Subjects and Methods
3. SUBJECTS AND METHODS

The aim of the study was to focus on the nutritional profiles of rural women belonging to extreme grades of CED, obesity and normal groups, existing in similar socio-cultural and environmental conditions. The study also intends to examine the relative value of BMI in particular and other selected indicators of nutritional status in the establishment of the malnourished conditions.

To achieve the aim and objectives of the study the tools and methods used are detailed in this section.

3.1. Selection of the study area:

Chittoor District comprises of rural areas that have striking socio-cultural and economic differences. Therefore, to minimize the socio-cultural variations, the Chandragiri mandal, Chittoor District of Andhra Pradesh state South India was purposively chosen for the present investigation. Further, laboratory investigations were part of the present research project and hence, proximity to the researchers operational area i.e. S.V. University, Tirupati, was also one of the aspects considered in the selection of the study area.

3.2. About the study area:

The villages in Chandragiri mandal of Chittoor District are 15-20 kilometers away from the Chandragiri town, which is a semi urban area and 10 - 40 km away from Tirupati, which is the nearest urban area. It was observed that the proximity to these two urban areas does influence the lifestyle of the inhabitants, in the socio-cultural and economic contexts. Though, the main occupation of a majority was agriculture, the crop pattern reflected the needs of the urban areas. Further, a majority of the high-income group families had business as another important occupation contributing significantly to the total family income. Each habitat was composed of a main village and small hamlets, where only scheduled caste families were the inhabitants. For a cluster of villages there was one hamlet inhabited by only scheduled tribes.
All villages had the facilities provided by the government such as electricity, roads and transport, protected water supply and primary schools. The total number of villages was 23 in the southeast, southwest, northeast and northwest areas of Chandragiri mandal. From among them six villages approximately covering 1/4 - 1/5th of the total number were selected randomly for the present study.

3.3. Selection of sample:

A household survey covering hundred percent of the households was conducted in the selected villages to identify apparently normal non-pregnant and non-lactating women in the age group of 18-50 years. The distribution of women according to their standard of living is given below--

<table>
<thead>
<tr>
<th>Standard of Living</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low standard of living (LSL)</td>
<td>310</td>
</tr>
<tr>
<td>Medium standard of living (MSL)</td>
<td>290</td>
</tr>
<tr>
<td>High standard of living (HSL)</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>629</td>
</tr>
</tbody>
</table>

It was observed that several research works done in the rural areas that the lifestyle of the women belonging to the HSL groups were significantly different from those of MSL and LSL groups of women. The HSL women were not directly involved in the forming activities and a majority had only very sedentary life style. Whereas, the MSL and LSL groups of women were directly involved in the different forming activities and were performing moderate to heavy categories of physical work and their life styles were observed to be more or less similar. Therefore, to minimize the variation in the group due to differences in the physical activity and food intake patterns the HSL group was purposively omitted and the households having 18-50 year old women from the MSL and LSL groups were only chosen for the present study.

Thus, the total number of women eligible for the present study was 600 distributed as 310 in the LSL and 290 in the MSL group. These women were further stratified into two groups on the basis of age as 18-30 and 30-50 years. The height and weight of all the 600 women were measured and the Body Mass Index was calculated. Based on this parameter the women in each SL and age group were divided into different categories of nutritional states as CED, Normal and Obese groups using the
BMI cut-off points proposed by James et al., (1988). Later from the above grades three groups of women belonging to extreme nutritional states viz., a) CED Grade III b) Obesity Grade II and c) Normal representing the plane of nutrition were selected. The sample size in each group was 40.

Biochemical investigations were part of the present study. All biochemical studies based on human beings require the cooperation of the subjects and also completion within a specified time period to have better comparison of the parameters. Hence, the sample size advocated is usually a small sample (n = 6 to 10). Further, all the investigations were planned to be conducted by the researcher herself within the specified time. Therefore, a sample amenable for relevant statistical treatments and drawing meaningful conclusions was aimed at. Care was taken to have the minimum required sample. With the sample size chosen, it was possible for the researcher to assess the wide range of parameters within a stipulated time. This resulted in minimizing the variations in food habits and physical activity patterns that may otherwise creep in when large samples were chosen and time period is extended, leading to changes in the seasons. The sample size is set as 40 in the three nutritional states chosen, as in grade II obese group the total number of women were only 41 (Refer table 8 and fig. 3 Experimental plan).

3.4. Selection of variables:

In the present context the rural women were categorized into differing plane of nutritional status using BMI. The major objectives of the project being a study of nutritional profiles of women in different plane of nutrition, a wide variety of nutritional status parameters were used. While focusing on the relative value of BMI, which is a secondary objective of the study, BMI is considered as a dependent variable (response variable) and all other parameters of nutritional status and age and income were treated as independent (influencing) variables.
Table 8: Distribution of the Rural Women Subjects into Different Nutritional States

Based on Body Mass Index

<table>
<thead>
<tr>
<th>BMI Classification</th>
<th>Nutritional Grade</th>
<th>LSL</th>
<th>MSL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 – 30</td>
<td>30 – 50</td>
<td></td>
<td>18 – 30</td>
</tr>
<tr>
<td>Chronic Energy Deficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 16 Grade - III</td>
<td>Severe</td>
<td>18</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>16 – 17 Grade - II</td>
<td>Moderate</td>
<td>13</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>17 – 18.5 Grade - I</td>
<td>Mild</td>
<td>22</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5 – 20.0 Low weight</td>
<td>Normal</td>
<td>24</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>20.0 – 25.0</td>
<td>Normal</td>
<td>41</td>
<td>68</td>
<td>109</td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0 – 30.0 Grade - I</td>
<td>Overweight</td>
<td>12</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>&gt; 30.0 Grade – II</td>
<td>Obese</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>140</td>
<td>170</td>
<td>310</td>
</tr>
</tbody>
</table>
Fig 3. PLAN OF THE STUDY
3.5. Tools for collection of data:

Complete data collection was done by the investigator only. However, the help of educated local residents in close proximity to the subjects of the study and educated subjects themselves was obtained in recording of the food intake data and the physical activities. Relevant schedules were prepared and the data was collected through personal interviews. As the investigator hails from the study region and familiar with the language used by the rural women, she did not encounter any problems while collecting information.

A general information survey schedule was administered to collect data on age and standard of living of the subject (schedule I). A nutrition survey schedule was used to collect information related to different nutritional status parameters such as anthropometry (schedule II) and diet survey (schedule III) and physical activity assessment (schedule IV). Refer appendix i for the details of each schedule.

3.6. Assessment of the variables:

The variables considered in the present study were age, income and nutritional status parameters, which include select physical, physiological and metabolic/biochemical variables. The techniques and procedures used for the assessment of these variables are presented here.

3.6.1. Assessment of Age:

Age of the women was elicited from the subjects themselves and was further confirmed from the primary and secondary sources of information available at the panchayat and mandal offices. Age was recorded in complete years.

The equation proposed by FAO/WHO/UNU(1985) and recommended by ICMR(1995) for calculation of BMR of Indians proposed the age categories as 18-30 and 30-60. As the present study intended to focus on BMR of the subjects the above mentioned age grouping was followed.

The earlier observations through research work done in the rural areas of Chittoor district reveal that 18-30 year women group is a fertile age group and only very meager percent of women above 30 years are observed to be in the physiological states of pregnancy and lactation. Further, wherever necessary while calculating BMR
and EE through physical activity assessment correction for body weight was carried out. Hence, with the intention to have homogeneity in the physical and physiological characteristics of the group the above age grouping of subjects was done in the present study.

3.6.2 Assessment of standard of living:

The summary household measure called the standard of living index (SLI) used in the National Family Health Survey- II (2000) was used in the present study. The SLI is calculated by adding the scores obtained for type of house, toilet facility, source of lighting, main fuel for cooking, source of drinking water, separate room for cooking, ownership of house, agricultural land, irrigated land, live stock and durable goods (Refer schedule I appendix I). Based on the total index scores the households were classified into three SLI categories as follows (table 9).

<table>
<thead>
<tr>
<th>Range of Index score</th>
<th>SLI category</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>Low</td>
</tr>
<tr>
<td>15-24</td>
<td>Medium</td>
</tr>
<tr>
<td>25-27</td>
<td>High</td>
</tr>
</tbody>
</table>

The women belonging to low and medium SLI categories were chosen in the present study. The research conducted will be of value when it caters to the needs of the community. It is well known that while nutritional deprivation and its consequences are prevalent mostly among the low and middle SL groups, the observations made on these groups reveal that the risk of obesity also is on the increase.

3.6.3. Anthropometric measurements and techniques:

It has been shown that nutritional anthropometry is a simple and reliable index of nutritional status. The somatic measurements chosen were height, weight, skinfold measurements and mid upper arm, waist and hip circumferences.
**Height:**

A standard vertical measuring rod called stadiometer that contained height measurements in centimeters was used. After removing the slippers the subjects were made to stand on a flat platform with the back touching the measuring rod and the heels touching each others, on the horizontal surface and with head, buttocks, shoulders and back of head held upright. The counter weighted board of the stadiometer is brought down till it touches gently to head. The subject was requested to come out without disturbing the headpiece. It was held in the same position and the weight was recorded to an accuracy of 0.1 cm.

**Weight:**

It is the simplest anthropometric measurement with least individual error. Amongst all other measurements, body weight is probably the best index of nutrition. A lever type balance with a platform was used for the estimation of weight, without slippers, with minimum clothing. The subjects were made to stand on the platform without touching anything else. The measurement was noted to the nearest 0.5 kg. Before starting each days work the balance was checked for zero error.

For the measurements of both height and weight, all precautions outlined by Jelliffe (1966) in his monograph were strictly followed.

**Skinfold thickness measurements:**

Most of the fat stored in the body lies under the skin (Edward, 1950). Thickness of a fat fold picked up at strategic sites, indicates the amount of subcutaneous fat (Montoye, 1965). Skinfold measurements are used in field circumstances to focus on the amount and distribution of subcutaneous fat and hence of calorie reserves. Despite the fact that increase or depletion of subcutaneous fat stores in not uniform all over the body the essence of the problem is to select one or two easily accessible sites that may be expected to give an approximate practical indication of calorie reserves. For this purpose for those with poor calorie stores and in obesity the triceps skinfold is the most practical measurement for all age groups.

The skinfolds measured consist of a double layer of skin and subcutaneous fat. The most appropriate “pinch” sites depend on the purpose of the study. In the present
study along with triceps skinfold three other skinfolds viz., biceps, subscapular and suprailliac were also measured as the study intends to focus on body fat in relation to different grades of nutrition.

The skinfold measurements were measured with standard skinfold calipers the ‘Harpenden calipers’ (Holtain Limited, UK). All the skinfolds were measured on the left side of the body. The SFT was measured thrice at each site and average value was taken as the final skinfold measurement. The measurements were made to nearest 0.2 mm.

Biceps:

Biceps skinfold is measured as thickness of a vertical fold in the front of the upper left arm, directly above the center of the cubital fossa at the same level as the triceps skin fold (Weiner and Lourie, 1969).

The measurement was taken approximately over the centre of the biceps of the muscle of the left upper arm. The arm of the subject was in a relaxed state and loosely hung. The skinfold was lifted about a cm below the mid point along the long areas of the muscles. The caliper, in a horizontal position was allowed to compress the skinfold about the point where the thumb and finger grasped the skinfolds.

Triceps skinfold:

Triceps skinfold is measured at the mid point of the back of the upper left arm (Weiner and Lourie, 1969).

As the fat deposition in the upper arm is not uniform in thickness, the site selected was the left mid upper arm between the tip of acromial process the scapula and the olecranon process of the ulna. The measurement was made with elbow slightly flexed and the site on the triceps was marked. The thickness of the fat fold was measured with the hand hanging freely at the side. The fat fold thickness was noted to the nearest 0.2 mm.

Subscapular skinfold:

Subscapular skinfold is measured just below and laterally to the angle of the left shoulder blade, with the shoulder and left arm relaxed. Placing the subjects arm behind the back may assist in the identification of the site. Skinfold is grasped at the marked
site with the fingers or top thumb below, and forefinger on the site at the lower tip of the scapula. The skin fold should angle 45° from horizontal, in the same direction as the inner border of the scapula medially upward and laterally downward according to Jette 1981 and Lohman et al., 1988.

Supra-iliac skinfold:

The skinfold is lifted just above the crest of the ilium. The fold is lifted to follow the natural diagonal line at this point (dorsally upward). Suprailiac skin fold is measured in the mid auxiliary line immediately superior to the iliac crest. The skin fold is picked up obliquely just posterior to the mid auxiliary line and parallel to the cleavage lines of the skin (Lohman, et al., 1988).

Mid upper arm circumference:

Poor muscle development or muscle wasting are a cardinal feature of all forms of protein calorie malnutrition. In older children and adults muscle mass is also related to general exercise and special increased use of certain muscle groups. Both mid upper arm circumference (MUAC) and calf circumference are recognized to indicate the status of muscle development. The mid upper arm is considered more feasible as it is simpler and easily accessible in any age and sex and so is practical to measure.

The mid-upper arm circumference is taken on the left hand. The mid-point between the tip of the acromion of scapula and tip of the olecranon of the forearm bone, ulna, is located with the arm flexed at the elbow and marked with a marker pen. The arm is allowed to hang freely and fiberglass tape is gently, but firmly placed embracing the arm without exerting too much pressure on the soft tissues. The reading is taken to the nearest millimeter with the tape still in position.

Waist and Hip circumferences:

The waist circumference was measured mid-way between the iliac crest and the lower most margins of the ribs. The hip circumference was measured at the maximum circumference of the buttocks, the subjects standing with feet placed together. Waist hip circumferences were measured with a measuring tape to the nearest 0.1cm. The readings of each circumference were taken for the calculation of the waist hip ratio.
3.6.4. Assessment of body composition:

Many diseases and disorders are accompanied by changes or abnormalities in body composition. Energy and protein malnutrition cause a decrease in the amount of fat and protein stores in the body, and many diseases are related to abnormalities in total body water or to its distribution among intra and extra cellular space (Forbes 1987, and Moore, et al., 1963). The most common problem may be obesity in which the excessive body fat probably causes abnormalities in lipid and carbohydrate metabolism, high blood pressure and different forms of cancer (Seidell, Deurenberg and Hautvast, 1987). There are direct, indirect and doubly indirect methods available to determine body composition. The determination of body composition using anthropometry is classified as doubly indirect method.

3.6.4.1. Weight/ height indexes – The body mass index (BMI):

As a measure of body composition, in fact body fat, a weight / height index has to have both high correlation with the amount of body fat, as well as a low correlation with body height, or else in short and tall people body composition would be systematically over or under estimated.

The most frequently used index today is the Quetlet or body mass index (BMI). The correlation of body mass index with body fat is relatively high (ranging from 0.6 to 0.8, depending on age) and the correlation with body height is generally low (Khosla and Lowe, 1967, Keys et al., 1972, Womersley and Durnin, 1977; Garrow and Webster, 1985, Deurenberg, Westrate and Seidell, 1991).

For predicting body composition in the general population e.g., in epidemiological studies this method is as good as other methods, which also have their limitations. Several studies have been published in which a good relationship between the Quetlet index and the amount of body fat were demonstrated, provided that the age-sex-specific prediction equations are used with such age-and-sex-specific prediction equations. The percentage of body fat can be predicted with an error of 3-5% (Womersley and Durnin, 1977; Deurenberg, Westrate and Seidell, 1991; Norgan and Ferro-Luzzi, 1982).
The BMI is a simple but objective anthropometric indicator of the nutritional status of the adult population and seems to be closely related to their food consumption level. It is relatively inexpensive, easy to collect and to analyse. The BMI is sensitive to socio-economic status and to seasonal fluctuations in food consumption relative to the level of physical activity.

Body mass index was calculated from weight (kg) and height (cm). It was calculated from the equation \( \text{wt in (kg)} / \text{ht(m)}^2 \). Khosla (1967) explained that this index gives a measure of weight for height that is highly independent of actual height (FAO/WHO / UNU, 1985). The BMI provides an estimate of present nutritional status. The justification for using this index is two fold and except in the very young and elderly, height appears to have little effect on energy requirements independently of its relation to weight. This index can also be used to assess the magnitude of potential health risks associated with chronic energy deficiency (underweight) or over-weight and as a guide to therapy.

In the present context the BMI was calculated using the formula \( \text{wt/ht}^2 \). The following cut-off points (table 10) were used to classify the subjects into different nutritional states.

<table>
<thead>
<tr>
<th>BMI Class</th>
<th>Presumptive diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 16.0</td>
<td>CED - Grade III severe</td>
</tr>
<tr>
<td>16.0-17.0</td>
<td>CED - Grade II moderate</td>
</tr>
<tr>
<td>17.0-18.5</td>
<td>CED - Grade I mild</td>
</tr>
<tr>
<td>18.5-20.0</td>
<td>Low weight normal</td>
</tr>
<tr>
<td>20.0-25.0</td>
<td>Normal</td>
</tr>
<tr>
<td>25.0-30.0</td>
<td>Obese grade I</td>
</tr>
<tr>
<td>&gt; 30.0</td>
<td>Obese grade II</td>
</tr>
</tbody>
</table>

Table 10: Cut-off points of BMI


3.6.4.2 Assessment of body fat using skinfold thickness measurement:

Body fat is located both internally and subcutaneously. There is a constant relationship between subcutaneous fat and total body fat. Total body fat can be
estimated by measuring the amount the subcutaneous adipose tissue. The amount of subcutaneous fat can be estimated by measuring the thickness of the subcutaneous fat layer at different sites of the body with a skinfold caliper (Durnin and Womersley, 1974). The relationship between subcutaneous fat and total fat is found to be relatively constant. It differs, however, between the two sexes (Lehman, 1981, Durnin and Womersley 1974). In adults the most frequently used formulas are those of Durnin and Womersley (1974) and Jackson and Pollock, (1978).

In the present study the sum of four SFTs viz. Triceps, Biceps, Subscapular and suprailliac was used to calculate body density and body fat and other indices following the formulas and reference values proposed by Durnin and Womersley (1974).

**Body density:**

The measurement of body density as an index of obesity was pioneered by Behnke (1942). This was later developed by Durnin and Rahmain (1967). Behnke suggested that the human body consisted of a lean body mass (LBM) of fixed density and a variable amount of fat, the fat could be quantitatively assessed by measuring body density. The body density can be calculated with the help of age and sex matched regression equation (Durnin and Womersley, 1974).

\[ \text{Body density} = c - (m \times \log \text{sum of SFTs}) \]

Where the values of \( c \) and \( m \) were taken from the tables of linear regression equations for the estimation of body density [table 11].

**Table 11: Age and specific matched regression equation for the calculation of body density**

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘c’</td>
</tr>
<tr>
<td>20-29</td>
<td>1.1599</td>
</tr>
<tr>
<td>30-39</td>
<td>1.1423</td>
</tr>
<tr>
<td>40-49</td>
<td>1.1333</td>
</tr>
<tr>
<td>50+</td>
<td>1.1339</td>
</tr>
</tbody>
</table>

Source: Durnin and Womersley, 1974
Body fat:

Body fat is calculated as percent body fat and later computed to body fat in kg.

Body fat percent:

Although the densitometric method has been used as the most accurate method of determining percent body fat, the formulae that translate body density to percent body fat assume a constant value for the density of lean tissue for all individuals.

Calculations for the percent body fat were based on equation given by Siri (1956).

\[ \text{Fat percent} = \left( \frac{4.451}{\text{Density}} - 4.5 \right) \times 100 \]

Body fat (kg):

The amount of fat in kg present in the body was calculated from the body fat percent. The formula used to calculate body fat in kg is:

\[ \text{Body fat (kg)} = \text{body weight (kg)} \times \text{fat \% / 100} \]

3.6.4.3. Assessment of lean body mass:

The lean body mass is composed of approximately 72% water, 20% protein, 7% minerals and 1% carbohydrate. The variability is less compared to body fat. Neutral fat does not bind water or electrolytes. Consequently, the measurement of total body water or total body potassium offers a means for estimating non-fat (kg) worked out earlier. The Durnin and Rahaman (1967) formula was used for the calculation of LBM.

\[ \text{LBM (kg)} = \text{Body wt (kg)} - \text{Body fat (kg)} \]

Assessment of muscle mass using arm muscle circumference and arm muscle area:

Mid upper arm muscle circumference:

Mid upper arm muscle circumference (AMC) can be used to assess total body muscle mass, and is frequently used for this purpose in field surveys. It is also used in hospitals to assess protein-energy malnutrition, as the size of the muscle mass is an index of protein reserves. Mid-upper arm muscle circumference is calculated using the following equation (Jelliffe, 1966).

\[ \text{AMC} = \text{MUAC} - (\pi \times \text{TSK}) \]
Where MUAC = mid upper arm circumference and TSK = triceps skinfold thickness.
This equation is only valid when all measurements are in the same units. Therefore, the
above measurements were recorded/converted to millimeters.

*Mid upper arm muscle area:* 

Mid upper arm muscle area (AMA) is preferable to mid upper arm muscle
circumference as an index of total body muscle tissue changes. Hence, along with AMC
this measurement was also included in the present study. The following equation was
used to estimate mid upper arm muscle area.

\[ AMA = \frac{(C - (\pi \times TSK))^2}{4 \pi} \]

Where C = mid upper arm circumference and TSK = triceps skinfold thickness
(Frisancho, 1990). Consistent units of measurement, millimeters were used throughout.
Arm muscle area was corrected for bone area by subtracting 6.5 cm² from calculated
arm muscle area (Frisancho, 1990).

3.6.4.4. Waist-hip-ratio:

The waist hip circumference ratio is a simple method for describing the
distribution of both subcutaneous and intra-abdominal adipose tissue (Larsson et al.,
1984; Jones et al., 1980). The reading of waist and hip circumferences were taken for
the calculation of waist to hip ratio. Willet et al., (1999) recommended a WHR >0.8 as
indicative of abdominal/central obesity.

3.7. Assessment of basal metabolic rate (BMR):

BMR which constitute nearly half or more of the total energy expenditure can
be defined as the rate of energy expenditure generally measured by indirect calorimetry
in the postabsorptive state under highly standardized conditions, i.e., at complete
physical rest lying down in thermoneutral state, 12-14 hours after the last meal, ½ hour
mandatory rest shortly after being woken up and without the presence of any disease or
fever.

Considerable data on BMR of Indians collected over several decades are
available (Banerjee, 1962; Patwardhan, 1958). These values were used to compute
energy requirements during sleep (Patwardhan, 1960). Recently, the FAO/WHO/UNU
expert consultation group (WHO, 1985) has provided equations for predicting the BMR from body weights based on world wide data on BMR which is applicable to different population groups. A comparison of BMR computed from FAO/WHO/UNU equation with actual measured BMR in a large number of well nourished Indians (Shetty, 1986) has indicated that the actual measured BMR of Indians is 5% lower than that predicted by the FAO/WHO/UNU equations proposed for international use. BMR of Indians can thus be computed using the equation of FAO/WHO/UNU but after lowering the values by 5%.

The modified equations applicable to Indians as recommended by the ICMR committee are given in table 12. For the women in the age group of 18-50yrs using these equations the BMR was calculated.

**Table 12: Equations for predicting BMR (k.cal/24 hours)**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age in yrs</th>
<th>Prediction equation</th>
<th>Proposed by FAO/WHO/UNU</th>
<th>Proposed by ICMR expert group for Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>18-30</td>
<td>14.7*B.W (kg)+496</td>
<td>14.0*B.W (kg)+471</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>8.7*B.W (kg)+829</td>
<td>8.3*B.W (kg)+788</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>10.5*B.W (kg)+596</td>
<td>10.0*B.W (kg)+565</td>
<td></td>
</tr>
</tbody>
</table>

3.8. Diet survey:

Diet surveys are essential part of all nutrition surveys. They provide useful information to interpret the existing nutritional situations in any community.

To elicit information pertaining to the quality and quantity of diets consumed by the rural women subjects a schedule (Schedule III) was formulated. The diet survey was conducted for 3 days in a week, selecting two week days and one weekend day. The data was collected in the combination of 24 hour recall and weighment method. The researcher herself measured the cooked food consumed by the subjects in randomized meal sessions, so that for each subject during the three day period all the meal sessions that would occur in a day were covered by the researcher.

92
Prior to the investigation the subjects were provided with a set of standardized cups and the use was demonstrated. The subjects were instructed to record the amount of food consumed by them at all the meals, which were not attended by the researcher. Both the subjects and the investigator measured the food using the standardized cups. Illiterate women were attached to literate subjects of the study or other members in the family or neighbours to record the dietary intake information.

The data collected on the cooked foods was converted to raw foods. Later the nutrient intake of the subjects was calculated using the nutritive value of Indian foods (ICMR, 1996).

3.9. Assessment of physical activity and energy expenditure pattern of the subjects:

Several methods of assessment of energy expenditure in free-living human populations covering periods extending from one day to several days are in vogue. Self-recording of various types of activities by the subject for 1440 min in a day in a record booklet is one of the popular methods used in the developed countries. Keeping in view the educational level and the nature of Indian subjects Satyanarayana et al., (1988) proposed a method suitable to rural women groups. In the present context this method was followed with the following modifications.

(i) The physical activity was assessed part by self-recording and recall and part by observation by the researcher.

(ii) Time spent for each activity was noted ignoring description related to intensity of the activity.

The subjects were explained regarding the importance of the information on physical activity. A schedule was prepared to enter the data on physical activity (Schedule IV). The schedule was divided into 96 periods of 15 min intervals in each day. The various physical activities were grouped into 9 activity zones (Appendix ii). The subject was familiarized with this grouping and the respective activity codes. Later they were asked to enter the corresponding activity code in the 15-min blocks from the time they awaken in the morning till they go to bed at night. Illiterate women were assisted by others to record the information. For each subject for a period of 18 hours
the researcher herself observed the activities at randomized sessions of 6 hour duration. The remaining 12 wake hours (approximately) were either recalled or recorded by the subjects themselves.

The physical activity of the subjects was obtained for a period of three days viz., two week days and one weekend day to cover the variations that may exist in the activity pattern of rural women. The physical activity assessment was done on the same days when diet survey was conducted to facilitate the calculation of energy balance of subjects.

The activity cost of energy is given for 60 kg person (Bouchard et al., 1983). Therefore, a correction for body weight was made for the energy cost of each activity zone. The correction was obtained by dividing the subjects actual weight by 60. This factor was multiplied with the energy cost of activity.

After recording the data the total time spent by the subject under each activity zone was obtained. This was multiplied with the energy cost of that category of activity to obtain the energy expenditure. The sum of the energy expended for each category of activity is the energy expended by the subject for a period of 24 hrs. In the present study the mean of energy expenditure of three days is presented as the subjects energy expenditure per day.

3.9.1 Energy balance:

This is an important indicator, which can explain the nutritional state and also can throw light on the existing body composition of a subject. In the present study the energy intake and energy expenditure data is collected for a period of 3 days in a week. For each day the energy balance is computed by subtracting the energy expenditure from energy intake. Later the mean of 3 days is presented as the energy balance (E) of the subject.

3.10. Bio chemical estimation:

In the development of any deficiency disease, biochemical changes can be expected to occur prior to clinical manifestations; therefore, bio chemical tests that can be conducted on easily accessible body fluids such as blood and urine can help to diagnose disease at the sub clinical stage. However, biochemical machinery being
complex, no test can fulfill all these criteria. While applying a test, one should be fully familiar with its limitations, particularly, the specificity and sensitivity.

The biochemical estimations chosen in the present investigation were haemoglobin, cholesterol and triglycerides.

3.10.1 Estimation of haemoglobin:

Anaemia is recognized as public health problem in India. Irrespective of age, sex and economic status a majority of population were shown to suffer from anaemia. Further, haemoglobin is used as a parameter to focus on the general nutritional situation in any community. Therefore in the present context Hb is included to examine its status in relation to differing nutritional states.

For the estimation of haemoglobin a pinprick was made on the tip of the finger of the subject. Using Lambda pipette 20μl of blood was collected. It was transferred into a coded whatman No.1 filter paper containing circles of 1cm diameter. While transferring the blood on to the area inside the circle, care was taken to avoid vigorous blowing which may result in bubble formation and loss of blood through splattering of the bubbles.

The cyanmethaemoglobin (Crossby Munn and Furth, 1954) method was employed to assess the haemoglobin content. The collection of blood samples was done following the field oriented techniques as given in the laboratory manual published by NIN (1983).

3.10.2 Estimation of triglycerides and cholesterol:

In the present context to focus on the metabolism of fat in two extreme conditions of malnutrition viz. CED and obesity, triglycerides and cholesterol were chosen as indicators.

The subjects were intimated about the exact date and time of the collection of blood samples. The sample was collected in a fasting state in the early hour of the day between (6:30 to 7:30 am). Sterile syringes and disposable needles were used to draw the blood samples with assistance of a laboratory technician. About 5ml of venous blood was drawn from the subject for the estimation of triglycerides and cholesterol. The blood samples were transferred into appropriately labeled, sterile glass vials and
transferred into the coded container and preserved in containers with ice packing. Twenty to thirty samples were collected at any one time. The approximate transit time between collection and reaching the laboratory for further treatment of sample and storage was around 2 hours.

**Serum preparation:**

The collected blood samples were allowed to stand at room temperature for 2-3 hours. After the clot was formed and the serum separated, the serum was transferred into centrifuge tubes and was centrifuged for twenty minutes at 3000 rpm. The clear serum was then transferred into clean dry labeled tubes. The serum was stored under refrigeration for the subsequent estimation of cholesterol and triglycerides. All the estimations were carried out within 24-48 hours after separating the serum.

Serum triglyceride levels were estimated by the method of Foster and Dunn (1973). The estimation of cholesterol in the serum was assayed by the method of Parekh and Jung (1970).

3.11 Clinical examination survey:

Clinical examination has always been and remains an important practical method for assessing the nutritional status of a community. Essentially the method is based on examination for changes, believed to be related to inadequate nutrition, that can be seen or felt in superficial epithelial tissues, especially skin, eyes, hair and buccal mucosa, or in organs near the surface of the body, such as the parotids and thyroid glands.

The clinical signs were observed and noted as per the guidelines provided by Jelliffe (1966). The checklist on clinical symptoms grouped according to the nutritional deficiency is used (Appendix iii). The results are expressed as percentage of the subjects showing the deficiency symptoms.

3.12 Statistical analysis:

- Means and standard deviations were calculated for all groups and for all the parameters chosen.
- The variation between groups for different parameters was analyzed through ANOVA.
• 't' test was conducted to know the significance of the difference between groups for all the parameters.

• To know the association between BMI and other parameters within the group and all groups combined correlation analysis was done.

• Stepwise multiple regression analysis was conducted taking all the parameters of nutritional status as independent variables and BMI as dependent variable. This was done within the different BMI group and for all groups combined.

• Statistical distributions were used wherever necessary.

3.13 Interpretation of the data:

• Since the women belong to three different stages of nutrition, for all parameters comparison was done across the groups.

• The mean values of all parameters were compared with the Indian standards, international standards and available research data.

• Though RDA are to be used as guidelines to show the relative differences between groups the group means are compared with RDA to present the nutrient intake profile of each nutritional state.

• As the purpose of the study is to focus on the value of BMI all parameters were discussed in terms of probability using distribution charts.