CHAPTER-III

Materials and Methods

(WITH 3 TABLES AND 1 FIGURE IN THE TEXT)

III

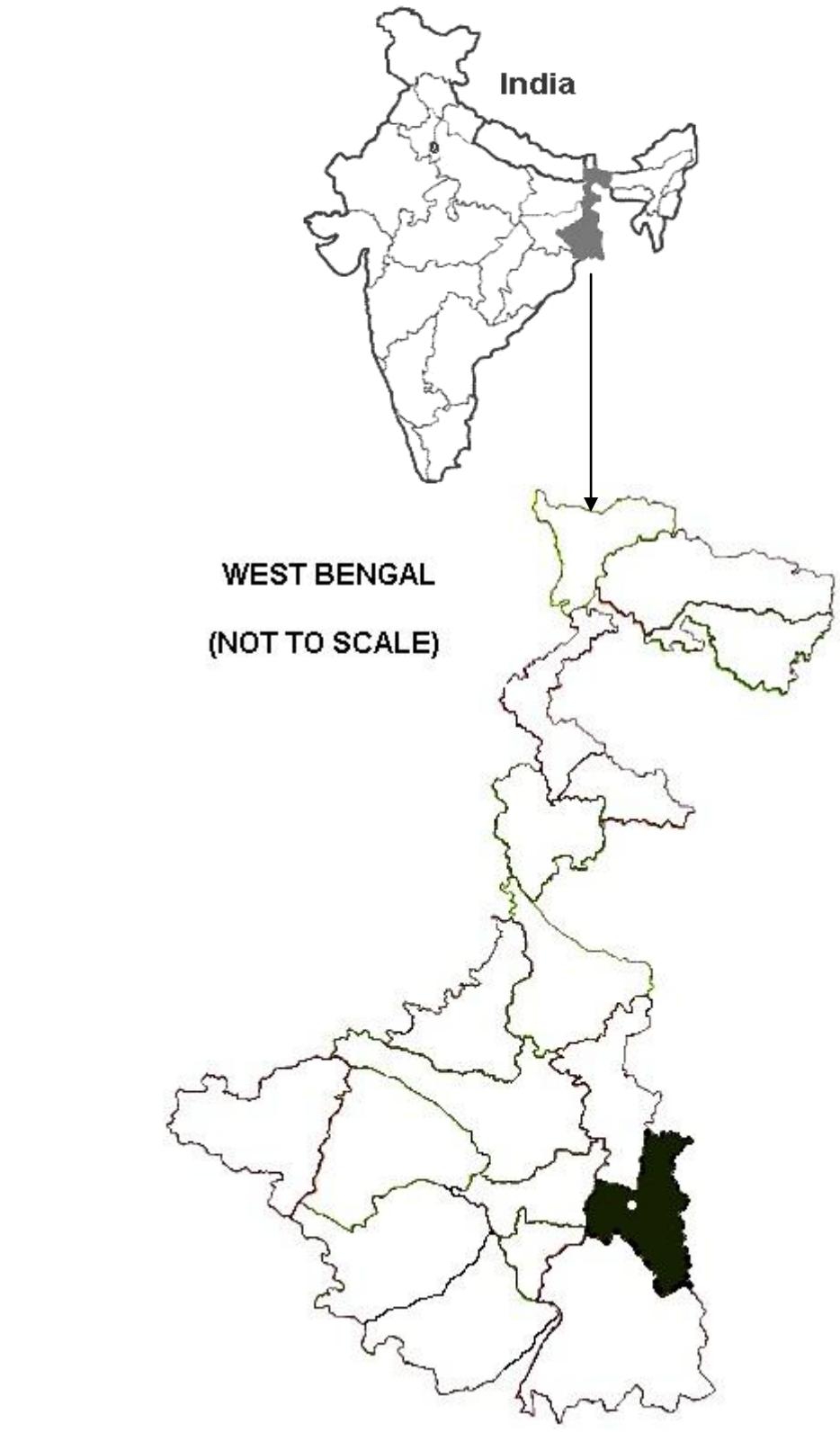
MATERIALS AND METHODS

3.1 AREA OF THE STUDY

The investigation was carried out among adolescent Muslim boys and girls in three secondary schools in Deganga block, North 24 Parganas, West Bengal. The schools are situated in a rural area approximately 38 km to the east from the heart of the city of Kolkata and approximately 17 km from the Barasat town. Barasat is the administratative headquarters of the district of North 24 Parganas, West Bengal, India (Figure 3.1).

The district North 24 Parganas lies between 21^{o}31' and 22^{o}57' north latitude and between 88^{o}2' and 89^{o}6' east longitude. The district is surrounded on the north by Bangladesh and the district of Nadia, on the south by the district of South 24 Parganas, on the east by Bangladesh and on the west by river Hooghly. The river Hooghly, flowing from north to south, separates the district from Hooghly and Howrah. Total area of the district is 4094 sq. km. As the district lies within the limits of the Gangetic delta, the physical features are like that of deltaic land.

Figure 3.1 : Outline Map of the Field Area at the National, State and District Level



3.2 SAMPLE OF THE STUDY

Arijullapur Siddikia High Madrasah, Arijullapur Siddikia Jr. High Madrasah (Unit - II) and Kumarpur Parasmoni Sikhabitan, Deganga, North 24 Parganas, West Bengal, India, were been chosen for present study. Official approval and ethical consent were obtained from the school authorities prior to the commencement of the study. All the students of the school were called to participate in the present research survey. The response rate was 70%. Thus, the sample size of this study was 1072. The age range of the subjects was 10-17 years, i.e., those who attained at least 9 years of age (i.e. from 10.0 years or above) but less than 18 years (i.e. up to 17.9 years or less). Four samples were excluded during the process of data management because of missing data. Thus, the final sample size of this investigation was 1068. The samples were classified into eight age groups by one year interval (Table 3.1).

3.3 NATURE OF THE SAMPLE

The students were mostly middle-class Bengalees who belonged to Bengali speaking Muslims (boys= 48.9 %, girls = 51.1 %) of West Bengal. The boys sample is 522 and girls sample is 546. The mean age and standard deviation of boys and girls are presented in table 3.1.

Table 3.1 : Studied Sample : An Overview

Field Setup			Secondary Schools
Age Range			10.0 – 17.9 Years
Mean Age	Boys	13.44	(SD ± 2.20) Years
	Girls	13.29	(SD ± 2.13) Years
Total Sample		522	Boys , 546 Girls
Age-wise Sample Distribution :		Boys	Girls
10.0 - 10.9		56	62
11.0 - 11.9		63	73
12.0 - 12.9		65	82
13.0 - 13.9		77	79
14.0 - 14.9		84	78
15.0 - 15.9		71	70
16.0 - 16.9		60	55
17.0 - 17.9		46	47

3.4 METHODS OF THE STUDY

The present research investigation was cross-sectional in nature and carried out during the period of July-October 2014. Requisite data were collected using a pre-tested Interview-Schedule (Annex - I). Signed consents were obtained from each participant. The Interview-Schedules were completed and the subjects measured during the working hours of the school.

Socio-demographic data for each subject included age, educational background, religion, parent's occupation, family income etc. Authentication of age (date of birth) was done from the school records.

A total of twelve anthropometric measurements were taken on each subject following standard protocols (Table 3.2). Furthermore, eighteen derivative variables on different aspects were incorporated for final analyses following the objectives of the study (Table 3.3).

3.5 ANTHROPOMETRIC MEASUREMENTS

The anthropometric measurements taken for each subjects were - height, weight, five circumferences and five skinfolds (two truncal and three extremity). All anthropometric measurements were taken by the researcher following standard anthropometric procedure as recommended by Lohman et al. (1988). All bilaterally represented measurements were taken on the left side of the subject.

Table 3.2: List of Measured Anthropometric Variables

01. Height (HT) [cm]
02. Weight (WT) [kg]

Circumferences [cm]	**Skinfolds** [mm]
03. Mid-Upper Arm (MUAC)	08. Biceps (BSF)
04. Chest (CC)	09. Triceps (TSF)
05. Minimum Waist (WC)	10. Subscapular (SUBSF)
06 Maximum Hip (HC)	11. Suprailiac (SUPSF)
07. Calf (CFC)	12. Medial Calf (CSF)

3.6 PROCEDURE OF ANTHROPOMETRIC MEASUREMENTS

3.6.1 HEIGHT

Height or stature is a major indicator of general body size and of skeletal length. It is important in screening for disease or malnutrition and in the interpretation of weight. Height was measured to the nearest 0.1 cm using Martin's anthropometer (movable type). Each subject was requested to stand in bare feet as straight as possible on the floor with heels together so that, weight of the subject was distributed evenly on both the feet. The subject's back was as straight as possible with his arms relaxed and was hanging freely by the sides of the trunk and palms were facing the thighs. Each subject was asked to inhale deeply and maintain a fully erect position without altering the load on the heels the head placed in a 'normal' position, with the eyes looking straight ahead. The anthropometer was placed behind the subject so that its lower ends stands between the heels and the beam passes vertically between the buttocks and touching the back of the head. The horizontal arm of the anthropometer was brought down on the top of the midsagittal plane on the head and the measurement was taken.

3.6.2 WEIGHT

Body weight is the most commonly recorded anthropometric variable. Weight is a composite measure of total body size. Body weight is universally accepted crude indicator for the general health status. It is particularly important in screening for unusual growth, obesity and undernutrition. Body weight was recorded to nearest 0.5 kg on a conventional weighing scale. Each subject was requested to stand without any support on a weighing machine placed on the floor. The subject was standing straight on the machine with the body weight evenly distributed between the feet. The participants were requested to remove their shoes prior to taking measurements and not to wear anything except underwear when they were on the weighing machine and eventually measurements were taken. The weighing machine was checked from time to time to set zero.

3.6.3 CIRCUMFERENCES

Circumferences are important measurements that record the size of cross-sectional and circumferential dimensions of the body. Circumferences used alone, in combination with skinfold measurement taken at the same location or in combination with other circumferences, are measures of growth and can provide indices of nutritional status and levels of fat patterning. During later childhood, adolescence and into adulthood, circumferences of the limbs, together with skinfold measures of subcutaneous adipose tissue thicknesses at corresponding levels, can provide cross-sectional areas of adipose tissue or the areas of the underlying “muscles plus bone”. Ratios between selected circumferences and with skinfolds of the trunk and of the limbs can provide indices of the patterning of subcutaneous adipose tissue and muscles (Lohman et al. 1988).

In the present research survey, circumference measurements were made to the nearest 0.1 cm using a flexible inelastic steel tape measure. The steel tape was applied lightly to the skin in order to avoid deforming the contour of the skin. Measurements were recorded with the zero end of the steel tape hold in the left hand above the remaining part of the tape hold by the right hand. Altogether seven circumferencial measurements were taken on each subject.

3.6.4 MID-UPPER ARM CIRCUMFERENCE

For this purpose, each subject was requested to stand erect with the arm hanging freely at the sides of the trunk and the palms were facing the thighs. Prior to taking this measurement the mid point of the upper arm was marked and was used to measure mid upper arm circumference. To locate the midpoint, the subject’s elbow was flexed to 90 degree with the palm facing superiorly. The lateral tip of the acromion by the palpating laterally along the superior surface of the spinous process of the scapula was traced by standing behind the subject. A small mark was made on the identified point. The most distal point on the acromial process was located and marked. A tape was placed over these two marks, and the mid point was then marked. After that, tape was placed around the arm in such a manner that it was touching the skin, but not

compressing the soft tissues. The tape was positioned perpendicular to the long axis of the arm at the marked midpoint. The measurement was taken with both biceps and triceps muscles were in relaxed condition. The measurements were taken by standing left side of the subject to get clear view of tape position.

3.6.5 CHEST CIRCUMFERENCE

During the measurement, each subject was requested to stand erect, relax, with the feet at shoulder width. To permit passage of the tape around the chest, subjects were instructed to abduct the arms and when the tape was placed round the chest, the arms were lowered to their natural position at the sides of the trunk. Chest circumference was measured at the level of nipple on the anterior aspect of the thorax which correspondence approximately to the fourth inter-costal space. The fourth inter-costal space is just inferior of the fourth costo-sternal joints. The measurement was made in a horizontal plane by standing in front of the subject but slight to left side. The tape housing was held in the right hand while the free end retrieved by the left hand as it passed around the subject's back. The free end of the tape was positioned between the right axilla and sternum. Precaution was taken to ensure that the tape was at the correct horizontal position, first at the back and then at the front. The reserve end of the tape then placed near the zero end and measurement was taken.

3.6.6 MINIMUM WAIST CIRCUMFERENCE

Waist circumference is an index of deep adipose tissue and it is related to fat-free mass. The subject stands erect with the abdomen relaxed, the arms at the sides and the feet together. The tape was placed around the subject, in a horizontal plane, at the level of the natural waist, which was the narrowest parts of the torso, as seen from the anterior aspect. The measurement was taken at the end of a normal expiration and without the tape compressing the skin.

3.6.7 MAXIMUM HIP CIRCUMFERENCE

Hip circumference is a measurement of external pelvic size that reflects the amount of adipose tissue in the region. Subjects were requested to stand erect, with arms at the sides and feet together. The measurement was recorded squatting at the left sides of the subject to get the clear vision of the greatest or maximum extension of the buttocks posteriorly. The steel tape was placed around the buttock in a horizontal plane at the level without compressing the skin and the measurement was taken. Precaution was taken for each subject to ensure the horizontal position of tape on the opposite side of the subject's body. The tape was in contact with the skin but did not indent the soft tissues. The zero end of the tape was placed below the measurement value. The buttock level was taken because it is easier to locate than the trochanteric level and buttock circumference is generally the maximum circumference of the hip area in a horizontal plane.

3.6.8 CALF CIRCUMFERENCE

Each subject was instructed to stand erect with the feet 20 cm apart and weight was distributed equally on both feet. The steel tape measure was positioned horizontally around the calf and moved up and down to locate the maximum circumference in a plane perpendicular to the long axis of the calf and measurement was eventually taken. The level was marked to measure the medial calf skinfold at the same level. The tape was in contact with the whole circumference but not indenting the skin. The zero end of the tape was placed below the measurement value.

3.6.9 SKINFOLDS

Skinfold thicknesses, sometimes called 'fatfold' thicknesses, are actually the thicknesses of double folds of skin and subcutaneous adipose tissue at specific sites on the body. The utility of skinfold thicknesses is twofold. First, they provide a relatively simple and non-invasive method of estimating general fatness. The second major use of skinfold thicknesses is in the characterization of the distribution of subcutaneous adipose tissue. Because subcutaneous fat may represent up to 50% of total body fat,

the measurement of skinfold thickness can provide a useful technique for evaluating body fat. Numerous equations for the prediction of body composition from anthropometric measurements have been developed that make use of skinfold thicknesses as essential components (Lohman et al. 1988).

In the present study, five truncal skinfolds (chest, midaxillary, subscapular, suprailiac and abdominal) and five extremity skinfolds (biceps, triceps, forearm, anterior thigh and medial calf) were measured to the nearest 0.2 mm using a Harpenden skinfold caliper. The thumb and index finger of the left hand were used to elevate a double fold of skin and the caliper jaws applied at exactly the level marked.

3.6.10 BICEPS SKINFOLD

During the measurement each subject was requested to stand erect with the upper extremity relaxed at the side, and palm directed anteriorly. The skinfold was picked up on the midline of the anterior aspect of the arm, over the biceps muscle, directly above the centre of the cubital fossa, at the same level as that at which the mid-upper arm circumference was measured. Standing in front of the subject the biceps skinfold was picked up with thumb and index finger. The caliper jaws were applied about 1 cm distal to the fingers holding the fold and measurement was taken.

3.6.11 TRICEPS SKINFOLD

During the measurement each subject was requested to stand erect with the arm suspended loosely. The triceps skinfold was measured on the midline of the posterior aspect of the arm, over the triceps muscle, at a point midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. The level of measurement was determined by measuring the distance between the lateral projection of the acromion process and the inferior border of the olecranon process of the ulna, using a tape measure, with the elbow flexed at the 90-degree angle. The tape was placed with its zero marks on the acromion and stretched along the upper arm, extending below the elbow. The midpoint was marked on the lateral side of the arm on the triceps muscle. Standing behind the subject the triceps skinfold was picked up with thumb and index finger, approximately 1

cm proximal to the marked level, and the jaws of the caliper were applied to the skinfold at the previously marked level and measurement was taken.

3.6.12 SUBSCAPULAR SKINFOLD

The subject was instructed to stand comfortably erect, with the upper extremities relaxed at the sides of the body. The site was just inferior to the inferior angle of the scapula. To locate the site, the investigator was palpating the scapula of the left side and was running the thumb and index finger of the left hand inferiorly and laterally, along the vertebral border until the inferior angle was identified. The subscapular skinfold was picked up on a diagonally inclined, infero-laterally, approximately 45-degree to the horizontal plane in the natural cleavage lines of the skin. The caliper jaws were then applied 1 cm infero-laterally to the thumb and finger, responsible to raising the oblique fold, and measurement was recorded subsequently.

3.6.13 SUPRAILIAC SKINFOLD

The subject stands with feet together and in an erect position. To improve access to the site, each subject was requested to abduct the left arm slightly. An oblique skinfold was grasped just posteriorly to midaxillary line following the natural cleavage lines of the skin. It was aligned inferomedially at 45 degree to the horizontal. The caliper jaws were applied about 1 cm from the fingers holding the skinfold, and thickness was eventually recorded.

3.6.14 MEDIAL CALF SKINFOLD

Each subject’s foot was placed on a platform for location of medial calf skinfold site. The level of the maximum calf circumference was marked on the medial aspect of the calf. From a position in front of the subject a skinfold was raised parallel to the long axis of the calf on its medial aspect and the thickness of the fold was measured. The caliper jaws were applied about 1 cm distal from the fingers holding the vertical skinfold, and thickness was eventually recorded.

3.7 ESTIMATION OF ADIPOSITY AND BODY FAT CONTENT

Body mass index (BMI) – a popular indicator of generalised adiposity was calculated following the formula of World Health Organization (1995). Conicity index (CI) and waist-hip ratio (WHR), two typical measures of central adiposity were derived using the equations given by Valdez et al. (1993) and, Yassin and Terry (1991) respectively. Waist-height ratio (WHTR), another measure of central adiposity was computed using standard equation.

Three regional adiposity indices were calculated following standard manner. They were subscapular-triceps ratio (STR), truncal-extremity fat ratio (TEFR) and centripetal fat ratio (CPFR). Three types of summation of skinfolds were estimated to point toward the subcutaneous fat content. They were sum of 5 skinfolds (S5S), sum of truncal skinfolds (STS) and sum of extremity skinfolds (SES).

3.8 ESTIMATION OF BODY COMPOSITION

Prediction of percent body fat from skinfold thicknesses is an acceptable method for the assessment of body composition in children and adolescents (Deurenberg et al. 1990, Al-Sendi et al. 2003, Mueller et al. 2003). Skinfold equation of Slaughter et al. (1988) for predicting body fat was utilized to estimate percent body fat (PBF). Four measures of body fat composition viz. fat mass (FM), fat free mass (FFM), fat mass index (FMI) and fat free mass index (FFMI) were estimated using standard formulae (van Itallie et al. 1990, Bose and Das Chaudhuri 2003). Three measures of arm muscle-fat composition viz. arm muscle circumference (AMC), arm muscle area (AMA) and arm fat area (AFA) were calculated using the equations given by Gibson (1990), Burr and Phillips (1984), and Himes et al. (1980) respectively.

Table 3.3 : List of Derived Metric Variables

Measures of Generalised and Central Adiposity

01. Body Mass Index (BMI) (kg/m^2) = Weight (kg) / { Height (m) } ^{2}

02. Conicity Index (CI) =
Minimum Waist Circumference (m) / [(0.109) × $\sqrt{\{ \text{Weight (kg)} / \text{Height (m)} \}}$]

03. Waist-Hip Ratio (WHR) =
Minimum Waist Circumference (cm) / Maximum Hip Circumference (cm)

04. Waist-Height Ratio (WHTR) =
Minimum Waist Circumference (cm) / Height (cm)

Measures of Regional Adiposity

05. Subscapular-Triceps Ratio (STR) =
Subscapular Skinfold (mm) / Triceps Skinfold (mm)

06. Truncal-Extremity Fat Ratio (TEFR) =
{ Sum of Truncal Skinfolds (mm) / Sum of Extremity Skinfolds (mm) }

07. Centripetal Fat Ratio (CPFR) =
{ Subscapular Skinfold (mm) × 100 } / { Subscapular Skinfold (mm) +
Triceps Skinfold (mm) }

Continued …

Table 3.3 : Continuation …

Measures of Subcutaneous Fat Content

08. Sum of 5 Skinfolds (S5S) (mm) =
{ Biceps + Triceps + Subscapular + Suprailiac + Medial Calf } (mm)

09. Sum of Truncal Skinfolds (STS) (mm) =
{ Subscapular + Suprailiac } (mm)

10. Sum of Extremity Skinfolds (SES) (mm) =
{ Biceps + Triceps + Medial Calf } (mm)

Measures of Body Composition

11. Percent Body Fat (PBF) =
[0.735 × { Triceps Skinfold (mm) + Medial Calf Skinfold (mm) }] + 1.0

12. Fat Mass (FM) (kg) = { (Percent Body Fat / 100) } × Weight (kg)

13. Fat Free Mass (FFM) (kg) = Weight (kg) – Fat Mass (kg)

14. Fat Mass Index (FMI) (kg/m^2) = Fat Mass (kg) / { Height (m) } ^{2}

15. Fat Free Mass Index (FFMI) (kg/m^2) = Fat Free Mass (kg) / { Height (m) } ^{2}

Measures of Arm Muscle-Fat Composition

16. Arm Muscle Circumference (AMC) (mm) =
Mid-Upper Arm Circumference (mm) – { π × Triceps Skinfold (mm) }

17. Arm Muscle Area (AMA) (mm^2) = { Arm Muscle Circumference (mm) } ^{2} / ($4 \times \pi$)

18. Arm Fat Area (AFA) (mm^2) =
[½ × { Triceps Skinfold (mm) × Mid-Upper Arm Circumference (mm) }] –
¼ × [π – { Triceps Skinfold (mm) } ^{2}]

3.9 EVALUATION OF NUTRITIONAL STATUS

Nutritional status was evaluated using body mass index (BMI).

The prevalence of undernutrition (UN) or thinness was further assessed using body mass index (BMI). The age and sex specific percentile values (< 5^{th}) of BMI based on the first National Health and Nutrition Examination Survey (NHANES I) in the United States of America were followed (WHO 1995). These nutritional indicators have been utilized by several recent studies worldwide among adolescents (Pawloski 2002, Venkaiah et al. 2002, Woodruff and Duffield 2002, Bener and Kamal 2005).

3.10 PROCEDURE OF PHYSICAL ACTIVITY

Information on physical activity is collected using a pre-tested interview schedule following the criteria of Global Physical Activity Questionnaire (GPAQ). The data on physical activity level is collected from each subject who comprises of physical exercise type, frequency per week, duration in minute etc.

3.11 RELIABILITY OF ANTHROPOMETRIC MEASUREMENTS

At the preliminary stage of the present interpretation, intra-observer technical errors of measurements (TEM) were calculated based on replicate measurements on 30 randomly selected subjects. But the results were fell within acceptable ranges when compared with other research (Cameron 1984, Mueller and Martorell 1988, Ulijaszek and Lourie 1994, Ulijaszek and Kerr 1999). Therefore, TEM was not incorporated in further statistical analyses.

3.12 TEST OF NORMALITY

The distribution of all the metric variables (total=30, measured=12 and derived=18) was checked for normality at the initial stage of data analyses. The distributions of most of the variables were normal except a few skinfolds and derived measures. However, to maintain consistency as well as for the simplicity in presentation of results all distributions were assumed to be normal. Therefore, parametric statistics were utilized.

3.13 AGE VARIATIONS IN METRIC VARIABLES

The total sample was classified into eight groups by one-year interval according to subject's chronological age and the age-trend was depicted by the mean values in each age group. Age trends in different anthropometric, adiposity and body composition measures were further presented by distance curve (frequency polygon). Net increase and percent increase from 10 to 17 years of age in all anthropometric measures were also estimated using the following formulae:

$$\text{Net increase} = (\text{Mean value at 17 years} - \text{Mean value at 10 years})$$

$$\text{Percent increase (\%)} = \frac{(\text{Mean value at 17 years} - \text{Mean value at 10 years}) \times 100}{\text{Mean value at 17 years}}$$

3.14 DATA MANAGEMENT AND STATISTICAL ANALYSES

The socio-demographic and anthropometric data were collected on a pre-designed 'Data Record Sheet' and transferred from raw data sheets onto a computer database package. All the entries were double-checked for any possible keyboard mistake. The data file was edited and revised methodically and then transformed into statistical program software.

All statistical analyses were performed using the Statistical Package for Social Sciences, program version 16.0 (SPSS Inc. 1999) and graphics were developed using MS Excel 2007, program version 9.0 (Microsoft Corp. 1999) on a desktop computer. Means, standard deviations (SD) and percentiles (5^{th}, 50^{th} and 95^{th}) were computed for all the metric variables. Necessary statistical tests were performed, as per requirements, following standard manner (Mascie-Taylor 1994a, 1994b; Madrigal 1998, Landau and Everitt 2004). Age variation was tested using ANOVA. Student's t-test was undertaken to check differences in adiposity and body composition characteristics between undernourished and normal samples. All the statistical tests were two-tailed; $p < 0.05$ was considered as statistically significant.