Material and Methods
MATERIALS AND METHODS

SAMPLE

The present study is based on 622 girls of Punjabi parentage, ranging in age from 6-16 years (312 from upper middle socio-economic group (UMSEG) and 310 from lower middle socio-economic group (LMSEG)) (Table 1). Subjects were selected randomly from schools of Chandigarh city and adjoining areas of Punjab. The field work was conducted during the period 1989 to 1993.

ASSESSMENT OF SOCIO-ECONOMIC STATUS

Classification of the two socio-economic groups was done on the basis of family income, per capita income, occupation of the head of the family, educational level of the head of the family and status of school attended by children. Each child's socio-economic status was rated accordingly (Table 2).

ASSESSMENT OF AGE AND AGE GROUPING

Date of birth of each child was noted down from the school records. When in doubt it was confirmed from the child’s parents and if the age was still not ascertained, the child was excluded from the study. All the ages were converted into decimal ages by the method provided by Tanner et al (1966). The subjects from 6 years to 6.9 years were grouped as 6+, those from 7 to 7.9 years as 7+ and so on.

NUTRITIONAL DEFICIENCY SURVEY

A survey of nutritional deficiency signs and diseases based on Jelliffe (1966) was conducted on a sample of 600 girls (300 girls from each socio-
# Table 1: Distribution of Mixed Longitudinal Sample and Percentage Longitudinality at Various Age Groups in Both the Socio-Economic Groups

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>N_h</th>
<th>L_h</th>
<th>C_h</th>
<th>(\lambda)</th>
<th>N_h</th>
<th>L_h</th>
<th>C_h</th>
<th>(\lambda)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMSEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6+</td>
<td>15</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>7+</td>
<td>31</td>
<td>19</td>
<td>12</td>
<td>61</td>
<td>18</td>
<td>10</td>
<td>08</td>
<td>56</td>
</tr>
<tr>
<td>8+</td>
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<td>07</td>
<td>83</td>
</tr>
<tr>
<td>10+</td>
<td>41</td>
<td>27</td>
<td>14</td>
<td>66</td>
<td>49</td>
<td>30</td>
<td>19</td>
<td>61</td>
</tr>
<tr>
<td>11+</td>
<td>35</td>
<td>19</td>
<td>16</td>
<td>54</td>
<td>18</td>
<td>09</td>
<td>09</td>
<td>50</td>
</tr>
<tr>
<td>12+</td>
<td>38</td>
<td>26</td>
<td>12</td>
<td>68</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>13+</td>
<td>40</td>
<td>27</td>
<td>13</td>
<td>68</td>
<td>50</td>
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<td>22</td>
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<tr>
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<td>-</td>
<td>100</td>
<td>36</td>
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<td>05</td>
<td>86</td>
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<tr>
<td>15+</td>
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<td>07</td>
<td>-</td>
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<td>18</td>
<td>18</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>16+</td>
<td>06</td>
<td>06</td>
<td>-</td>
<td>100</td>
<td>18</td>
<td>18</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Total 312

310

\(N_h\): Total number of subjects present at time \(t_h\)

\(L_h\): Total number of subjects present at time \(t_h\) and also present at time \(t_{h-1}\)

\(C_h\): Total number of subjects present at time \(t_h\) and absent at time \(t_{h-1}\)

\(\lambda\): Percentage Longitudinality
### TABLE 2: CLASSIFICATION USED FOR DEFINING THE SOCIO-ECONOMIC STATUS

<table>
<thead>
<tr>
<th>Category</th>
<th>Total family income (p.m.)</th>
<th>Per capita income</th>
<th>Occupation of head of the family</th>
<th>Education of head of the family</th>
<th>Status of School of the children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper middle socio-economic group (UMSEG)</td>
<td>&gt;Rs. 3500-10,000 &gt;Rs. 600-1200</td>
<td>&gt;Rs. 600-1200</td>
<td>a) Govt. Class 1 Officers b) College &amp; University teachers c) Lawyers d) Doctors e) Engineers f) Bank Officers g) Medium business community</td>
<td>Postgraduates &amp; above and/or professional degrees</td>
<td>Public and Private Schools</td>
</tr>
<tr>
<td>Lower middle socio-economic group (LMSEG)</td>
<td>&gt;Rs. 1500-3500 &gt;Rs. 200-600</td>
<td>&gt;Rs. 200-600</td>
<td>a) Office Clerks b) Petty shopkeepers</td>
<td>Matric or Graduation</td>
<td>Govt. Schools</td>
</tr>
</tbody>
</table>
economic group) so as to arrive at the nutritional status of the two groups. 
(The present investigator had undergone a rigorous training in diagnosing 
various deficiency signs in children at the Postgraduate Institute of Medical 
Education and Research, Chandigarh).

**Deficiency signs** (Rapid clinical survey)

The following deficiency signs were examined:

1. Hair : Lack of lustre, dyspigmentation, easy pluckability, 
          sparseness of head hair, flag sign.
2. Eyes  : Bitot’s spot, conjunctival xerosis, pale conjunctiva, 
          corneal xerosis, angular palpebritis, keratomalacia.
3. Face  : Moon-face, diffuse depigmentation, naso-labial 
          dyssebacea.
5. Teeth : Mottled enamel.
7. Tongue: Oedema, scarlet and raw tongue, magenta tongue, 
          atrophic papillae.
8. Glands: Thyroid enlargement (Goitre), parotid enlargement.
9. Skin  : Xerosis, follicular hyperkeratosis (type 1 and 2), 
          petechiae, pellagrous dermatosis, flaky-paint 
          dermatosis, scrotal and vulval dermatosis.
12. Muscular & Skeletal system: Muscular wasting, craniotabes, frontal and parietal skeletal system bossing, epiphyseal enlargement (tender or painless), beading of ribs, persistently open anterior fontanelle, knock knees or bow-legs, diffuse or local pelvic skeletal deformities, deformities of thorax, musculo-skeletal haemorrhages.

**Deficiency diseases** (Interpretation of clinical signs)

The deficiency signs were interpreted and grouped as following deficiency diseases:

1. **Protein - Calorie malnutrition in school children**:
   - Symptoms: a) Diminished subcutaneous fat, b) Muscle wasting, c) Oedema, d) Dyspigmentation of hair, e) Easy pluckability of hair, f) Thin sparse hair, g) Straight hair (pathological), h) Diffuse depigmentation of skin, i) Psychomotor changes, j) Moon-face, k) Flaky-paint dermatosis, l) Parotid enlargement, m) Oedema of ankles.

2. **Vitamin A deficiency**
   - Symptoms: a) Xerosis of skin, b) Follicular hyperkeratosis type-1, c) Bitot’s spots, d) Conjunctival xerosis, e) Corneal xerosis, f) Keratomalacia.

3. **Thiamine deficiency**
4. **Riboflavin deficiency**
   **Symptoms**: a) Angular stomatitis or scars, b) Dyssebacea, c) Angular palpebritis, d) Scrotal or vulval dermatosis, e) Corneal vascularization f) Atrophic lingual papillae, g) Magenta tongue, h) Cheilosis.

5. **Niacin deficiency**
   **Symptoms**: a) Pellagrous dermatosis, b) Scarlet and raw tongue, c) Tongue fissuring, d) Atrophic lingual papillae, e) Malar and supraorbital pigmentation.

6. **Vitamin C deficiency**
   **Symptoms**: a) Petechiae, b) Ecchymoses, c) Follicular hyperkeratosis (type 2), d) Spongy and bleeding gums, e) Epiphyseal enlargement (painfull), f) Intramuscular or subperiosteal haematoma.

7. **Vitamin D deficiency**
   **Symptoms**: (i) Active rickets (in young children)
   a) Epiphyseal enlargement (painless) (over 6 months of age) b) Beading of ribs, c) Persistently open anterior fontanella (after 18 months of age), d) Craniotabes (under 1 year of age), e) Muscular hypotonia.
   (ii) Healed rickets (in older children)
   a) Knock-knees or bow legs, b) Deformities of thorax (Harrison’s sulcus, pigeon chest), c) Frontal or parietal bossing.

8. **Iodine deficiency**
   **Symptoms**: (i) Enlargement of Thyroid gland. (Size assessed by the technique of Perez et al, 1960).
9. Iron deficiency

10. Excess of fluorine
    Symptoms: Mottled dental enamel.

11. Folic acid deficiency
    Symptoms: Pale conjunctiva due to anaemia.

**DIETARY SURVEY (HOUSEHOLD FOOD CONSUMPTION SURVEY)**

Assessment of food and nutrients consumption

A dietary survey was conducted following the method of Jelliffe (1966). 410 households including both socio-economic groups were visited for 7 days. Quantity of foods consumed by way of breakfast, lunch and dinner during this period was recorded by weighing and measuring them in raw state. Also details of family composition in terms of age and sex of individuals were noted down.

The data on the food consumed was converted into different nutrients i.e. protein, calories etc. as calculated from the food consumption tables (NIN, 1989). Nutrient consumption per capita per day was then calculated by the method recommended by ICMR (1966, 1974). This method is based on the total family diet divided in different family members using coefficients where an healthy young adult male was considered as 1.0 consumption unit and other family members as fraction of this unit depending upon their age. In the present study healthy young adult females and adolescent girls (11-16 years) were given the coefficient 0.90 and the
prepubescent (6-10 yrs) as 0.75 and the older people as 0.30-0.50. The individual consumption of vitamins and minerals was calculated on the basis of actual amount of milk, butter, fruits eggs and vegetables consumed by them, because very often the parents provided these items to their children by depriving themselves of these.

The subjects were grouped into broad age groups of 6-9+, 10-13+ and 14-16+ years while presenting their nutrition consumption.

ANTHROPOMETRIC MEASUREMENTS

All the anthropometric measurements were taken on apparently physically and mentally normal subjects and who did not suffer from any major ailment within the last two years of examination.

Each subject was measured for twelve body dimensions (listed below) six times at half yearly intervals within fifteen days of the target date. Any missing data was obtained by linear interpolation according to Patterson (1950).

The following body measurements were taken on each subject:
- Body height (cm)
- Sitting height vertex (cm)
- Biacromial diameter (cm)
- Maximum hip width (cm)
- Chest circumference(cm)
- Upper arm circumference (cm)
- Body weight (kg)
- Biceps skinfold (mm)
- Triceps skinfold (mm)
- Suprailiac skinfold (mm)
- Subscapular skinfold (mm)
- Calf skinfold (mm)

The standard instruments used for the collection of data included Martin’s anthropometer for measuring body height and sitting height vertex, a standard flexible steel tape for measuring the body circumferences, Martin’s rod compass for measuring widths & diameters and Lange’s skinfold calipers for measuring skinfold thicknesses. Weight was measured with the help of a portable spring loaded weighing machine. The precise anatomical landmarks were located by the standard techniques given by Wiener and Lourie (1969).

Skinfolds at various sites were measured in triplicate and the average of 3 readings was taken. The linear measurements were recorded in centimeters, body weight in kilograms and skinfolds in millimeters. Height was recorded to the nearest 0.1 cm and weight to the nearest 0.1 kilogram. All the measurements were taken by the author and recorded herself.

Quality control was maintained during data collection to reduce systematic or random error in the data. The following precautions were taken:

1. Instruments were checked for their accuracy before each session of measurements with the help of a standard verificator.
2. The investigator measured the subjects as well as noted down the values herself without taking any other persons' help to avoid any error.

3. Personal error or measurement error was assessed by taking a measurement on the same child, twice, but at different times and the error was brought to as low as 0.5 percent.

All the linear measurements were taken on the left side of the subjects. While measuring circumferences, the tape was kept in continuous contact with the skin and not pressed too hard to distort the body contour. All well defined landmarks used in the measurements, were first marked on the body before taking the measurements.

**Techniques of various measurements**

**Body height**

The subject was made to stand on a horizontal surface, without shoes, with his heels together in touch with the surface and body stretching upwards to the fullest extent, aided by a gentle traction on the mastoid process. Back was kept straight with the head placed in frankfurt horizontal plane (F-H plane) or eye-ear plane. The anthropometer was placed straight at the back of the subject and the movable horizontal arm was gradually brought in contact with the subject's head in the mid-sagittal plane.

**Sitting height vertex**

The subject was made to sit on a table top, feet hanging down, unsupported over the edge. A gentle push was given to the back to straighten it. The subject was asked not to contract the muscles of the
thigh and buttocks. The head was kept in F-H plane and gentle traction
applied under the chin. The anthropometer was held vertically in contact
with the back, at the sacral and inter-scapular regions and the horizontal bar
of anthropometer was gently brought in contact with the head in the mid-
sagittal plane.

Biacromial diameter

The subject was made to stand with her shoulders relaxed, to give
the maximum shoulder width. Measurement was taken by standing behind
the subject and placing the fixed horizontal arm of the anthropometer on the
external border of one acromian process and the other movable arm was
brought in touch with the opposite acromial external border.

Maximum hip width

The subject was made to stand with his heels together. The
measurement was taken from behind the subject by placing the horizontal
bars of the anthropometer compass at the level of the greater trochanters
on either side.

Chest circumference

A measuring flexible steel tape was used for this measurement. It
was taken at the union of 3rd and 4th sternebrae, at right angles to the long
axis, of the body, at the end of normal expiration.

Upper arm circumference

This was also measured with the help of a flexible steel tape. The
circumference was taken horizontally at the maximum circumference over
the contracted biceps, with the elbow flexed.
Body weight

Weight was taken on a portable spring-loaded weighing scale with the subject wearing very light clothes. Corrections for weight of the clothes were also made. Before using, the scale was corrected by adjusting the machine to read zero whenever required.

Skinfold thicknesses

All skinfolds were picked up between thumb and forefinger and the jaws of the skinfold caliper were applied at the marked level. The measurement was read immediately within 2 seconds after applying the full pressure of the jaws of the caliper. (If a longer interval is allowed the jaws may ‘creep’ and the reading may become inaccurate). The accuracy of skinfold measurements is influenced strongly by the manner of locating and picking up the skinfold (Weiner and Lourie, 1969). Skinfolds were measured to nearest 10th of a mm when exerted a uniform pressure of 10 g/mm² at contact surface area.

Biceps skinfold

This fold was picked up on anterior surface of the upper arm, directly above the center of the cubital fossa at the same level as marked for upper arm circumference.

Triceps skinfold

This was taken at the back of the arm, about 1 cm above the same level as marked for upper arm circumference and directly in line with the point of elbow or olecranon process of ulna.
Suprailiac skinfold

This skinfold was picked up approximately 1 cm above and 2 cm medial to anterior superior iliac spine.

Subscapular skinfold

The measurement was taken just below the angle of the left scapula. The fold was picked up vertically, pointing slightly downwards and outwards.

Calf skinfold

This skinfold was picked up at the level of the maximum circumference of the calf, along the longitudinal axis on the posterior border of the leg.

ASSESSMENT OF SECONDARY SEXUAL DEVELOPMENT

Following sexual characteristics were observed on a subsample of 214 girls (87 in UMSEG and 127 in LMSEG).

a) Development of breasts
b) Growth of pubic hair
c) Age at the onset of Menarche
d) Growth of axillary hair

Sexual maturation was determined by visual assessment of sexual characteristics on the naked bodies or naked photographs of the subjects, wherever possible. The 5 point grading system given by Tanner (1962) was used to describe the stages of breast development and pubic hair growth.

Breast development stages are given below:
Stage 1 (B₁) : Pre-adolescent, elevation of papilla only.
Stage 2 (B₂): Breast bud stage; elevation of breast and papilla as a small mound, enlargement of areolar diameter.

Stage 3 (B₃): Further enlargement of breast and areola, with no separation of their contours.

Stage 4 (B₄): Projection of areola and papilla to form a secondary mound above the level of the breast.

Stage 5 (B₅): Mature stage; projection of papilla only, due to recession of the areola to the general contour of the breast.

Mean breast stage at each age group was calculated by the formula given by Lee et al (1963). The method used for obtaining mean breast stage at each age group was, by first assuming that the intervals between the four stages to be all equal e.g. if 100, 11 year old girls were taken in a study and distribution among them was 31 in B₁, 33 in B₂, 35 in B₃ and 1 in B₄ then mean breast stage was calculated as follows:

\[
\frac{(31 \times 1) + (33 \times 2) + (35 \times 3) + (1 \times 4)}{100} = 2.06
\]

Percentage of girls present at different stages of breast development according to age levels was also calculated.

Pubic hair growth was classified as follows:

Stage 1 (PH₁): Pre-adolescent; the vellus over the pubes is not further developed than that over the anterior abdominal wall i.e. no pubic hair

Stage 2 (PH₂): Sparse growth of long, slightly pigmented, downy hair, straight or only slightly curled, appearing chiefly along the labia.
Stage 3 (PH₃):

Considerably darker, coarser, and more curled hair, spreading sparsely over the junction of the pubes.

Stage 4 (PH₄):

Hair is now adult in type, but the area covered by it is still considerably smaller than in most adults. There is no spread to the medial surface of the thighs.

Stage 5 (PH₅):

Hair is adult in quantity and type, distributed as an inverse triangle of the classically 'feminine' pattern.

Incidences of girls present at different stages of pubic hair development, was calculated age-wise.

**Age at menarche** was obtained by recall method. Individually each girl was questioned at three monthly visit whether or not she had begun her menstrual cycle. In case where exact date of first menstrual period was not available the period of onset was related to any major event or social function in the family or any festival or school examination or school vacations so that an approximation of plus minus a week or ten days was mediated and the middle of this period was taken as the time of onset of menarche. Mean ages of menarche for the two socio-economic groups were estimated by logit and probit analysis. Frequency of girls who had menstruated or not, at a particular age group, was also calculated.

Growth of axillary hair was recorded as present or absent without attempting a division into stages.
METHOD OF ANALYSIS OF MIXED LONGITUDINAL DATA

The statistical procedure used for analysing the present data is same as provided by Tanner (1951a) which utilised both crossectional and longitudinal elements present in the series so as to derive maximum information from it. The notations used in the analysis and their descriptions are given below:

- \( N_h \) - Total number of subjects present at time \( t_h \)
- \( L_h \) - Total number of subjects present at time \( t_h \) who were also present at time \( t_{h-1} \)
- \( C_h \) - Total number of subjects present at time \( t_h \) but not present at time \( t_{h-1} \)
- \( m_h \) - Mean of \( N_h \) (crossectional mean)
- \( V(m_h) \) - Variance of \( m_h \)
- \( I_h \) - Mean of \( L_h \)
- \( V(I_h) \) - Variance of \( I_h \)
- \( \lambda \) - Percentage longitudinally from time \( t_1 \) to \( t_2 \) i.e. proportion of children present at time \( t_2 \) who were also present at \( t_1 \)
- \( M_h \) - Best estimate of population mean at time \( t_h \) taking into account all the years prior to \( t_h \)
Several ways of estimating sample means have been given above \((m_h, l_h, C_h, M_h)\), but, only best estimate of population mean \((h+1M_h)\) and crossectional mean \((M_h)\) and variances of these means \(V(h+1M_h)\) and \(V(M_h)\) have been calculated. The best estimate of annual mean increment \((h+1M_h - lM_h)\) is calculated. Regression and correlation-coefficients have also been obtained. Percentage of longitudinally \(\%\) has been presented in order to show the degree of longitudinal and cross-sectionality of the sample. The above results have been given for all the anthropometric parameters according to age groups and socio-economic status.
The skinfold measurements were transformed into logs to overcome the non-gaussian frequency distribution so that the distributions approximate normality. Their mean values are presented in log values as well as in millimeters in the tables and only as log values in graphs. While the mean annual increments are given only in mm in both tables and graphs. The data was analysed at the Computer Center, Panjab University, Chandigarh.