.05) kg per SD increase in birth weight, p<0.001
10	(Kuzawa, McDade et al. 2010)	The Philippines	M 770 F 655	20.9	M: 1.66 (0.03, 3.28),p =0.046 F : 0.09 (−1.19, 1.39), p= 0.880
11	(Ridgway, Sharp et al. 2011)	UK	M/F 783	25.6	2.60 (95%CI: 1.52, 3.67) kg per birth weight SD score, p<0.001
12	(Ridgway, Ong et al. 2009)	Finland	M/F 4273	31.0	0.080 kg per birth weight SD score, p < 0.001
13	(te Velde, Twisk et al. 2004)	The Netherlands	M/F 273	36.6	-0.02 (-2.55, 2.51)
14	(Robinson, Jameson et al. 2008)	The UK	M/F 2983	66.2	M:2.06 (1.38, 2.74) F :1.50 (0.91, 2.10)
16	Ortega, Labayen et al. 2009)	Spain	M 818 F 983	15.4	M:1.152 (0.278, 2.025), p <0.01 F : 1.018 (0.505, 1.532) p<0.001
17	(Moura-Dos-Santos, Wellington-Barros et al. 2013)	Brazil	M/F 356	8.8 y	NBW 14.0 , LBW 12.9; p= 0.001

Unless stated otherwise, the values are β coefficients (95% CI) indicating change in grip strength in Kg per Kg difference in birth weight.

After adjustment for age and height

Adapted from (Sayer, Syddall et al. 2008) and (Dodds, Denison et al. 2012)

2.6.3 Relationship between early nutrition and muscle morphology

Animal studies have shown that an adverse intrauterine environment can affect muscle histology(Rehfeldt and Kuhn 2006, Costello, Rowlerson et al. 2008). Muscle fiber numbers, size, and metabolic phenotypes develop at distinct fetal stages and thus these aspects of muscle formation and growth are affected differently depending upon the timing of the nutritional insult during gestation (Yates, Macko et al. 2012). Nutritional insults during early or mid-gestation are known to interfere with myotube formation and reduce fiber density in skeletal muscle as indicated by a study in sheep (Zhu, Ford et al. 2004). In addition, fetal developmental adaptations reduce muscle oxidative metabolism as indicated by histological measurements which revealed a smaller proportion of oxidative-to-glycolytic muscle fibers in some skeletal muscles(Costello, Rowlerson et al. 2008).

Only a few studies on the relationship between early under nutrition and skeletal muscle morphology in humans have been reported till date which have shown that low birth weight is associated with altered skeletal muscle morphology and and decreased oxidative capacity (Jensen, Storgaard et al. 2007, Patel, Jameson et al. 2011).

2.6.4 Early life influences on muscle mass and strength: possible mechanisms

Studies in animal models provide valuable evidence on the developmental plasticity of the muscle. Muscle fibre number is a critical determinant of muscle mass and strength. Impaired myogenesis resulting in low skeletal myofibre numbers is considered as one of the main reasons for negative long-term consequences of intrauterine growth retardation (Rehfeldt, Te Pas et al. 2011). A number of other studies have also shown that prenatal maternal diet restriction is associated with reduced neonatal muscle weight in sheep

(Greenwood, Hunt et al. 2000) as well as a reduction in postnatal muscle fibre number in the pig (Oksbjerg, Nissen et al. 2013). Moreover, experimental nutritional restriction during pregnancy in sheep resulted in altered offspring muscle mass and composition along with increased lipid accumulation and reduced oxidative capacity in muscle (Zhu, Ford et al. 2004). A study in mice also showed that prenatal under nutrition was associated with reduced frequency of myogenic stem cells (Woo, Isganaitis et al. 2011).

Other line of evidence is provided by in vitro studies which showed that maternal under nutrition during peri-implantation and late gestation periods affected the slow-twitch myofibre and capillary density in the fetal triceps (Costello, Rowlerson et al. 2008). This reduction in the fibre density was associated with higher insulin receptor, glucose transporter GLUT-4 and IGF-1receptor mRNA levels. It was postulated that these findings reflect redistribution of resources at the expense of specific peripheral tissues which may be mediated by a decrease in capillary density.

Fetal under nutrition is associated with fetal hypoxia and hypoglycaemia which are in turn associated with increase in circulating nor-epinephrine and epinephrine along with decrease in glucose-stimulated insulin secretion (Harwell, Padbury et al. 1990). As a result, endocrine and metabolic adaptations develop to conserve fetal nutrients by lowering skeletal muscle energy requirements for protein synthesis and growth (Regnault, de Vrijer et al. 2007). Nutritional insults during early or mid-gestation interfere with myotube formation and reduce fiber density in skeletal muscle (Yates, Macko et al. 2012). In addition, fetal developmental adaptations reduce muscle oxidative metabolism (Quigley, Kleemann et al. 2005). There is thus multifaceted defect in skeletal muscle development in the growth

retarded fetuses, which manifests in lowered myonuclei content, altered fiber phenotypes and impaired metabolic regulation.

Fetal adaptations to IUGR such as increased peripheral glucose and insulin sensitivity, decreased β-cell mass and insulin secretion, diminished skeletal muscle cell number and capacity for net protein synthesis are likely to persist into postnatal life and into late childhood and adulthood. These adaptations, especially with increased energy intake during later life increase the risk of obesity and type 2 diabetes during adulthood in IUGR infants (Thorn, Rozance et al. 2011). Increased insulin sensitivity predisposes the IUGR infant to have abnormally increased rates of fatty acid deposition, leading to increased risk of obesity and insulin resistance. In addition, diminution of myocyte number could reduce whole body insulin action.

2.6.5 Postnatal catch-up growth and adult body composition

As mentioned earlier, the majority of the studies indicate that the association between birth weight and body composition is dependent on adjustment for the adult BMI. A number of researchers have, therefore, argued that the appropriate interpretation of these findings is that the growth patterns between birth and follow-up are responsible for this relationship rather than the low birth weight itself. This is consistent with the observations that, for many aspects of the metabolic syndrome, greatest risk is observed in those who were born small and had higher weight gain subsequently (Bavdekar, Yajnik et al. 1999, Adair and Cole 2003).

Finnish birth cohort studies have significantly contributed to the understanding of the interrelationships between size at birth, childhood growth and adult health outcomes (Eriksson and Forsén 2002). The study included 13,345 men and women born in the Helsinki during 1934–1944 who had their height and weight measurements recorded from birth till 7 years. The results indicated that a pattern of slow growth during foetal life and infancy followed by increase in BMI thereafter was associated with coronary heart disease and type 2 diabetes (Barker, Osmond et al. 2009). A study in a subset of participants from the Helsinki cohort indicated that the increase in the risk of cardiovascular disease may be mediated by deposition of body fat with rapid childhood growth. This study included 885 men and 1032 women aged 56-70 years and examined the relationship of the change in BMI in childhood with adult body composition using bioelectrical impedance analysis (BIA) (Ylihärsilä, Kajantie et al. 2008). It was observed that increase in BMI from birth to 1 year was positively associated with the LBM whereas the increase in BMI between 2 and 11 y of age was positively associated with fat mass during adulthood. Studies from the Netherlands and Sweden corroborate these observations. The Netherlands (PROGRAM) study assessed the relationship of size at birth and weight gain during childhood with body composition, using DXA, in a cohort of 312 young adults (Leunissen, Stijnen et al. 2009). The study showed that weight gain during childhood was related positively to the fat mass and negatively to the LBM. Another study from Sweden in 248 participants, whose height and weight were measured at birth, 6 mo, and 3 and 6 y, examined the relationship of childhood growth with body composition at 17 years of age using air-displacement plethysmography (Ekelund, Ong et al. 2006). The results indicated that rapid weight gain during childhood was associated with larger BMI, fat mass, and waist circumference at 17 years.

Studies from non-Western populations also indicate similar findings. For example, a study in 172 Brazilian boys who were followed up since birth and had measurements of weight and height recorded at birth, 6 months, 1 and 4 years assessed the relationship of childhood growth with body composition measured by BIA at 9 years (Wells, Hallal et al. 2005). The relationship of fat mass and LBM at 9 years varied with the growth during the discrete time points. Weight gain during 0–6 months was positively associated with later height and LBM, but not with fatness. Weight gain during 1–4 years was positively associated with both LBM and fat mass at 9 years whereas weight gain during 4–9 years was strongly positively associated with fatness but not LBM at 9 years. Another study in Guatemala assessed body composition of 358 women and 352 men (mean age 32.7 years) using anthropometry, whose weight and height was measured at birth and then periodically during first 7 years (Corvalán, Gregory et al. 2007). BMI at birth and increases in length prior to the age of 3 years were most strongly associated with increases in LBM whereas increases in BMI between 3 and 7 years had stronger (positive) associations with adult fat mass and abdominal fat than the LBM. These observations are in line with the findings of the New Delhi birth cohort study mentioned earlier (Sachdev, Fall et al. 2005). A recent study which reported an estimate with pooled data from five well characterized birth cohorts from low- and middle-income countries also confirmed the positive association of early growth with LBM during adulthood (Kuzawa, Hallal et al. 2012)

2.6.6 Birth weight: an inadequate measure of early nutrition

Majority of the studies assessing the relationship of early under nutrition and later disease risk have used birth weight or weight during childhood as a proxy for nutritional status. However, the major criticism of these studies is that birth weight does not completely

capture the degree of nutritional adversity and may not be the most appropriate indicator of nutritional status. Birth size is considered as a surrogate for summing the interaction between environmental and genetic influences and despite being a convenient marker in epidemiological research; it does not adequately describe the phenotypic characteristics of a baby with regard to long-term health outcomes (Gluckman and Hanson 2006). Several paths of foetal growth can achieve the same birth size and the effect of programming does not necessarily affect the size at birth. Findings from the Dutch Famine study indicate that programming of the disease risk can occur without growth failure. In this study, the prevalence of coronary heart disease was higher in those exposed to famine in early gestation than in non-exposed people but this effect was independent of birth weight (Roseboom, van der Meulen et al. 2000). Therefore, it has been suggested that a more direct measure of nutritional status during early life is desirable to assess the role of early nutrition in the programming of adult health and disease.

2.6.7 Relationship between maternal nutrition and health outcomes in the offspring

As the majority of the studies mentioned above were retrospective cohort studies, the only measure of intrauterine under nutrition available was birth weight. Obviously, it is not the low birth itself *per se* which causes these problems but what low birth weight represents, that is, foetal under nutrition and impaired growth. As foetal growth depends on the uptake of nutrients at the end of a complex maternal-foetal supply line, understanding how maternal nutrition influences the programming of disease risk is vital for better understanding of the process and developing effective interventions.

Very few studies, however, have prospectively assessed the indicators of maternal nutritional status such as dietary intake during pregnancy or maternal anthropometry and related it to the health outcomes in the offspring.

2.6.7.1 Indicators of maternal nutritional status and body composition of the offspring

A prospective cohort study in Southampton assessed the relationship of maternal body composition with the body composition of children at 9 years. Results indicated that mothers with a higher pre-pregnant BMI or a larger mid-upper arm circumference during pregnancy, tended to have children with greater adiposity at age 9 years (Gale, Javaid et al. 2007).

The Pune Maternal Nutrition Study, a prospective study that assessed maternal anthropometry, physical activity and nutritional intake during pregnancy, also provided information on neonatal outcomes and follow-up measurements. Analyses exploring the relationship of maternal nutritional status with the body composition of the offspring at 6 years in more than 650 mother-child pairs, indicated that women who were short, and had lower head circumference and higher fat mass (indicative of poor growth in early life and relative energy excess in later years), had children with a higher body fat percentage (Yajnik 2000) .

A few studies have also explored the relationship of maternal weight gain during pregnancy and offspring body composition in later life. The Southampton Women's Survey examined the relationship of maternal weight gain during pregnancy (measured by weight before pregnancy and at 34 weeks gestation) and related it to the body composition of children (measured by DXA) at birth, 4 years and 6 years in 948 mother-child pairs (Crozier,

Inskip et al. 2010). It was observed that children whose mothers had excessive weight gain during pregnancy had greater fat mass at birth, 4 and 6 years when compared to the children of mothers who had adequate weight gain. In addition, at 6 y of age, evidence of a U-shaped relationship was found, such that adiposity was greater among both children born to mothers whose pregnancy weight gain was inadequate and those with excessive gains. Similarly, the Motherwell study from the UK which assessed the relationship of maternal weight gain with offspring body composition (using anthropometry) at age 30 years in 276 individuals, showed that higher maternal pregnancy weight gain was associated with higher fat mass and waist circumference in the offspring (Reynolds, Osmond et al. 2010). Studies assessing the relationship of maternal weight gain during pregnancy and offspring body composition, however, have not been reported from developing countries. It is possible that this relationship differs in the case of undernourished women from developing countries because higher weight gain during pregnancy is known to be associated with a higher birth weight (Ludwig and Currie 2010) which, in turn, is associated with reduced adiposity.

A few studies have used other indicators of maternal nutritional status to explore its relationship with the body composition of offspring. For example, a study from Pune showed that higher maternal erythrocyte folate concentrations at 28 weeks of pregnancy predicted higher offspring adiposity and higher insulin resistance at 6 y (Yajnik, Deshpande et al. 2008). The authors speculated that high folate intake in vitamin B12 deficient mothers may lead to defects in one-carbon metabolism that may be responsible for lower lean tissue deposition and higher lipogenesis. Another example is based on the recently reported findings of the Mysore cohort study (Krishnaveni, Veena et al. 2011) which assessed the relationship of maternal serum 25-hydroxyvitamin D concentrations at 28-32 wk gestation with body composition (measured by anthropometry) of the offspring at 5 and 9.5 y in 568

mother-infant pairs. At both the time-points, children born to mothers who had vitamin D deficiency had smaller arm-muscle area in comparison with children born to mothers who were not deficient. In addition, the children of vitamin D-deficient mothers had lower grip strength and higher fasting insulin resistance than children of non-deficient women. Vitamin D is known to influence the growth and differentiation of muscle tissue (Pasco, Wark et al. 2008, Harvey, Moon et al. 2014) as well as insulin secretion (Chiu, Chu et al. 2004). As fetal vitamin D concentrations are mainly dependent on maternal vitamin D concentrations, maternal deficiency may lead adverse outcomes in the offspring (Lapillonne 2010).

2.6.7.2 Maternal dietary intakes and body composition of the offspring

2.6.7.2.1 Famine studies

Some of the earliest evidence of the relationship of maternal nutritional status and adult body composition was derived from two natural experiments at the time of Second World War when some population groups were exposed to substantially reduced food intake for specific time periods. The1944–1945 Dutch Famine was a 5-month period of extreme food shortage that struck a previously (and subsequently) well-nourished population. The official daily rations fell below 1000 kcal during this period and at the peak of famine, rations were between 400–800 kcal/day. This tragic event provided a unique opportunity to study the effects of a short but severe period of maternal under nutrition during different stages of gestation on the offspring. The Dutch Famine birth cohort study included the survivors who were exposed to famine *in utero* and compared them with controls born either before the famine or conceived after the famine. The study indicated that maternal under nutrition during pregnancy was associated with a high fat body composition and increased risk of

cardiovascular disease in the offspring at a later age (Ravelli, van der Meulen et al. 1999). The effect of prenatal exposure to famine also depended on its timing. For example, the rate of obesity was higher in men exposed to famine in the first half of gestation and lower in men exposed in the later trimester of gestation. However, these findings were not replicated in another famine study, the 'Siege of Leningrad', which found no association between prenatal exposure to famine and subsequent risk of obesity (Stanner and Yudkin 2001). The null findings in the later study have been attributed to the lack of catch-up growth in the Leningrad cohort, in contrast to the Dutch cohort.

A recent study from China, which investigated the association between exposure to famine in early life and the risk of overweight and obesity in adulthood, also indicated that women exposed to famine in early life tended to be shorter and overweight. Famine exposure, however, had no impact on the BMI of men (Wang, Wang et al. 2009).

2.6.7.2.2 Follow-up studies of nutrition intervention trials

Apart from the famine studies identified above, evidence of the effect of early life nutrition interventions on body composition in adulthood is scarce. Two community-based studies, one from Guatemala (Martorell 1995) and another from the Gambia (Hawkesworth, Prentice et al. 2008) explored these relationships using the opportunity provided by the maternal nutrition supplementation trials during pregnancy, although these trials were primarily conducted to evaluate the interventions for improving pregnancy and birth outcomes. In the INCAP study in Guatemala, villages were randomized to receive either Atole (a high energy, high protein drink which provided 163 Kcal and 11.2 g protein per cup (180 ml)) or Fresco (lower energy, no protein drink which provided 59 Kcal per cup) which

served as a control. The supplemental drinks were provided *ad libitum* to pregnant women and their children up to the age of 7 years. The results of the follow-up investigation showed that the intervention was associated with a taller height and a higher LBM, especially in females during adolescence (Rivera, Martorell et al. 1995). The Gambian study, however, provided conflicting results. This study examined body composition (using BIA) of 1270 children in the age group of 11-17 years whose mothers had participated in a cluster-randomized trial of protein-energy supplementation during pregnancy (Hawkesworth, Prentice et al. 2008). The supplement which was provided daily included two biscuits prepared from local ingradients that provided about 1016 Kcal 22 g protein, 56 g fat, 47mg calcium and 1.8mg iron. The intervention group received supplementation during pregnancy (from 20 weeks of gestation until term) while the control group received the supplement in the postpartum period for 20 weeks. The supplementation was associated with a significantly higher birth weight in the intervention group. However, the follow-up study showed that the body composition of the adolescents in the two groups including body fat, trunk fat or LBM, did not differ. The authors speculated that as the control women received the supplement during lactation, this may have reduced the between-group difference.

Only one study from India has assessed the effect of a protein-energy supplement provided to pregnant women and children on the body composition and cardiovascular risk factors of offspring in adolescence (Kinra, Sarma et al. 2008). The study examined a cohort of 1165 adolescents aged 13-18 years and found that despite participants in the intervention group being taller than controls, they had a similar body composition.

Two studies have reported the effect of maternal micronutrient supplementation on the body composition of offspring. A study in Nepal assessed the relationship of maternal

micronutrient supplementation during pregnancy and body composition (measured by anthropometry) of their children (n=3771) at 6-8 y using a randomized controlled trial (Stewart, Christian et al. 2009). Maternal supplementation with folic acid + iron + zinc was associated with an increase in mean height and a decrease in arm fat area. No significant differences were found between groups in mean weight or BMI-for-age z-scores, waist circumference, or arm muscle area. Another study in Peru, which was a randomized controlled trial of prenatal zinc supplementation, assessed the effect of supplementation on the growth and body composition of infants (n=546) at 1 y (Iannotti, Zavaleta et al. 2008). The results showed that, after adjustment for a range of covariates, the supplementation was associated with greater weight, calf circumference, chest circumference, and calf muscle area, suggestive of lean tissue mass accretion.

2.6.8 Link between early under nutrition and later adiposity: possible mechanisms

Developing countries like India that are undergoing demographic and nutrition transition are currently facing a "double burden" of two contrasting forms of malnutrition – under nutrition in children and adiposity in adults. Environmental influences such as increased dietary intakes and decreased physical activity cannot explain these two contrasting phenomena and therefore this double burden of malnutrition calls for an additional explanation. Studies on the association between the childhood under nutrition and later body composition suggest a possibility of a common underlying mechanism that can explain the link between under nutrition in early life and adiposity related chronic diseases in later life.

Evidence on the 'programming' of higher LBM in adulthood by higher birth weight and better growth during infancy is consistent as shown by the studies described earlier. This

is reflected in the high rates of childhood under nutrition and higher proportion of adults with low LBM in the low income settings especially in south Asian countries (Gupta, Misra et al. 2009, Kim, Park et al. 2013). Lifestyle changes associated with nutrition transition, especially the excessive intake of processed foods and reduced energy expenditure may further enhance fat deposition. The impact of the obesogenic environment in the transitioning societies is likely to be greater in population groups that experienced nutritional insults in early life (Hoffman and Klein 2012).

In addition, evidence suggests that the low LBM itself may predispose to fat accretion by influencing the energy balance. It is well recognized that synthesis and breakdown of muscle protein are principally responsible for the energy expenditure of resting muscle. Low muscle mass and LBM could, therefore, have a significant effect on energy balance (Zurlo, Larson et al. 1990). It has been estimated that a decrease of 10 kg in muscle mass translates to a conservation of ≈100 kcal/day in energy expenditure which in turn translates to the accumulation of 4.7 kg fat mass/year (Wolfe 2006). It may therefore be argued that poor muscle mass may be an important reason for the high fat phenotype in this population. In addition, a number of energy-sparing mechanisms take place in adults who were under-nourished and stunted in childhood. For example, a study from Brazil showed that nutritionally stunted children had impaired fat oxidation and preferential oxidation of carbohydrate as indicated by a higher respiratory quotient (RQ) in the fasting state and at 30-min after a meal (Hoffman, Sawaya et al. 2000, Martins, Toledo Florencio et al. 2011). As oxidation of 1 g of carbohydrate is equivalent to 4 KCal compared to 9 KCal with the oxidation of 1 g of fat, tendency to store fat is enhanced with this adaptation, especially in an environment where physical activity is low (Frisancho 2003). However, epidemiological evidence on this is not consistent. For instance, in studies from Guatemala (Wren, Blume et

al. 1997), as well as from Cameroon (Said-Mohamed, Bernard et al. 2012), mean RQ and weight-adjusted resting energy expenditure (REE) did not differ between stunted children and non-stunted children. However, in the Cameroon study, stunted children had lower physical activity measured using accelerometers which corroborates the findings of a few past studies (Hoffman, Sawaya et al. 2000, Mamabolo, Kruger et al. 2007).

A number of studies have demonstrated that Cortisol hormone plays a key role in "programming" after intra-uterine under nutrition (Phillips, Barker et al. 1998). Under nutrition is a powerful stimulator of stress and can prompt an increased secretion of Cortisol leading to an increase in the Cortisol-to-Insulin ratio which helps direct the energy in the form of glucose to the brain (Sawaya, Martins et al. 2009). This hormonal imbalance also leads to a reduction in key hormones responsible for growth, such as insulin-like growth factor 1 (IGF-1), and thyroid hormones, leading to lower linear growth as well as lower energy expenditure (Fowden and Forhead 2004). An excess of Cortisol is also associated with profound changes in intermediate metabolism, resulting in long-term changes in lipid metabolism and an increase in the concentration of tumour necrosis factor-alpha (TNF-α) (Enwonwu, Phillips et al. 2005). Moreover, recent evidence on epigenetic programming suggests that early under nutrition can influence phenotype by modulation of genes that control DNA methylation and by histone acetylation (Sebert, Sharkey et al. 2011). It thus appears that early under nutrition during the critical stages of development induces a cascade of adaptive processes with short-term survival benefits but these become maladaptive in the face of lifestyle changes associated with nutrition transition.

2.7 Life-course determinants of muscle mass and strength

Skeletal muscle mass is the predominant component of LBM and represents a relatively fixed proportion of LBM (Wang, Heo et al. 2001, Abe, Bemben et al. 2012). Variation in the LBM is thus primarily linked to variation in muscle mass. Age-related decrease of muscle mass is known as sarcopenia, a term proposed by Rosenberg in 1989 (Greek 'sarx' or flesh + 'penia' or loss). Increasing healthcare costs associated with the growing numbers of elderly individuals with disabilities primarily linked to age-related decline in muscle mass and function have stimulated interest in both the determinants of muscle mass and strength and potential strategies to improve these characteristics. Focussing on the determinants that operate throughout the lifecourse helps to better understand the opportunities to maximize peak muscle mass and strength during the growing years and also minimize the age-related losses in later life.

2.7.1 Non-modifiable determinants of muscle mass and strength

2.7.1.1 Age and sex

Age is the most important non-modifiable determinant of muscle mass and strength. Muscle mass increases steadily during childhood followed by marked changes during adolescence. After reaching a peak in early adult years, skeletal muscle mass as well as strength decline gradually (Figure 2.2).

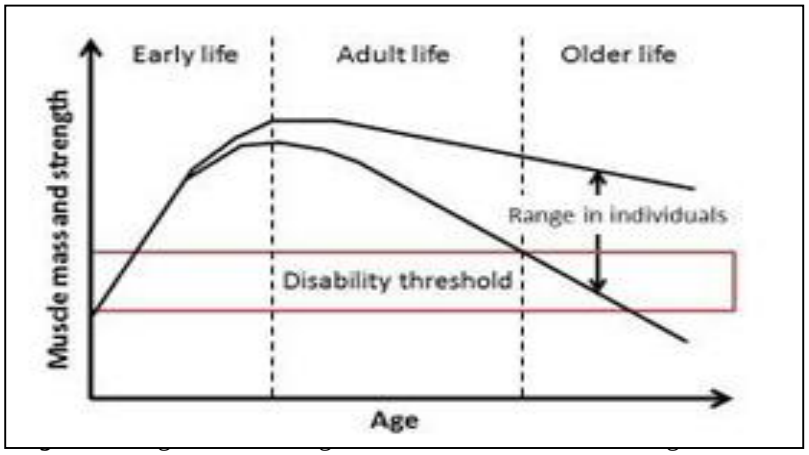


Figure 2.2 Age related changes in the muscle mass and strength**.**

Adapted from (Sayer, Syddall et al. 2008)

Although several studies have assessed the influence of ageing on muscle mass and strength, limited information is available on how these characteristics develop during the growing years. Muscle mass and strength increase steadily during childhood with marked increases in adolescence. Although girls tend to have relatively lower muscle mass and strength than boys throughout childhood, the differences are modest and relatively constant. In general, from age 5 to 10 years, boys have 1-3 kilograms more LBM than girls with the sex differences in LBM primarily determined by differences in the muscle mass (Fomon, Haschke et al. 1982, Roemmich, Clark et al. 1997). During puberty, boys accrue LBM (and muscle mass) at a much greater rate and for a longer period of time such that the young adult complement of LBM is attained at age 15-16 years for girls, but 19-20 years for boys (Leonard, Elmi et al. 2010). In boys, the significant increase in LBM exceeds the total gain in weight as there is a concomitant loss of adipose tissue (Rogol, Roemmich et al. 2002).

Although the changes in the muscle strength are closely related to muscle mass throughout growth, the increase in muscle strength during growth and maturation cannot be

fully explained by the increase in the muscle size (Bouchant, Martin et al. 2011). Structural and morphological changes in the muscle tissue during growth could be responsible for the marked increase in muscle strength during pubertal growth (BA De Ste Croix, Armstrong et al. 2002, Van Praagh, Dor et al. 2002). Moreover, muscle strength is influenced by neural activation as well as contractile properties of the muscle apart from the muscle mass (Sacchetti, Balducci et al. 2012). Peak gain in muscle mass and muscle strength occur during puberty but the peak muscle mass and strength are attained at a later age following a period of tissue maturation (Hulthén, Bengtsson et al. 2001). Peak values are reached between 25 and 35 years of age, and are maintained or are slightly lower between 40 and 49 years of age before the age-related decline starts after 50 years of age (Lindle, Metter et al. 1997, Janssen, Heymsfield et al. 2000, Doherty 2001). However, in a cross-sectional study in Afro-Caribbean men, grip strength appeared to increase until 50 years of age (Forrest, Bunker et al. 2012). These differences could partly be explained by study design as cross-sectional studies tend to show changes at a later age and with attenuated magnitude than longitudinal studies. For example, longitudinal changes in muscle strength were found to be almost 60% higher than those reported by cross-sectional studies (Hughes, Frontera et al. 2001). The age-related loss of muscle strength is usually higher than the loss of muscle tissue indicating that neuronal changes may mediate the changes in muscle strength (Luff 1998, Delmonico, Harris et al. 2009). A longitudinal study in American older adults aged 70-79 years showed that maintenance or even gain of LBM in men and women did not necessarily prevent the loss of strength (Goodpaster, Park et al. 2006).

Recently, a term ‘dynapenia’ has been coined to describe age-associated loss of muscle strength that is not caused by neurologic or muscular diseases (Clark and Manini 2012). The rate of loss of strength with advancing age has been estimated to be about 2-4%

per year. This is 2-5 times faster than the loss of muscle mass indicating that the factors other than loss of muscle contribute to the loss of strength (Mitchell, Williams et al. 2012). The biological contributors to dynapenia are likely to be multi-factorial including both the nervous and muscle systems because the muscle force production depends on the ability of the nervous system's ability to activate skeletal muscle as well as the muscle size and muscle protein metabolism (Clark and Manini 2012).

Across the studies, muscle mass in men was higher than in women by about 36% (Gallagher, Visser et al. 1997, Janssen, Heymsfield et al. 2000) although the number of muscle cell nuclei was found to be similar in men and women (Miller, MacDougall et al. 1993). The annualized rates of strength decline were also estimated to be higher in men (about 3-4%) than in women (2-3%) (Goodpaster, Park et al. 2006). Although men have a higher loss of muscle mass than women, sarcopenia is a greater public health problem for women due to their longer life span.

2.7.1.2 Ethnicity

A number of studies investigating the ethnic variation in muscle mass and strength have consistently shown that blacks have a higher muscle mass than whites whereas Asians tend to have a smaller muscle mass than the above ethnic groups (Silva, Shen et al. 2009). Surprisingly, higher muscle mass among the blacks was not associated with higher grip strength in a population-based, cross-sectional study from Boston (Araujo, Chiu et al. 2010). Moreover, African-Americans were found to have a higher age-related loss of muscle mass than white men and women in a longitudinal study (Silva, Shen et al. 2009). The ethnic differences in muscle mass are evident even at birth. A recent study which compared the

FFM in European and South Asian infants using air-displacement plethysmography showed that South Asians have reduced FFM in infancy. The authors speculated that this early manifestation of this phenotype suggests that it is either genetic and/or determined through exposure to maternal physiology, rather than a consequence of behaviours or diet in childhood or at older ages (Stanfield, Wells et al. 2012). Similar findings have been reported earlier from a study that compared the birth measurements of babies born in Pune, India with babies born in Southampton, UK. The study demonstrated that although the Indian babies were smaller in all aspects, the lean tissues (skeletal muscle and abdominal viscera) were the most affected whereas fat tissue was relatively preserved (Yajnik, Fall et al. 2003).

2.7.2 Modifiable determinants

Undoubtedly, nutrition and exercise are the two most important modifiable influences on muscle mass and strength. A combination of nutrition and exercise interventions results in marked improvements in muscle mass and strength than either of these interventions used in isolation (Breen and Phillips 2012).

2.7.2.1 Nutrition

Nutritional influences operate throughout the lifecourse and are major determinants of muscle growth during childhood and muscle loss during older age. The nutrients that are consistently linked to muscle mass and strength include proteins, essential fatty acids, vitamin D and a number of other micronutrients.

2.7.2.1.1 Dietary protein

Dietary protein, when supplemented with resistance type exercise, is known to increase muscle protein synthesis rates and inhibit muscle protein breakdown, thereby allowing net protein accretion (Phillips, Tang et al. 2009, Evans, Boccardi et al. 2013). On the other hand, inadequate protein intake is known to influence muscle tissue mainly by reducing the synthesis rather than increasing the degradation of muscle protein. Essential amino acids are especially important in promoting muscle protein synthesis and Leucine seems to be most influential in initiating the molecular events associated with muscle protein synthesis (Millward 2012). Higher intake of protein, particularly milk-based protein, has been associated with higher muscle mass development during childhood, puberty and adulthood (van Vught, Heitmann et al. 2009, Papadaki, Linardakis et al. 2010, Morris and Jacques 2012). Higher Leucine content along with the whey and casein components in the milk influences development and sustenance of muscle mass. Protein ingestion within two hours after resistance exercise, when muscle protein synthesis is stimulated almost 40-100% over and above the resting levels, has been shown to be especially effective in increasing muscle accretion in young adults (Burd, Tang et al. 2009). Higher protein intake may be especially important in the elderly as indicated by observational studies that have shown reduced loss of lean body mass in men and women with higher energy-adjusted protein intakes (Evans, Boccardi et al. 2013).

Studies assessing the efficacy of essential amino acid (EAA) supplementation for increasing muscle mass in the elderly individuals have, however, shown equivocal results. A small study in 12 glucose intolerant subjects which assessed the impact of EAA supplementation for 16 weeks demonstrated a substantial increase in muscle mass as well as

muscle strength whereas a study in 28 elderly European men with EAA supplementation for 12 weeks along with resistance exercise failed to show any increase in skeletal muscle mass and strength (Verdijk, Jonkers et al. 2009). A recent meta-analysis which pooled data from 22 randomized controlled trials that included 680 subjects showed that dietary protein supplementation during resistance type exercise training, increased LBM as well as muscle strength in both younger and older subjects (Cermak, Res et al. 2012).

A number of factors can explain the differences in the results seen in the intervention studies. These include: participants' age and health status, baseline protein consumption; degree of oxidative stress, concomitant intake of creatine, carbohydrate, or antioxidant; quality of protein (hydrolysate versus intact) and/or amino acid cocktail (essential or branched-chain including leucine, an insulin secretion stimulator) supplementation and its daily distribution etc. In addition, whether or not supplementation was accompanied by resistance exercise training could be an important factor influencing the response to protein or amino acid supplementation. Moreover, majority of the studies assessing the anabolic response of skeletal muscle to various protein supplements in the elderly have been carried out over a relatively short period. The duration of supplementation may not be adequate to produce a detectable difference in the muscle mass or strength.

Table 2.3 Intervention studies (10 weeks+) with protein supplementation

Reference	Sex	Age (y)	Supplement type	+RET	Protein dose	Duration (wks)	Outcomes	Result
(Andrews, MacLean et al. 2006)	M / W	60–69	Protein drink	Yes	0.4 g/LBM 3 times a week	12	LBM	Significant increase in LBM in M & W
(Bemben, Witten et al. 2010)	M	48–72	Whey and / or creatine	Yes	35 g 3 times a week	14	LBM, Muscle strength	Increased strength and lean body mass in each group with no between – group differences
(Candow, Chilibeck et al. 2006)	M	59–76	Protein before or after RET	Yes	0.3 g/kg 3 times a week	12	LBM, Muscle strength	Supplementation (before or after training) had no effect on muscle mass and strength
(Dillon, Sheffield-Moore et al. 2009)	W	66-70	EAA	No	15 g/day	12	LBM, Muscle strength Muscle protein fractional synthesis rate	Increase in LBM and basal muscle protein synthesis
(Eliot, Knehans et al. 2008)	M	48–72	Whey± creatine	Yes	35 g 3 times a week	14	LBM	No added benefit of protein supplementation in addition to RET
(Holm, Olesen et al. 2008)	W	55±4 (PM)	Whey+ CHO+Ca+ Vit D	Yes	10 g 3 times a week	24	Muscle mass, Muscle strength	Increase in muscle mass and muscle strength

(Onambele-Pearson, Breen et al. 2010)	M/ W		AA mixture+ CHO	Yes	22 g 3 times a week	12	Muscle mass, Muscle strength	Increase in muscle mass and muscle strength
(Solerte, Gazzaruso et al. 2008)	NA	66–84	AA mixture	No	8 g/ 2 times a week	24	LBM	Significant increase in LBM

NA: not available, M: men, W: women, PM: postmenopausal, +RET: coupled to resistance exercise training, LBM: lean body mass, AA: amino acid, CHO: carbohydrate, BIA: bioelectric impedance analysis, FSR: fractional synthesis rate, US: ultrasonography, 1RM: one repetition maximum, DXA: dual-energy X-ray absorptiometry

Adapted from (Mithal, Bonjour et al. 2013)

2.7.2.1.2 Fatty acids

Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) influence cell membrane integrity and may play a role in the maintenance of muscle mass. The ratio of omega-3 and omega-6 PUFAs is especially important because the role of omega-3 PUFAs in enhancing cell membrane fluidity may favour muscle hypertrophy whereas omega-6 PUFAs, that promote inflammatory process may contribute to muscle catabolism (Simopoulos 2002). Omega-3 PUFAs, by their potent anti-inflammatory function, may prevent the age-related loss of muscle mass and strength stimulated by pro-inflammatory cytokines (Simopoulos 2008, Kiecolt-Glaser, Belury et al. 2012). Relatively larger number of studies has examined the association of Omega-3 PUFAs with muscle strength than with muscle mass. Omega-3 PUFAs can influence muscle strength through a number of pathways. These fatty acids not only influence the membrane composition of skeletal muscle but may also affect the nervous tissue and thereby influence neuromuscular function and muscle strength (Figueroa, Cordero et al. 2013).

A number of observational studies have shown a positive association between intake of omega-3 PUFAs and muscle strength. For example, in a study including about 3000 older men and women (aged 59-73 years) from the Hertfordshire cohort, fatty fish consumption was the most influential dietary component that had a positive effect on grip strength (Robinson, Jameson et al. 2008). Fish oil also contains vitamin D which influences muscle strength; but the level of fatty fish intake had a stronger association than vitamin D intake with grip strength in this study, indicating that the greater effect of fatty fish was due to components other than vitamin D. In addition, evidence suggests that omega-3 fatty acid supplementation may be more effective if combined with other interventions that stimulate protein synthesis such as higher protein intake and resistance exercise. For example,

supplementation of alpha-linolenic acid (ALA) in flax oil for 12 weeks during resistance exercise in older adults was found to enhance the lean mass, muscle strength and functional ability more than resistance exercise alone (Cornish and Chilibeck 2009). Another recent study in 16 healthy older adults showed that supplementation with omega-3 fatty acids resulted in augmented muscle protein synthesis in response to amino acid feeding without change in muscle basal protein synthesis. Muscle protein synthesis was accompanied by greater increases in muscle mTOR signalling, which is believed to act as major regulator of skeletal muscle growth (Smith, Atherton et al. 2011). There is some evidence that omega-3 supplementation may also decrease proteolysis by down regulating the ubiquitin-proteasome pathway (Whitehouse and Tisdale 2001).

2.7.2.1.3 Acid producing diets

An acidic environment is an established stimulus for muscle catabolism (May, Kelly et al. 1986). It has been suggested that chronic intake of excess acid-producing nutrients such as meat and cereal grains in combination with a low intake of the alkalizing fruits and vegetables may lead to a chronic acid challenge which is known to have negative effects on muscle. Recent studies have shown that diets high in alkali-producing foods (fruits and vegetables) are associated with the preservation of lean tissue mass in older adults (Dawson-Hughes, Harris et al. 2008, Welch, Macgregor et al. 2013). Moreover, a few prospective studies have demonstrated that intake of excess alkali in the form of potassium or sodium bicarbonate reduces urinary nitrogen excretion and thus potentially spares body protein stores in healthy older adults (Ceglia, Harris et al. 2009, Buehlmeier, Frings-Meuthen et al. 2012)

There is some evidence that acid–base balance and vitamin D may be interdependent in their effects on muscle. Chronic metabolic acidosis is known to increase the serum 1,25-(OH)2D concentrations and may thus influence the muscle indirectly(Krapf, Vetsch et al. 1992). On the other hand, animal studies suggest that vitamin D deficiency results in a metabolic acidosis whereas repletion with vitamin D results in metabolic alkalosis (Hulter 1985). However, clinical evidence for interaction of acid–base with vitamin D in their effects on muscle is currently inadequate.

2.7.2.1.4 Vitamin D

Proximal muscle weakness is an important feature of vitamin D deficiency and several observational studies suggest a positive association between serum 25-hydroxyvitamin D levels and muscle strength or lower extremity function in older persons (Visser, Deeg et al. 2003, von Hurst, Conlon et al. 2012). Further evidence on the role of vitamin D in muscle function is based on the finding that vitamin D receptor (VDR) is expressed in human muscle tissue (Ceglia, da Silva Morais et al. 2010) and VDR activation may promote de novo protein synthesis in muscle (Freedman 1999, Bischoff-Ferrari 2013) .

A large number of studies in adolescents have shown a positive association of vitamin D status with muscle strength and force (El-Hajj Fuleihan and Vieth 2007, Foo, Zhang et al. 2009, Ward, Das et al. 2010). In addition, vitamin D supplementation in adolescents has also been shown to have beneficial effects on LBM and muscle strength. For example, a study from Lebanon with vitamin D supplementation for one year in 10-17 year-old girls demonstrated a positive effect with either low (5 µg/day) or high (50 µg/day) doses of vitamin D_3 on LBM (approx. 4 kg or 9%, as measured by dual-energy X-ray absortiometry)

when compared to the control group. The effect was more pronounced in pre-menarcheal girls and surprisingly, a beneficial effect was even seen in the low-dose supplement group which did not show an increase in serum 25(OH)D levels (El-Hajj Fuleihan and Vieth 2007). On the other hand, in a randomized clinical trial in girls (12 to 13 years) from Manchester, UK, supplementation of vitamin D_2 over 12 months could not detect positive effects on muscle strength or power although the supplement increased the serum vitamin D levels. Nonetheless, vitamin D supplementation increased movement efficiency, and there was a trend towards increases in jump height and jump velocity, which depend predominantly on the fast-twitch fibres of the quadriceps muscle (Ward, Das et al. 2010).

A few studies carried out in adults, however, did not show any association between vitamin D status and muscle mass. For example, in a cross-sectional study in young women from Canada, vitamin D status was not associated with thigh muscle cross-sectional area although low vitamin D status was related to higher fat infiltration of the muscle (Gilsanz, Kremer et al. 2010). On the other hand, a large number of studies have consistently reported a positive relationship between vitamin D status and muscle strength, function, and balance in older individuals (Grimaldi, Parker et al. 2012). The beneficial effect of vitamin D, however, may be limited to individuals with baseline vitamin D deficiency. Two excellent reviews have been published recently summarizing the evidence (Bischoff-Ferrari 2012, Girgis, Clifton-Bligh et al. 2012). A meta-analysis collated the evidence from 17 RCTs involving 5,072 participants and showed no significant effect of vitamin D supplementation on grip strength or proximal lower limb strength in adults with no vitamin D deficiency at baseline whereas studies in vitamin D deficient participants demonstrated a large effect of vitamin D supplementation on hip muscle strength (Stockton, Mengersen et al. 2011). Furthermore, vitamin D use was associated with a statistically significant reduction in the risk of falls (odds

ratio for suffering at least one fall, 0.86; 95% confidence interval, 0.77–0.96) as indicated by a meta-analysis which included data from 26 trials that enrolled 45,782 participants (Murad, Elamin et al. 2011). This effect was more prominent in patients who were vitamin D deficient at baseline and in studies in which calcium was administered along with vitamin D.

2.7.2.2 Exercise

Strong evidence exists on the positive effect of resistance exercise on muscle mass and strength (Oates, Glover et al. 2010, Van der Heijden, Wang et al. 2010, Candow, Chilibeck et al. 2011). Resistance exercise can help muscle accretion in a number of ways including activation of the mTOR muscle protein synthetic pathway (Dreyer, Fujita et al. 2010), stem cell (satellite cell) activation and proliferation (Walker, Fry et al. 2012), anabolic hormone production (Arazi, Damirchi et al. 2012), as well as decrease in catabolic cytokine activity (Gatta, Russell et al. 2012, Ho, Dhaliwal et al. 2012). Exercise influences the GH-IGF1 axis and can stimulate growth of muscle and other tissues in children and adolescents (Frystyk 2010). However, acute exercise bouts are also known to exert a pro-inflammatory effect, reflected by transient elevations of inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Moon, Cho et al. 2012). The influence of resistance exercise on the muscle mass thus depends on the balance between these two opposing effects of exercise. Improved muscle mass and function with resistance training may in turn enhance glucose sensitivity and could be an effective strategy for prevention of diabetes mellitus (Hills, Shultz et al. 2009). On the other hand, muscle disuse due to lack of mobility can cause drastic reduction in muscle strength and muscle atrophy. As little as 4 days of disuse led to marked impairment in muscle force in younger as well as older participants in a study from Denmark (Hvid, Suetta et al. 2012).

2.7.2.2.1 Observational studies

Several cross-sectional studies demonstrated significantly greater muscle mass as well as improved architecture and function in strength-trained master athletes compared to age-matched sedentary controls (Krustrup, Christensen et al. 2010, Sundstrup, Jakobsen et al. 2010). Furthermore, a few studies demonstrated that muscle mass, strength, contraction characteristics, and histology in elderly resistance-trained athletes were equivalent to young untrained participants (Hawkins, Wiswell et al. 2003). Apart from studies in sports participants, a number of community-based studies have demonstrated the positive association between physical activity and muscle mass and strength (Hughes, Frontera et al. 2001, Goodpaster, Park et al. 2006, Genton, Karsegard et al. 2011).

2.7.2.2.2 Intervention studies

A few studies in children and adolescents have assessed the impact of exercise intervention on muscle mass and strength. For example, in a 12-week resistance exercise program, body composition of 12 adolescents was measured using DXA at baseline and at completion. The exercise program resulted in significant increase in muscle mass as well as strength gain in both upper and lower body muscle groups (Van Der Heijden, Wang et al. 2010). In addition, the exercise resulted in improved hepatic insulin sensitivity and decreased glucose production rate, even though it did not affect total fat mass or visceral, hepatic and intramyocellular fat content. Another longitudinal study from Canada in adolescent boys and girls also showed that physical activity had a significant effect on LBM and muscle mass accrual and for the same level of activity; boys accrued about 20-120% more absolute LBM (Baxter-Jones, Eisenmann et al. 2008). Similarly, in a 7-yr prospective longitudinal study

from Finland, which included 202 girls aged 10–13 years at baseline, consistent high physical activity was associated with a higher LBM and muscle mass at the end of the follow-up (Völgyi, Alén et al. 2011).

A large number of studies in young as well as older adults have demonstrated the positive influence of resistance exercise on muscle mass and strength. For example, a prospective randomized controlled study that involved 63 men and women in younger (20-30 y) and older (65-80 y) age groups showed that progressive resistance training for 14 weeks resulted in increase in the size (cross sectional area) of type 2 muscle fibres, which in turn, produced increases in muscle force and power in both the age groups (Claflin, Larkin et al. 2011). Although exercise regimens can improve muscle function and muscle mass in older subjects by hypertrophy of existing muscle fibers, it is not clear if they can prevent the age-related loss of muscle fibers (Wilborn, Taylor et al. 2009). Active older adults, including veteran athletes, also develop age-related decline in muscle mass and function (Korhonen, Cristea et al. 2006).

Although the positive effect of resistance training on muscle mass and muscle strength has been confirmed by a large number of epidemiological and intervention studies, evidence on the impact of aerobic exercise on muscle mass and strength is equivocal. A cross-sectional study in men and women from different age groups showed that long-term aerobic exercise could attenuate age-related reductions in muscle strength in addition to its cardio-respiratory and metabolic benefits (Crane, MacNeil et al. 2012). However, in a prospective study from Korea where participants were randomized to a moderate intensity aerobic exercise group for 12 weeks, those in the intervention group did not have a higher muscle mass or strength at the end of the study when compared to the control group (Kwon,

Min et al. 2010). The duration of intervention may not be adequate to produce the desired effect.

Majority of the above studies have assessed the relationship between leisure time physical activity with muscle mass and muscle strength. Occupational activity largely accounts for physical activity in majority of individuals in the developing countries like India. But information on the influence of occupational physical activity on the muscle mass and strength is currently lacking.

2.8 Possible causes of low lean body mass in Indians

Based on the evidence presented above, the factors that could contribute to low LBM and high adiposity in Indians can be classified into early life and later life factors.

2.8.1 Early life factors

The evidence on the relationship between low birth weight and low LBM and higher adiposity is consistent as indicated by studies from developed as well as developing countries. A high prevalence of low birth weight and under nutrition in early childhood years in India could therefore be an important factor underlying the adipose body composition observed in adulthood.

2.8.2 Later life factors

2.8.2.1 Diet

Data from large scale nutritional surveys in India indicate that the majority of Indians consume diets that are cereal based with low intakes of protective foods such as animal foods,

dairy products, fruits and vegetables (NNMB 2006). These diets are therefore deficient in important nutrients such as proteins, essential fatty acids, micronutrients including zinc, calcium etc.

2.8.2.2 Vitamin D deficiency

A number of studies from India have highlighted a high prevalence of vitamin D deficiency in different population groups: urban and rural adults (Harinarayan, Ramalakshmi et al. 2007), paramilitary personnel (Tandon, Marwaha et al. 2003), pregnant women (Marwaha, Tandon et al. 2011) as well as children and adolescents (Sahu, Bhatia et al. 2009, Khadgawat, Marwaha et al. 2013). A number of reasons have been postulated for this high prevalence of vitamin D deficiency including pollution, skin pigmentation, low sunlight exposure levels in the population, high body fat which sequestrates the vitamin D etc(Harinarayan and Joshi 2009).

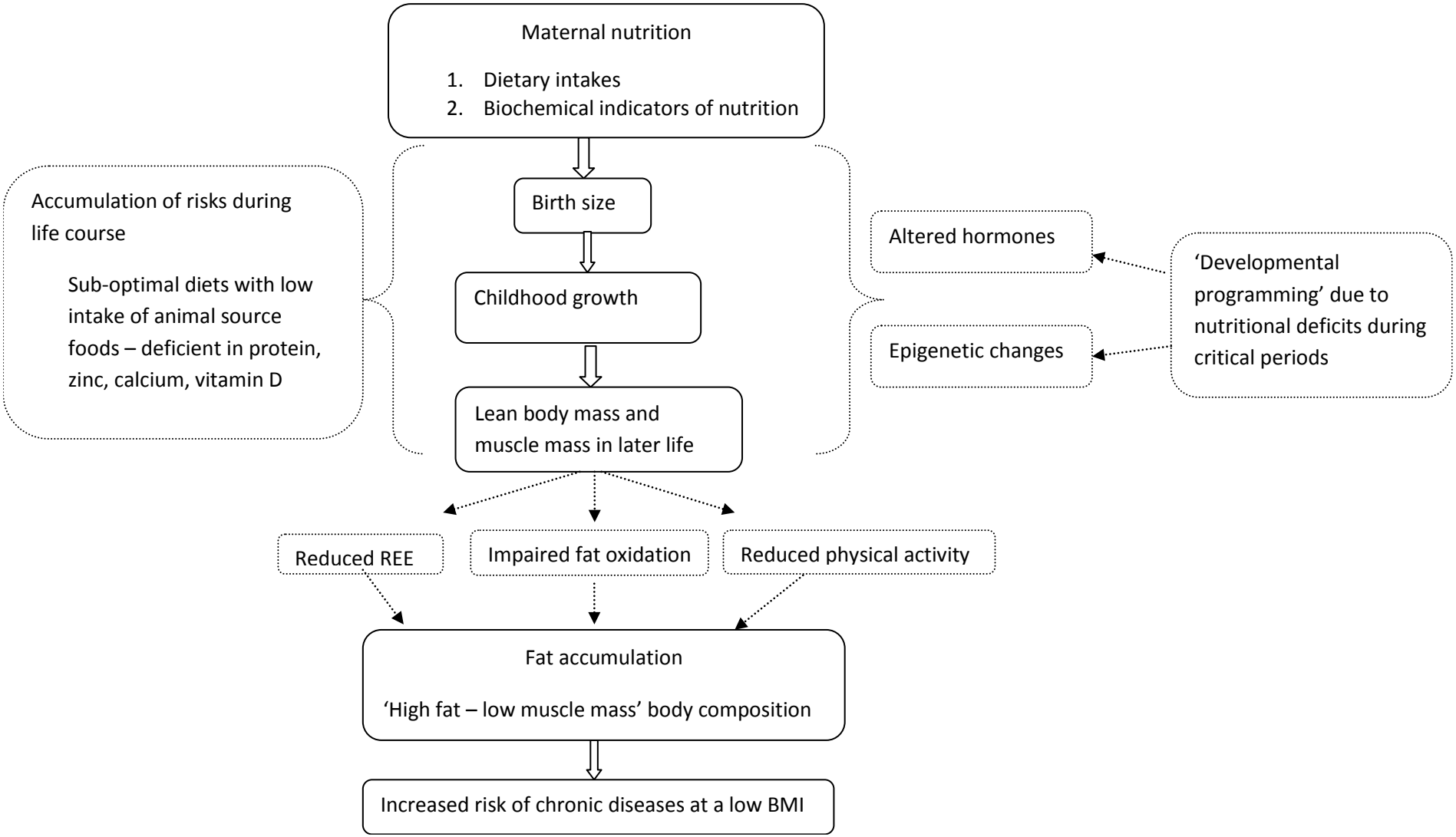
2.8.2.3 Physical activity

Level of physical activity is also known to be lower in many South Asian people compared to other population groups. Studies have demonstrated that physical inactivity makes a significant contribution to the excess CVD mortality observed in the South Asian population in the UK (Williams, Stamatakis et al. 2011). In a study from Delhi, India, approximately 75% of urban adolescents and young adults were observed to be sedentary (Dhingra, Chatterjee et al. 2002). Low levels of physical activity could contribute to adiposity and low muscle mass in Indian populations as exercise is one of the important determinants of muscle mass development.

It thus appears that both under nutrition in early life as well as the diet and physical activity patterns in later life could influence the LBM and muscle mass. Estimation of the relative contribution of early *vs* later life factors is important in order to direct the strategies for improving the LBM and muscle mass.

Sub-optimal LBM may in turn increase propensity for fat accumulation and adipose body composition as discussed in previous sections. This peculiar body composition with high fat and low LBM is considered to be an important determinant of elevated risk of chronic diseases at a relatively low BMI in Indians as summarized in the conceptual framework (Figure 2.3).

Figure 2.3 Life course determinants of body composition in under nourished populations



2.9 Assessment of body composition

Body composition assessment has become an integral part of nutritional assessment because of the understanding that alterations in body composition may mediate morbidity and mortality. Though scientific inquiry in the area of body composition assessment began many centuries ago, modern body composition assessment techniques have mainly evolved in the past few decades depending on developments in particular scientific disciplines such as chemistry, anatomy and nutrition. Since 1960, the study of human body composition accelerated with the development of imaging techniques such as ultrasound, DXA, CT scan, MRI and in vivo neutron activation analysis (Heymsfield 2008, Ahtiainen, Hoffren et al. 2010, Gallagher, Thornton et al. 2010, Wang, Heymsfield et al. 2010) and has become a distinct branch of human biology (Wang and Heymsfield 1999).

2.9.1 Models for body composition assessment

Body composition assessment involves the study of structure, function and physiological significance of the components forming living organisms. Wang et al. (Wang, Pierson et al. 1992) proposed a model in which the body can be characterized at five levels to provide a structural framework for studying human body composition (Table 2.4) based on separate but integrated levels beginning with atomic and moving to molecular, cellular, tissue-organ, and whole body levels.

Theoretically the body mass can be divided into multiple compartments to the atomic level, but in reality, body composition assessment is limited by the availability of techniques. Therefore, different models of body composition assessment have been proposed based on the availability of techniques to measure different body compartments.

Table 2.4 Representative multi-component model at the five-body composition levels

Level	Body composition model	Number of components
Atomic	BM = H + O + N + C + Na + K + Cl + P + Ca + Mg + S	11
Molecular	BM = FM + TBW + TBPro + Mo + Ms + CHO	6
	BM = FM + TBW + TBPro + M	4
	BM = FM + TBW + non-fat solids	3
	BM = FM + Mo + residual	3
	BM = FM + FFM	2
Cellular	BM = cells + ECF + ECS	3
	BM = FM + BCM + ECF + ECS	4
Tissue-organ	BW = AT + SM + bone + visceral organs + other tissues	5
Whole body	BW = head + trunk + appendages	3

AT- adipose tissue; BCM- body cell mass; BM- body mass; CHO- carbohydrates; ECF- extracellular fluid; ECS- extracellular solids; FFM- fat-free mass; FM- fat mass; M- mineral; Mo- bone mineral; Ms- soft-tissue mineral; SM- skeletal muscle; TBPro- total body protein; TBW- total body water.
Adapted from (Shen, St-Onge et al. 2005).

2.9.1.1 Two compartment models

The classic two compartment (2C) models describe the body as fat mass and FFM (Brožek, Grande et al. 1963)and is the most widely used approach. The earliest reported 2C model was based on the measurement of total body density using hydrodensitometry or underwater weighing (UWW). Later, two nuclear-based methods, ^{40}K counting and dilution with radioactive water, emerged for use with the 2C body composition model. Radioactive tracers were later replaced by stable isotopes to estimate total body water (TBW), and in

recent years, the UWW technique has been replaced by air-displacement plethysmography, where the subject is enclosed in an air-filled chamber instead of water.

Methods based on the measurement of body density assumed a constant density of the FFM whereas methods based on the estimation of potassium or TBW, assumed that the relative concentration of water in FFM is constant. These assumptions, however, are not valid in many population sub-groups such as the very young or elderly subjects, those from different ethnic groups, or subjects with certain diseases. Models which involve fewer assumptions and measure additional body compartments were therefore considered necessary.

2.9.1.2 Three compartment models

To reduce the limitations encountered with the 2C models, three-component (3C) models were developed which included dividing the FFM into 2 parts. For example, the 3C model suggested by Siri includes the measurement of TBW, in addition to UWW, to divide the FFM into a water content and remaining solids (Siri 1961). The DXA technique which provides a measurement of fat mass, fat-free soft tissue and bone mineral content can also be considered as a 3C model. The 3C model provides some improvements over the basic 2C model for healthy adults and older children. However, for patients with significantly depleted body protein mass or bone mineral mass, the estimated values for the density of solids would be incorrect; thus the final estimate of body fat mass would also be inaccurate. An additional estimate of the components of the solids compartment was therefore considered necessary.

2.9.1.3 Four compartment models

The 3C model was later expanded with the addition of bone mineral measurement to the estimation of body density and TBW. Thus the 4C model measures body mass, body volume, TBW and bone mineral (Wang, Shen et al. 2005). An alternate 4C model which does not require the UWW measurement has also been developed. In this model, the body's FFM is divided into three basic cellular or physiological compartments: body cell mass (BCM), extracellular water fluid or water (ECW), and extracellular solids (ECS) (Ellis 2000). In summary, the FFM, a heterogeneous compartment, can be further divided into its constituents (water, protein, mineral etc.) and then combined to form a variety of three compartment (3C), four compartment (4C) or even six compartment (6C) models. Importantly, no single technique allows for the measurement of all tissues and organs, and no technique is error free.

2.9.2 Estimation of lean body mass

Recent evidence emphasizes the significance of lean body mass (which mainly includes skeletal muscle and organs) for various health outcomes and this has stimulated a reappraisal of *in vivo* assessment of lean body mass (Wolfe 2006). There is also a growing interest in the measurement of components of the lean mass including skeletal muscle mass and organ masses. One of the reasons for this heightened interest is the increasing prevalence of sarcopenia with a rapidly aging population. Sarcopenia and its functional consequences have become important public health concerns in the developed as well as developing world (Szulc, Munoz et al. 2010).

There has been rapid technological improvement in the area of *in vivo* measurement of lean body mass since Shaffer and Coleman measured total body skeletal muscle mass for the first time using urinary creatinine excretion (Shaffer, Coleman et al. 1909). A number of techniques such as the isotope dilution technique, hydrodensitometry and ^{40}K counting evolved later with the advancement of the understanding of human metabolism (Behnke, Feen et al. 1942, Forbes, Gallup et al. 1961).

Methods for the assessment of lean body mass/muscle mass can be broadly classified as:

a) Anthropometric measurements - to calculate total LBM and regional muscle mass such as arm muscle area.
b) Methods based on muscle metabolites such as urinary creatinine clearance and 3 Methylhistidine estimation.
c) Radiographic methods such as DXA, computed tomography (CT) and Magnetic Resonance Imaging (MRI).
d) Methods based on assessment of TBW such as the isotope dilution technique and bioelectrical impedance analysis (BIA).
e) Nuclear techniques such as total body potassium estimation, total body nitrogen estimation using neutron activation analysis etc.

All methods have inherent strengths and limitations and the choice of method depends on the purpose, cost, access etc.

2.9.3 Dual energy X-ray absorptiometry (DXA)

The original and primary purpose of DXA was to measure bone mineral density but in addition, the technique has increasingly been used to assess soft tissue. Before the development of DXA, single photon absorptiometry (SPA) and dual photon absorptiometry (DPA) were used to measure bone mineral density and soft tissue composition. However, limitations of these techniques led to the development of DXA in which the radioactive source was replaced by an X-ray tube with a filter to convert the poly-chromatic X-ray beam into low and high energy peaks. This improved the precision of the measurement of soft tissue composition and led to widespread use of DXA.

2.9.3.1 Principle of absorptiometry

The fundamental principle related to DXA is that, when an X-ray source is placed on one side of an object, the intensity of the beam on the opposite side of the object is related to its thickness, density, and chemical composition. The attenuation of energy through bone, lean tissue, and fat is different, reflecting differences in their densities and chemical composition. DXA devices consist of a generator emitting the X-rays of two energies – high and low. The generation of a high and low energy emission by an X-ray source produces a better discrimination between bone and soft tissue mass. Prior knowledge of the coefficient of attenuation for bone and soft tissue enables the calculation of bone mineral density. In bone-free pixels, distinction can be made between fat mass and fat free mass. DXA derives the proportion of FM and LBM by comparing the ratio of the energy attenuation of soft tissue mass, with experimental attenuation values obtained from the measurement of phantoms with

100% fat and 100% lean tissue mass. These equations are solved mathematically to derive mass and density of different components (Genton, Hans et al. 2002).

2.9.3.2 Advantages and limitations of DXA

DXA measurements are typically quick (5-10 minutes), non-invasive, precise and operator independent (Lohman and Chen 2005). Moreover, the dose of radiation exposure is low – about 2 to 5 µSv, which is less than the daily background radiation of 5-7 µSv (Laskey 1996). In addition, the ease of operation as well as relatively low cost of measurements has resulted in increased popularity of DXA. A major advantage of DXA is its ability to measure regional body composition, an important determinant of cardiovascular risk (Vega, Adams-Huet et al. 2006). Total and regional skeletal muscle mass can be accurately measured using CT and MRI scans, however these techniques are expensive and impractical for large epidemiologic studies. DXA allows measurement of skeletal muscle mass based on estimates of appendicular lean soft tissue. A number of studies have validated DXA skeletal muscle mass estimates using MRI as the reference method and confirmed that the accurate prediction of skeletal muscle mass is possible with DXA (Kim, Wang et al. 2002).

Like all body composition techniques, DXA has a number of limitations and some researchers have argued that it is not a 'gold standard' approach (Roubenoff, Kehayias et al. 1993, Williams, Wells et al. 2006). As DXA was primarily designed for the measurement of bone mineral, the technique can only use non-bone pixels to calculate the fat-to-lean ratio and extrapolate these measurements to the bone-containing pixels (Pietrobelli, Formica et al. 1996, IAEA 2010). It has been estimated that 40-45% of the 21,000 pixels in a typical whole body scan contain bone in addition to soft tissue, and these pixels are excluded from the

calculation of values for soft tissues. Soft tissue determination, therefore, may not be accurate in arms, legs and thorax. In addition, DXA measurements are also influenced by the thickness of the tissue due to 'beam hardening' effect and by the assumption of a constant hydration of lean tissue mass (Lohman and Chen 2005). Modest systematic variations in the absolute estimates of body composition have also been reported due to different hardware and software combinations (Wang, Deurenberg et al. 1999). In the case of obese participants, the scanner table may be too small for complete acquisition of the whole body, resulting in inaccurate results. In addition, the higher anteroposterior thickness of the body may affect the accuracy of estimates in obese individuals (Lohman and Chen 2005).

2.9.3.3Precision and accuracy of the DXA measurements of body composition

Precision

The precision or repeatability of the total and regional body composition estimates from DXA can be evaluated over the short- or long-term. The short-term precision of body composition estimates is usually evaluated by repeatedly scanning subjects and calculating the average intra-individual standard deviation. Standard deviation for the LBM and per cent fat estimates by DXA is usually about 1% for each variable, which is comparable to the precision reported for other methods like hydrodensitometry (Lohman and Chen 2005).

Keibzak and colleagues assessed the precision of total and regional body composition measurements using DXA in 20 volunteers who were scanned once each day for 4 consecutive days using a Lunar DPX-L densitometer. It was observed that regional measurements were less precise than the total body measurements, with coefficients of

variation (CVs) of 1-3% for total body, 4.3% for fat mass of arm and 3.1% for trunk (Kiebzak, Leamy et al. 2000). Therefore, when regional body composition changes are assessed, it is recommended that DXA be carried out two times within each measurement period (within 7-10 days) to decrease the measurement error (Lohman and Chen 2005).

Accuracy

The accuracy of the estimate reflects how close the estimate is with respect to the true value. Accuracy of DXA for the measurement of body composition can be evaluated in a number of ways. Animal studies that compare the DXA estimates of body composition with that measured by a direct method like carcass analysis provide the most accurate estimate of the accuracy of the technique. Human studies typically compare the body composition estimates by DXA with other reference methods. Since different measurement techniques involve different assumptions, there may be systematic errors in comparing the body composition estimates using different methods. It is well known that the magnitude of these errors may vary in different populations and with different DXA instruments (Genton, Hans et al. 2002). Many methods tend to overestimate fatness in lean populations and underestimate fatness in the obese (Schoeller, Tylavsky et al. 2005). The systematic errors may be attributable to the inaccuracies of the reference method, inaccuracies of the DXA estimate, or the combination of the two.

2.9.3.4 Validation studies in animals

A large number of studies have assessed the accuracy of body composition estimates using DXA in animal models. For example, Black and colleagues compared the body

composition estimates by DXA with direct chemical analysis in 10 adult rhesus monkeys (Black, Tilmont et al. 2001). DXA measurements of fat and lean mass were highly correlated ($r > 0.95$) with direct analyses and there were no significant differences in the estimates by the two methods. Another study validated body composition estimates by DXA in chickens by chemical analysis. A very good agreement was found between the two methods for the estimation of lean mass, fat mass and fat percentage (Swennen, Janssens et al. 2004). However, another study evaluated body composition estimates by DXA in small free living rodents by comparing with carcass analysis and found that despite the excellence of the accuracy of DXA for prediction of lean mass, variation in fat mass measurement was high (Stevenson and Van Tets 2008).

2.9.3.5 Validation studies in humans

2.9.3.5.1 Comparison of DXA with reference methods

A large number of studies have compared body composition estimates by DXA with other techniques and Lohman and colleagues have reviewed validation studies comparing DXA with multi-component criterion methods (Lohman, Harris et al. 2000). In general, the mean values of estimates by DXA were in good agreement with criterion methods. However, the majority of the studies reviewed were performed using pencil beam instruments. Many studies using fan beam instruments have reported overestimation of LBM by DXA. For example, a large study by Schoeller et al. collated data sets from seven US centres. Data included fat mass and LBM measured with a QDR 4500A and criteria measurements of body composition from TBW by dilution at 4 centres, densitometry from 1 centre, and 4C analysis at 2 centres. In a total of 1195 participants (602 men and 593 women aged 19–82 y with a

BMI of 16–44), DXA overestimated LBM by about 5% (mean (±SE) 5 ± 1%). The authors recommended that the lean soft tissue mass estimate with the fan-beam QDR 4500A be reduced by 5% and that for fat mass be increased by the same mass (Schoeller, Tylavsky et al. 2005). Two other smaller studies conducted earlier in the USA support these findings. A study by Tylavsky et al. compared the body composition estimates by DXA (Hologic QDR 4500 A) with that measured by 4C model in 58 men and women (age 70–79 y). The results indicated that DXA systematically underestimated LBM but applying a correction factor of 0.964, the differences between the two methods could be alleviated (Tylavsky, Lohman et al. 2003). Another study by Visser et al. compared LBM mass and leg muscle mass measurements using DXA with those measured by 4C model and multi-slice CT in 60 men and women aged 70-79 years and BMI 17.5–39.8. LBM by DXA was significantly higher [53.5 ± 12.0 (SD) kg] than LBM by 4C (51.6 ± 11.9 kg; $P< 0.001$). Leg muscle mass measured by DXA was also higher than by CT, indicating systematic overestimation by DXA (Visser, Fuerst et al. 1999).

Although fewer studies have reported validation of fat mass and LBM by DXA with criterion 4C techniques, a large number of studies have compared body fat percent estimates by DXA with the reference technique as shown in Table 2.5.

2.9.3.5.2 Comparison of body composition estimates by different DXA devices

Differences in body composition estimates have also been noted between the DXA instruments by different manufacturers as shown by studies included in Table 2.6. Although based on the same physical principles, differences exist in the generation of the high and low energy x-ray beams, the x-ray detectors, the calibration methodology etc. Additional

differences exist in the algorithms used for selective tissue imaging, edge detection, region-of-interest definition and system calibration, which may contribute to the differences in body composition estimates by different machines (Genton, Hans et al. 2002).

A study by Soriano et al. compared body composition estimates between two pencil-beam (Lunar DPX and DPX-L) and two fan-beam (Lunar Prodigy, Hologic Delphi A) DXA systems in 78 healthy adults. The results indicated that, whilst the body composition estimates across the four DXA machines were highly correlated, significant differences were observed between the instruments, especially between different manufacturers. DPX-L estimates of LBM were highest whereas the Prodigy estimates were the lowest. Most of the comparisons were sex-dependent (Soriano, Ioannidou et al. 2004). A recent study in the UK carried out *in vivo* cross calibration of body composition between a Hologic QDR2000 and a GE Healthcare Lunar Prodigy in 21 subjects (Pearson, Horton et al. 2011). There was no significant difference in whole body lean body mass between the QDR2000 and the Prodigy, however, fat mass and %fat were significantly higher on the QDR2000.

Table 2.5 Comparison of percent body fat (%BF) measured by DXA and the four-compartment model (4-C)

Study	Sex	Age	%BF (from 4C)	DXA system	Type of X-ray beam	Mean Difference in %BF vs. 4-C
(Fuller, Jebb et al. 1992)	16 M	18–59	12.0–25.0	GE Lunar DPX	Pencil	−1.4% (NR)
	12 F		19.6–38.0			
(Bergsma-Kadijk, Baumeister et al. 1996)	20 F	19–27	29.4 ± 3.2	GE Lunar DPX	Pencil	−3.1%*
	18 F	65–78	38.8 ± 5.9			−5.3%*
(Prior, Cureton et al. 1997)	91 M	21 ± 2	12.5 ± 5.9	Hologic QDR	Pencil	0.6%
	81 F		22.3 ± 7.6	1000W		0.2%
(Withers, LaForgia et al. 1998)	24 M	18–36	Athletes: 12.1 ± 2.8	GE Lunar DPX-L	Pencil	Athletes: −3.5%*
			Non-athletes: 21.8 ± 8.2			Non-athletes: −1.3%*
	24 F		Athletes: 16.4 ± 2.4			Athletes: −1.3%*
			Non-athletes: 28.9 ± 4.7			Non-athletes: −0.4%
(Modlesky, Evans et al. 1999)	14 M	27 ± 6	20.7 ± 10.2	Hologic QDR 1000W	Pencil	−2.1%
	10 F					
				GE Lunar DPX-L	Pencil	−1.1%

(Arngrimsson, Evans et al. 2000)	22 M	21 ± 3	Athletes: 9.5 ± 2.5	Hologic QDR 1000W	Pencil	Athletes: −2.9%*
			Non-athletes: 17.2 ± 4.6			Non-athletes: −2.9%*
	22 F		Athletes: 21.1 ± 4.0			Athletes: −4.0% *
			Non-athletes: 26.6 ± 3.6			Non-athletes: −2.2% *
(Deurenberg-Yap, Schmidt et al. 2001)	144 M	18–75	26.2 ± 6.5	Hologic QDR 4500	Fan	−3.8%*
	147 F		36.2 ± 7.4			−2.3%*
(Van Der Ploeg, Withers et al. 2003)	118 M	18–59	6.5–36.6	GE Lunar DPX-L	Pencil	−1.9% (NR)
	34 F		12.3–37.5			−1.7% (NR)
(van Marken Lichtenbelt, Hartgens et al. 2004)	27 M	19–44	7.5–23.2	GE Lunar DPX-L	Pencil	0.9%
(Williams, Wells et al. 2006)	26 M	19–21	15.6 ± 6.3	GE Lunar	Narrow	1.7%*
	44 F		29.9 ± 6.1	Prodigy	Fan	2.0%*
(Santos, Silva et al. 2010)	24 M	22 ± 3	9.2 ± 4.1	Hologic QDR 4500A	Fan	2.9%*

The table includes studies with weight-stable healthy adults.

Percent body fat using the four compartment model was determined using underwater weighing or BodPod, DXA and deuteriumdilution.

*Statistically significant difference between DXA and 4-C model ($P < 0.05$).

NR, statistical significance not reported.

Adapted from (Toombs, Ducher et al. 2012)

Table 2.6 Differences of body composition between various DXA systems according to the type of X-ray beam

Study	Population			DXA systems		Differences in between the two systems		
	Sex	Age (y)	BMI	*Pencil beam vs. Pencil beam*		LBM (kg)	FM (kg)	%BF
(Tothill, Avenell et al. 1994)	5 M	NR	19–28	GE Lunar DPX	Hologic QDR 1000W	NR	NR	3.7*
	6 F			Norland XR 26	Hologic QDR 1000W	NR	NR	6.3*
				Norland XR 26	GE Lunar DPX	NR	NR	2.6*
Modlesky *et al*. (73)	13 M	22.2 ±3.6	NR	GE Lunar DPXL	Hologic QDR 1000W	−1.4*	1.2*	1.5*
Modlesky *et al*. (39)	14 M, 10 F	26.7± 6.0	24.4 ± 3.5	GE Lunar DPXL	Hologic QDR 1000W	NR	1.2*	1.7*
				Pencil beam vs. Fan beam				
Hull *et al*.(10)	47 M, 52 F	18–81	18.1–46.4	GE Lunar DPXL	GE Lunar Prodigy	M: 2.4*, F: 2.0*	M: −3.0*, F: −2.4*	NR
				GE Lunar DPXL	GE Lunar iDXA	M: 3.2*, F: 1.4*	M: −3.6*, F: −2.1*	NR

				Fan beam vs. Fan beam				
Genton *et al*. 51	36	Nr	>25	GE Lunar Prodigy (narrow fan)	Hologic QDR 4500A NPM	−2.1	4.0	3.4*
				GE Lunar Prodigy (narrow fan)	Hologic QDR 4500A HPM	−5.0*	3.2	4.2*
Hull *et al*. (10)	47 M, 52 F	18–81	18.1–46.4	GE Lunar Prodigy (narrow fan)	GE Lunar iDXA (narrow fan)	M: 0.8*, F: -0.6*	M: −0.6*, F: 0.3*	NR

*Statistically significant difference ($P < 0.05$).
%BF: percent body fat; F: female; HPM: high power mode; M:male; NPM: normal power mode; NR: not reported.

Adapted from (Toombs, Ducher et al. 2012)

2.9.3.5.3 DXA for the estimation of muscle mass

Since there is an increased awareness regarding the importance of skeletal muscle in a number of metabolic processes, there is a compelling need to estimate the total and regional muscle mass. Though techniques such as CT and MRI scanning allow accurate quantification of muscle mass, both the methods are expensive and CT scanning involves high radiation exposure. DXA systems provide a measure of appendicular lean soft tissue (ALST) (also called appendicular skeletal muscle mass or ASM), a fat- and bone mineral-free component. A large proportion of total-body skeletal muscle is found in the extremities, and a large proportion of ALST is skeletal muscle (Figure 2.4).

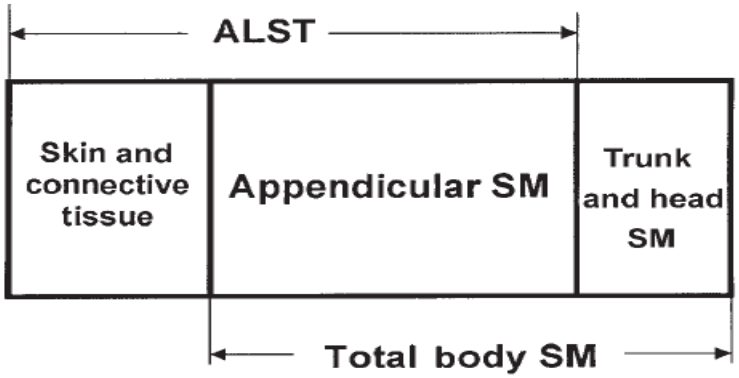


Figure 2.4. **Relation between ALST and total SM**

A number of studies have used DXA to predict skeletal muscle mass. For example, Heymsfield and colleagues compared the estimates of limb muscle mass using dual photon absorptiometry in healthy men and women with measurements of TBK, TBN, and anthropometric assessment of regional muscle mass. Significant correlations were found between appendicular muscle mass and TBK ($r = 0.94$), TBN ($r = 0.78$) and upper arm ($r = 0.82$) and thigh muscle-plus-bone areas ($r = 0.88$) (Heymsfield, Smith et al. 1990).

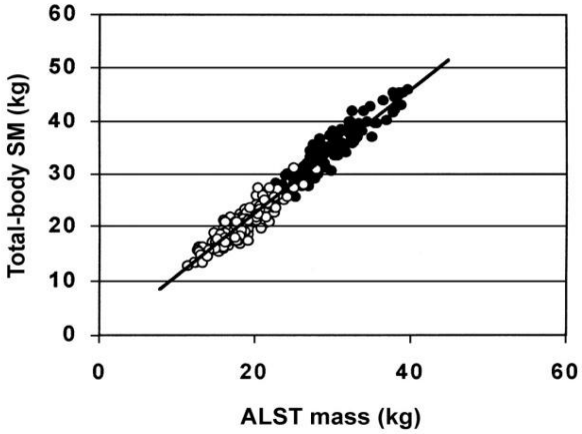


Figure2.5. **Correlation between total-body skeletal muscle (SM) mass estimated by magnetic resonance imaging and appendicular lean soft tissue (ALST) mass estimated by dual-energy X-ray absorptiometry in men (•) and women (○) in the model-development group.**

Total-body SM mass = (1.19 x ALST) - 1.01. R^2 = 0.96, P< 0.001, SEE = 1.63 kg. n = 321.

From (Kim, Wang et al. 2002)

In a widely cited study, Kim et al. developed prediction models in healthy adults based on ALST estimated by DXA using total-body skeletal muscle quantified by multi-slice MRI as reference method. ALST was found to be highly correlated with the skeletal muscle mass measured by MRI (R^2 = 0.96, SEE = 1.63 kg, P < 0.001) (Figure 2.5). Addition of age and sex to the model improved the prediction (Kim, Wang et al. 2002). A number of later studies have demonstrated that DXA estimation of ALST is a valid tool for the assessment of skeletal muscle mass in older women (Chen, Wang et al. 2007) as well as in children and adolescents (Kim, Shen et al. 2006). Table 2.7 lists the studies which validated the DXA based prediction equations against a reference method of MRI.

Pencil-beam systems (Lunar DPX and DPX-L) are usually considered more accurate but slower than fan-beam systems (Lunar Prodigy and Hologic Delphi A). However, a study which compared ALST estimates in 35 healthy adults across these four systems showed that the estimates were highly correlated. There was no significant bias detected across the four

systems and therefore the authors concluded that these systems appear interchangeable as methods for quantifying total body skeletal muscle mass in vivo (Ioannidou, Padilla et al. 2003).

Table 2.7 Studies comparing DXA estimates of skeletal muscle mass with reference methods.

Reference	N	Sex	Age group (y)	Reference method	Difference
(Wang, Wang et al. 1999)	27	M	36 ± 12	Multi-scan CT	3.9%
(Fuller, Laskey et al. 1992)	28	M/F		TBK	
(Wang, Visser et al. 1996)	25	M		CT	5.8%
(Kim, Wang et al. 2002)	414	M/F	41± 18	Multi-slice MRI	
(Shih, Wang et al. 2000)	207	M/F	43± 16	MRI	< 1%
(Zhao, Wang et al. 2013)	66	M/F	52 ± 13	MRI	

2.9.3.5.4 Assessment of sarcopenia using DXA

Ageing is associated with progressive decline in skeletal muscle mass leading to decreased strength and functionality. Although sarcopenia is associated with significant disability and enormous financial costs, consensus regarding the definition of this important public health problem is still lacking. A number of criteria based on various indices of appendicular skeletal muscle mass measured using DXA have been suggested. Baumgartner and colleagues developed one of the definitions of sarcopenia using the ASM index (Baumgartner, Koehler et al. 1998). The ASM measured using DXA is divided by body

height (in meters) squared (analogous to BMI) to adjust for the strong association between height and ASM. To define the cut-point for low muscle mass, an approach similar to that of osteoporosis was taken. Older persons with an ASM index less than -2 SD from the mean of a young reference population were classified as sarcopenic. The developed cut-points were 7.26 kg/m^2 for men and 5.45 kg/m^2 for women. DXA data from a volunteer sample of 229 white men and women aged 18-40 years were used to determine the young reference values.

In 2003, an alternative method was proposed by Newman et al. (Newman, Kupelian et al. 2003). The findings of the Health, Aging and Body composition study suggested that the prevalence of sarcopenia using the Baumgartner cut-points was very high (>50%) in normal weight older persons but zero in obese older persons. Therefore, a new criterion was devleoped which included both height and total body fat. ASM index was calculated as proposed by Baumgartner et al. but instead of comparing index values with a cut-off from a younger population, participants were classified as sarcopenic if their value was less than the sex-specific lowest 20th percentile. This definition was examined for prediction of 5 year incident lower extremity mobility limitation in the Health Aging and Body composition study and was also found to be a better predictor of lower extremity functional limitations (Delmonico, Harris et al. 2007).

The European Working Group on Sarcopenia in Older People (EWGSOP) has recently proposed a working definition of sarcopenia which recommends using the presence of both low muscle mass and low muscle function (strength or performance) for the diagnosis of sarcopenia (Cruz-Jentoft, Baeyens et al. 2010). However, the consensus document did not recommend a particular reference standard highlighting the need for more research in order to obtain good reference values for populations around the world.

2.9.4 Isotope dilution technique

2.9.4.1 Principles and assumptions

Assessment of body composition using the isotope dilution technique is based on calculation of TBW which is the most abundant constituent of body. As lipids are hydrophobic and thus free from water, body water is restricted to the fat free compartment and accordingly, FFM can be calculated from TBW using a hydration constant. The technique relies on the principle that the volume of the TBW can be determined as the ratio of the dose of a tracer, administered orally or intravenously, to its concentration in that body compartment within a short time after the dose is administered. Typically, two samples (blood, saliva, or urine) are collected: one just before administration of the dose, to determine the natural background levels, and the second sample after waiting for a sufficient amount of time for penetration of the tracer within the compartment of interest. The commonly used tracers include deuterium and oxygen 18 (^{18}O) (IAEA 2009).

The assessment of body water *in vivo* by the isotope dilution technique depends on a number of assumptions. The basic assumptions are:

1) The tracer is distributed only in body water,
2) It is equally distributed in all anatomical water compartments,
3) It is not metabolized during the equilibration time, and
4) Tracer equilibration is achieved relatively rapidly.

However, in reality, the tracers used for this method do not behave in an ideal manner and deviations from the basic assumptions are possible.

1) Assumption 1: Tracer is distributed only in body water.

None of the isotopic tracers are distributed only in body water. Each tracer exchanges to a small degree with non-aqueous molecules and thus, the dilution space of the isotope is slightly greater than TBW. Specifically, hydrogen exchanges with exchangeable atoms in body protein whereas oxygen undergoes exchange with organic matter, but to a lesser extent than hydrogen (IAEA 2009). A number of studies have attempted calculation of the *in vivo* isotope dilution space in animal models. Together, this evidence indicates that oxygen exchange is about 1% of the TBW and the deuterium exchange is about 4% of TBW (Racette, Schoeller et al. 1994, Schoeller 2005). The consensus is that the 2H space is approximately 1.041 times that of TBW, whereas the ^{18}O space is approximately 1.007 times that of TBW (IAEA 2009).

2) Assumption 2: The tracer is equally distributed in all anatomical water compartments.

Although isotopic tracers are almost identical to body water, differences in molecular weight can lead to isotopic fractionation. Comparisons of the isotopic concentration in various anatomical water compartments have demonstrated that there is very little isotopic fractionation within the body. But isotopic fractionation has been observed in water leaving the body by evaporation (IAEA 2009).

3) Assumption 3: Neither the tracer nor body water undergoes metabolism during equilibration.

Body water is in a constant state of flux. In temperate climates, the average fractional turnover rate in adults is about 8% each day (Schoeller 1988). In tropical climates, the

turnover is 50% to 100% greater because of increased insensible water loss. It is recommended to reduce water intake during equilibration time to minimize water turnover.

The validity of these assumptions also depends on the tracer used for the measurement of TBW by dilution as the tracers are also not free from metabolic complications. Different tracers have been used in the past including antipyrine, ethanol, urea, and isotopically labelled water etc. The non-isotopic tracers are rapidly metabolized, and therefore significant elimination from the body occurs during the equilibration time. The isotopic tracers of water (radioactive tracer tritium oxide and the 2 stable isotope tracers-deuterium oxide and ^{18}O) offer advantage over the non-isotopic tracers.

In view of the violations of the assumptions, appropriate adjustments in the ratio of administered dose to fluid concentration must be made. In mathematical terms, the equation that describes the dilution principle is:

$$V = k1 \,.k2.\, k3.\, k4[\ (D-E) / (\ [d_t] - [d_0]\ )\ ]$$

where the *k* values are constants used to correct for differences with each of the basic model, D is the tracer dose, E is the amount excreted during the equilibration period, $[d_t]$ is the tracer concentration in the fluid sampled after time t following the administration of the dose, and $[d_0]$ is the baseline concentration before the tracer.

4) Assumption 4: The rate of equilibration of the tracer is rapid.

The definition of equilibration time depends on the precision of the measurement method as a more precise method requires a closer approach to equilibration. Sampling before complete equilibration affects the TBW estimates. For example, Scholler et al.

reported that in 63 subjects receiving either deuterium or ^{18}O , at 3 hours, TBW estimate was smaller by 0.3% than at 4 hour after oral administration (Schoeller 2005). Another study by Colley et al. in 20 participants, who were dosed with deuterium oxide (0.05 g/kg body weight), indicated that isotopic equilibrium was reached by 50, 80 and 100% of participants at 4, 6 and 8 h, respectively (Colley, Byrne et al. 2007). Even a small disequilibrium is of concern in subjects with excess body water in poorly vascularised compartments such as oedema, ascites, pleural effusion etc. Generally equilibration times of 4 to 5 hours after oral administration of the isotope are considered to be adequate in case of healthy subjects.

2.9.4.2 Hydration of fat free mass

The water content or hydration of fat-free mass (FFM) is among the best known and most widely applied of the body-composition constants. The assumed "constancy" of fat-free body mass hydration is a cornerstone in the body-composition research field. Pace and Rathbun first proposed that total body water (TBW) is a constant fraction of FFM (x ± SD:0.724 ± 0.021) on the basis of experiments in guinea pigs (Pace and Rathbun 1945). Subsequent chemical analysis of mature animals supported a range of a hydration magnitude between 0.70 and 0.76 for several mammal species with widely varying body size (Sheng and Huggins 1979). A number of studies on whole-body chemical analysis of human cadavers showed a mean TBW:FFM ratio of 0.737 with a range between 0.684 and 0.808 (Sheng and Huggins 1979). Although the assumption of a constant hydration factor is clearly incorrect in subjects who are dehydrated or have abnormal water accumulation leading to oedema, hydration is relatively constant in healthy subjects and the most commonly used constant is 73.2% in adults aged 21 years and above (IAEA 2009).

This hydration constant, however, is not constant across the life span. In healthy newborns it is about 80-83% and rapidly decreases over the next 3–5 y until the hydration fraction reaches that observed for adults. The change in hydration reflects a change in the ratio of water between the intracellular and extracellular compartments. A study by Bossingham showed that FFM hydration (TBW:FFM) was significantly higher ($P < 0.05$) in the older subjects than in the younger subjects (Bossingham, Carnell et al. 2005). However, another study which included about 703 adults in the age-group of 20-94 yr, did not show age related differences in the hydration of the FFM (Gallagher, Visser et al. 1997). In addition, malnourished patients with protein depletion have been reported to have hydration factors as high as 75% (Beddoe, Streat et al. 1985). Bodybuilders with expanded skeletal muscle compartments also have hydration factors elevated by 2% to 3% (Modlesky, Cureton et al. 1996). Hydration of FFM is also known to be affected by disease conditions (Ellis 2000).

2.9.4.3 Measurement procedures

To achieve precise measurement of TBW, careful attention to detail is required. Each aspect of the measurement including subject preparation, dosing, sample collection, and isotope analysis must be controlled such that systematic and random errors are less than 0.5%.

The subject should be euvolemic because both over-hydration and dehydration reduce the precision of the estimates. For this, subject should have normal fluid and food intake on the day before the measurement and avoid vigorous exercise after the final meal of the previous day. The final meal should be eaten between 12 and 15 hours before the dose to

minimize the water content of the intestine and the subject should not drink for several hours before the test to avoid over-hydration. The ambient conditions should be such that the subject should not sweat excessively.

The dose of the isotope should be aliquoted by weight using a balance with a precision and accuracy such that the relative uncertainty in the dose of labelled water is less than 0.3% (Schoeller 2005). Furthermore, the dose should be stored in a screw-capped container to minimize evaporation. Although the dose can be given intravenously, oral dosing is quite effective and less invasive. Physiological samples must be collected over a period long enough to ensure equilibration. Typically, blood, saliva or urine is collected: one pre-dose, to determine the natural background levels, and the second after waiting a sufficient amount of time for equilibration (3 h for saliva, and 5 h for urine).

The dose should be given to the subject in the fasting state to maximize the rate of absorption. Usually the subjects are prevented from eating or drinking during the equilibration period to minimize the changes in body pool size. In cases where the fluid intake is unavoidable, the volume of water intake should be carefully recorded and subtracted from the dilution space. The dose should be administered with great care to avoid losses. In addition, the sample of the dose should be saved and analysed for isotope enrichment using the same procedure used to analyse the physiologic samples (IAEA 2009).

2.9.4.4 Sample handling and storage

The major concern during sample handling and storage is the minimization of evaporation and contamination as these processes will alter the isotopic abundance of the

specimen and introduce error into the calculation of TBW. The specimens therefore should be collected in capped containers and should be kept frozen till analysis.

2.9.4.5 Sample analysis

Analysis of ^{18}O currently requires Isotope Ratio Mass Spectroscopy (IRMS) whereas deuterium analyses can be carried out using either IRMS or Fourier transform infrared (FTIR) spectrometry.

An IRMS is the most versatile analytical instrument for measuring stable isotopes in terms of sample throughput, sensitivity and selectivity. This instrument separates ions in a high vacuum according to their mass to charge ratio (m/z). The major components of a mass spectrometer are the inlet system, high vacuum, ion source, mass analyser and detector. Modern mass spectrometers are computer controlled and have sophisticated data processing software. The abundance of the stable isotopes of C, H, O and N are measured in simple gases using IRMS with individual detectors for each isotope. IRMS has a high degree of isotope specificity and highly sensitive detection limits (IAEA 2009).

2.9.4.6 Calculation of Total Body Water

As described by Coward et al. (Coward 1990), both a sample of the diluted dose and the physiological samples should be measured in the same analytical run and dilution space can be calculated from mass and instrumental units as follows:

$$N = (WA/a)\,(S_a - S_t)\,f/\,(S_s - S_p)$$

where N is expressed in grams, W is the mass of the water used to dilute the dose, A is the dose administered to the subject, a is the mass of the dose used in preparing the diluted dose,

f is the fractional factor for the physiological sample relative to the body water, S_a is the measured value for the diluted dose, S_t is the value for the tap water used in dilution, S_s is the value for the physiological sample, and S_p is the value for the pre-dose physiological sample. The value of S_s, the isotopic abundance, can be obtained by the plateau or back-extrapolation to the time of the dose.

Finally, to determine the TBW from the isotope dilution, correction of the overestimation due to exchange with the non-aqueous compartment is necessary. In case of deuterium, the overestimation is about 4% in adults. Therefore, the following correction is necessary:

$$TBW = V/1.04$$

As mentioned previously, the overestimate for ^{18}O is smaller and estimated to be 0.7% in adults.

Using TBW to estimate body composition, the FFM is then calculated as:

$$FFM\ (kg) = TBW\ /\text{hydration constant}$$

Subsequently, fat mass (FM) and per cent body fat (%BF) can be calculated based on the two compartment model of body composition by subtracting FFM from body weight (BW):

$$FM\ (kg) = BW - FFM$$

$$\%BF = (BW - TBW/\text{constant}) / BW*100$$

The assumption of a constant hydration of FFM used for calculation of fat and FFM has raised concerns (Kinnamon, Lipsitz et al. 2010) and is considered to be the major limitation of isotope dilution technique by many researchers.

2.9.4.7 Precision of the estimate

The precision of the TBW measurement depends on the analytical method as well as the dose of the tracer administered to the subject. In general, the mass spectrometric methods are the most precise. IRMS can detect very small excesses of deuterium or ^{18}O, and thus the investigator can administer a dose in which the increase in enrichment of body water exceeds the random measurement error by a factor of 200 to 500. Under these conditions, the precision of the estimate is between 1% and 2%. The TBW values obtained using the dilution technique are, therefore, considered as the reference or criterion values for comparison with alternate measurement techniques (Schoeller, Tylavsky et al. 2005)

2.9.4.8 Accuracy

Accuracy of the isotope dilution method for the measurement of TBW is considered to be excellent unless there is a bias caused by failure to reach equilibrium. The accuracy depends on the uncertainties related to the estimate of the non-aqueous exchange and the hydration constant, which are estimated to be 1-2%.

2.9.4.9 Advantages

Isotope dilution is simple to carry out and requires minimal subject cooperation. It has proved particularly valuable in infants and toddlers because of the low compliance required,

and can easily be used in field studies. Isotope dilution technique is the most accurate technique of body composition measurement for use in the field settings.

2.9.4.10 Validation of the isotope dilution technique

As the isotope dilution technique is considered the criterion technique for the assessment of TBW and the most accurate technique for 2C model body composition assessment, very few studies have compared the isotope dilution technique with other criterion methods. However, a few studies have compared this technique with the 4C method and reported excellent precision and accuracy. For example, a study from Singapore measured body composition in 108 adult Chinese, 76 Malays, and 107 Indians by densitometry, deuterium oxide dilution, DXA and a chemical 4C model. Biases were found between %BF estimates by 4C model and that measured by the other 3 methods namely, isotope dilution, densitometry and DXA. Among these 3 methods, isotope dilution had the lowest bias while DXA gave the highest bias (Deurenberg-Yap and Deurenberg 2002).

Another study from Mexico compared the LBM estimates by isotope dilution technique with that measured using 4C model in 60 adolescents (Ramírez, Valencia et al. 2009). LBM estimates by the 4C and 2C models were 26.9 and 25.7 kg, respectively. Bias for the difference in LBM was not significant (−1.27, 95% confidence interval −1.5 to 0.9) and no association between the mean of the differences and the magnitude of the measurements was found ($P > 0.05$). The authors concluded that the isotope dilution technique was accurate and precise method for body composition assessment in Mexican children and adolescents compared with the 4C model.

2.9.5 Anthropometry for assessment of lean body mass

Anthropometry is one of the oldest techniques used for body composition assessment. As early as 1921, Matiegka developed equations for predicting body fat from the measurements of body length, width, circumference, and skin-fold thickness (Matiegka 1921). The newer methods such as the imaging techniques (DXA, CT and MRI scans) as well as the methods based on calculation of TBW such as isotope dilution are expensive, labour intensive and are available at a limited number of centres. The availability of more sophisticated techniques has not reduced the popularity of anthropometry because of its distinct advantages of being portable, non-invasive, inexpensive and thus useful in large scale epidemiological field studies. The use of anthropometry as a predictor of body composition therefore continues to play an important role in clinical practice and in large population-based studies.

2.9.5.1 Measurement methods

Anthropometric assessment of body composition relies on the measurements of length and breadth as well as circumferences of different body parts and skin-fold thickness at various anatomical sites, which are then converted to body fat and LBM values using prediction equations derived using reference or 'gold standard' methods.

<u>Lengths and breadths</u>

Skeletal lengths and breadths including stature usually have low correlation with %BF but a higher correlation with LBM. Forbes collated data from various sources and analysed

the relationship of stature to the lean body mass (Forbes 1974). The slope of the linear regression was observed to be 0.69 kg/cm for adult males and 0.48 kg/cm for females.

Apart from stature, combined indices based on selected measures of length, breadth, and depth of the skeleton have been developed to represent 'frame size'. For example, Katch and Freedson developed a frame size model which included stature and the sum of biacromial and bitrochanteric breadths. The relationship of body fat and FFM with the frame size was different for males and females. For males, FFM increased with increase in frame size, while fat weight per frame size remained constant. In females, there was a significant increase in fat weight with increase in frame size where as the LBM did not increase with increase in frame size (Katch and Freedson 1982).

Circumferences

Circumferences are more reliable than skin-fold measurements because they are easier to measure precisely. Circumferences at the mid-arm, mid-thigh, waist, and hip are used more frequently than others. Reproducibility of the measurement can be increased by giving special attention to positioning the subject, using anatomical landmarks to locate the sites, taking reading in millimetres with a non-flexible tape measuring directly in contact with the subject's skin without compression, and keeping the tape at 90 degrees to the long axis of the region of the body under the measured circumference (WHO 1995, Wang, Thornton et al. 2000, Bellisari and Roche 2005).

Skinfold thickness

Skinfold thickness is accepted as a predictor of body fatness because about 40-60% of total body fat is in the subcutaneous region of the body and skin fold thickness can be directly measured using a well-calibrated calliper. A number of anatomical sites have been used for assessment of skin fold thickness but no single site is an accurate predictor of body fat per cent because of individual variations in the distribution of subcutaneous adipose tissue and the proportion of adipose tissue that is subcutaneous (Bellisari and Roche 2005). Generally, three or four skin fold thicknesses (triceps, biceps, suprailiac, subscapular) are required in the prediction equations. The high correlations among the sites can make the regression coefficients unstable and therefore, log of the sum of skin fold thicknesses is used to reduce this instability (Durnin and Womersley 1974).

2.9.5.2 Standardisation of anthropometric measurements

Anthropometric measurements involve inter and intra-observer errors and are therefore observer dependent. Standardization of the methodology including proper positioning of the subject and the instrument and well trained data collector is essential to obtain reproducible measurements (WHO 1995). A number of attempts have been made for standardization of anthropometric techniques. Publication of 'Anthropometrica' was a significant advance in this area (Norton and Olds 1996) and this publication formed a basis of the current guide for anthropometric procedures – ISAK's International Standards for Anthropometric Assessment (Marfell-Jones, Stewart et al. 2011). Use of standard protocols is important to achieve several objectives: it ensures that the measurements are comparable with reference data, facilitates interpretation of results, provides a basis for training and

standardization of investigators and maximizes the reliability of the measurements (WHO 1995). Special attention to locating the site, grasping the skin fold, and assuring that the caliper is at 90-degree angle relative to the grasped skin-fold are essential for high reproducibility (Wang, Thornton et al. 2000).

2.9.5.3Prediction of body density and body fat using anthropometry

Numerous equations have been reported to predict body density and body fat from anthropometric measurements. Jackson and Pollock's sex-specific generalized equations (Jackson and Pollock 1978, Jackson, Pollock et al. 1980) and Durnin and Womersley's sex- and age-specific equations using the sum of four skinfolds (Durnin and Womersley 1974) are the most commonly used equations for predicting body density from anthropometric measurements. As hydrostatic weighing was the only criterion technique available at the time for 2C body composition assessment, both the equations were developed using hydrostatic weighing as the criterion method. From the values of body density, fat and FFM can be predicted (Siri, Brozeck et al. 1961).

The use of skinfold thickness for prediction of body density or body fat is based on implicit assumptions that (a) measurement of skinfold thickness at a few sites provides an adequate description of subcutaneous adipose tissue (SAT) compartment and (b) there is a fixed relationship between subcutaneous and deep adipose tissue (DAT) depots (Bellisari and Roche 2005). However, the assumptions may not be accurate because age and sex differences in the relationships between SAT and DAT compartments have been noted (Enzi, Gasparo et al. 1986). Equations developed by regression analyses to predict body fat per cent commonly

overestimate the low values and underestimate the high values, but this tendency is reduced if power terms are included (Jackson and Pollock 1978).

2.9.5.4 Ethnic specific equations for estimation of body composition

Ethnic differences in the distribution of body fat and density of the FFM are known to exist and therefore equations developed in one ethnic group may not be valid in other ethnic groups (Deurenberg and Deurenberg-Yap 2003). For example, density of the FFM has been reported to be higher in African Americans than Caucasians and, therefore, commonly used prediction equations based on anthropometry were found to underestimate the FFM density in this group (Schutte, Townsend et al. 1984). In addition, ethnic differences in the distribution of SAT (He, Horlick et al. 2002) indicate that skinfold equations are likely to perform poorly when applied to ethnic groups other than those from which they were developed. These considerations have led to development of equations for specific ethnic groups (Nagamine and Suzuki 1964).

The above pattern is, however, not consistent across different studies that have assessed the validity of body composition estimation from anthropometric measures in different ethnic groups. For example, a study in Bangalore, India assessed body composition of 99 men and 89 women using three methods - hydrodensitometry, bioelectrical impedance and skinfold thickness. Comparison of the hydrodensitometry (reference method) and skinfold method showed that there were no significant differences between the methods, for estimates of LBM and %BF. Durnin and Womersley's equation, though developed in Caucasians, was found to be valid for Indian men and women (Kuriyan, Petracchi et al. 1998). On the other hand, a study from the USA failed to demonstrate the validity of

commonly used anthropometric prediction equations in adults. The study compared the body composition estimates by anthropometry with criterion 4C model in 681 healthy white adults (Peterson, Czerwinski et al. 2003) and observed that the commonly used prediction equations (Durnin-Womersley and Jackson-Pollock) underestimated per cent body fat, although the equations were developed in the same ethnic group. Bland-Altman plots showed limited agreement between Durnin-Wormersley, Jackson-Pollock, and the 4-compartment model in this study. The authors speculated that development of earlier equations using hydrometry (in contrast to the 4C model in Peterson study) as the criterion method may be the reason for the discrepancy. The results suggest that the two commonly used equations require further evaluation with criterion 4C models of body composition assessment. A study from Japan also indicated that anthropometric measurement of body fat per cent using Japanese prediction equation underestimated body fat per cent when compared to DXA (Kagawa, Kuroiwa et al. 2007).

In general, prediction equations should not be applied to a group that is markedly different from the group used to develop the equation. Important group differences may relate to age, sex, ethnicity, and level of body fatness. A large number of studies have therefore been carried out in different settings in order to develop population specific prediction equations to estimate body fat using different reference techniques (Table 2.8)

Table 2.8 Anthropometric equations for prediction of BF% in adults using different reference methods

No	Study	Country	Age group	N	Sex	Reference method	Dependent Variable	Anthropometric variables	R^2	SEE
3	(Van der Ploeg, Gunn et al. 2003)	Australia	19-59 y	79	M	4C	BF %	nine skinfolds, five girths and two bone breadths	0.94	1.8%
4	(Garcia, Wagner et al. 2005)	Germany	26-67 y	117	M/F	DXA	BF %	skinfolds, circumferences, bone breadth measurements	0.90	NA
6	(Pongchaiyakul, Kosulwat et al. 2005)	Thailand	20-84 y	436	M/F	DXA	BF %	weight, height, Waist and hip circumferences, skinfolds	0.83	NA
8	(Ramirez-Zea, Torun et al. 2006)	Guatemala	18-56 y	237	M/F	UWW	BF %	Weight, height, limb circumferences, waist circumference, skinfolds	0.86	3.0
9	(Minematsu, Takamura et al. 2011)	Japan	18-59 y	810	M/F	UWW	BF %	height, weight, 7 circumferences, 8 skinfold thicknesses	0.69	NA
10	(Leahy, O'Neill et al. 2012)	Ireland	18-81 y	1136	M/F	DXA	BF %	height, weight, circumferences, skinfold thicknesses	0.90	2.5%
11	(Kagawa, Kuroiwa et al. 2007)	Japan	18-27 y	139	F	DXA	BF %	height, weight, circumferences, skinfold thicknesses	0.88	2.1%
12	(Ball, Altena et al. 2004)	USA	18-62 y	160	M	DXA	BF %	height, weight, circumferences, skinfold thicknesses	0.90	2.2%

SEE: Standard error of the estimate

2.9.5.5 Prediction of LBM, muscle mass and regional body composition using anthropometry

Prediction of LBM

As most of the prediction equations based on anthropometry typically calculate %BF, LBM can be calculated indirectly by subtracting fat mass (fat mass = body weight x percentage body fat) value from total mass (body weight). However, a few studies have specifically attempted prediction of LBM from simple anthropometric equations. For example, Fuchs and colleagues reported an equation to predict LBM in men from two anthropometric measurements: height and the circumference of the flexed biceps (Fuchs, Theis et al. 1978). This was developed using values for LBM from body density (using hydrodensitometry), total body water (by tritium dilution technique) and total body potassium (by 40K whole body counter) in men from the U.S. Air Force.

Prediction of muscle mass

There are a few anthropometric equations to predict total body muscle mass because it is difficult to measure it accurately. The whole body CT or MRI scans are considered to be the reference methods for the measurement of total body skeletal muscle mass and these methods being expensive are not applicable to large samples. Indices of muscle mass from creatinine excretion or the potassium-nitrogen ratio are difficult to measure and may not be useful for development of prediction equations (Heymsfield, Arteaga et al. 1983).

As mentioned earlier, Mateigka first developed an anthropometric approach for quantifying whole-body skeletal muscle mass (Matiegka 1921). Martin et al. (Martin, Spenst

et al. 1990) and Doupe et al. (Doupe, Martin et al. 1997) later extended Matiegka's approach and developed anthropometric prediction formulas for skeletal muscle mass based on the Brussels Cadaver Study. In 2000, Lee and colleagues conducted a prospective study to develop anthropometric prediction models for total-body skeletal muscle mass, using MRI as the reference method(Lee, Wang et al. 2000). The study included non-obese (135 men and 109 women) as well as obese (39 men and 41 women) participants. Two separate equations, one based on limb circumferences and skinfold thicknesses and the other based on body weight and height, were developed and cross-validated in non-obese and obese groups of adults. The skinfold thicknesses-circumference model had a higher accuracy than did the body weight and height model in predicting total body SM in healthy adult populations, and both models were sex, age, and race specific. Table 2.9 includes studies for anthropometric prediction of skeletal muscle mass using different reference methods.

Table 2.9 Anthropometric equations for prediction of muscle mass in adults using different reference methods

No	Study	Country	Age group	N	sex	Reference method	Dependent Variable	Anthropometric variables	R^2	SEE
1	(Lee, Wang et al. 2000)	USA (multi-ethnic)		244	M/F	MRI	SM	Height, Weight, Circumferences, skinfolds	0.91	2.2 kg
2	(Quiterio, Carnero et al. 2009)	Portugal	15±2.8 y	168	M/F	DXA	ALST	Height, Weight, Circumferences, skinfolds	0.86	1.8 kg
3	(Wen, Wang et al. 2011)	China	18-69 y	763	M/F	DXA	ALST	Weight, height, limb circumferences, waist circumference, skinfolds	0.93	1.33 kg
4	(Kuriyan and Kurpad 2004)	India	18-45 y	67	M	24 h creatinine excretion	SM	Height, weight, arm muscle area, thigh muscle area and calf muscle area	0.55	2.58 Kg
5	(Galvao, da Silva et al. 2013)	Portugal	60-89 y	101	F	DXA	ALST	Height, weight, right forearm perimeter, hip circumference, thigh skinfold thickness	0.77	1.52 kg
6	(Scafoglieri, Tresignie et al. 2012)	Belgium	30±10 y	117	M/F	DXA	SLM	Weight, height, 14 circumferences, 13 skinfolds and 4 bone breadths	0.96	0.72 kg

SLM: Segmental lean tissue mass (lean mass of both arms, trunk and both legs)

A few other studies have reported anthropometric prediction equations for estimation of skeletal muscle mass using DXA as a reference technique whereas one study from Bangalore, India has reported equations using 24 hour urinary creatinine excretion as a reference technique. A large study from China developed anthropometric prediction equations to estimate appendicular lean soft tissue in a sample of 763 adults with excellent predictive value (coefficient of determination: 0.93 and SEE 1.33 kg) (Wen, Wang et al. 2011). Another study from Belgium which developed equations to predict segmental lean tissue mass, also showed very good predictive value of the equations (Scafoglieri, Tresignie et al. 2012).

Prediction of regional muscle mass

Historically, the use of anthropometric measurements of the upper arm to estimate muscle mass has dominated the literature (Gurney and Jelliffe 1973). Estimates of upper arm muscle circumference are also used as a functional index of protein-energy malnutrition in children (Scholl, Johnston et al. 1980).

A commonly used method for regional muscle mass estimation includes arm circumference corrected for subcutaneous adipose tissue thickness and muscle cross-sectional area is estimated from the corrected circumference. The basic assumptions of this index are: a) the mid-arm is circular, b) the triceps skin-fold thickness is twice the average adipose rim diameter at the middle of the upper arm c) the mid-arm muscle compartment is circular. Heymsfield and colleagues examined the validity of the anthropometric estimation of upper-arm muscle area by comparing with the reference values determined by CT and reported that each of the assumptions was in

error to some degree (Heymsfield, Olafson et al. 1979). They subsequently developed revised sex-specific equations to account for the errors in each of the assumptions (Heymsfield, McManus et al. 1982). Corrected arm muscle area (CAMA) has been found to be a valid indicator of under nutrition and an independent predictor of mortality in community dwelling older adults in Australia (Miller, Crotty et al. 2002).

2.10 Assessment of muscle function

The gold standard for measuring muscle strength is the use of fixed dynamometers which enable the isometric, isotonic, isoinertial, dynamic and isokinetic strength, as well as the muscle power to be determined. However, the use of these large devices is costly, inaccessible and uncomfortable, so the use of hand-held hydraulic or electronic devices for these measurements is common.

Grip strength is the simplest method for assessment of muscle function in clinical practice. A study which included Dutch children, adolescents and young adults showed that grip strength was strongly correlated with total muscle strength, with correlation coefficients between 0.736 and 0.890 ($p<0.01$) (Wind, Takken et al. 2010).Consensus approach proposed by the European Working Party on Sarcopenia in Older People (EWGSOP) recommends use of grip strength for assessment of muscle strength (Cruz-Jentoft, Baeyens et al. 2010). Moreover, hand grip dynamometers are portable and inexpensive and therefore suitable for clinical practice as well as large epidemiological studies.

A number of studies have shown that low hand grip strength predicts disability, hospitalization, and mortality (Ferrucci, Penninx et al. 2002, Rantanen, Masaki et al. 2012, Roberts, Syddall et al. 2012). Among a sample of 2500 independent living Mexican Americans over 65 years followed up for 7 years, incident disability was more common in the lowest quartile compared to the highest hand grip strength quartile even after adjustment for confounding factors (Al Snih, Markides et al. 2004). Low knee extensor strength, which indicates lower limb function, was also found to be associated with increased risk of incident disability and increased mortality in the Health, Aging and Body Composition (HABC) study cohort (Newman, Kupelian et al. 2006).

Factors known to affect muscle strength include age, gender, body size and physical activity. Muscle mass and strength increase steadily during childhood with marked increases in adolescence. During puberty, boys accrue LBM (and muscle mass) at a much greater rate and for a longer period of time such that the young adult complement of LBM is attained at age 15-16 years for girls, but 19-20 years for boys (Leonard, Elmi et al. 2010). Peak gain in muscle mass and muscle strength occur during puberty but the peak muscle mass and strength are attained at a later age following a period of tissue maturation (Hulthén, Bengtsson et al. 2001). Peak values are reached between 25 and 35 years of age, and are maintained or are slightly lower between 40 and 49 years of age before the age-related decline starts after 50 years of age (Lindle, Metter et al. 1997, Janssen, Heymsfield et al. 2000, Doherty 2001). As expected, studies have shown that muscle strength is directly related to lean body mass rather than fat mass in men and women (Bunout, Barrera et al. 2004).

2.10 Key features of hand dynamometers

Instrument type	Hydraulic	Pneumatic	Mechanical	Strain
Measures Based on	Grip strength Measurement using a sealed hydraulic system	Grip pressure measurement by compression of an air-filled compartment	Grip strength measurement from the tension produced in a spring	Grip strength Measurement from the variation in electrical resistance of a length of wire due to the strain applied to it
Example of instrument	Jamar	Martin vigorimeter	Harpenden dynamometer	Isometric strength testing unit
Units	Kg or pounds of force (lbf)	mmHg or pounds per square inch (psi) (lb/in^2)	Kg or pounds of force (lbf)	Newtons of force (N)
Advantages	Portable, economical, large amount of normative data available	Gentler on weak or painful joints	No evidence for superiority presented in the literature	Not subject to leaks (of oil/water/air), which can compromise accuracy
Limitations	Can cause stress on weak joints. Can develop slow leaks.	Grip pressure measurements are dependent on the surface area over which the force is applied. Hand size can therefore influence the measurement.	Reproducibility of the grip force measurements is limited due to difficulties in exactly replicating the grip position and in calibrating the device	Can be expensive and heavy

In addition, it has been suggested that the grip strength may vary between dominant and non-dominant hands. However, studies in American and Greek

volunteers showed that in case of right handed people, the grip strength was higher in the right hand compared to left hand by 10% whereas whereas among left dominant subjects the results were equivocal(Bohannon 2003). A number of other factors influence muscle strength measurements including: forearm position (Richards, Olson et al. 1996), position of elbow and shoulder (Su, Lin et al. 1994), handle position of the dynamometer (Trampisch, Franke et al. 2012), verbal encouragement (McNair, Depledge et al. 1996), interval between the measurements (Watanabe, Owashi et al. 2005). The American Society of Hand Therapists (ASHT) protocol recommends use of the mean of three trials of grip strength in each hand which had higher test–retest reliability (Mathiowetz, Weber et al. 1984). As a number of factors illustrated above can influence these measurements, standardized protocols are essential in order to obtain precise and reproducible estimates. Differences in protocol also affect the ability to compare absolute values reported for grip strength between different study populations.

The major limitation in adoption of hand grip strength as a tool for nutritional evaluation in general population is the absence of reference values based on representative samples of the population. A study from Taiwan which included grip strength measurements in 482 adults concluded that the mean grip strength in this sample was significantly (male 25%, female 27%) lower than norms derived from Caucasian populations (Wu, Wu et al. 2009). After controlling for age and sex, palm length was the important determinant of grip strength and the study recommended ethnic specific reference standards for grip strength. A few other studies have attempted generation of reference values for specific population groups including Brazilians (Schlussel, dos Anjos et al. 2008), Germans (Gunther, Burger et al. 2008),

Koreans (Shim, Roh et al. 2013), Spanish children and adolescents (Marrodan Serrano, Romero Collazos et al. 2009), Malaysians (Kamarul, Ahmad et al. 2006) as well as in Danes (Frederiksen, Hjelmborg et al. 2006). These studies have, however, used different sampling designs, different equipment (dynamometers) and protocols for data collection and therefore the data are not directly comparable.

2.11 Summary and Implications

Appraisal of evidence cited above indicates that developmental "programming" of LBM by early under nutrition may be an important mechanism which can explain the double burden of childhood under nutrition and high prevalence of adult onset adiposity related disorders in India. However, this evidence is largely based on animal studies and human epidemiological studies that have used an indirect (i.e. birth weight) indicator of early nutrition to demonstrate its relationship with LBM in later life. A few studies using direct indicators (i.e. maternal nutritional intakes or biochemical indicators of maternal nutrition) of early nutrition in order to assess this relationship have shown inconsistent results. The overall evidence suggests that the studies that have assessed the relationship of birth size and childhood growth with body composition during adulthood, i.e. after completion of pubertal growth have shown a consistent positive association with LBM. On the other hand, studies assessing these relationships using direct indicators of maternal dietary intakes have failed to show any consistent relationship. Further research to examine the relationship between early nutritional intakes and adult LBM and muscle strength is therefore necessary.

This research would, however, require precise and valid methods of body composition assessment. The literature cited above shows that although a large number of studies have validated different body composition assessment techniques in different population groups, very few have been reported from India. As body composition differences in relation to ethnicity are well known, population specific validation studies carried out in Indian participants are required. DXA is a promising technique with high precision, relatively low cost and low radiation exposure. Validation studies comparing estimates of body composition by DXA with other precise technique in Indian participants are thus required.

Moreover, body composition estimates reported by majority of the studies are restricted to body fat percentage with scant attention to the LBM and muscle mass. Large scale epidemiological studies focussing on these metabolically important body compartments of lean and muscle mass would require availability of simple and inexpensive measurement techniques. Anthropometry fulfils these criteria but requires population specific prediction equations to estimate LBM and muscle mass from the anthropometric measurements. Such equations developed in a large sample of Indian adults are not reported till date.

Thus, from the literature cited above, it is evident that important knowledge gaps in this area exist and more research in the following areas is necessary:

a) In the context of the developmental origins of health and disease, information on the relationship between maternal nutritional status and adult body composition based on more direct indicators of maternal nutritional status

such as dietary intakes is required. Particularly, more information is needed on the role of maternal nutrition on the LBM and muscle strength of the offspring.

b) Evaluation of different techniques of body composition assessment such as DXA and isotope dilution technique in the Indian population is required to facilitate future studies on the LBM and its determinants in Indians.

c) Development of population specific anthropometric prediction equations for estimation of LBM and muscle mass in Indian men and women is necessary as it would help future population based studies focussing on these important measures of human capital.

The three studies elaborated in the following chapters (Chapters 3-5) attempt to fulfil the above mentioned research gaps.

Chapter 3: Study 1

The Association of Early Life Supplemental Nutrition With Lean Body Mass and Grip Strength in Adulthood: Evidence From APCAPS

Modified from

Bharati Kulkarni, Hannah Kuper, KV Radhakrishna, Andrew P Hills, Nuala M Byrne, Amy Taylor, Ruth Sullivan, Liza Bowen, Jonathan C Wells, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Sanjay Kinra. The Association of Early Life Supplemental Nutrition With Lean Body Mass and Grip Strength in Adulthood: Evidence From APCAPS**. American Journal of Epidemiology** 2014;179: 700-9.

And

Sanjay Kinra, KV Radha Krishna, Hannah Kuper, KV Rameshwar Sarma, Poornima Prabhakaran,Vipin Gupta, Gagandeep Kaur Walia,Santhi Bhogadi, **Bharati Kulkarni**, Aniket Kumar, Aastha Aggarwal, Ruby Gupta, D Prabhakaran, K Srinath Reddy, George Davey Smith, Yoav Ben-Shlomo, Shah Ebrahim. Cohort Profile: Andhra Pradesh Children and Parents Study (APCAPS). **International Journal of Epidemiology**2013 Sep 9. [Epub ahead of print].

Statement of Contribution of Co-Authors for Thesis by Published Paper

The following is the format for the required declaration provided at the start of any thesis chapter which includes a co-authored publication.

The authors listed below have certified* that:

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2. they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. there are no other authors of the publication according to these criteria;
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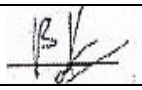
Bharati Kulkarni 	Study design, data collection , data analysis, manuscript writing
Hannah Kuper	Aided study design, data collection and revision of draft manuscript
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Principal Supervisor Confirmation

I have sighted email or other correspondence from all Co-authors confirming their certifying authorship.

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Name Signature Date

3.1 Introduction

Indians are particularly vulnerable to diabetes and cardiovascular disease at a relatively young age (Chan, Malik et al. 2009). Body composition with relatively high body fat and low LBM, compared to other ethnic groups may be partly responsible for this higher risk (Deurenberg Yap, Chew et al. 2002, Mohan, Sandeep et al. 2007). The majority of studies exploring the relationship between body composition and risk of metabolic syndrome have focussed on role of adiposity with less attention on the role of LBM (Misra and Vikram 2004, Després and Lemieux 2006, Lorenzo, Serrano-Ríos et al. 2012). Muscle mass, a predominant component of LBM, is important for insulin-stimulated plasma glucose uptake and has an independent association with insulin sensitivity (Wolfe 2006, Unni, Ramakrishnan et al. 2009). Functional competence of muscle tissue (measured using grip strength) is indicative of improved general health and is associated with decreased risk of chronic diseases and premature mortality (Rantanen, Masaki et al. 2012). Moreover, LBM and muscle strength are important measures of human capital and understanding their determinants is important. Understanding the determinants of lean body mass and its functional competence is therefore important.

Evidence suggests that early life nutrition may 'programme' LBM and muscle strength. Low birth weight was associated with lower LBM and grip strength during adulthood in observational studies from the UK, Finland and India (Phillips 1995, Sachdev, Fall et al. 2005, Yliharsila, Kajantie et al. 2007, Sayer, Dennison et al. 2008).This may be especially relevant in Indian settings where almost a third of the babies are born with low birth weight (Rantanen 2003). A study from Pune, India that compared the birth measurements of babies born in Pune

with babies born in Southampton, UK, demonstrated that although the Indian babies were smaller in all aspects, the lean tissues (skeletal muscle and abdominal viscera) were the most affected (Yajnik, Fall et al. 2003).The major criticism of these studies is the use of birth weight, which may be a poor measure of intrauterine nutrition because birth weight is also influenced by non-nutritional factors(Kuzawa 2004). More direct evidence of exposure is desirable but such evidence is scarce.

A few studies have investigated associations between early nutrition intervention and later body composition in follow-up studies of nutrition supplementation trials in pregnant women conducted a few decades ago, but their results are inconsistent. A study from Guatemala showed that maternal nutrition supplementation was associated with a taller height and higher LBM in the offspring (Rivera, Martorell et al. 1995). Another study from Gambia, however, did not find any association between maternal nutrition supplementation and offspring body composition (Hawkesworth, Prentice et al. 2008). Measurement errors due to imprecise techniques of body composition assessment and large variations in the body composition parameters related to pubertal maturity could have masked the relationship, if any.

Apart from early life influences, nutrition and physical activity in later years are major modifiable determinants of LBM (Wackerhage and Rennie 2006, Morris and Jacques 2012). Higher intake of protein is associated with higher LBM and many studies have demonstrated a beneficial effect of protein and amino acid supplementation on muscle protein synthesis and LBM (Houston, Nicklas et al. 2008, Tang, Moore et al. 2009). Physical activity, particularly resistance exercise, is known to have a strong positive effect on LBM especially when

complemented with higher intake of protein (Yang, Breen et al. , Pennings, Koopman et al. 2011).

Assessment of the relative importance of early nutrition and later lifestyle as determinants of LBM and muscle strength would help determine the focus of interventions to improve health outcomes. We therefore, examined the determinants of LBM (assessed by DXA) and muscle strength in young adults born within an earlier community trial of protein-energy supplementation conducted in Hyderabad, India (Kinra, Radhakrishna et al. , Kinra, Sarma et al. 2008). We hypothesized that early nutrition would be a more important determinant of LBM and its functional competence than current lifestyle in these rural young adults and that intervention group participants would have higher LBM and muscle strength than controls.

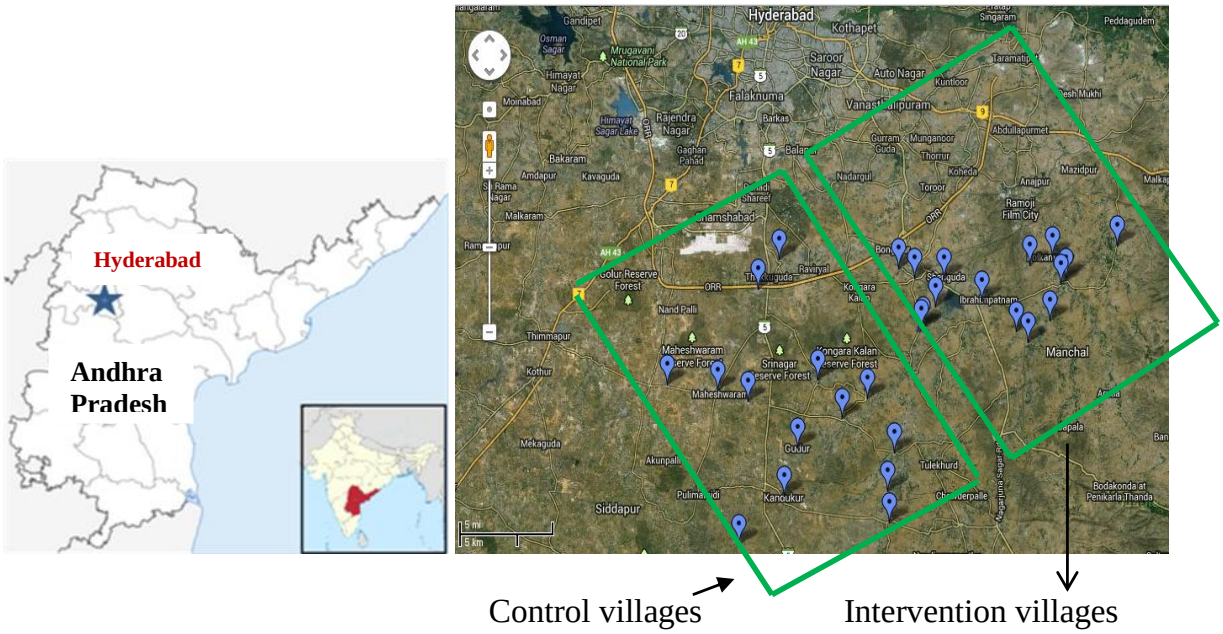
3.2 Methods

3.2.1 Study design

The present study represents a second follow-up of the cohort developed as a part of the Andhra Pradesh Children and Parents study (APCAPS) which was originally established to study the long-term effects of early-life under nutrition on risk of cardiovascular disease. It builds on the Hyderabad Nutrition Trial (HNT) conducted in 1987–90 to compare the effects of a protein-calorie supplement for pregnant women and children on birth weight. Details of the initial trial conducted during 1987-90 and the first follow-up study in 2003-5 have been reported earlier (Kinra, Sarma et al. 2008, Kinra, Radha Krishna et al. 2013)

Initial trial (The Hyderabad Nutrition Trial, 1987-90)

The Hyderabad Nutrition Trial (HNT) evaluated the impact of Integrated Child Development Services (ICDS) scheme, a national community outreach program, initiated in 1975 to improve the health, nutrition and development of children in India. The central focus of the ICDS programme is the improvement of nutritional status of pregnant and lactating women and children less than 6 years of age. It also includes early childhood education, health, hygiene and nutrition education for the mothers and delivery of other national programs (immunisation, anaemia control and basic health care) from the ICDS centres. Provision of free food to pregnant and lactating women and young children is the major component of the program. During the period of initial trial, the nutritional supplement provided by the programme was in the form of a cereal-based meal prepared from corn-soya blend and soybean oil. On average, the meal provided 2.09 MJ and 20-25 g protein to pregnant /lactating women and about 1.25MJ and 8-10g protein to children up to 6 years of age. The Hyderabad Nutrition Trial was conducted by the National Institute for Nutrition (Hyderabad) during 1987-90, and was funded by the Indian Council for Medical Research and the United States Assistance for International Development.



Two adjacent administrative areas 50 to 100 km away from Hyderabad city (one with the ICDS program, and the other awaiting implementation at that time) were identified to allow a 'controlled' stepped wedge study design involving 29 villages (15 intervention and 14 control villages) of Ranga Reddy district in Andhra Pradesh, India. All women in the reproductive age group of 13-45 years were identified in the initial household enumeration. Women "at risk" of pregnancy were monitored and those who became pregnant were followed closely during pregnancy until childbirth. A clinical examination was carried out at each trimester; attempts were made to weigh the newborns within 48 hours of delivery and follow them for the first year of life. No published findings exist, but historical records suggest that approximately 4,338 births took place during these 3 years. An abstract published in an internal publication of National Institute of Nutrition reported a modest 61 g (95% CI 18-104, p=0.007) higher birth weight in the intervention village children.

A large amount of data was collected on the pregnant women and ensuing offspring during the trial (socio-demographics, medical and obstetric history for women, feeding and immunisation history for offspring, nutritional supplementation and anthropometrics for both). However, the data collected at different time points was recorded in separate questionnaires which subsequently could not be linked due to a lack of reliable linking identifier and so is unavailable for use. Only birth weight data (which as the primary outcome of the study was recorded in additional files) is available for 603 (40%) of the 1,492 children whose identities could be reliably linked to the historical records.

Long-term follow-up of the Hyderabad Nutrition Trial

As HNT was not planned for long-term follow-up, the records available could not be linked comprehensively to individuals. An ecological approach was therefore used in which children of ages corresponding to 1987-90 births and permanently resident in the villages were recruited and categorised as living in intervention or control villages. This approach posed the pragmatic policy question: what could be achieved in the long-term from a nutritional intervention applied to villages?

In each village the names of the couples (i.e. parents) available from trial records were used to trace the trial women. A brief socio-demographic questionnaire was completed for women who could be contacted, eliciting information about each child ever borne by her. Local event calendar recall was used to determine the date of birth in many cases. Restricting the households to those with at least one live child born during 1987-90 provided the sampling frame

for the long-term follow-up study. Of the 2765 names of trial couples available from historical records, 1963 (71%) could be contacted in 2003-05, of which 1826 had at least one child born during the trial period (1987-90) who was still alive in 2003-05: these constitute the APCAPS trial households. Children born during 1987-90 and still alive in 2003-05 constitute the APCAPS birth cohort (N=2,601). A probability algorithm matching on family name pairs (women and her spouse) and child information (date of birth within 6-12 months, birth order, sex, and maternal recall of child involvement in the study) was used to retrospectively link the children from the 2003-05 survey to the historical records from the trial. A total of 1,492 (57%) children could be reliably linked.

First follow-up (mothers and children of the APCAPS birth cohort in adolescence, 2003-2005)

Only those children who could be reliably linked to their historical records (N=1,492) and their mothers were invited to participate in this follow-up. An interviewer administered questionnaire and clinical examination on 1,165 (45% of trial births) adolescent cohort members (Intervention=654 and control=511) along with a brief questionnaire and examination of 1,064 mothers (out of 1090 alive, 97% response) was carried out at clinics conducted in the study villages. Comparison of data collected on households surveyed in 2003-05 suggested that children who took part in the clinics at the first and second follow-ups were more likely to be males and students than those who did not. However, there was no difference in birth weights of the two groups (among the sub-group for whom birth weight data were available).

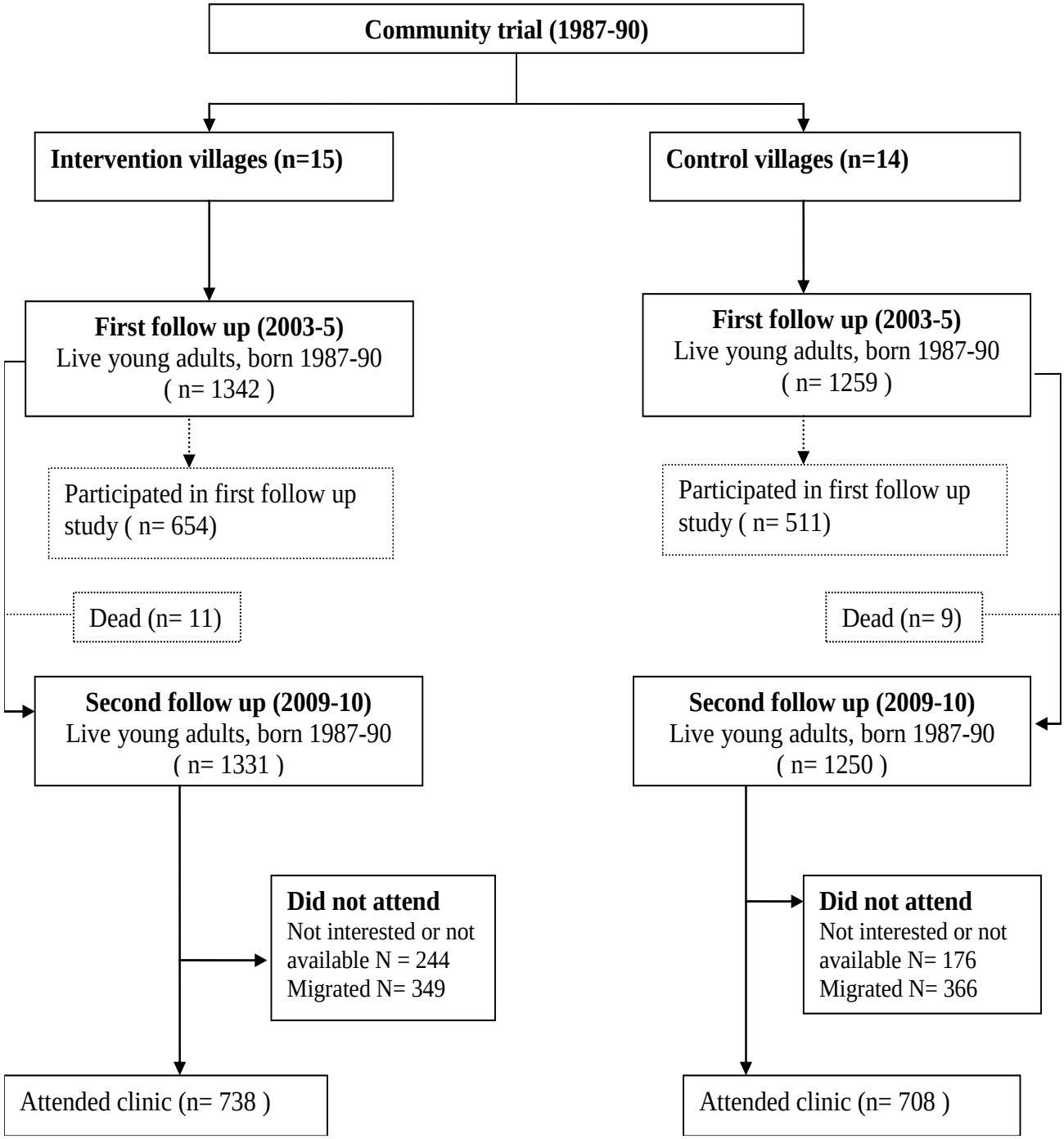
The results of this first follow-up study were consistent with the developmental origins of adult disease hypothesis, suggesting a lower risk of cardiovascular disease (as evidenced by arterial stiffness and insulin resistance) in adolescents from the intervention villages, as compared to controls (Kinra, Rameshwar Sarma et al. 2008). Participants in the intervention group were also 14 mm taller than controls, but the body composition of participants from the two groups was similar.

Second follow-up (Children of the APCAPS birth cohort in young adulthood, 2009-2010)

All 2,601 trial children irrespective of historical linkage were eligible to participate during this follow up which evaluated DXA measures of body composition and markers of chronic disease risk. A total of 1,446 (56% of trial births) who were now young adults aged 18-21 years were examined during this phase. Figure 3.1 represents the flow chart of participant recruitment in the present study.

One of the reasons for the relatively low response rate at the second follow-up (56%) was related to the distance to the clinic. Clinics at the second follow-upwere conducted 1-2 hours drive away at the National Institute of Nutrition because of DXA equipment. The response rate was particularly low among the girls, since many of them had migrated out of the area consequent to marriage (girls frequently marry before the age of 20 years in this setting) (Table 3.1).

Figure 3.1. **Flow chart of participant recruitment in the Andhra Pradesh Children and Parents Study (APCAPS)**



Ethics approval for the study

Ethical approval for the study was obtained from the ethics committees of the National Institute of Nutrition, Hyderabad, India, the London School of Hygiene and Tropical Medicine, UK and Queensland University of Technology, Australia (Appendix 1). Approval was also sought from village heads and their committees in each of the 29 villages. Written informed consent (witnessed thumbprint if illiterate) was obtained from the participants prior to their inclusion in the study.

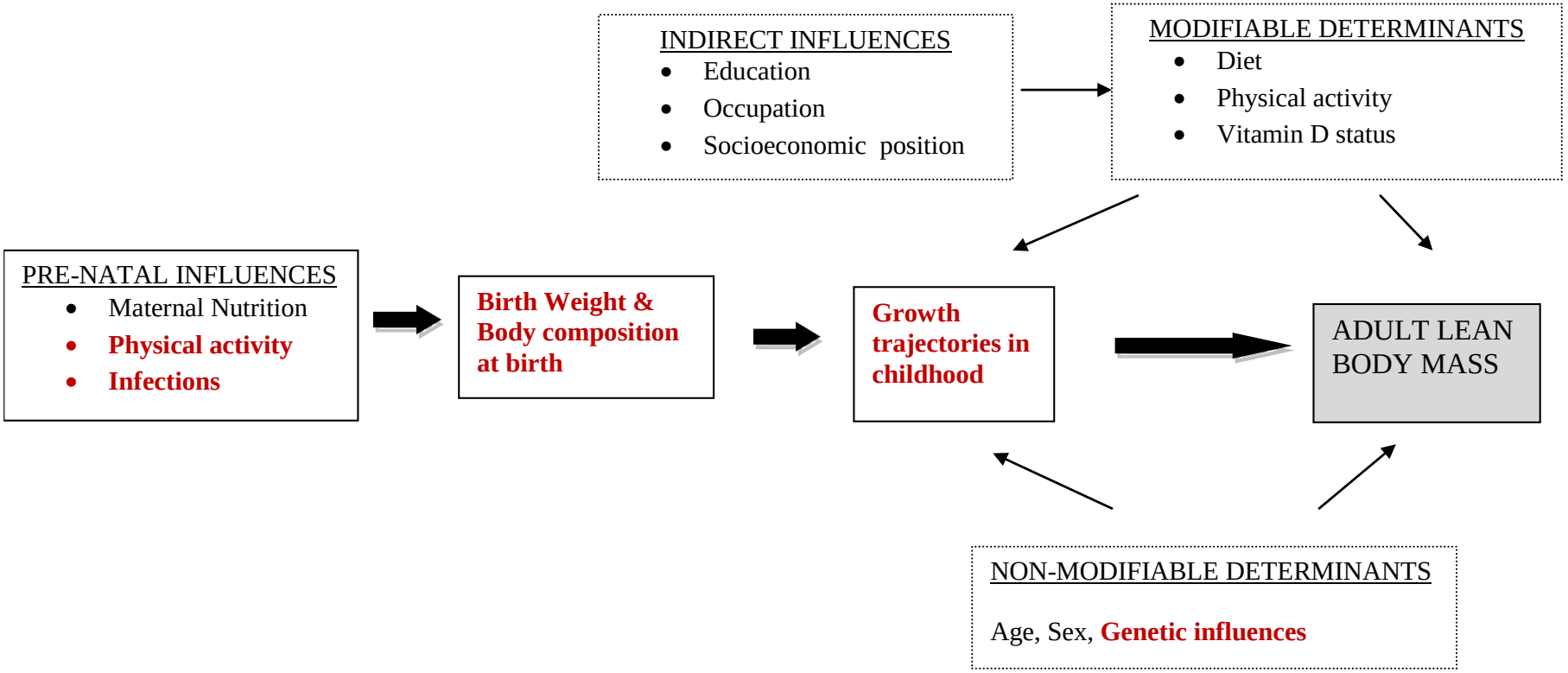


Figure 3.2. **Conceptual framework of determinants of adult lean body mass**

Information not available in the present study is indicated in bold letters.

Table 3.1 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.

Characteristic	Intervention area (n = 1342)			Control area (n = 1259)		
	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)		(n = 695)	(n = 532)	
Full time student	608 (82.5)	415 (69.4)	<0.001	534 (76.8)	328 (60.3)	<0.001
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)	2716(416)	2730(381)	0.74	2640 (432)	2592(427)	0.26
	(n=198)	(n=136)		(n=273)	(n=165)	

* 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.

3.2.2 Measurements

All consenting participants visited a clinic at the National Institute of Nutrition, Hyderabad. A semi-structured questionnaire was administered to all participants by a trained interviewer to assess background information including education, occupation, socioeconomic position etc.

3.2.2.1 Socio-economic position

Socioeconomic position was examined using a standard of living index (SLI), a household level asset-based scale devised for Indian surveys selecting those we believed the most informative for our study population. These included: quality of house, toilet facilities, source of lighting and drinking water, whether the household collected rations using a ration card, possession of clock, radio, television, bicycle, motorcycle, car, refrigerator and telephone, number of rooms in the household and whether the household owned agricultural land. These items were weighted to give a maximum score of 34, using weights developed by the International Institute of Population Sciences in India(2007).This index has been widely used in epidemiological studies from India (Subramanyam, Kawachi et al. 2010) and was found to correlate highly with income data (Filmer and Pritchett 2001).

3.2.2.2Dietary intakes

Dietary intakes over the past year were estimated using a semi-quantitative food frequency questionnaire (FFQ) which assessed frequency of intake (daily, weekly, monthly, yearly/never) of 98 commonly consumed food items. Indian food composition tables were then used to estimate nutrient content of a single portion of each food item (Narasinga Rao, Deosthale

et al. 1989). Where nutrient values were unavailable from the Indian food composition tables (e.g. for foods such as manufactured 'western' snacks and sweetened drinks), the United States Department of Agriculture nutrient database (USDA, Release No. 14) (Agriculture) or McCance and Widdowsons Composition of Foods were used(Welch, Unwin et al. 1995). The FFQ was validated against a 24 hour dietary recalls measured on three different days (Bowen, Bharathi et al. 2012).The validation study showed that although the FFQ showed acceptable validity for measuring intakes of groups, it overestimated dietary intakes (mean difference in energy intake = 1743 kJ or 413 Kcal).

3.2.2.3 Physical activity

Physical activity of previous week was assessed across the following domains: work, travel, sports/games/exercise, household, and sedentary. For each activity, information was collected on its frequency and duration. Metabolic equivalent of tasks (METs) were then calculated as the multiples of resting metabolic rate (1 MET is equivalent to the energy expenditure value of sitting quietly) using the Compendium of Physical Activity and WHO/FAO/UN guidelines, supplemented with country specific values(Ainsworth, Haskell et al. 2000, Organization 2005, Vaz, Karaolis et al. 2005). Total activity was calculated as total METs (hr/day) by summing daily MET values of all activities (Ainsworth, Haskell et al. 2000, Vaz, Karaolis et al. 2005). In addition, we calculated time (min/day) spent in four categories of activity intensity using previously published cut-points; sedentary 1.5 METs; light 1.5 to 3 METs; moderate 3 to 6 METs; vigorous > 6 METs (Pate, Pratt et al. 1995). Time spent in moderate and vigorous intensity activities was subsequently combined as only 3% of the sample reported activity of a vigorous intensity. Reliability and validity of this questionnaire was

examined in a separate study which included 479 participants with different levels of physical activity (sedentary, light, moderate and vigorous) (Sullivan, Kinra et al. 2012). Physical activity estimated by the questionnaire was compared to that measured using uni-axial accelerometers. This study showed that the questionnaire can be an appropriate tool for ranking physical activity of individuals in India.

3.2.2.4 Anthropometry

Weight was measured (to the nearest 0.1 kg) using a digital SECA balance (Hamburg, Germany) and standing height was measured (to the nearest 1 mm) with a stadiometer (Leicester height measure; Chasmors Ltd, Camden, London, UK). Each measure was assessed twice, and the average of two values was used in the analysis. Body mass index (BMI) was calculated by dividing weight in kg by height in meters squared.

3.2.2.5 Muscle strength

Grip strength was measured using a grip dynamometer (Grip D, Takei, Tokyo, Japan), separately for each arm. This measurement was done three times, with a rest of at least 10 seconds between attempts and the maximal estimate of force displayed by the machine was recorded. These measurements were done in the morning after participants had consumed their breakfast. The field investigators provided standard verbal encouragement to the participants as different methods of instruction and/or verbal encouragement can affect the performance(Roberts, Denison et al. 2011).Reproducibility of grip strength measurements assessed in a sub-sample of 20 adults showed high reliability (Intra-class correlation coefficient:

0.95). As the results of the analyses using dominant and nondominant arm were similar, analyses using the dominant arm values have been reported.

3.2.2.6 Body composition

Body composition measurements including whole body lean mass, fat mass and percentage of fat mass as well as regional fat and lean mass were assessed with DXA (using either Hologic Discovery A model (91% of scans) or Hologic 4500W, Waltham, MA, USA (9% of scans)). The scanner was calibrated daily using a phantom, and its performance was monitored as per the quality assurance protocol. No sign of scanner drift was observed during the study period. The in vivo precision (coefficient of variation) was < 1% for the lean body mass measurement. Pregnant women were excluded from the DXA scanning. Standard Hologic software options were used to define regions of the body (head, arms, trunk and legs). Appendicular skeletal muscle mass (ASM) was calculated as the sum of bone free lean tissue in arms and legs (Kim, Wang et al. 2002).

3.2.3 Quality control

We produced detailed protocols and used them regularly to standardise work of the fieldwork team. Masking the group assignment from fieldworkers was not an option but the key outcome measures including DXA estimates of LBM and ASM were automated reducing possibility of bias. Anthropometric and grip strength measurements were undertaken by two observers and the inter-observer bias was estimated periodically. DXA scans were analysed by a single trained technician. Incomplete scans or those with major movement artefacts were

excluded from the analyses. Scans with minor movement artefacts were included in the analyses. Sensitivity analyses showed that their exclusion did not make a difference to the results.

3.2.4 Statistical analyses

All analyses were conducted using Stata, version 11.2 (Stata corp, Texas, US). In case of dietary energy and protein intakes, extreme values (below 1st percentile and above 99th percentile were adjusted and made equivalent to the values of 1st and 99th percentile, respectively. Protein intake was examined using the nutrient residual energy adjustment method, in which protein residuals obtained by regressing absolute protein intake on total energy intake were used as the independent variable(Willett, Howe et al. 1997). An advantage of this method is that it provides a measure of protein intake independent of total energy intake. Differences in participant characteristics in relation to the supplemental nutrition were assessed separately for men and women with Student's t test for continuous variables and chi-square test for trend for categorical variables with appropriate degrees of freedom. Unadjusted associations between supplemental nutrition and outcome variables (total lean body mass, ASM and grip strength) were assessed using linear regression models with robust standard errors to account for clustering by village and sibling pairs (as 38% of the participants were sibling pairs). Simultaneous adjustment for clustering by village and sibling pairs was done by dividing the 29 village clusters further into two categories: village clusters without any sibling pair (n=29) and village clusters with sibling pairs (n=27), thus forming a total of 56 clusters. To examine the role of various determinants on the lean mass indicators, linear regression models were used with each of the lean mass indicators (LBM, ASM and grip strength) as a dependent variable and the physiological (age and sex), socioeconomic (education, occupation and household standard of living index) and lifestyle

indicators (dietary intakes and physical activity) as predictor variables. Continuous predictor variables (SLI, physical activity and dietary energy and protein intakes) were divided into tertiles due to their non-linear relationship with the outcome variables and imprecise nature. Tests for linear trends across tertiles of these predictors were conducted by using the median value in each tertile as a continuous variable in the linear regression models. These models were additionally adjusted for early nutrition supplementation. Finally, to examine the association between supplemental nutrition and the LBM indicators, multiple linear regression models were constructed after adjusting for the potential confounders described earlier. Three predefined models were fitted to adjust incrementally for the main domains of potential confounding or intermediary variables mentioned above: model 1 - physiological variables; model 2 - socioeconomic indicators; model 3 - lifestyle indicators. As height was related to all the lean mass indicators (LBM: 0.87, $p < 0.001$; ASM: 0.84, $p < 0.001$; Grip strength: 0.70, $p < 0.001$), an additional model (model 4) which included height in addition to the variables included in model 3 was constructed in order to assess the extent to which the variation in lean mass indicators in relation to the confounders is mediated by change in height. DXA estimated measures of LBM and ASM in all of the above analyses were additionally adjusted for the scanner. We pooled the sexes for the multiple regression analyses as there was no evidence of interaction between nutrition intervention and sex. Robust standard errors were used throughout to account for clustering of the data by village and sibling pairs as described above. Examination of residuals after fitting the regression models for the main outcome variables showed a normal distribution eliminating the possibility of bias. Missing data were handled with list-wise deletion.

Sample size calculations undertaken before the study commenced suggested that the anticipated sample (about 1400) would provide adequate power to detect relatively small differences (about 0.17 of a standard deviation) in total LBM, ASM and grip strength with 90% power and 5% level of significance.

3.3 Results

Of the 2,601 children eligible for inclusion in the study (i.e. born in these villages during 1987-90), a total of 1,446 individuals (32% women) participated in the clinics: 738 in the intervention and 708 in the control area. A larger proportion of women were lost to follow-up mainly due to migration out of the study area consequent to marriage. Body composition estimates by DXA were available for 1,387 (96%) participants with missing data in 49 women and 13 men. The main reason for missing DXA data in case of women was pregnancy and for men, exclusion of DXA scans due to major movement artefacts. Information on other variables including grip strength, physical activity and dietary intake estimates was available for >99% of participants.

Table 3.2 shows participant characteristics and distribution of the key exposures in the intervention and control groups of men and women. Although the mean age and physical activity were similar in the two groups of men and women, dietary intakes including energy and protein intakes (in men only) were higher in men and women from the intervention group than the control group. Overall dietary energy and protein intakes were higher than the average intakes of rural adults (especially in men) reported by the National Nutrition Monitoring Bureau (NNMB)

surveys (2007) probably due to over-estimation by FFQ as observed in a number of other studies from India (Sudha, Radhika et al. 2006, Bowen, Bharathi et al. 2012).

The majority of the men were either students or engaged in manual occupations while the majority of the women were home-makers. A higher proportion of men and women from the intervention group had received higher education than the control men and women and a larger proportion of control women were engaged in manual occupations (33.9% vs 25.8%), providing indirect evidence of the relatively lower socio-economic position and rurality of the control group participants. Socio-economic position as assessed by the SLI was, however, not different in the two groups of participants.

Overall, the participants were of relatively short stature (Men: 166.7 cm; Women: 152.6 cm) with a low BMI (Men: 19.7 kg/m^2; Women: 19.1 kg/m^2) and about 37% men and 50% women suffered from chronic energy deficiency (BMI < 18.5 kg/m^2). The height, weight and BMI were not different in the two groups of participants.

Table 3.2. Characteristics of participants included in the follow-up of Andhra Pradesh Children and Parents Study (APCAPS)

	Men Intervention (n=499)	Control (n=483)	*P* value	Women Intervention (n=239)	Control (n=227)	*P* value
Age (y)	20.8 (1.1)	20.8 (1.2)	0.37	21.0 (1.1)	21.1 (1.2)	0.33
Dietary intakes						
Energy intake (Kcal/d)	3366 (1146)	3186 (1020)	<0.01	2149 (690)	2024 (547)	0.03
Protein intake (g/day)	82 (30)	78 (27)	0.03	51 (17)	49 (15)	0.17
Physical activity						
Physical activity (MET-hr/day)[1]	40.2 (6.4)	40.0 (6.7)	0.80	36.6 (5.3)	36.8 (5.4)	0.66
Time spent in MVPA[2] (min/day)	220 (135)	223 (151)	0.76	118 (123)	104 (122)	0.22
Occupation						
No paid employment[3]	44.1	42.1	0.30	71.7	62.6	0.11
Manual work (skilled / unskilled)	47.9	51.9		25.8	33.9	
Professional	8.0	6.0		2.5	3.5	
Education						
≤ Primary school	15.4	19.5	0.01	19.6	37.4	<0.01
Secondary school	76.7	76.6		71.2	59.5	
College	7.8	3.9		9.2	3.1	
SLI[4]	18.7 (4.2)	18.6 (4.2)	0.57	17.9 (4.2)	17.2 (4.7)	0.09
Anthropometry						
Height (cm)	166.6 (6.4)	166.7 (6.0)	0.63	152.7 (5.1)	152.5 (5.4)	0.69
Weight (kg)	54.8 (8.8)	54.9 (8.6)	0.88	44.2 (7.8)	45.0 (7.2)	0.23
BMI[5] (kg/m^2)	19.7 (2.8)	19.7 (2.8)	0.92	18.9 (3.1)	19.3 (2.7)	0.15

[1]MET-hr/day: Metabolic equivalents of tasks (hr/day)
[2] MVPA: Moderate and vigorous intensity physical activity
[3]This categoryincluded homemakers, students and unemployed
[4]SLI: Standard of living index. Higher value indicates higher socioeconomic position.
[5] BMI: Body mass index
Values are means (SD) for continuous variables or percentages for categorical variables.
P values are based on unpaired t tests or $\chi 2$ tests for trend

Figures3.3, 3.4 and 3.5 show distribution of outcome variables in men and women from the intervention and control groups.

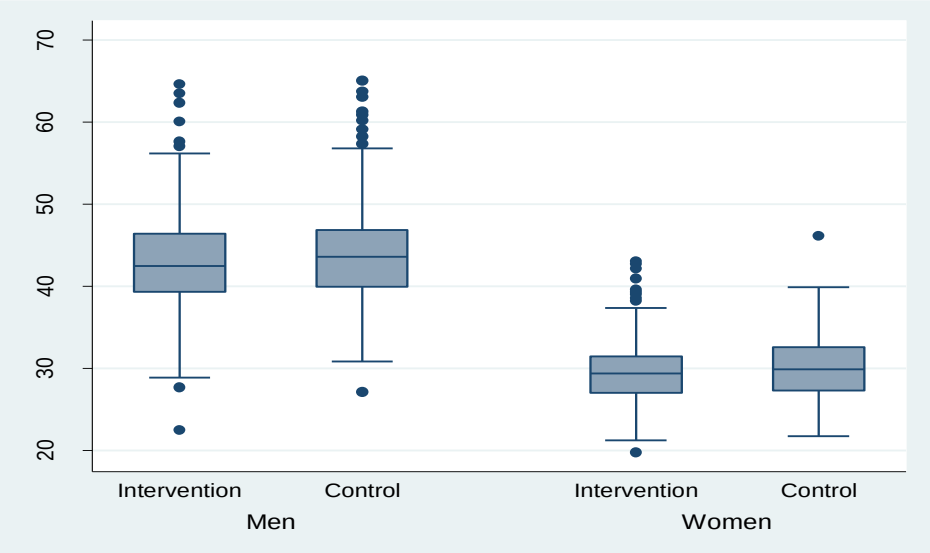


Figure 3.3. **Lean body mass in men and women from the intervention and control groups.**

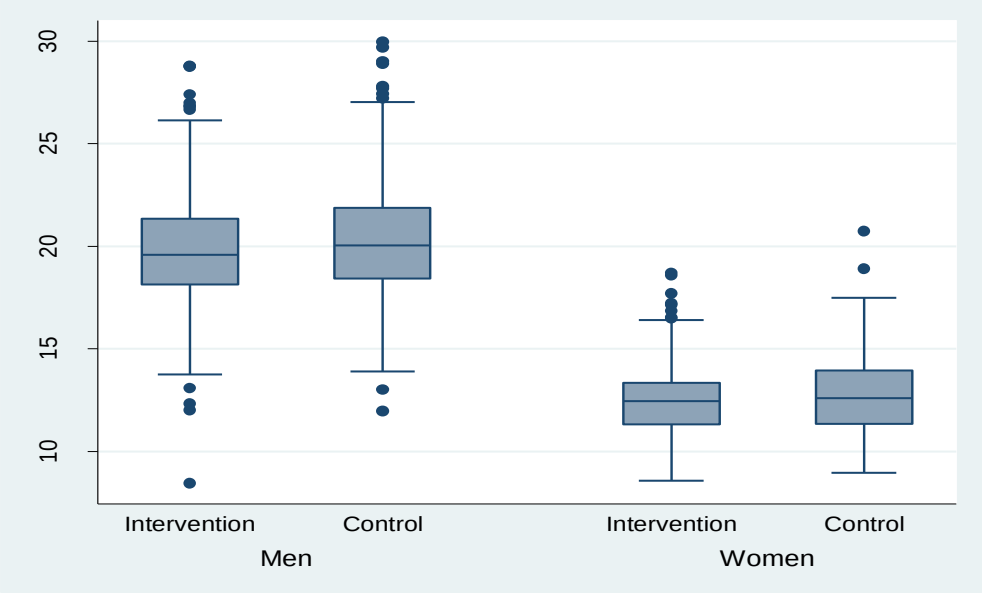


Figure 3.4. **Appendicular skeletal muscle mass in men and women from the intervention and control groups.**

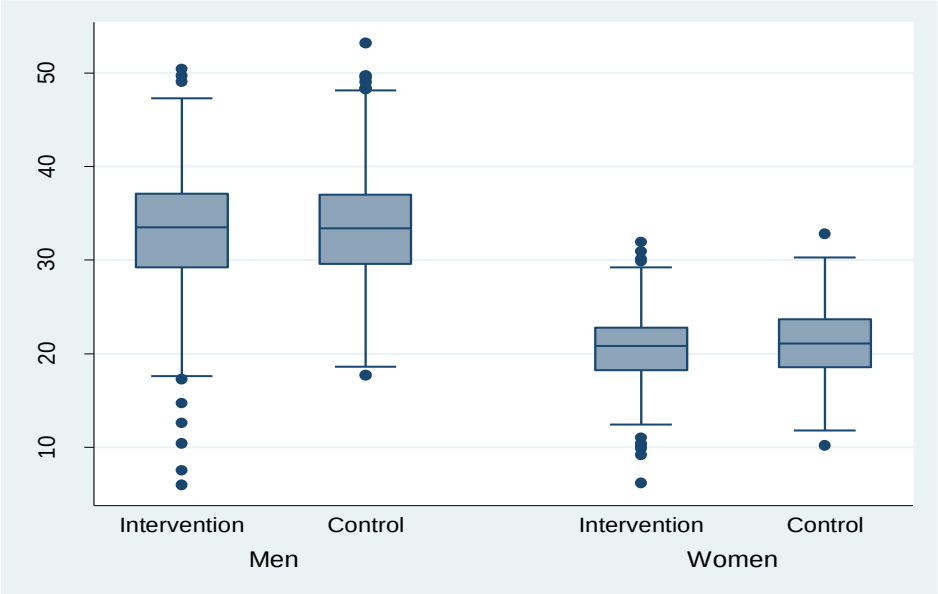


Figure 3.5. **Grip strength (dominant hand) in men and women from the intervention and control groups.**

Table 3.3 shows the differences in outcome variables in men and women from the intervention and control groups assessed using linear regression models with robust standard errors to account for clustering by village and sibling pair.

The LBM and grip strength (both dominant as well as non-dominant hand) were largely similar in the two groups but ASM (kg) was lower in the intervention group men compared to the control group men (mean (SD): 19.78 (2.71) vs 20.24 (2.67); p =0.03).

Table 3.4 shows the matrix of the correlation coefficients of the univariate relationship between predictor and outcome variables.

Table 3.3 Distribution of Outcome Variables by Area of Intervention in Participants of the Andhra Pradesh Children and Parents Study

	Men				Women			
	Intervention (n=490)	**Control (n=479)**	**Difference (95% CI)**	***P* value**	**Interventio n(n=218)**	**Control (n=200)**	**Difference (95% CI)**	***P* value**
[1]LBM (kg)	43.03 (5.49)	43.78 (5.37)	-0.74 (-1.58 to 0.10)	0.08	29.60 (3.87)	30.20 (3.95)	-0.67 (-1.53 to 0.19)	0.13
[2]ASM (kg)	19.78 (2.71)	20.24 (2.67)	-0.45 (-0.86 to -0.04)	0.03	12.56 (1.81)	12.87 (1.91)	-0.34 (-0.79 to 0.10)	0.13
Dominant hand grip strength (kg)	33.11 (6.24)	33.65 (5.78)	-0.59 (-1.44 to 0.27)	0.18	20.60 (3.97)	21.10 (3.68)	-0.39 (-1.09 to 0.31)	0.27
Non-dominant hand grip strength (kg)	32.75 (6.12)	33.51 (6.02)	-0.76 (-1.70 to 0.17)	0.11	19.47 (3.80)	19.62 (3.71)	-0.15 (-0.78 to 0.49)	0.65

Values are means (SD) unless indicated otherwise.

[1]LBM: Lean body mass; [2]ASM: Appendicular skeletal muscle mass

Sample size for grip strength- men: 499 (intervention), 483 (control) and women: 240 (intervention) and 225 (control)

P values are based on linear regression models with robust standard errors to account for clustering by village and sibling pair. DXA estimates of LBM and ASM are additionally adjusted for DXA scanner.

Table3.4 Correlation matrix of outcome and predictor variables

	Lean body mass	ASM	Grip Strength	Age	SLI	Energy intake	Protein intake	Physical activity
Lean body mass	-							
ASM	0.9826*	1.000						
Grip strength	0.7926*	0.8055*	-					
Age	0.0053	-0.0318	-0.0131	-				
SLI	0.1844*	0.1678*	0.1724*	-0.0375	-			
Energy intake	0.5093*	0.5042*	0.4735*	-0.0202	0.1854*	-		
Protein intake	0.4993*	0.4887*	0.4509*	-0.0061	0.2016*	0.9717*	-	
Physical activity	0.2498*	0.2559*	0.2018*	0.0008	-0.0885*	0.2317*	0.2240*	-

*P < 0.001

ASM: Appendicular skeletal muscle mass; SLI: Standard of living index

Table 3.5 shows multivariable associations between important exposure variables (confounders or potential determinants) and the lean mass indicators. Age had a positive association with all the lean mass indices showing that participants had probably not achieved their peak muscle mass and strength. As expected, there were marked differences in the lean mass indices of men and women. Although socio-economic position had a positive association with the lean mass indices, education and occupation group were largely unrelated to the lean mass indicators. Physical activity, however, had a positive association with all the indicators except grip strength. Dietary energy intake had a strong positive association with total lean mass, ASM as well as grip strength. Compared to the participants with energy intakes in the lowest tertile, participants with energy intakes in the middle tertile had about 1.2 kg higher LBM and those with energy intake in the uppermost tertile had about 2.65 kg higher LBM. Energy-adjusted protein intakes were not associated with any of the lean mass indices. The positive relationship between SLI and lean mass indices was probably mediated by energy intake as energy intakes were positively related to the SLI whereas physical activity had negative association with the SLI (data not shown).

Table 3.5 Multiple regression analyses to examine the association of outcome variables with various determinants in the long-term follow-up of Hyderabad Nutrition Trial.

	LBM[1] (kg) (n = 1375) β (95% CI)	***P***	**ASM[2] (kg) (n = 1375) β (95% CI)**	***P***	**Grip strength (kg) (n = 1435) β (95% CI)**	***P***
Age (y)	0.43 (0.21 to 0.65)	<0.01	0.12 (0.02 to 0.22)	0.02	0.40 (0.20 to 0.61)	<0.01
Sex						
Male	Reference		Reference		Reference	
Female	-12.04 (-12.69 to -11.39)	<0.01	-6.59 (-6.90 to -6.28)	<0.01	-11.29 (-11.91 to -10.67)	<0.01
Standard of living index						
Tertile 1	Reference		Reference		Reference	
Tertile 2	0.30 (-0.32 to 0.91)		0.11 (-0.20 to 0.41)		0.22 (- 0.48 to 0.93)	
Tertile 3	1.69 (1.09 to 2.29)	<0. 01	0.74 (0.45 to 1.02)	<0.01	1.16 (0.49 to 1.83)	<0.01
Education						
≤ Primary school	Reference		Reference		Reference	
Secondary school	0.07 (-0.64 to 0.77)		0.10 (-0.25 to 0.46)		1.03 (0.09 to 1.98)	
College	0.78 (-1.10 to 2.66)	0.78	0.32 (-0.44 to 1.09)	0.45	1.05 (-0.49 to 2.63)	0.07
Occupation						
No paid employment	Reference		Reference		Reference	
Manual work (Unskilled /skilled)	0.34 (-0.34 to 1.02)		0.08 (-0.24 to 0.42)		0.51 (0.01 to 1.02)	
Professional	-0.05 (-1.17 to 1.28)	0.33	-0.22 (-0.95 to 0.37)	0.38	1.05 (-0.50 to 2.60)	0.32

Physical activity

Tertile 1	Reference		Reference		Reference	
Tertile 2	0.10 (-0.63 to 0.84)		0.10 (- 0.27 to 0.47)		0.06 (-0.75 to 0.89)	
Tertile 3	0.71 (0.03 to 1.39)	<0. 01	0.45 (0.12 to 0.77)	<0.01	0.14 (- 0.67 to 0.94)	0.45

Dietary energy intake

Tertile 1	Reference		Reference		Reference	
Tertile 2	1.20 (0.53 to 1.86)		0.59 (0.25 to 0.94)		0.81 (0.22 to 1.40)	
Tertile 3	2.65 (0.53 to 1.86)	<0. 01	1.26 (0.80 to 1.71)	<0.01	2.17 (1.37 to 2.96)	<0.01

Energy- adjusted protein intake

Tertile 1	Reference		Reference		Reference	
Tertile 2	0.40 (-0.30 to 1.11)		0.23 (-0.15 to 0.62)		- 0.05 (- 0.85 to 0.74)	
Tertile 3	0.23 (-0.48 to 0.94)	0.75	0.07 (-0.30 to 0.44)	0.71	- 0.36 (- 1.11 to 0.38)	0.35
R square	0.63		0.67		0.56	

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[1]LBM: Lean body mass; [2]ASM: Appendicular skeletal muscle mass

Associations of individual predictors with the lean body mass indices were examined by multivariable linear regression after adjusting for all the other predictors and nutrition supplementation. *P* values are based on the robust standard errors to account for clustering by village and sibling pair. *P* values for trend are reported for categorical variables (tertiles of SLI, physical activity, energy intake, energy adjusted protein intake as well as education and occupation).

Standard of Living Index tertiles: T1 < 17; T2 17 to 20; T3 > 20

Physical activity (MET-hr /day) tertiles: T1 < 35.3; T2 35.3 to 40.7; T3 > 40.7

Energy intake (Kcal/day) tertiles: T1 < 2273; T2 2274 to 3316; T3 > 3316

Energy adjusted protein intake (g/day): T1 < 69.3; T2 69.3 to 73.5; T3 > 73.5

Table 3.6 shows the differences in lean body mass indicators in relation to the nutrition supplementation after adjustment for the potential confounders mentioned above. Model 1, which was adjusted for physiological variables (age and sex), indicated lower ASM (β coefficient, 95% CI: -0.40 kg, -0.75 to -0.05 kg, *p* =0.02) in the intervention group with a similar trend in case of total LBM (β coefficient, 95% CI: - 0.66, - 1.36 to 0.04 kg; *p* =0.06) with no difference in grip strength between the two groups. After further adjustment for socio-economic indicators (SLI, education and occupation group) in model 2, intervention group participants had a lower grip strength than the control group (β coefficient, 95% CI: -0.71kg, -1.37 to -0.05 kg, *p*=0.04) while the differences in LBM and ASM were largely similar to those in model 1. Model 3, which was additionally adjusted for behavioural/ life style determinants (dietary intakes and physical activity), showed slightly higher differences with lower values of all the lean mass indicators in the intervention group. Magnitude of the differences in LBM, ASM as well as grip strength in relation to early nutrition supplementation in all three models was, however, small (<0.1 SD). We also re-examined Model 3 by including intensity of physical activity (time spent in moderate and vigorous intensity physical activity in min/day) instead of total physical activity in MET-hr/day. Results were similar for both the indicators of physical activity and therefore, only the results with total physical activity are shown in tables 3 and 4. As all the outcome variables had significant positive relationships with height (LBM: 0.82, p <0.01: ASM: 0.84, p <0.01; Grip strength: 0.70, p <0.01), we additionally adjusted for height (model 4) in order to assess the extent to which the variation in the lean mass indicators in relation to the supplemental nutrition was mediated by differences in height. Adjustment for height resulted in only slight reductions in differences in total lean mass, ASM and the grip strength in relation to supplementation, indicating that these differences were largely unrelated to height.

Table3.6 Multivariable association between supplemental nutrition and lean body mass indicators in the long-term follow-up of Hyderabad Nutrition Trial.

	Model 1 (n = 1388)		**Model 2 (n=1375)**		**Model 3 (n=1375)**	
	B (95% CI)	***P* Value**	**B (95% CI)**	***P* Value**	**B (95% CI)**	***P* Value**
LBM (kg)	- 0.66 (- 1.36 to 0.04)	0.06	-0.75 (-1.41 to -0.09)	0.03	-0.64 (-1.20 to -0.08)	0.03
R Square	0.60		0.64		0.75	
ASM (kg)	-0.40 (- 0.75 to -0.05)	0.02	- 0.50 (- 0.82 to -0.12)	<0.01	-0.41 (- 0.69 to - 0.13)	<0.01
R Square	0.65		0.67		0.80	
Grip strength (kg)	-0.50 (- 1.22 to 0.22)	0.17	- 0.81 (-1.41 to -0.21)	<0.01	- 0.70 (- 1.27 to - 0.12)	0.02
R Square	0.54		0.56		0.60	

LBM: Lean body mass ; ASM: Appendicular skeletal muscle mass

Differences are intervention – control.

Sample size for grip strength: Model 1-1447, Model 2 – 1402, Model 3 – 1435, Model 4- 1434

Model 1 – adjusted for age and sex

Model 2 – adjusted for variables in model 1 + education + occupation + tertile of standard of living index (SLI) + village urbanization (village population <2000, 2000-5000, >5000)) + tertiles of physical activity + tertiles of dietary energy and energy adjusted protein intake

Model 3 - adjusted for variables in model 2 + height

All the p values are based on are based on linear regression models with robust standard errors to account for clustering by village and household (sibling pair). Additional adjustment for the DXA scanner was done in case of total lean body mass and ASM.

SLI tertiles : T1- < 17; T2 – 17- 20; T3 - < 20; Physical activity (MET-hr /day) tertiles: T1 < 35.3; T2 35.3 to 40.7; T3 > 40.7 ; Energy intake (Kcal/day) tertiles: T1 < 2273; T2 2274 to 3316; T3 > 3316 ;

Energy adjusted protein intake: T1 < 69.3; T2 69.3 to 73.5; T3 > 73.5

3.4 Discussion

In this long-term follow-up of a community trial of nutrition supplementation in rural young adults, exposure to early nutrition supplementation was not associated with a positive effect on the LBM and muscle strength in adulthood. Consistent with existing evidence, current dietary energy intake, physical activity and socio-economic position were important determinants of lean mass indices.

A number of studies based on long-term follow-up of birth cohorts from high as well as low income countries have shown a positive relationship between birth weight (an indirect indicator of early nutrition) and adult LBM and muscle strength (Singhal, Wells et al. 2003, Sachdev, Fall et al. 2005, Yliharsila, Kajantie et al. 2007, Kuzawa, Hallal et al. 2012). On the other hand, follow-up studies of nutrition intervention trials in pregnant women have shown either positive or no relationship between early nutrition and LBM of the offspring (Rivera, Martorell et al. 1995, Hawkesworth, Prentice et al. 2008, Khan, Kabir et al. 2013). A widely cited study from Guatemala conducted by the Institute of Nutrition of Central America and Panama (INCAP) showed a positive impact of a high energy, high protein supplement provided to pregnant women and young children on the LBM of the offspring (girls only) during adolescence (age 14-20 y)(Rivera, Martorell et al. 1995). However, another study from the Gambia, a cluster-randomized trial of protein-energy supplementation during pregnancy (from 20 weeks of gestation to term), could not confirm the findings of the Guatemalan study(Hawkesworth, Prentice et al. 2008). Similarly, in a study from Bangladesh, early invitation (at around 9 weeks) to start food and/or multiple micronutrient supplementation compared to usual invitation (at around 20 weeks) to start supplementation during

pregnancy did not affect offspring body composition at 5 y of age (Khan, Kabir et al. 2013). Our study corroborates the findings of the Gambian and the Bangladeshi studies that relatively short term nutrition supplementation in early life may not be effective in improving the offspring lean mass.

The negative association of early nutrition supplementation with the adult lean mass indices in our study is difficult to explain. Differences in the results of our study and the INCAP study (only study which found a positive association between early nutrition and later LBM) may be partly related to differences in the study design. The INCAP study was a randomized controlled trial with supervised nutrition supplementation whereas exposure in our study was ecological as participants born in the intervention villages were considered to be exposed to the supplement. In addition, difference in the age at follow up measurements may have influenced the outcome. Previous follow up assessment of our cohort members when they were adolescents showed an indirect positive effect of supplementation on LBM (taller height) which is qualitatively similar to the findings of the INCAP study (Kinra, Sarma et al. 2008). This beneficial effect of early nutrition intervention on LBM probably did not persist beyond adolescence due to "dilution" of the programming effect of early nutrition by diet and other lifestyle changes over the years. Other explanation of this unexpected finding may be possible. From an evolutionary life-history perspective, it may be hypothesized that in a chronically under nourished population, supplemental energy was not utilized for laying muscle tissue but diverted to the growth of other tissues such as fat which may confer survival or reproductive benefits to the offspring (Wells 2009). However, such a shift in the allocation of energy from lean to fat tissue is less likely to explain the above findings

as supplementation was associated with a taller height during adolescence in this cohort.

A more plausible explanation could be a confounding influence exerted by imperfectly measured or unmeasured confounders on the observed relationship. Studies based on long term follow up of birth cohorts in transitioning communities are faced with challenges of dealing with complex confounding influences related to rapid socio-economic and lifestyle changes. Direction of bias resulting from such confounding effect may be either towards or away from the null, depending on the correlation structure and the distribution of the confounders in the two study groups (Greenwood, Gilthorpe et al. 2006). Anecdotal evidence from our study area suggests that the intervention villages have undergone relatively rapid urbanization than the control villages in the past few years. Lower muscle mass and strength in the intervention participants could be attributed to their relatively urbanized lifestyles and a higher level of education which increases the likelihood of sedentary occupations. In spite of our efforts of measuring the possible confounders with rigorous quality control, inherent inaccuracies in the measurement of some of the exposures (e.g. socio-economic position, urbanization, dietary intakes, physical activity etc.) may have resulted in bias due to inadequate adjustment for these confounders (Fewell, Davey Smith et al. 2007). Moreover, the effect size of the negative relationship between early nutrition and later lean mass indices was small (<0.1 SD) and possibility of this being a chance finding cannot be ruled out (Zakzanis 2001).

The positive relationship of lifestyle determinants including dietary energy intake and physical activity with the LBM indicators observed in the present study is largely consistent with the existing evidence (Koopman, Saris et al. 2007, Beelen, Burke et al. 2010). Energy adjusted protein intake was, however, unrelated to the lean mass indicators unlike some of the previous studies showing a beneficial effect of protein intake on the lean mass (Houston, Nicklas et al. 2008, Scott, Blizzard et al. 2010). This could be attributed to the differences in the protein quality: animal protein intake in the previous studies *vs.* cereal protein in the present study. Moreover, the lack of variation in the protein intake of this homogeneous population group may have precluded the detection of association between protein intake and LBM. Socio-economic position had a significant positive influence on the LBM indicators probably due to its positive influence on the energy intakes. Meaningful comparison of the relative importance of early *vs* later life factors as determinants of adult LBM is, however, not possible due to imprecise estimates of these exposures.

A major strength of this study is the comprehensive assessment of early and later life influences on the LBM and muscle strength. On other hand, majority of previous studies have examined "programming" of the LBM by early nutrition or the role of lifestyle determinants in isolation. In addition, the nutrition intervention in the present study mimics real life situation as the intervention was a part of an ongoing nutrition programme in India (as against a controlled nutrition supplementation trial) and therefore allows realistic estimation of the long-term impact of the programme. Other strengths include use of a precise technique for body composition assessment and a large sample size.

The study also has some important limitations that need to be acknowledged. Major limitations include non-randomisation of villages in the baseline study, and losses to follow-up (about 44% of the original sample). Age and sex distribution of the participants and non-participants among the eligible cohort members was similar in the intervention and control groups. As data on distribution of other potential confounders in the participants and non-participants are not available, it is not possible to assess whether the losses to follow up could have biased the results. Another important limitation of the study is the lack of direct evidence on who took the nutritional supplement and the amount of supplement consumed and the primary analyses are therefore ecological, based on the area and time of birth. However, considerable efforts were made to ensure uptake of the supplement in the initial trial. This coupled with a higher birth weight of children born in the intervention area, comparable in magnitude to other trials of nutritional supplementation, suggests that the intervention was effective. Although the ecological design could lead to an underestimation of the efficacy of nutritional supplement, it provides a more pragmatic estimate of the effectiveness of such nutrition supplementation programmes in real-world situations, which has important policy implications. Bias could also have arisen from differing rates of urbanization in the intervention and control groups. The study area has over the past ten years shown definite trends in urbanization, with a large number of educational institutes (schools, colleges and vocational/industrial training centres) mushrooming in the area. The state government also built a new international airport in the vicinity, buying up agricultural land and contributing to a sudden increase in the socio-economic status of many nearby villages and their inhabitants. Although this may be a limitation for trial-based analyses, the natural experiment arising from the rapid but uneven

economic development of the area also provides unique opportunities to investigate the mechanisms by which socio-economic development and urbanization increase the risk of chronic diseases. Finally, despite relative automation of the major outcome measure (LBM) used in this study, possibility of bias arising from the lack of blinding of the fieldworkers cannot be ruled out completely.

In summary, these data do not support the role of "programming" by early nutrition on adult LBM and muscle strength. Consistent with existing evidence, current socio-economic position and lifestyles including dietary energy intake and physical activity were found to be important determinants of the LBM and muscle strength in this setting. Based on these data, until better evidence emerges, strategies to improve lean mass and muscle strength should focus on improving lifestyles among the youth.

Chapter 4: Study 2

Comparison of body composition estimation by Dual-energy X-ray absorptiometry and isotope dilution technique in Indian men and women

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Modified from: Bharati Kulkarni, Hannah Kuper, Amy Taylor, Jonathan C Wells, KV Radhakrishna, Sanjay Kinra, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, AV Kurpad, Nuala M Byrne, Andrew P Hills. Comparison of body composition estimation by Dual-energy X-ray absorptiometry and isotope dilution technique in Indian men and women. **British Journal of Nutrition** [In press]

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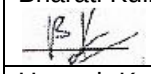
Bharati Kulkarni 	Study design, data collection , data analysis, manuscript writing
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I have sighted email or other correspondence from all Co-authors confirming their certifying authorship.

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Name Signature Date

4.1 Introduction

Assessment of body composition is an important part of nutritional assessment as fat and lean compartments of body mass have different health implications. Fat mass is closely linked to metabolic complications of obesity because adipose tissue functions as an endocrine organ releasing bioactive substances that have pro-inflammatory properties (Ouchi, Parker et al. 2011). On the other hand, lean body mass plays a protective role against the risk of chronic diseases including diabetes and osteoporosis (Wolfe 2006). Skeletal muscle, the major component of lean mass, is the primary site for glucose and triglyceride disposal and influences insulin sensitivity (Rattarasarn, Leelawattana et al. 2010). In addition, the muscle contractions play an important role in bone mass and strength and changes in bone mass and muscle strength usually track together over the life span (Rikkonen, Sirola et al. 2012). Ethnic differences in the relationship between BMI and disease risk have been associated with differences in body composition (Dulloo, Jacquet et al. 2010, Nightingale, Rudnicka et al. 2013).

A number of techniques are available for body composition assessment and the choice of technique usually depends on precision, accuracy, ease of application, as well as the cost. DXA is increasingly used for body composition assessment because of its high precision and low dose of radiation exposure. Moreover, DXA scans are quick, non-invasive, operator-independent, require little subject compliance and are relatively cheap. A number of studies have validated other less precise techniques such as anthropometry, bioelectrical impedance analysis (BIA) etc. against DXA, using it as a reference method(Bousbiat, Jaffrin et al. 2011, Loveday, Thompson et al. 2012, Karelis, Chamberland et al. 2013). DXA, however, is not without limitations. Although studies have shown that DXA estimates of body composition

are highly correlated with those derived from more accurate methods, variation between the estimates have been reported. Compared to the criterion 4 compartment model, regarded as the most accurate in vivo method, both over-estimation (Schoeller, Tylavsky et al. 2005)and underestimation (Visser, Fuerst et al. 1999) of lean body mass by DXA has been observed.

Validation studies comparing DXA against other techniques tend to be population-specific due to ethnic variations in body composition (Haroun, Taylor et al. 2010). Studies comparing DXA estimates of body composition with other reference methods have not been reported from India. This study was therefore carried out to compare the body composition estimates by DXA with that using the isotope dilution technique.

4.2 Methods

Ethics statement

The study was approved by the ethics committees of the London School of Hygiene & Tropical Medicine, National Institute of Nutrition and Queensland University of Technology. Written informed written consent was obtained from all participants.

4.2.1 Study participants

Healthy volunteers (n=152) were enrolled in this study from two pre-established cohorts living around the city of Hyderabad, India. Younger participants (n=58; age range: 19 to 23 y) were part of a birth cohort established to assess the long-term impact of early nutrition supplementation provided to pregnant women and young children (Hyderabad

Nutrition Trial). Older participants were enrolled from the Hyderabad arm of the Indian Migration Study (IMS) that was established to examine the association between rural to urban migration and cardio-metabolic risk (n=28; age range: 26 to 72 y). However, due to inability to recruit adequate number of participants from the IMS study, older participants were enrolled from two other sources: a) parents of the younger participants from the Hyderabad Nutrition Trial (n=23; age range: 40 to 72 y); b) employees of the National Institute of Nutrition, Hyderabad, India (n=43; age range: 26 to 52 y).

In order to obtain a sample representing a broad BMI range, 2 to 4 men and equal number of women were chosen in each of the age (<50 y and ≥50 y) and BMI categories (<17, 17-20.9, 21-23.9, 24- 27.9, 28-32.9 and >33).

Table 4.1. BMI distribution of older participants from the Indian Migrants Study (n=100; Age=35-60 y)

Males						Females					
Rural non-migrants			**Urban (migrants and non-migrants)**			**Rural non-migrants**			**Urban (migrants and non-migrants)**		
Age (y)	**<50**	**50+**		**<50**	**50+**	**Age**	**<50**	**50+**	**Age**	**<50**	**50+**
BMI			**BMI**			**BMI**			**BMI**		
<20	2	3	**<23**	4	3	**<20**	2	3	**<24**	4	3
20-23.9	3	2	**23-25.9**	4	4	**20-23.9**	3	2	**24-27.9**	4	4
24-27.9	2	3	**26-29.9**	4	4	**24-27.9**	2	3	**28-32.9**	4	4
≥28	3	2	**≥30**	3	4	**≥28**	3	2	**≥33**	3	4

Table 4.2. BMI distribution of participants from the Andhra Pradesh Children And Parents Study (APCAPS) (N=60; age=18-21 y)

	Intervention arm								**Control arm**							
	Males				**Females**				**Males**				**Females**			
BMI category	**<17**	**17-20.9**	**21-24.9**	**≥25**	**<16.5**	**16.5-19.4**	**19.5-21.9**	**≥22.0**	**<17**	**17-20.9**	**21-24.9**	**≥25**	**<16.5**	**16.5-19.4**	**19.5-21.9**	**≥22.0**
Number of participants	4	4	4	3	4	4	4	3	3	4	4	4	3	4	4	4

4.2.2 Demographic and anthropometric data

Demographic information was collected on all study participants using an interviewer administered questionnaire. Weight was measured to the nearest 0.1kg in light clothes without footwear using a digital Seca scale (Hamburg, Germany). Standing height was measured using a portable stadiometer (Leicester height measure; Chasmors Ltd, Camden, London, UK). Anthropometric measurements were taken twice and the average of the two values for each was used in the analysis.BMI was calculated as weight (kg)/height $(m)^2$.

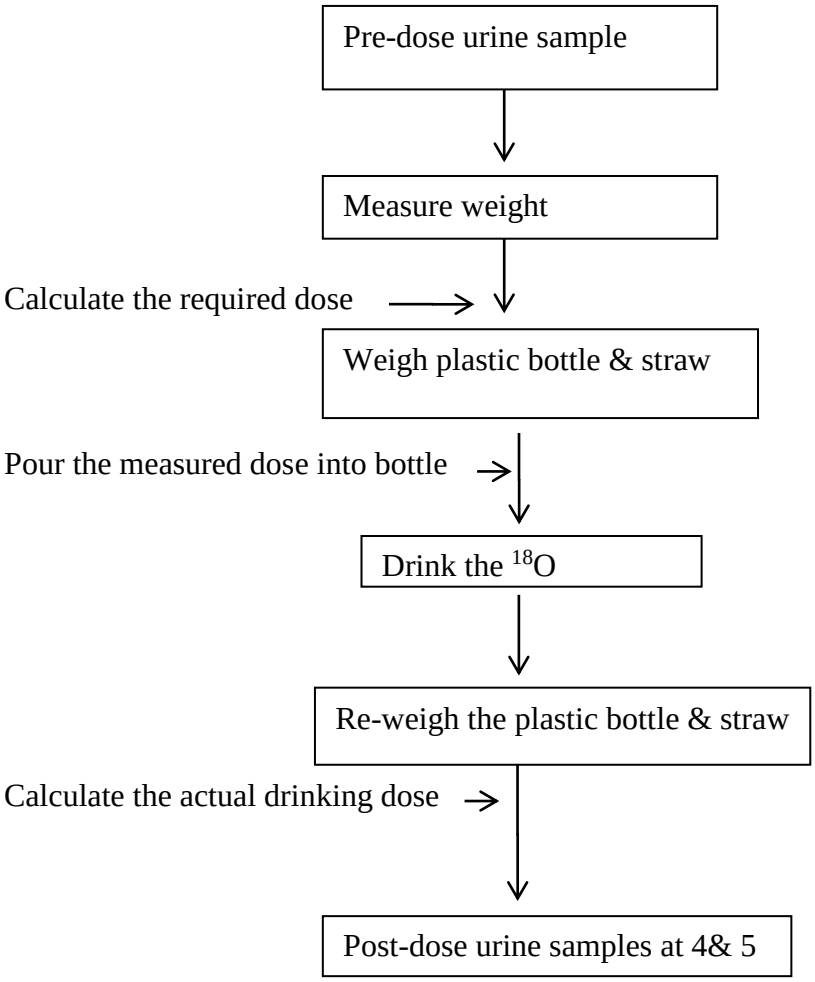
Body composition of each participant was assessed by DXA and isotope dilution technique on the same day.

4.2.3 Isotope dilution technique

Protocol for dosing

Participants arrived in the morning following an overnight fast. After measuring their height and weight, a baseline urine sample was collected after complete emptying of bladder. A baseline sample enables the estimation of the basal levels of ^{18}O in the body. The dose of ^{18}O was calculated @ 0.2 g / kg body weight for each participant and was measured using a weighing scale (Model EG0620-3NM, Laboratory Supply Company GmbH & Co. KG, Germany) to the nearest 0.001 gram. This dose was diluted with 50 mL of water and given to the participant to drink from a clean dry plastic bottle with the help of a straw at about 09:00 hour. The bottle containing the dose was rinsed with 50mLof deionised water which was consumed by the participant. To ensure a complete intake of the dose, the bottle used to administer the dose was weighed before and after the intake of the dose. A light breakfast

was provided with 50 mL water at about 10:00 hour. Any subsequent oral intake was avoided. The participant was advised not to perform heavy activities before the follow-up urine samples were collected. Follow-up urine samples were collected 4 and 5 hours after the intake of dose to allow complete equilibration of the isotope within the body water compartments. The administration steps are summarised as follows:



Record of administration of ^{18}O

Stable Isotope Administration

Date of dosing	

Name of the Subject		Subject ID	

Subject Ht.		Subject Wt.		Dose @ 0.2 g / Kg	

Wt. of the empty container	
Required volume of Isotope	
Measured volume of Isotope	

Time of Basal urine collection	
Time of Dosing	
Time of 4 Hr Collection	
Time of 5 Hr Collection	

Wt. of the empty container (after dose)	

Storage and analysis of samples

Each urine sample was labelled with participant's ID, name, the time of collection and dose number, and then stored at -20 degree C for later analysis. The samples were transported to Bangalore for analysis at the end of the study.

Analyses of isotope enrichment

Isotope enrichment in the pre-dose and post-dose urine samples, the dose given and the local tap water were measured using Isotope Ratio Mass Spectrometry (Hydra 20-20, SerCon) at St John's Research Institute, Bangalore, India, one of the International Atomic Energy Agency´s select collaborating centres worldwide.

TBW was calculated as follows: Both a sample of the diluted dose and the physiological samples were measured in the same analytical run and dilution space was calculated from mass and instrumental units as follows (Coward 1990) :

$$N= (WA/a) (S_a – S_t) f/ (S_s – S_p)$$

Where N is expressed in grams, W is the mass of the water used to dilute the dose, A is the dose administered to the subject, a is the mass of the dose used in preparing the diluted dose, f is the fractional factor for the physiological sample relative to the body water, S_a is the measured value for the diluted dose, S_t is the value for the tap water used in dilution, S_s is the value for the physiological sample, and S_p is the value for the pre-dose physiological sample. The value of S_s, the isotopic abundance, was obtained by the plateau method.

Each sample was analysed in duplicate and the mean was used for analysis. Total body water (TBW) was calculated allowing a correction by 0.7% for in vivo exchange (Racette, Schoeller et al. 1994).

Estimation of FFM from TBW requires consideration of hydration constant of FFM. In healthy adults, TBW constitutes 73.2% of the FFM (IAEA 2009). Using TBW, the FFM was then calculated as:

$$\text{FFM (kg)} = \text{TBW} / \text{hydration constant}$$

Subsequently, fat mass and body fat percent was calculated as follows:

$$\text{Fat mass} = \text{Body weight} - \text{FFM}$$

$$\text{Body fat percent} = (\text{Body weight} - \text{FFM}) / \text{Body weight} \times 100$$

4.2.4 DXA scans

Body composition was assessed by a whole body DXA scan using a fan beam Hologic DXA machine (Discovery A model:www.hologic.com). DXA scans were done after the participant had taken the dose in the morning. The scanner was calibrated daily with a spine phantom and its performance was monitored as per quality assurance protocol. During the scan, the participants were asked to lie supine on the scanning bed with their arms at their sides. Standard software options were used to calculate the total fat mass and fat free mass. The FFM was the sum of lean soft tissue mass and bone mineral content. Precision estimates (CV %) of DXA measurements of body composition based on repeat measurements in 30 participants were 0.7% and 1.4% for fat free mass and fat mass, respectively.

4.2.5Statistical analyses

All analyses were conducted using Stata, version 11.2 (Statacorp, Texas, US). As lean and fat mass showed a skewed distribution, these variables were log transformed prior to analysis and therefore the mean differences between them are expressed as ratios. Other continuous variables were used in the original scale. Differences between the body composition estimates (fat free mass, fat mass and body fat percent) by DXA and isotope dilution technique were assessed using paired t tests. The method of Bland and Altman was used to assess agreement between the two techniques (Martin Bland and Altman 1986). The mean difference between techniques (bias) and their 95% limits of agreement (mean difference ±1.96 standard deviations of the difference) were calculated.As the bias and limits of agreement for lean and fat mass were on a log scale, these values are presented as ratios. Correlation coefficients were calculated to examine the association between the average values of body composition measurements by two methods and the difference between the methods which indicates the proportional bias. All analyses were conducted for the sample as a whole and additionally stratified by sex, and the amount of fat free mass, fat mass or body fat percent values (above and below the median). Finally, linear regression was used to examine factors predicting bias in fat free mass, fat mass and body fat percent estimates including age, sex, BMI or the average values of the fat mass, fat free mass or body fat percent by the two methods as independent variables in the respective models. For the purpose of this analysis, age was classified into 3 categories: < 30 y, 30.1 to 50.0 y and > 50 y and Z scores of BMI and average fat free mass and fat mass values were used.

4.3 Results

A total of 73 men and 79 women participated in the study. Their characteristics are presented in **Table 4.3**. As participants were chosen to represent a broad range of BMI, their BMI varied between 13.8 to 39.7 kg/m^2. Total mass value measured by DXA showed a strong correlation with the weight measured by scale (0.99, p <0.01). Although the body composition estimates by DXA and isotope dilution technique correlated strongly (lean mass: 0.95, fat mass: 0.95, body fat percent: 0.89, all p <0.01) (**Figure 4.1 a, b and c**), DXA estimates of lean mass were higher than those by isotope dilution technique in the whole sample as well as in the subgroups stratified by sex and lean mass values (above and below median) (**Table 4.4**). DXA estimates of fat mass and body fat percent were lower than those measured by isotope dilution technique.

Table 4.3 Participant characteristics

	Men (n= 73)				Women (n =79)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Age (y)	37	15	19	70	37	14	19	62
BMI (kg/m^2)	23.3	5.1	14.5	37.6	24.1	5.8	13.8	39.7
Height (cm)	165.5	6.3	149.1	183.2	151.7	5.6	136.0	162.5
Weight (kg)	64.1	15.1	38.7	108.0	55.6	14.3	31.2	103.7
TM by DXA (Kg)	64.0	15.0	39.2	107.6	55.7	14.2	31.4	102.6

TM: Total mass

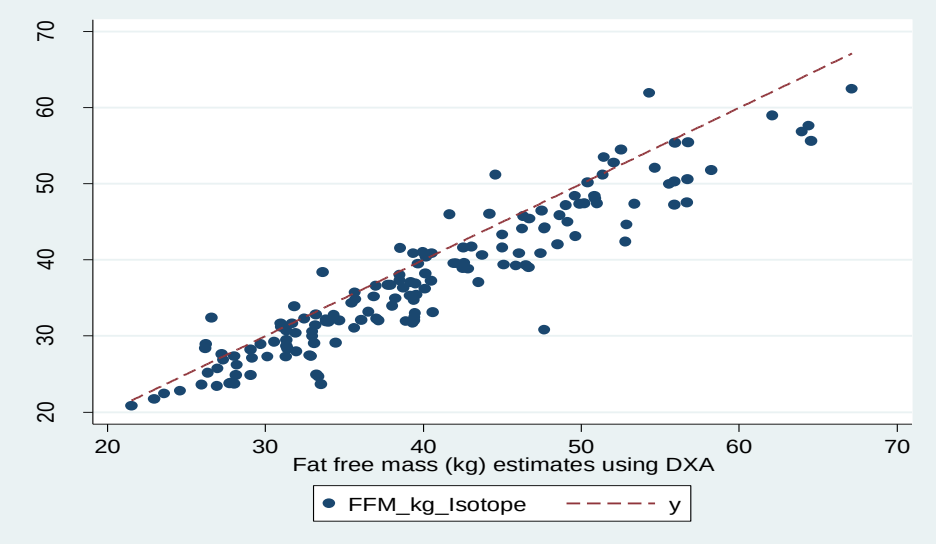


Figure 4.1 (a). **Scatter plot of fat free mass estimates by isotope dilution technique *vs* DXA**

Values are on log scale. Line is line of identity for fat free mass estimate by isotope dilution technique.

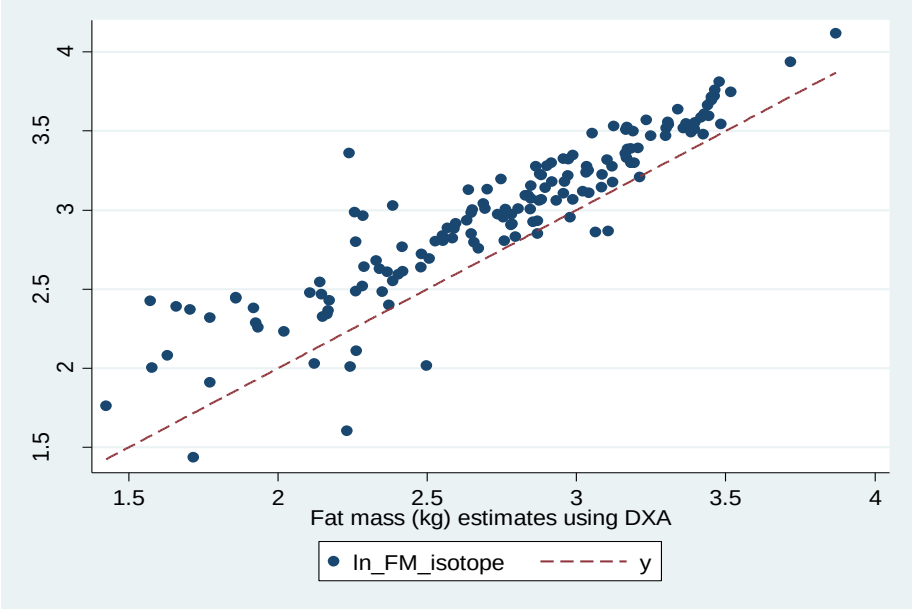


Figure 4.1 (b). **Scatter plot of fat mass estimates by isotope dilution technique *vs* DXA**

Values are on log scale. Line is line of identity for fat mass estimate by isotope dilution technique.

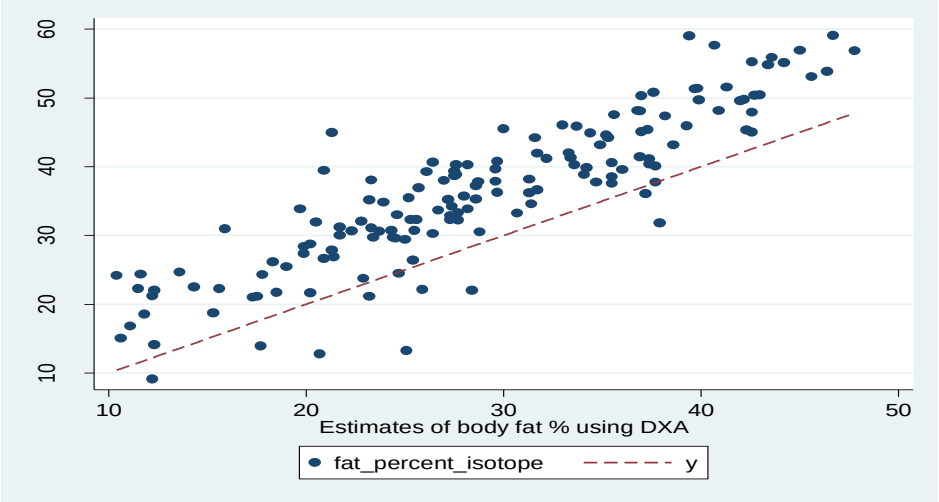


Figure 4.1 (c). **Scatter plot of body fat % estimates by isotope dilution technique *vs* DXA**

Line is line of identity for body fat % estimate by isotope dilution technique.

On average, DXA overestimated the fat free mass values by about 7% (95% CI: 6 to 9%) (**Table 4.5**). However, the limits of agreement showed that 95% of the DXA estimates of fat free mass are expected to be between 9% lower and 26% higher than the values measured by isotope dilution technique. In the case of fat mass, the bias was greater and on average, the estimates by DXA were about 21% lower than those by isotope dilution technique. The limits of agreement for the fat mass by the two methods were much larger (-54% to 17%) than that for the fat free mass. There was no correlation between the bias and the average values of the estimates by the two methods in case of fat free mass as well as fat mass indicating that the bias in the fat free mass and the fat mass estimates did not change with the amount of fat free mass and fat mass respectively. On average, the estimates of body fat percent by DXA were lower than the estimates by isotope dilution technique by 7.4% (95% CI: -8.2% to -6.6%). In the case of body fat percent, the bias was negatively correlated to the average body fat percent indicating that the difference between the two methods was higher in case of individuals with lower values of body fat percent (**Table 4.6**). The estimates of fat free mass, fat mass and body fat percent by DXA explained about 89%, 85% and 78% variation in respective values estimated by the isotope dilution technique.

Table 4.4 Fat free mass and fat mass estimates by DXA and isotope dilution technique

		Isotope dilution		DXA		
	N	**Mean**	**SD**	**Mean**	**SD**	***p* value**[1]
Fat free mass						
Whole sample	152	37.42	9.45	40.09	9.84	<0.01
Males	73	44.18	7.98	46.89	8.28	<0.01
Females	79	31.17	5.65	33.79	6.39	<0.01
Above median[2]	76	45.13	6.58	47.93	7.08	<0.01
Below median[2]	76	29.71	4.03	32.23	4.48	<0.01
Fat mass						
Whole sample	152	22.27	10.20	17.78	8.3	<0.01
Males	73	19.93	9.58	15.09	7.49	<0.01
Females	79	24.43	10.34	20.27	8.28	<0.01
Above median[3]	76	30.17	7.86	24.41	6.03	<0.01
Below median[3]	76	14.37	4.61	11.16	3.68	<0.01
Body fat %						
Whole sample	152	36.3	10.9	28.9	9.2	<0.01
Males	73	29.8	8.7	22.3	6.6	<0.01
Females	79	42.3	9.1	35.1	6.6	<0.01
Above median[4]	76	42.3	9.5	35.0	6.8	<0.01
Below median[4]	76	30.4	8.7	22.9	7.1	<0.01

[1] P value from paired t test of the difference

[2]Median average fat free mass value (by DXA and isotope dilution technique) was 37.3 kg.

[3]Median average fat mass value (by DXA and isotope dilution technique) was 19.2 kg.

[4]Median average fat mass value (by DXA and isotope dilution technique) was 33.0%.

Table 4.5 Bias and 95% limits of agreement for DXA measures of body composition compared with isotope dilution technique

	N	Bias[1]	95% CI		Limits of agreement[2]		R[3]	p value[4]
Fat free mass								
Whole sample	152	1.07	1.06	1.09	0.91	1.26	-0.077	0.35
Males	73	1.06	1.04	1.08	0.92	1.23	-0.127	0.28
Females	79	1.08	1.06	1.10	0.91	1.29	0.083	0.47
Above median[5]	76	1.06	1.04	1.08	0.92	1.22	-0.035	0.76
Below median[5]	76	1.08	1.06	1.11	0.91	1.30	0.049	0.67
Fat mass								
Whole sample	152	0.79	0.77	0.82	0.54	1.17	0.045	0.58
Males	73	0.75	0.71	0.79	0.48	1.17	0.043	0.71
Females	79	0.84	0.81	0.86	0.63	1.12	-0.181	0.11
Above median[6]	76	0.81	0.79	0.84	0.64	1.04	-0.136	0.24
Below median[6]	76	0.78	0.73	0.82	0.48	1.26	-0.098	0.39
Body fat %								
Whole sample	152	-7.4	-8.2	-6.6	-17.3	2.6	-0.345	<0.01
Males	73	-7.5	-8.7	-6.3	-17.7	2.8	-0.428	<0.01
Females	79	-7.3	-8.4	-6.2	-17.0	2.4	-0.513	0.03
Above median[7]	76	-7.3	-8.5	-6.1	-17.6	3.1	-0.537	0.05
Below median[7]	76	-7.5	-8.6	-6.4	-17.0	2.1	-0.357	<0.01

[1]Mean bias and 95% CI for fat free mass and fat mass are expressed as ratio of DXA: Isotope dilution technique values. Bias is the difference (DXA minus isotope dilution) between log-transformed values of the fat free mass and fat mass from the two techniques. All the values related to body fat % are on original scale.

[2]95% Limits of agreement (mean difference ± 2SD) expressed as ratio of DXA: isotope dilution values of fat free mass and fat mass. Values for body fat % are on original scale.

[3]Correlation coefficient is for the difference between DXA and isotope dilution technique against the average of DXA and isotope measures of fat free mass, fat mass and body fat %. In case of fat free mass and fat mass, log transformed values were used.

[4] Significance of correlation coefficient

[5]Median average fat free mass value (by DXA and isotope dilution technique) was 37.3 kg.

[6]Median average fat mass value (by DXA and isotope dilution technique) was 19.2 kg.

[7]Median average fat mass value (by DXA and isotope dilution technique) was 33.0%.

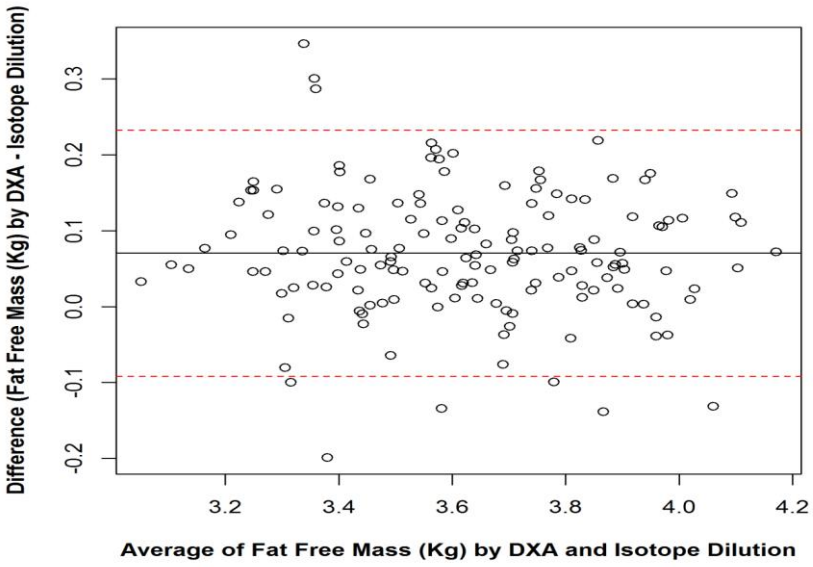


Figure 4.2 (a). **Bland Altman plot of fat free mass estimates by DXA and isotope dilution technique**

Values are presented on log scale. The central dashed line represents the mean difference between measures. The upper and lower dashed lines represent the 95% limits of agreement (± 2 SDs of the mean difference).

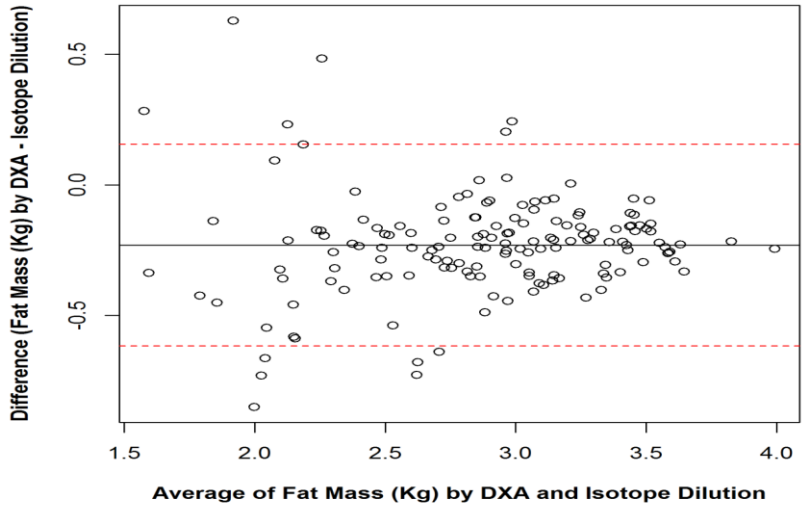


Figure 4.2 (b). **Bland Altman plot of fat mass estimates by DXA and isotope dilution technique**

Values are on log scale. The central dashed line represents the mean difference between measures. The upper and lower dashed lines represent the 95% limits of agreement (± 2 SDs of the mean difference).

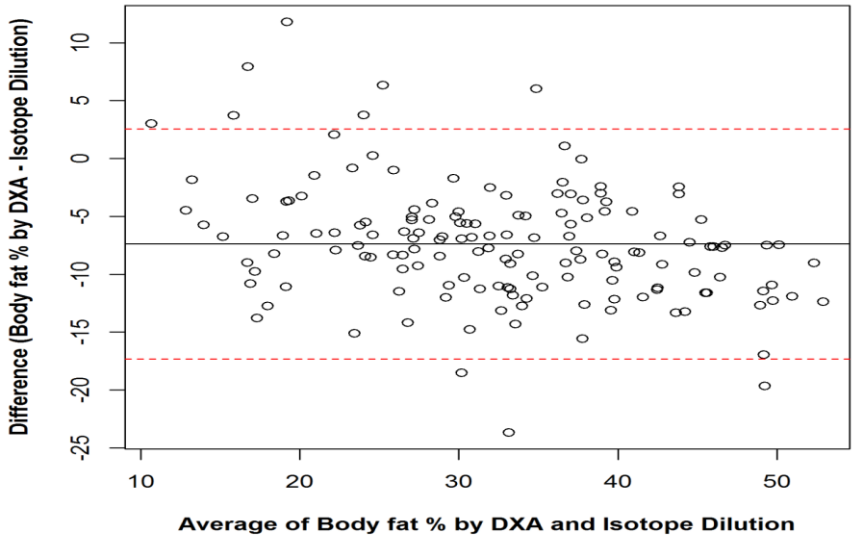


Figure 4.2 (c). **Bland Altman plot of body fat percent estimates by DXA and isotope dilution technique**

The central dashed line represents the mean difference between measures. The upper and lower dashed lines represent the 95% limits of agreement (± 2 SDs of the mean difference).

Table 4.6 shows the linear regression analyses to examine the predictors of the observed bias in the estimates of body composition by the two methods. After adjusting for other variables in the models, older age and higher BMI predicted a higher bias in the fat free mass estimates whereas younger age and lower body fat percent predicted a larger bias in the body fat percent estimates. Bias in the fat mass estimates was higher in case of women compared to men, when adjusted for age and BMI.

Table 4.6 Predictors of the difference (DXA-isotope dilution technique) between measures of body composition

	Univariate				Multivariate[3]			
	β	95% C. I		*p* value	β	95% C. I		*p* value
Bias in fat free mass[1]								
Age[4]	1.03	1.01	1.05	<0.01	1.02	1.00	1.04	<0.01
Sex[5]	1.02	0.99	1.05	0.34	1.02	0.99	1.04	0.18
BMI-Z[6]	1.03	1.01	1.04	<0.01	1.02	1.00	1.03	<0.01
Fat free mass-Z[7]	0.99	0.98	1.00	0.35	0.99	0.98	1.01	0.60
Bias in fat mass[1]								
Age[4]	0.99	0.95	1.20	0.93	0.98	0.94	1.02	0.34
Sex[5]	1.03	1.00	1.06	<0.01	1.11	1.04	1.18	<0.01
BMI-Z [6]	1.01	1.00	1.01	0.08	1.03	1.00	1.06	0.08
Fat mass-Z[7]	1.01	0.98	1.04	0.50	1.00	1.00	1.00	0.86
Bias in body fat %[2]								
Age[4]	-1.40	-2.41	-0.39	0.01	-1.23	-2.33	-0.15	0.03
Sex[5]	0.20	-1.42	1.84	0.54	0.19	-1.42	1.80	0.81
BMI-Z[6]	-0.68	-1.50	0.13	0.10	-0.34	-1.20	0.52	0.43
Body fat %	-0.18	-0.26	-0.10	<0.01	-0.32	-0.43	-0.21	<0.01

[1]The outcome variables - bias in fat free mass and bias in fat mass were log transformed. The coefficients and 95% CI therefore represent ratios (DXA: isotope dilution technique).

[2]Values related to body fat% are on original scale.

[3] Adjusted for all other variables in the model. BMI Zscore or body composition variables (fat mass, fat free mass or body fat percent) were entered separately in the respective models.

[5] Sex: 1= Male, 2 = Female

[4] Age (y) was classified into 3 categories: < 30 y, 30.1 to 50.0 y and > 50 y

[6] BMI-Z : Z score ofBMI

[7] Fat free mass-Z and Fat mass-Z: Z scores of fat free mass and fat mass were used in respective analyses

4.4 Discussion

This study compared body composition estimates by two precise techniques – DXA and isotope dilution technique - in healthy and weight stable Indian men and women with a broad range of BMI. Fat free mass estimates by DXA were higher than that using isotope dilution technique whereas the fat mass and body fat percent estimates by DXA were lower than that by the other technique in this sample. Agreement between the methods was not good as indicated by significant bias between methods and wide limits of agreement especially for the estimates of fat mass and body fat percent. However, there was no evidence of any systematic bias for either fat free mass or fat mass although body fat percent showed a negative correlation with the bias estimates. The study indicates that these methods cannot be used interchangeably as systematic differences exist between body composition estimates.

These findings are consistent with a few other studies that compared body composition estimates by DXA with isotope dilution technique. For example, a large study from the United States which compared body composition by DXA with other reference techniques in 1195 men and women (DXA was compared with isotope dilution technique in 395 participants) showed that DXA overestimated FFM by about 5% (Schoeller, Tylavsky et al. 2005).The authors recommended that lean soft tissue mass estimates with fan-beam DXA be reduced by 5% and that for fat mass be increased by the same mass. Another smaller study in 95 children aged 6-12 y also reported underestimation of body fat by DXA compared to the isotope dilution technique (Robotham, Schoeller et al. 2006). These findings are corroborated by a few animal studies also. For example, a study comparing body composition of 61 rhesus monkeys by DXA and isotope dilution also showed that that DXA underestimated fat mass by (mean ± SD) 0.67 ± 0.26 kg (7.5%, $P < 0.01$) and overestimated

fat-free mass by (mean ± SD) 0.57 ± 0.29 kg (20%, $P < 0.01$) when compared with isotope dilution(Blanc, Colman et al. 2005).

A large number of studies have compared DXA with multi-component criterion methods for assessment of body composition (Fuller, Jebb et al. 1992, Bergsma-Kadijk, Baumeister et al. 1996, Withers, LaForgia et al. 1998, Arngrimsson, Evans et al. 2000, Deurenberg-Yap, Schmidt et al. 2001, Van Der Ploeg, Withers et al. 2003). The majority of these studies reported underestimation of percent body fat by DXA when compared to the criterion technique and our study corroborates this finding. A few studies have, however, reported a bias in the opposite direction. For example, a study by William et al. compared DXA with a 4 compartment (4C) model and reported overestimation of fat mass and percent fat by DXA in non-obese adults (Williams, Wells et al. 2006). Although the majority of the studies cited above were performed using pencil beam instruments, studies using fan beam instruments have also shown similar results. For example, a study by Tylavsky et al. which compared the body composition estimates by fan beam DXA with those by 4C model also indicated systematic overestimation of fat free mass and underestimation of fat mass by DXA. Authors suggested that by applying a correction factor of 0.964, differences between the two methods could be alleviated (Tylavsky, Lohman et al. 2003).Furthermore, a study comparing muscle mass, a major component of fat free mass, measured by DXA with that measured by multi-slice computerized tomography (CT) in elderly men and women also showed overestimation of muscle mass by DXA compared to CT (Visser, Fuerst et al. 1999).

On average, DXA overestimated the fat free mass by about 7% (≈3 kg) and underestimated fat mass by about 21% (≈4.2 kg) in our study sample. This magnitude of bias is within the range of values reported by some of the previous studies. For example, in a

study by Schoeller et al. which collated data from 7 studies, mean differences in the fat free mass estimates between DXA and criterion techniques ranged from 1.8 to 4.7 kg and those in fat mass ranged from 1.3 to 5.1 kg. A number of other studies have reported bias values within this range (Bergsma-Kadijk, Baumeister et al. 1996, Visser, Fuerst et al. 1999). A larger bias in fat mass estimates compared to that in fat free mass estimates is not surprising considering that the value of fat mass was an indirect estimate derived by subtracting fat free mass from body weight measured by scale. Although the total mass by DXA and body weight measured by scale were highly correlated (r = 0.99, $p<0.01$), there was a statistically robust difference between these two measures ranging from (weight – DXA measured mass) – 3.1 to 2.3 kg. Difference between these two estimates of total mass may have accentuated the differences in fat mass.

Limits of agreement between the two methods observed in our study were, however, substantially wider (Fat free mass: - 9% to + 26%; fat mass: -46% to +17%; body fat %: -17.3 to 2.6%) than those reported by other studies. The majority of the studies have reported the limits of agreement between ±10% of the mean (Mahon, Flynn et al. 2007, Toombs, Ducher et al. 2012). However, a few other studies have reported that DXA could under- or overestimate a person's fat mass by almost 28% (Fuller, Jebb et al. 1992). One of the reasons for the narrow limits of agreement by other studies could be the exclusion of extreme values of the differences. For example, Schoeller et al. excluded observations where difference in the lean mass estimates by DXA and isotope dilution was > 6 kg. Our study did not exclude observations with larger differences between the measurements and this may have resulted in larger bias between the measurements.

Interestingly, bias in body composition estimates by DXA compared to isotope dilution technique was higher at lower values of the estimates as indicated by the negative relationship between the bias and the average of the body fat percent estimates by two methods (Table 4.6). Multiple regression analyses showed that after adjustment for age and sex, higher BMI predicted a larger bias in the fat free mass estimates whereas a lower body fat percent predicted a larger bias in the body fat percent estimates. In addition, the magnitude of bias in these two estimates was influenced by the age of the participant. Previous studies have also shown that bias of DXA estimates varied according to a number of factors including age, body size, body fatness, sex, health status, type of instrument etc. (Williams, Wells et al. 2006). A study comparing abdominal fat estimates by DXA with those using magnetic resonance imaging (MRI) in this sample also showed that overestimation of abdominal fat by DXA was greater in individuals with less abdominal fat (Taylor, Kuper et al. 2012). It is possible that the algorithms used for body composition estimation by DXA produce a larger error at very low levels of body fat.

A number of factors can influence the body composition estimates by DXA including assumption of a constant hydration of FFM, beam hardening with increase in the tissue depth, exclusion of the head soft tissue from the body composition estimates etc. Moreover, DXA can use only non-bone pixels to calculate the fat-to-lean ratio and extrapolates these estimates to the bone-containing pixels (Pietrobelli, Formica et al. 1996). Differences in body size can therefore lead to differential bias in the estimates of body composition by two methods. In addition, variations in the body composition estimates with machines by different manufacturers and even with different models of the same manufacturer are reported (Robotham, Schoeller et al. 2006, Sakai, Ito et al. 2006). Similarly, isotope dilution technique also has a number of limitations because the body composition estimates are based on a

number of assumptions including equal distribution of tracer in body water and constant hydration of fat-free mass(Krumbiegel 2010). Both these techniques are thus error prone and lack of agreement between the methods for estimation of body composition could be related to a number of factors that can lead to inaccuracies in the estimates.

Strengths of this study include a large sample with representation from a broad range of age and BMI. In addition, the study used Oxygen-18 as the isotope tracer which may provide a more accurate estimate of total body water than the more commonly used deuterium oxide because it exchanges to a smaller degree with non-aqueous molecules (Krumbiegel 2010). A limitation of the study is the use of an isotope dilution technique for validating DXA measurements of body composition instead of a multi-component criterion technique. However, body composition estimates using isotope dilution techniques are highly correlated with those using the criterion technique of the 4 compartment model (Deurenberg-Yap, Schmidt et al. 2001). A study comparing body composition estimates by densitometry, DXA and isotope dilution with criterion 4 compartment model in Asian adults also showed that body fat percent estimates by isotope dilution had the lowest bias while those by DXA showed the highest bias suggesting that isotope dilution technique may be the best 2-compartment model for measuring body fat (Deurenberg-Yap and Deurenberg 2002). Moreover, currently there is no true criterion technique for body composition assessment and most studies in humans have used reference methods that may not have been accurate. In addition, most researchers do not have access to four compartment models for body composition assessment.

The study thus shows that although the average estimates of fat free and fat mass by DXA and isotope dilution technique were fairly close, considerable differences in the

estimates were seen at individual level. However, these differences in the absolute values at individual level may not affect the results of the studies exploring the relationship of body composition using either of these methods with health outcomes as these values were highly correlated. The variable bias in the estimates of fat free mass and body fat percent in relation to participant characteristics including BMI and body fat percent, however, has implications for longitudinal studies assessing body composition changes as these changes may be confounded by the method used for estimation of body composition.

In conclusion, the study shows that the body composition estimates by the two commonly used reference methods of DXA and isotope dilution may be considerably different at individual level with particularly larger differences in the body fat percent estimates. The two methods are not directly interchangeable. Additional studies are required to develop correction factors that could be used to calibrate DXA in order to alleviate the differences in these two methods.

Chapter 5: Study 3

Development and validation of anthropometric prediction equations for estimation of lean body mass and appendicular lean soft tissue in Indian men and women

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Modified from: Bharati Kulkarni, Hannah Kuper, Amy Taylor, Jonathan C Wells, KV Radhakrishna, Sanjay Kinra, Yoav Ben-Shlomo, George Davey Smith, Shah Ebrahim, Nuala M Byrne, Andrew P Hills. Development and validation of anthropometric prediction equations for estimation of lean body mass and appendicular lean soft tissue in Indian men and women. **Journal of Applied Physiology** 2013; 115: 1156–1162

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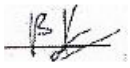
Bharati Kulkarni 	Study design, data collection , data analysis, manuscript writing
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5.1 Introduction

Lean body mass, the metabolically active compartment of the body, plays a central role in a number of physiologic processes. Muscle mass, which is a major component of LBM, is particularly important for insulin sensitivity and plays a protective role against chronic diseases like osteoporosis(Wolfe 2006). Estimation of muscle mass and LBM during nutritional assessment, therefore, provides important insights.

Magnetic resonance imaging (MRI) is considered to be a gold standard for evaluating skeletal muscle mass due to its high accuracy and lack of radiation to the subjects. However, MRI is expensive and not widely available for use in research and clinical practice. Dual energy X-ray absorptiometry (DXA) is an attractive alternative approach to estimate LBM and skeletal muscle mass because of its good precision, less radiation exposure and substantially lower cost. DXA can estimate appendicular lean soft tissue (ALST) which is considered to be a surrogate of skeletal muscle mass and majority of the operative definitions of sarcopenia use cut points based on estimation of ALST by DXA(Cruz-Jentoft, Baeyens et al. 2010, Fielding, Vellas et al. 2011) .

DXA, however, is not portable and impractical for use in large scale epidemiological studies. Anthropometry is one of the oldest techniques of body composition assessment which has been validated by cadaver studies and other 'gold standard' methods (Matiegka 1921, Martin, Spenst et al. 1990, Doupe, Martin et al. 1997). It offers distinct advantages of being simple, portable, non-invasive, and inexpensive. The use of anthropometry to assess body composition, therefore, continues to play an important role in clinical practice and in large population-based studies.

Anthropometric assessment of body composition relies on prediction equations derived from 'gold standard' methods. Commonly used prediction equations including Durnin and Womersley's (Durnin and Womersley 1974) and Jackson and Pollock's equations(Jackson and Pollock 1978, Jackson, Pollock et al. 1980) have been developed in Caucasian populations. Population-specific prediction equations are, however, desirable due to ethnic differences in body composition. We, therefore, developed equations to predict LBM and ALST based on anthropometric variables using DXA as a reference method in a large sample of Indian adults.

5.2Methods

The study was approved by the ethics committees of the National Institute of Nutrition, London School of Hygiene & Tropical Medicine and Queensland University of Technology.

5.2.1 Participant enrolment

Healthy volunteers (n=2364) were enrolled in the study from two pre-established cohorts living around the city of Hyderabad, India. The first group of participants (n= 1448; 32% women; age range: 18 to 23 y) were members of a birth cohort established to assess the long-term impact of early nutrition supplementation provided to pregnant women and young children (Andhra Pradesh Children and Parents Study (APCAPS))(Kinra, Radhakrishna et al.). The second group included participants of the Hyderabad arm of the Indian Migration Study that was established to examine the association between rural to urban migration and cardio-metabolic risk (n= 916; 46 % women; age range: 21 to 79 y). Of these, 108 participants (including 26 women who were pregnant) did not undergo DXA scanning. In

addition, data on 36 participants were excluded due to major artefacts in the DXA scans. Finally, data on 2220 participants who had outcome measurements done by anthropometry as well as DXA were included in the analyses. Demographic information was collected on all study participants using a standardized interviewer administered questionnaire.

5.2.2 Anthropometric measurements

These measurements were carried out by two trained investigators using standardized procedures(Lohman, Roche et al.). Weight was measured to the nearest 0.1kg in light clothes without footwear using a digital Seca scale (Hamburg, Germany).Standing height was measured using a portable stadiometer (Leicester height measure; Chasmors Ltd, Camden, London, UK). Circumferences (mid-arm, calf and hip) were measured to the nearest mm using a non-stretch narrow metal tape (Chasmors metallic tape). Mid-arm circumference was measured at the midpoint between the tip of acromion and olecranon process with the participant's arm flexed at 90 degrees.

Calf circumference was measured at the widest part of the lower leg. Hip circumference was measured at the widest part of the buttock. Skinfold thickness was measured at four sites (biceps, triceps, subscapular and suprailiac) to the nearest 0.1 mm using a Holtain caliper (Chasmors, London). Skinfold thickness measurements were recorded three times and rest of the measurements were done twice. The average of the measured values for each was used in the analysis.

Corrected arm muscle area (CAMA) in cm^2 was calculated using the mid-arm circumference and triceps skinfold measurements with the following formula –

$$CAMA = \frac{(MAC - (\pi \times TSF))^2}{4\pi} - BA$$

where MAC = mid-arm circumference (cm), TSF = triceps skinfold thickness (cm) and BA = correction for bone area which was considered to be 10 cm for men and 6.5cm for women(Heymsfield, McManus et al. 1982). Anthropometric variables were also used to calculate body fat percent using equations developed by Durnin and Womersley (Durnin and Womersley 1974). Body fat percent values using these equations were then converted to lean body mass estimates.

5.2.3 DXA measurements

A whole body DXA scan was carried out for each participant using either Hologic Discovery A model (90% of scans) or Hologic 4500W , Waltham, MA, USA (10% of scans), on the same day as anthropometry. The scanners were calibrated daily with a phantom and their performance was monitored as per quality assurance protocol. During the scan, participants were asked to lie supine on the scanning table with their arms at their sides. Pregnant women were excluded from the DXA scanning. Standard Hologic software options were used to define regions of the body (head, arms, trunk and legs). ALST was calculated as the sum of bone free lean tissue in arms and legs.

5.2.4 Statistical analyses

All analyses were conducted using Stata, version 11.2 (Stata corp, Texas, US). Skinfold thickness measurements were log- transformed to reduce the skewness of the distribution. Participants were divided randomly into prediction (60%) and validation (40%) groups. To reduce the influence of outliers in case of the anthropometric and DXA variables, extreme values below 1st percentile and above 99th percentile were adjusted and made equivalent to 1st and 99th percentile, respectively. Participant characteristics in the prediction and validation groups were compared using student's t-test. In the prediction data set, two sets of stepwise multiple linear regression analyses were performed to predict LBM and ALST using anthropometric variables as predictor variables. Anthropometric variables (weight, height, circumferences at arm, calf and hip, sum of four skinfolds) were entered in different combinations as predictors of LBM and ALST. The models were additionally adjusted for the DXA scanner and participant age. Coefficient of determination (adjusted R^2), standard error of the estimate (SEE), and Akaike information criterion (AIC) (Akaike 1974) were used to evaluate the precision of the equations. Equations with a high R^2, a small SEE, and the smallest AIC value were considered to be the best "fit" models.

The equations developed using the prediction set data were applied to the validation set data to calculate the predicted values of LBM and ALST. The predicted values were compared with the values measured by DXA using a paired sample *t*-test in the validation group. The pure error (PE) was calculated as the square root of the mean of squares of differences between measured and predicted values of the LBM and ALST. A smaller pure error value indicated greater accuracy of the equation. The predicted and measured values were also compared using Bland and Altman method (Martin Bland and Altman 1986).

5.3 Results

Characteristics of participants in the prediction and validation groups are presented in **Table 5.1**. There were no significant differences in age and physical characteristics of men in the two groups but women in the validation group were younger and lighter and had lower LBM and ALST compared to women in the prediction group.

Table 5.1 Characteristics of participants in the prediction and validation group

	Men							Women						
	Prediction group			Validation group				Prediction group			Validation group			
	N	Mean	SD	N	Mean	SD	*p* value	N	Mean	SD	N	Mean	SD	*p* value
Age (y)	851	30.1	14.7	570	30.2	14.5	0.86	481	34.7	14.3	318	31.9	14.0	0.01
Height (cm)	851	166.0	6.2	570	166	6.33	0.51	481	152.6	5.4	318	152.1	5.6	0.18
Weight (Kg)	851	58.6	11.6	570	58.8	10.7	0.70	481	54.1	13.8	318	51.5	13	0.01
BMI (Kg/m^2)	851	21.2	4.0	570	21.3	3.6	0.92	481	23.2	5.6	318	22.3	5.3	0.03
LBM (Kg)	851	44.84	6.39	570	45.16	6.17	0.35	481	33.56	6.01	318	32.52	5.45	0.01
ALST (Kg)	851	20.22	2.84	570	20.33	2.84	0.44	481	13.93	2.54	318	13.46	2.25	0.01

BMI: Body mass index; LBM: Lean body mass; ALST: Appendicular lean soft tissue

Prediction and validation of LBM

Table 5.2 shows the proposed prediction equations for estimation of LBM (Kg). The simplest model with age, weight and height as predictor variables (equation 1) explained about 90% of the variation in LBM in case of men and women. Addition of circumferences at hip, calf and arm and / or sum of skinfold thickness measurements at 4 sites (equations 2-4) resulted in improved coefficients of determination (adjusted R^2), reduced SEE and AIC values indicating better predictive qualities of the models with added variables. Increase in the value of adjusted R^2 (from 0.90 to 0.94 in men and 0.91 to 0.92 in women) and decrease in SEE (from 1.92 to 1.47 kg in men and from 1.84 to 1.63 kg in women) from equation 1 to equation 4 was, however, marginal. Based on the AIC, the equation 4 which had the lowest value can be considered as the best fit model.

Table 5.2 Proposed anthorpometric equations for estimation of lean body mass (Kg)

	Predictor variables	Sex	*N*	Proposed equations	*Adjusted* R^2	SEE [1]	AIC [2]
Equation 1	Height[3], Weight[4]	M	851	Lean mass = -15.605 - (0.032 x age[5]) + (0.192 x height) + (0.502 x weight)	0.90	1.92	3530
		F	481	Lean mass = -13.034 - (0.018 x age) + (0.165 x height) + (0.409 x weight)	0.91	1.84	1953
Equation 2	Height, Weight, Circumferences [6]	M	851	Lean mass = - 9.326 – (0.015 x age) + (0.207 x height) + (0.574 x weight) + (0.285 x arm circumference) + (0.182 x calf circumference) - (0.305 x hip circumference)	0.92	1.76	3382
		F	481	Lean mass = 3.191 - (0.013 x age) + (0.122 x height) + (0.581 x weight) - (0.093 x arm circumference) + (0.023 x calf circumference) - (0.188 x hip circumference)	0.91	1.76	1918
Equation 3	Height, Weight, Skinfold thickness [7]	M	851	Lean mass =13.782 - (0.018 x age) + (0.064 x height) + (0.697 x weight) - (5.842 x logarithm of sum of 4 skinfolds)	0.94	1.57	3190
		F	481	Lean mass = 1.689 -(0.014 x age) + (0.120 x height) +(0.499 x weight) -(3.315 x logarithm of sum of 4 skinfolds)	0.92	1.68	1871
Equation 4	Height, Weight, Circumferences at 3 sites , Skinfold thickness at 4 sites	M	851	Lean mass = 10.385 – (0.005 x age) + (0.103 x height) + (0.680 x weight) + (0.288 x arm circumference) + (0.130 x calf circumference) - (0.183 x hip circumference) – (5.278 x logarithm of sum of 4 skinfolds)	0.94	1.47	3081
		F	481	Lean mass = 10.632 – (0.009 x age) + (0.102 x height) + (0.592 x weight) + (0.055 x arm circumference) + (0.043 x calf circumference) - (0.158 x hip circumference) - (3.174 logarithm of sum of 4 skinfolds)	0.92	1.63	1845

[1] SEE: Standard error of the estimate (Kg) ;[2] AIC: Akaike's information criterion; [3] Height in cm; [4] Weight in Kg [5] Age in y
[6] Circumferences at arm, calf and hip in cm; [7] Skinfold thickness measurements at biceps, triceps, subscapular and suprailliac regions in mm

The above four equations were then validated in the validation group participants. The mean differences between the LBM measured by DXA and equation 1 based on age, height and weight in case of men and women were about 0.28 kg and 0.02 kg respectively (**Table3**). Although this difference was statistically robust in case of men, it was < 1% of the mean LBM. The pure error and limits of agreement were also relatively narrow. Inclusion of circumferences and skinfold thicknesses as predictors in models 2-4 reduced the difference between the measured and predicted LBM value along with reduction in pure error and narrowed the limits of agreement. The Bland Altman plot comparing the LBM estimates measured by DXA and those predicted by equation 4 also showed that the mean estimates by the two methods were similar (**Figure 5.1**).

When the values of LBM measured by DXA were compared with the LBM estimates derived by commonly used Durnin's equation, the values predicted by the Durnin's equation were substantially higher than the DXA estimates with a mean (SD) difference of 4.32 (2.18) kg in men and 4.03 (1.86) kg in women. Although the values predicted by this equation explained more than 88% of the variation in the DXA measured LBM, the limits of agreement were wider than those using the equations developed in the present study.

Table 5.3 Validation of anthropometric equations for estimation of lean body mass (Kg) in the validation group

	Sex	N	Difference (DXA-equation)[1]	SD	*p* value[2]	*Adjusted R²*	Pure error[3]	Limits of agreement[4]	
Equation 1[5]	M	570	0.28	1.96	<0.01	0.90	1.96	-3.57	4.13
	F	318	0.02	1.64	0.83	0.91	1.64	-3.20	3.24
Equation 2[6]	M	569	0.23	1.91	<0.01	0.90	1.91	-3.52	3.98
	F	318	0.01	1.58	0.91	0.91	1.59	-3.09	3.11
Equation 3[7]	M	568	0.17	1.64	0.01	0.93	1.64	-3.04	3.39
	F	309	0.05	1.46	0.59	0.92	1.46	-2.82	2.91
Equation 4[8]	M	567	0.10	1.56	0.14	0.94	1.56	-2.96	3.16
	F	309	0.05	1.39	0.51	0.93	1.39	-2.68	2.79
Durnin Womersley equation (Ref 7)	M	568	-4.32	2.18	<0.01	0.88	2.11	-8.61	-0.03
	F	309	-4.03	1.86	<0.01	0.89	1.76	-7.68	-.39

1 Difference in the estimates of lean body mass by DXA – proposed anthropometric equation in Kg ;[2] p valuebased on paired t test

[3] Pure error (kg): calculated as square root of the mean of squares of differences between the lean body mass estimates by DXA and proposed equations

[4] Limits of agreement: 95% Limits of agreement (mean difference ± 2SD) by DXA and proposed equations calculated by Bland Altman technique

[5] Equation 1: based on height and weight; [6]Equation 2: based on height, weight, circumferences (arm, calf and hip)

[7] Equation 3: based on height, weight, skinfold thickness at biceps, triceps, subscapular and supraillic regions

[8] Equation 4: based on height, weight, circumferences (arm, calf and hip) and skinfold thickness (biceps, triceps, subscapular and supraillic)

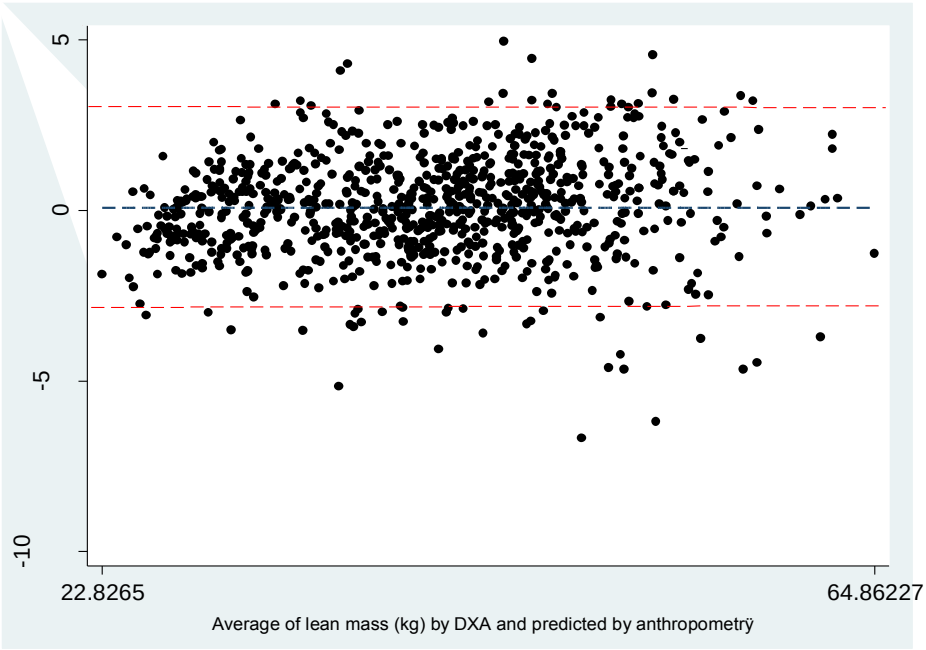

***Figure 5.1.*Bland Altman plot of lean body mass (Kg) estimates by DXA and prediction equation based on sex- specific anthropometric variables**

Equations used for prediction of lean body mass:

Men: Lean mass = 10.385 – (0.005 x age) + (0.103 x height) + (0.680 x weight) + (0.288 x arm circumference) + (0.130 x calf circumference) - (0.183 x hip circumference) – (5.278 x logarithm of sum of 4 skinfolds)

Women: Lean mass = 10.632 – (0.009 x age) + (0.102 x height) + (0.592 x weight) + (0.055 x arm circumference) + (0.043 x calf circumference) - (0.158 x hip circumference) - (3.174 logarithm of sum of 4 skinfolds)

Prediction and validation of ALST

Table 5.4 shows the proposed prediction equations for estimation of ALST (Kg). Compared to equations for estimation of LBM (table 3), variation in ALST explained by these equations was lower ranging from 0.78 by the simplest model (equation 1 – including age, height and weight) to 0.86 by equation 4 which additionally included circumferences and skinfold thicknesses. SEE and AIC values were lowest with equation 4 compared to simpler models proposed by equations 1, 2 and 3 indicating better prediction quality of the model compared to the equations 1-3. Equation 3, which included a derived index of CAMA in addition to height and weight, showed better prediction ability compared to equation 1 indicating that inclusion of this index improved the precision of the estimate.

Table 5.4 Proposed anthorpometric equations for estimation of ALST[1] (Kg)

	Predictor variables	Sex	N	Proposed equations	*Adjusted R^2*	SEE[2]	AIC[3]
Equation 1	Height[4], Weight[5]	M	851	ALST = -13.432 - (0.0445 x age[6]) + (0.200 x weight) + (0.140 x height)	0.78	1.28	2842
		F	481	ALST= -9.852 - (0.028 x age) + (0.170 x weight) + (0.102 x height)	0.82	1.05	1420
Equation 2	Height, Weight circumferences[7]	M	851	ALST= -12.81 - (0.029 x age)+ (0.211 x weight) + (0.153 x height) + (0.255 x calf circumference) + (0.141 x arm circumference) - (0 .178 x hip circumference)	0.82	1.17	2687
		F	481	ALST = -2.658 -(0.023 x age) + (0.244 x weight) + (0.082 x height) + (0.087 x calf circumference) - (0.058 x arm circumference) - (0.102 x hip circumference)	0.84	1.01	1386
Equation 3	Height, Weight, CAMA[8]	M	851	ALST = -16.270 - (0.037 x age) + (0.143 x weight) + (0.159 x height) + (0.087 x CAMA)	0.82	1.18	2696
		F	481	ALST = -10.818 - (0.027 x age) + (0.142 x weight) + (0.109 x height) + (0.051 x CAMA)	0.83	1.02	1394
Equation 4	Height, Weight Circumferences, Skinfolds[9]	M	851	ALST= -0.996 - (0.023 x age) + (0.274 x weight) + (0.090 x height) + (0.223 x calf circumference) + (0.143 x arm circumference) - (0.104 x hip circumference) - (3.163 x logarithm of sum of 4 skinfolds)	0.86	1.02	2452
		F	481	ALST = 1.609 - (0.021 x age) + (0.250 x weight) + (0.070 x height) + (0.098 x calf circumference) + (0.027 x arm circumference) - (0.085 x hip circumference)- (1.821 x logarithm of sum of 4 skinfolds)	0.86	0.94	1314

ALST: Appendicular lean soft tissue (Kg); [2] SEE: Standard error of the estimate (Kg) ;[3] AIC: Akaike's information criterion;

[4] Height in cm; [5] Weight in Kg; [6] Age in y; [7] Circumferences at arm, calf and hip in cm; [8] CAMA: Corrected arm muscle area in cm;

[9] Skinfold thickness measurements at biceps, triceps, subscapular and suprailliac regions in mm

When the above models were applied to the validation group for prediction of ALST, the mean values of ALST estimates by DXA and prediction equations were similar as the differences between the estimates by two methods were not statistically robust (**Table 5**). Pure error reduced from equation 1 (men: 1.33 kg; women: 0.97 kg) to equation 4 (men: 1.09 kg to 0.83 kg) as a result of additional variables added to the model used in equation 4. Limits of agreement were also narrower in case of equation 4 compared to equation 1. The Bland Altman plot comparing the two ALST estimates (measured by DXA and predicted by sex-specific equation 4) also showed that the mean estimates by the two methods were similar (**Figure 5.2**).

Table 5.5 Validation of anthropometric equations for estimation of ALST[1] (Kg) in the validation group

	Sex	N	Difference (DXA-equation)[2]	SD	*p* value[3]	*Adjusted* R^2	Pure error[4]	Limits of agreement[5]	
Equation 1[6]	M	570	0.05	1.33	0.36	0.78	1.33	-2.56	2.67
	F	318	-0.06	0.97	0.31	0.82	0.97	-1.96	1.85
Equation 2[7]	M	569	0.01	1.30	0.89	0.79	1.30	-2.53	2.55
	F	318	-0.04	0.93	0.44	0.83	0.93	-1.86	1.78
Equation 3[8]	M	567	0.02	1.09	0.67	0.85	1.09	-2.11	2.15
	F	309	-0.02	0.83	0.69	0.86	0.83	-1.65	1.61
Equation 4[9]	M	570	0.00	1.20	0.96	0.82	1.20	-2.36	2.36
	F	318	-0.05	0.93	0.34	0.83	0.93	-1.87	1.77

[1] ALST: Appendicular lean soft tissue in Kg

[2] Difference in the estimates of ALST by DXA – proposed anthropometric equation in Kg ;[3] *p* valuebased on paired t test

[4] Pure error (kg): calculated as square root of the mean of squares of differences between the ALST estimates by DXA and proposed equations

[5] Limits of agreement: 95% Limits of agreement (mean difference ± 2SD) by DXA and proposed equations calculated by Bland Altman technique

[6] Equation 1: based on height and weight;

[7] Equation 2: based on height, weight, circumferences (arm, calf and hip)

[8] Equation 3: based on height, weight and corrected arm muscle area

[9] Equation 4: based on height, weight, circumferences (arm, calf and hip) and skinfold thickness (biceps, triceps, subscapular and supraillic)

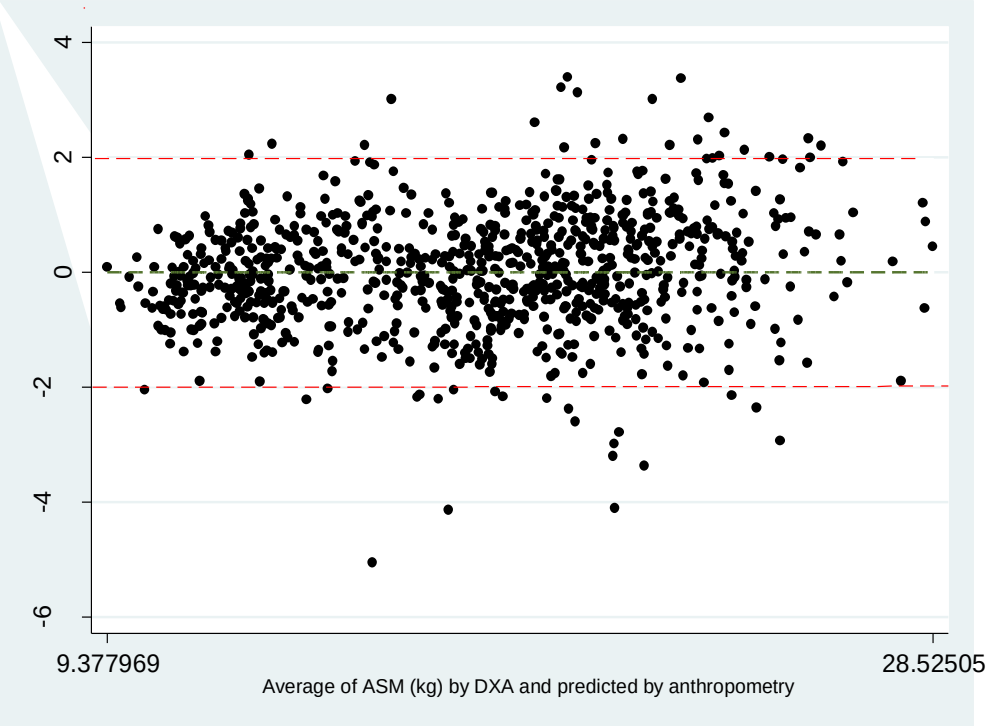

***Figure 5.2.*Bland Altman plot of Appendicular lean soft tissue (Kg) estimates by DXA and prediction equation based on sex- specific anthropometric variables.**

Equations used for prediction ofAppendicular lean soft tissue:

Men:ALST= -0.996 - (0.023 x age) + (0.274 x weight) + (0.090 x height) + (0.223 x calf circumference) + (0.143 x arm circumference) - (0.104 x hip circumference) - (3.163 x logarithm of sum of 4 skinfolds)

Women: ALST = 1.609 - (0.021 x age) + (0.250 x weight) + (0.070 x height) + (0.098 x calf circumference) + (0.027 x arm circumference) - (0.085 x hip circumference)- (1.821 x logarithm of sum of 4 skinfolds)

5.4 Discussion

In the present study, we developed anthropometric prediction equations to estimate LBM and ALST in a large sample of healthy Indian adults using DXA as a reference method. The newly developed models were validated in a validation sub-sample which showed that the equations predicted the LBM and ALST with high precision and low error. Commonly used Durnin and Womersley's equations, on the other hand, showed high prediction error with substantial overestimation of LBM in this sample. The prediction equations developed in the present study using simple, commonly used anthropometric measurements could be a valuable tool in population based studies assessing these important body mass compartments in Indians and other ethnic groups with similar body composition.

Anthropometry is one of the oldest techniques used for body composition assessment. Matiegka first developed equations for predicting body composition from the measurements of body length, width, circumference, and skin-fold thicknesses (Matiegka 1921). A number of researchers later extended this approach and developed anthropometric prediction formulas and validated them with cadaver studies(Martin, Spenst et al. 1990, Doupe, Martin et al. 1997) and other criterion techniques such as hydrodensitometry (Durnin and Womersley 1974, Jackson and Pollock 1978, Jackson, Pollock et al. 1980), MRI (Rolland-Cachera, Brambilla et al. 1997) and four compartment model(Peterson, Czerwinski et al. 2003, Van der Ploeg, Gunn et al. 2003). However, majority of the equations based on anthropometry have typically calculated body fat percentage and only a few studies have specifically attempted prediction of LBM or skeletal muscle mass.

Results of our study are comparable to those of a similar study in Chinese adults which developed anthropometric prediction equations for estimation of ALST using DXA as a reference technique in a large sample (n = 763)(Wen, Wang et al. 2011). The R^2 of prediction models based on different combinations of height, weight and limb circumferences ranged from 0.90 to 0.93 with SEE of around 1.5 kg. These values are similar to those observed in our study indicating that combinations of different anthropometric measurements can be used for accurate and precise estimation of LBM and ALST.

A few other studies that developed anthropometric prediction equations for estimation of LBM and ALST using different reference techniques have reported variable prediction qualities. One of the early studies from the United States developed an anthopometric equation to predict LBM using isotope dilution and whole body ^{40}K counting as reference techniques in 198 men from the Air Force (Fuchs, Theis et al. 1978). Although the LBM predicted using this equation correlated with that measured by the reference techniques, significant prediction bias was detected (pure error 3.3 kg). With recently increased interest in the estimation of muscle mass, a few more studies have developed anthropometric prediction equations to estimate skeletal muscle mass (SM). For example, a study from the U.S. developed two anthropometric prediction equations in a multi-ethnic sample using MRI as a reference technique. A simpler equation using height and weight could estimate the SM with a SEE of 2.8 kg whereas a relatively complex equation which included skinfold corrected limb circumferences showed improved prediction quality with SEE of 2.2 kg (Lee, Wang et al. 2000). Equations for LBM and ALST

estimation in the present study showed relatively higher precision (SEE < 2 kg and < 1.3 kg for estimation of LBM and ALST, respectively) compared to the above mentioned studies probably due to larger sample size and homogeneous study sample. The SEEs of the equations in our study were also lower than that reported by equations in a study in young Indian men (n=66) which used 24 h urinary creatinine excretion as a reference technique (Kuriyan and Kurpad 2004).

In the present study, we have developed a number of equations for prediction of LBM and ALST using different sets of anthropometric variables. Age had a negative association with LBM and ALST in all the models indicating age related reduction in the lean tissue. The prediction error in case of equations for women was lower compared to that in men. These differences could be related to the participant characteristics in the prediction and validation groups of men and women. Prediction equations appeared to underestimate the LBM and ALST compared to DXA in the validation group of men who had characterstics similar to the prediction group. On the other hand, although validation group women had lower LBM and ALST compared to prediction group women, their values predicted by equations were similar to DXA values probably indicating underestimation by the equations. Among the anthropometric variables, limb circumferences had positive association whereas hip circumference had a negative association with these outcomes. This suggests that although arm and calf circumferences can be considered as indicators of muscle mass, hip circumference may be an indicator of glueto-femoral fat rather than the muscle mass in this region. As expected, sum of SFTs was negatively associated with LBM and ALST due to its close association with adiposity.

The equations using simple anthropometric variables of height and weight (equation 1 in **Tables 5.2** and **5.4**) had lower prediction qualities (higher SEE, higher pure error and wider limits of agreement) compared to the equations with additional variables such as hip and limb circumferences and skinfold thicknesses. However, the contribution of these added variables to the explained variation in the measured LBM and ALST was only about 5-8%. (**Table5.2**). For example, equation 1 using age, height and weight explained 90% of the variation in measured LBM of men. Addition of hip and limb circumferences and SFTs to this equation (equation 4), increased the variation explained to 94%. In case of women, equation 1 explained 91% of the variation in LBM which did not increase substantially after addition of other anthropometric measures to the model. Similarly, the contribution of circumferences and SFTs to the variation explained by simple anthropometric measurements of height and weight for prediction of ALST was modest. This shows that equations based on simple measures of height and weight can provide reasonably precise estimates of LBM and ALST if applied to a population groups similar to the study sample. The use of corrected arm muscle area (CAMA) along with height and weight increased the adjusted R^2 value and reduced the SEE (equation 3, **Table 5.4**) in prediction of ALST. Thus, this model with commonly measured anthropometric variables of arm circumference and triceps SFT can provide a fairly accurate estimation of ALST. However, the use of fully adjusted models that included height, weight, hip and limb circumferences and SFT measurements alleviated the differences in the mean estimates of measured *v*spredicted values of LBM and ALST (**Figure 5.1** and **5.2**) showing excellent prediction quality of these models. Validity of these equations in other ethnic groups or in groups with different body composition patterns needs to be examined.

Commonly used Durnin and Womersley's equations based on SFT measurements did not predict LBM accurately in this study sample. The mean LBM values predicted by these equations were higher by about 4.3 kg and 4.0 kg compared to DXA measures in men and women respectively. On the other hand, the prediction equations developed in the present study using similar predictors (age, height, weight and SFTs) could estimate the LBM with high accuracy with mean differences (DXA- equation) of 0.17 kg and 0.05 kg in men and women, respectively (equation 3, **Table 2**). A number of reasons including the reference technique as well as the study sample used for developing the equations could explain the differences in the prediction quality of these two sets of equations. Durnin and Womersley's equation was developed using hydrodensitometry as a reference method in Caucasian men and women. A study from the U.S. also reported that Durnin's equations did not accurately predict body composition when compared to 4 compartment model although this study included healthy, white participants with characteristics comparable to those included in the Durnin and Wormersley' study (Peterson, Czerwinski et al. 2003). The differences were therefore probably related to the reference technique used for developing these equations.

We used ALST as an indicator of muscle mass in this study. This is based on a concept that about three-quarters of total body muscle mass exists in the extremities and that appendicular lean tissue is primarily skeletal muscle (Lee, Wang et al. 2000). ALST therefore is commonly used as a surrogate of skeletal muscle mass and DXA estimates of ALST have been validated using the skeletal muscle mass measurements by MRI and CT (Shih, Wang et al. 2000, Kim, Wang et al. 2002,

Bridge, Pocock et al. 2009, Zhao, Wang et al. 2013). The guidelines on the diagnosis of sarcopenia also use cut points based on ALST estimates (Fielding, Vellas et al. 2011). Moreover, cut points based on ALST predicted physical disability in elderly men and women independent of other covariates such as age, physical activity and prevalent morbidity in a longitudinal study from the U.S. (Baumgartner, Wayne et al. 2004). As access to DXA is limited in resource poor settings, the anthropometric prediction equations developed in our study would be a valuable tool to detect low muscle mass in different population groups.

The strengths of our study include a study sample representing a broad range of age and BMI and a large sample size which allowed development of equations with high prediction quality. To our knowledge, this is the first study from India to develop such equations using a precise technique of DXA as a reference method. Limitation of the study includes lack of data on SFT measurements at thigh and chest regions which precluded comparison of our equations with some of the other commonly used equations like Jackson and Pollock's equations (Jackson and Pollock 1978, Jackson, Pollock et al. 1980).

In summary, in the present study, a number of sex-specific anthropometric prediction equations for estimation of LBM and ALST were developed and then cross-validated in an independent sample of healthy adults. Fully adjusted models which included hip and limb circumferences and SFTs along with weight and height predicted these outcomes with high accuracy. Simple models including age, height and weight also predicted LBM and ALST with a reasonably low prediction error. These equations based on commonly measured anthropometric variables used in our

study can be used in a wide range of epidemiological studies collecting anthropometric data and could be a valuable tool in resource poor settings. Additional validation studies are, however, needed to test their validity in different settings.

Chapter 6: General discussion and Conclusions

6.1 General discussion

Rising prevalence of adiposity related chronic diseases on the background of widely prevalent chronic under nutrition is a significant public health concern in India and other developing countries undergoing epidemiological and nutrition transition. A large number of studies in Indians and other Asians have shown that their peculiar body composition with high fat mass, and low lean body mass may be an important determinant of the elevated risk of metabolic syndrome in this population.

Majority of the studies exploring the link between body composition and risk of chronic diseases have focused on the fat mass with little attention to the LBM. However, it is being increasingly recognized that the LBM (which includes skeletal muscle mass) plays a central role in a number of physiologic processes and energy metabolism. A lower LBM is independently associated with increased risk of insulin resistance (Braith and Stewart 2006, Atlantis, Martin et al. 2009) as well as a number of chronic diseases (Wolfe 2006). Moreover, muscle mass is an important determinant of resting metabolic rate and a higher muscle mass may contribute to the prevention of obesity by influencing energy balance (Wolfe 2006).

The focus of the present thesis was to evaluate important aspects related to the determinants and measurement of lean body mass in Indian adults and the three

studies described in previous chapters were conducted as a part of this PhD programme. These studies together make significant contribution to the advancement of knowledge in this area.

The first study (Chapter 3) examined the determinants (both early nutrition as well as current life factors) of the lean body mass, muscle mass and functional competence of muscle mass, operating during lifecourse in rural young adults residing in a transitioning community. Although the information on the relative importance of early nutrition and later lifestyle for adult LBM and muscle strength helps determine the focus of interventions to improve these health outcomes, majority of the previous studies have examined the early and later life determinants of the LBM in isolation. Studies which include comprehensive assessment of early as well as later life influences on the LBM and muscle strength are scarce. The first study in this thesis attempts to fill this important knowledge gap and makes a significant contribution to the evidence base in this area.

The study shows that the early nutrition exposure was not associated with a higher lean mass, muscle mass or muscle strength in this cohort of rural young adults born within an earlier community trial of balanced protein-calorie supplementation. On the other hand, current socioeconomic position, dietary energy intakes and physical activity were important determinants of lean mass indices in these rural participants living in rapidly urbanizing environments.

A number of explanations for the lack of hypothesized association between early nutrition exposure and later lean mass and muscle strength observed in this

study are possible. The nutrition intervention offered in the present study was a part of a government programme in India and the actual intake of the supplement was not supervised in the initial trial. It is possible that the effective supplemental dose was too low to demonstrate a lasting impact on the adult characteristics. Another important reason could be the differences in the current lifestyle (particularly diet and physical activity patterns) of the participants in the intervention and control groups. Anecdotal evidence from the study area suggested that the intervention villages have undergone relatively rapid urbanization than the control villages in the past few years. Lower muscle mass and strength in the intervention participants, therefore, could be attributed to their relatively urbanized lifestyles and likelihood of sedentary occupations. Interestingly, the previous follow up of this cohort (when the participants were adolescents) had shown a positive impact of early nutritional supplementation on the height and cardio-metabolic risk indicators of the participants. The positive influence of early supplementation appeared to decrease over time probably due to urbanization-related changes in the diet and physical activity patterns of the participants.

The results of the study thus suggest that, until better evidence emerges, strategies to improve lean mass and muscle strength should focus on improving lifestyles among the youth. These findings have important implications for the policy development in developing countries. To our knowledge, this is the first study from India that assessed the long term impact of nutrition supplementation provided through a government programme on important measures of human capital. Moreover, the study provides an interesting example to the students of epidemiology

because it highlights a number of challenges faced by epidemiological studies involving long term follow up of birth cohorts.

The first study used DXA for the outcome (LBM and muscle mass) assessment due to its advantages such as high precision and low dose of radiation exposure. DXA, however, is not without limitations. Although DXA estimates of body composition have been found to be highly correlated with those derived using a more accurate method (four compartment model), variations between the estimates were observed (Schoeller, Tylavsky et al. 2005). Such method-dependent variation in the estimates of LBM could have influenced the results of the first study. The second study in this thesis therefore compared the body composition estimates by DXA with the estimates using isotope dilution technique in a sample of 152 adults representing a wide range of age (19-70 years) and BMI (13.8 to 39.7 kg/m^2). Isotope dilution technique was chosen for the validation of DXA because the body composition estimates by isotope dilution technique have been found to be highly correlated with the estimates using the criterion technique of the 4 compartment model (Deurenberg-Yap and Deurenberg 2002). The analyses of the present study showed that the two commonly used reference methods for body composition assessment provide substantially different estimates of body composition. Compared to the isotope dilution technique, lean mass estimates by DXA were higher by about 7%, fat mass estimates were lower by about 21% and body fat percent estimates were lower by about 7.4%. The study thus showed that the body composition estimates are method-dependent and caution should be exercised while interpreting the results of studies using different techniques of body composition assessment. The LBM estimates by DXA and isotope dilution technique were, however, highly correlated

suggesting that the differences in absolute values of LBM at individual level may not have affected the results of the first study that assessed the relationship of various early and current life determinants with the lean body mass of young adults.

Similar studies from different parts of the country are, however, required for enhancing our understanding on the determinants of lean and muscle mass in Indians. This information, although critical for developing intervention strategies focused on improvement of the muscle and lean mass, is however scarce. The paucity of information is mainly due to difficulties involved in quantifying these body compartments in resource-poor settings. Inexpensive methods for LBM and muscle mass measurement suitable for large scale epidemiological studies are not available.The third study in this PhD programme therefore developed anthropometric prediction equations for estimation of lean body mass and appendicular lean soft tissue (ALST, an indicator of skeletal muscle mass) in a large sample of healthy volunteers. Participants (n= 2,220; 36% females; age 18-79 y) representing a wide range of BMI (14 - 44 kg/m^2) were enrolled in this study and sex-specific equations were developed using DXA as a reference technique. The equations were cross-validated in a sub-sample of 40% participants. Simple equations using age, height and weight explained > 90% variation in the LBM and ALST in both men and women. More complex equations using all the above anthropometric variables could predict the DXA measured LBM and ALST accurately as indicated by low standard error of the estimate as well as the Bland Altman analyses indicating good agreement with the reference method. Commonly used Durnin and Womersley's equations were not found suitable for prediction of LBM in this study sample as these equations grossly overestimated LBM compared to DXA in both men and women. The

population specific equations developed in the present study therefore could be a valuable tool in future epidemiological studies assessing the LBM and muscle mass in Indians and other population groups with similar body composition.

6.2 Conclusions and implications

Thus the three studies together contribute richly to the existing body of knowledge on the determinants and measurement of lean mass and muscle mass of Indian adults.

Based on the findings of these studies, following conclusions can be drawn –

1. Modest protein energy supplementation provided through a government programme did not have long term positive impact on the lean mass, muscle mass or muscle strength of the young adults who were beneficiaries of the programme. On the other hand, current life factors including socioeconomic position, dietary energy intakes and physical activity were important determinants of lean mass and muscle strength in these settings.

2. Although the average estimates of lean mass and fat mass by DXA and isotope dilution technique were fairly close, considerable differences in the estimates were seen at individual level. Lean mass estimates by DXA were higher than those by isotope dilution technique whereas the fat mass and body fat percent estimates by DXA were lower than that by the isotope dilution technique in this sample. However, there was no evidence of any systematic bias for either lean or fat mass measurements by DXA compared to the isotope dilution technique.

3. Anthropometric prediction equations for estimation of lean body mass and appendicular lean soft tissue (an indicator of skeletal muscle mass) have been developed in the present study. These equations would be a useful tool to measure these important body compartments in resource poor settings.

Findings of the current thesis have potential to contribute to theory and practice in the following ways:

1. Although birth weight is known to have a strong positive relationship with adult lean body mass and muscle strength, short term nutrition supplementation may not have a lasting positive impact on these adult characteristics as indicated by the first study. On the other hand, nutrition and physical activity are known to exert influence throughout lifecourse. The study thus indicates that interventions throughout the lifecourse would be helpful in improving the lean mass, muscle mass and muscle strength of the populations in developing countries.

2. One of the reasons for the lack of impact of early nutrition intervention on the adult lean body mass could be related to the low dose of supplemental nutrition. Future studies are therefore required to assess the long term impact of interventions that provide nutrients in quantities adequate for influencing growth.

3. Urbanization-related lifestyle changes in participants residing in the transitioning rural communities pose significant challenges for long term cohort studies assessing the impact of early life factors on adult health

outcomes. Studies assessing the relationship of early life factors with adult health outcomes should include precise measures of current life factors for adequate adjustment for their potential confounding influence.

4. Imperfect measurement of potential confounders can bias the association between early nutrition exposure and later health outcomes. These limitations should be considered while drawing conclusions of studies that involve a large range of confounding influences. In addition, more research is required for improving the methods for assessment of lifestyle indicators including dietary intakes and physical activity.

5. As different methods of body composition assessment can produce substantially different estimates, these methods cannot be used interchangeably. Results of studies using different methods of body composition measurement should, therefore, be compared with caution.

6. Results of validation studies comparing body composition methods in different population groups vary considerably. Population specific validation studies comparing different methods are therefore required.

7. The prediction equations developed in specific population groups have better prediction quality and are therefore desirable. Equations developed in other ethnic groups, although widely used in epidemiological studies, can produce estimates with substantial error.

6.3 Contribution of the study to existing knowledge and future research needs

1. The first study on the determinants of the lean body mass in young adults (Chapter 3) is the only study from India and other developing countries which provides information on the long term health impact of a public-funded nutrition supplementation programme. In addition, the study provides valuable insight into the relative importance of early nutrition and current lifestyle (mainly diet and physical activity) in rural communities that are undergoing nutrition transition associated with rapid urbanization. The findings of the study showed that the positive health impact of the modest protein-calorie supplementation that was observed in this cohort during adolescence did not persist till adulthood probably due to adoption of unhealthy lifestyle by these young adults. The findings of the present study, therefore, prompt future studies to examine the impact of lifestyle modifications on the adult health outcomes.

2. The second study (Chapter 4) which compared two methods of body composition measurement (DXA and isotope dilution technique) is the first such study in Indian population. The study provides population specific evidence on the validity of DXA compared to another precise technique of body composition measurement based on a large sample. The study would be helpful for interpretation of other studies on body composition that use different measurement techniques. The study stimulates similar studies in other parts of India. The results of these studies could be collated in order

to derive a correction factor that can be applied to make the body composition estimates by different methods comparable.

3. The study on the anthropometric prediction equations for lean mass and muscle mass estimation (Chapter 5) is the first study in Indian population that has developed accurate and precise equations based on a large sample. The study also proved that widely used prediction equations developed in other ethnic groups lead to large errors in the body composition estimates and are therefore not suitable. These population specific equations provide a valuable tool for inexpensive and precise estimates of the lean mass and muscle mass in Indians and would thus facilitate future research in this area.

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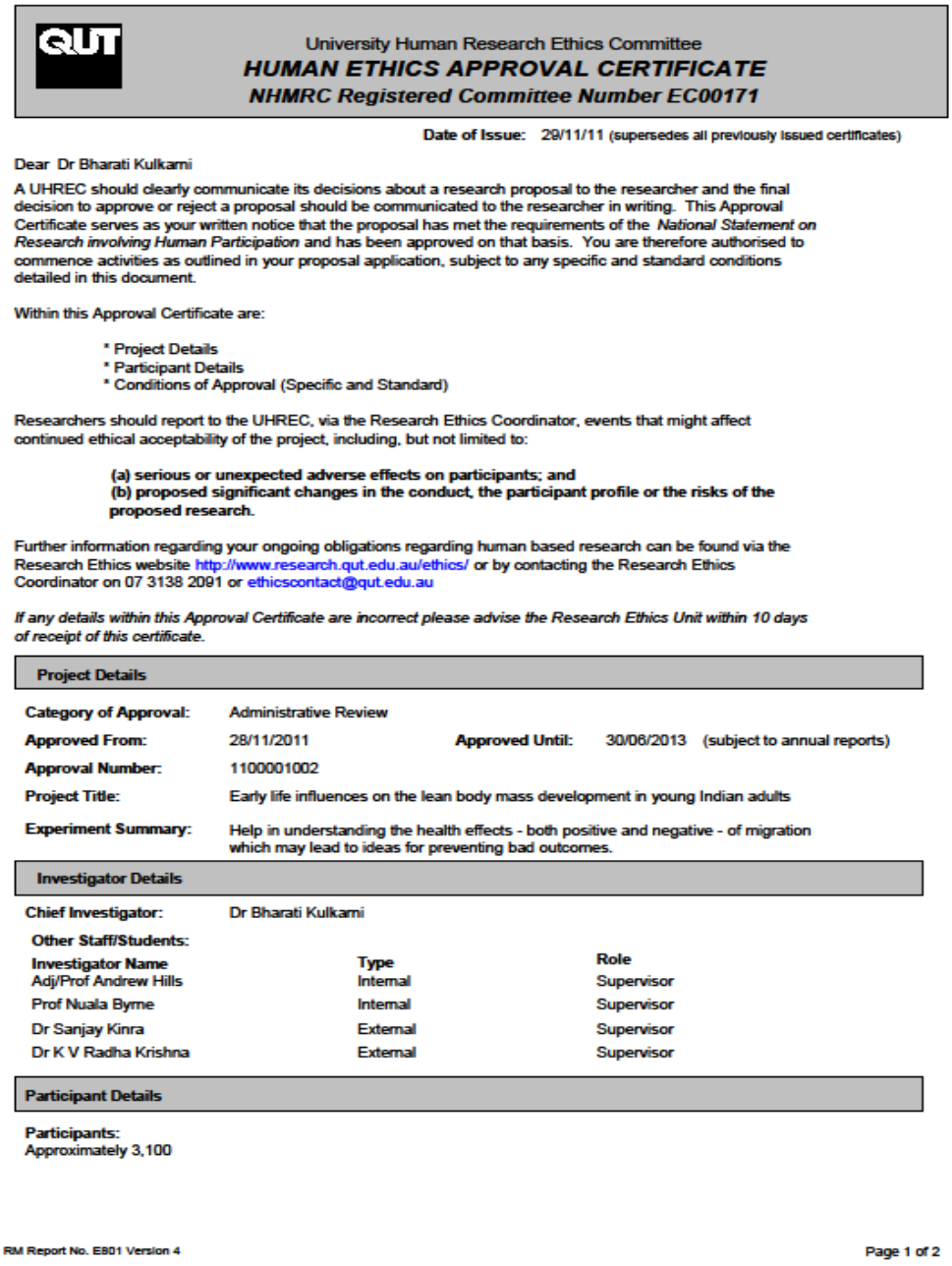
Appendix

Ethics approval



University Human Research Ethics Committee

HUMAN ETHICS APPROVAL CERTIFICATE

NHMRC Registered Committee Number EC00171

Date of Issue: 29/11/11 (supersedes all previously issued certificates)

Dear Dr Bharati Kulkarni

A UHREC should clearly communicate its decisions about a research proposal to the researcher and the final decision to approve or reject a proposal should be communicated to the researcher in writing. This Approval Certificate serves as your written notice that the proposal has met the requirements of the *National Statement on Research involving Human Participation* and has been approved on that basis. You are therefore authorised to commence activities as outlined in your proposal application, subject to any specific and standard conditions detailed in this document.

Within this Approval Certificate are:

* Project Details
* Participant Details
* Conditions of Approval (Specific and Standard)

Researchers should report to the UHREC, via the Research Ethics Coordinator, events that might affect continued ethical acceptability of the project, including, but not limited to:

(a) serious or unexpected adverse effects on participants; and
(b) proposed significant changes in the conduct, the participant profile or the risks of the proposed research.

Further information regarding your ongoing obligations regarding human based research can be found via the Research Ethics website http://www.research.qut.edu.au/ethics/ or by contacting the Research Ethics Coordinator on 07 3138 2091 or ethicscontact@qut.edu.au

If any details within this Approval Certificate are incorrect please advise the Research Ethics Unit within 10 days of receipt of this certificate.

Project Details

Category of Approval:	Administrative Review		
Approved From:	28/11/2011	**Approved Until:**	30/06/2013 (subject to annual reports)
Approval Number:	1100001002		
Project Title:	Early life influences on the lean body mass development in young Indian adults		
Experiment Summary:	Help in understanding the health effects - both positive and negative - of migration which may lead to ideas for preventing bad outcomes.		

Investigator Details

Chief Investigator: Dr Bharati Kulkarni

Other Staff/Students:

Investigator Name	Type	Role
Adj/Prof Andrew Hills	Internal	Supervisor
Prof Nuala Byrne	Internal	Supervisor
Dr Sanjay Kinra	External	Supervisor
Dr K V Radha Krishna	External	Supervisor

Participant Details

Participants:
Approximately 3,100

RM Report No. E801 Version 4

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Date of Issue: 29/11/11 (supersedes all previously issued certificates)

Location/s of the Work:
Hyderabad, India

Conditions of Approval

Specific Conditions of Approval:
No special conditions placed on approval by the UHREC. Standard conditions apply.

Standard Conditions of Approval:
The University's standard conditions of approval require the research team to:

1. Conduct the project in accordance with University policy, NHMRC / AVCC guidelines and regulations, and the provisions of any relevant State / Territory or Commonwealth regulations or legislation;

2. Respond to the requests and instructions of the University Human Research Ethics Committee (UHREC);

3. Advise the Research Ethics Coordinator immediately if any complaints are made, or expressions of concern are raised, in relation to the project;

4. Suspend or modify the project if the risks to participants are found to be disproportionate to the benefits, and immediately advise the Research Ethics Coordinator of this action;

5. Stop any involvement of any participant if continuation of the research may be harmful to that person, and immediately advise the Research Ethics Coordinator of this action;

6. Advise the Research Ethics Coordinator of any unforeseen development or events that might affect the continued ethical acceptability of the project;

7. Report on the progress of the approved project at least annually, or at intervals determined by the Committee;

8. (Where the research is publicly or privately funded) publish the results of the project is such a way to permit scrutiny and contribute to public knowledge; and

9. Ensure that the results of the research are made available to the participants.

Modifying your Ethical Clearance:
Requests for variations must be made via submission of a Request for Variation to Existing Clearance Form (http://www.research.qut.edu.au/ethics/forms/hum/var/var.jsp) to the Research Ethics Coordinator. Minor changes will be assessed on a case by case basis.

It generally takes 7-14 days to process and notify the Chief Investigator of the outcome of a request for a variation.

Major changes, depending upon the nature of your request, may require submission of a new application.

Audits:
All active ethical clearances are subject to random audit by the UHREC, which will include the review of the signed consent forms for participants, whether any modifications / variations to the project have been approved, and the data storage arrangements.

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