CHAPTER III

SUBJECTS AND METHODS
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A striking change in the developing countries, including India, has been the rapid growth of urban affluent societies accompanied with an increased adoption of the western pattern of diet and lifestyle. Globalization of food markets has resulted in an advent of high energy foods rich in saturated fats, refined foods, animal products, sugar-rich carbonated beverages and alcohol. Consequently, the food basket of urban Indian households has changed from the traditional complex carbohydrates and protein foods to those rich in fats and sugar. With increased automation, there has been a decrease in the physical activity in the already sedentary lifestyles. As a result, these populations have become increasingly vulnerable to developing obesity, especially abdominal obesity and associated Noncommunicable Diseases (NCDs) (Gopalan, 1998). The present study was therefore designed to study obesity, abdominal obesity and NCD risks in association with the diet and activity patterns among urban affluent adults living in Pune city, Maharashtra, India.

3.1 Study design:

Cross-sectional epidemiological studies, also known as “prevalence surveys” provide information on the frequency of disease in a population. Often the population is defined geographically, but it may also be defined by employment in a company or participation in a socio-cultural activity for instance. In a cross-sectional study, the epidemiologist randomly selects a sample to determine the frequency of the disease of interest in that population, as well as factors that may be associated with the presence or absence of the disease (Lilienfield and Stolley, 1994). The present study also adopted a cross-sectional design. Data was collected as a part of an ongoing project undertaken in the Biometry and Nutrition Unit at Agharkar Research Institute, Pune. The study protocol was approved ethically by the Institutional Committee.

Study population: Social life in Pune is dominated by socio-cultural organizations like Rotary Club and Lions Club which are established with the purpose of community service. Members of these clubs represent urban higher income class of the population and have good educational and professional background. People from diverse professions like doctors, architects, builders, lawyers, businessmen, I.T. professionals and so on come together through such clubs to devote their time, effort
and money to socially relevant causes. These clubs were approached for enrollment of study subjects. It was planned that the surveys would be conducted as ‘Obesity Assessment Camps’ for these clubs. Contact details of the club presidents were obtained and they were approached through telephone calls, emails and personal meetings. Information brochures were developed for explaining the purpose of the activity. Several clubs were approached in this manner. After a persistent follow-up, few of the clubs agreed for the activity.

Prior to organizing the assessment survey, a lecture was arranged for the club members, usually during their weekly club meetings. Issues such as obesity, its causes and consequences, role of diet and exercise in its prevention and general guidelines for a healthy lifestyle were discussed. The aim was to create health awareness among people and thus ensure their participation. The activity was explained giving detailed instructions such as wearing light and loose clothing and maintaining a 12-14 hours of fast for the blood collection. Mutually suitable dates were then decided for the survey, usually in the mornings on weekends which would be a relatively free time for the working professionals and so sufficient time would be obtained for collection of fasting blood, anthropometric measurements and other required data. Members voluntarily registered for the study and a written consent (Annexure VI) was taken prior to data collection. The assessment included physical measurements, fasting blood examination and filling a questionnaire. The questionnaire was designed to obtain information on the socio-economic status, dietary habits and physical activity patterns of the subjects. Confidentiality was maintained while distributing the reports of the obesity assessment and blood examination of the participants.

Active search for disease among apparently healthy individuals is a fundamental aspect of prevention. This is embodied in ‘Screening’, which has been defined as ‘the search for unrecognized disease of defect by means of rapidly applied tests, examinations or other procedures in apparently healthy individuals’. A screening test is not intended to be a diagnostic test. It is only an initial examination (Park and Park, 1991). Our study was also planned with the purpose of screening apparently healthy individuals in the urban affluent community for the presence of obesity, abdominal obesity and NCD risk. There were several instances during the course of the study when a subject was identified with a previously undiagnosed disease and in such a situation, it was advised that the subject goes for a medical check up with his family physician.
Since our interest was to observe the adult population, the age group was restricted between 30-60 years. Although data on both men and women were collected through the surveys as a part of the ongoing departmental project, the sample selection for the present study was restricted to males. Intrinsically, abdominal adiposity reflects the male pattern of fat deposition (Vague, 1956). Women have higher percentage of body fat to facilitate for reproduction and related activities, but are prone to deposit this fat in the gluteo-femoral area. Accordingly, the risk for various NCDs varies among the sexes Apart from the differences in diet and physical activity, a woman’s body is affected by several physiological states such as pregnancy, lactation and menopause causing hormonal changes which could alter her metabolism (Lovejoy, 2003). It would have been difficult to control all known confounding factors among women. Hence the present study included only the men in the study population. From the ongoing study, we selected sample of 302 men, which was appropriate sample size assuming 30% prevalence of abdominal obesity (from literature) with 5% tolerance. Although, ideally it would have been appropriate to have 350 sample size but could get 302 men who satisfied the criteria of age and had a record of all anthropometric and blood measurements.

3.2 Survey tools:

Various survey tools employed for collecting data on socio-economic variables, anthropometric measurements, dietary and physical activity patterns and NCD risk factors are described below.

3.2.1 Socio-economic information: Obesity and related NCDs are being termed as problems of affluence. A prominent study by Nutrition Foundation of India has confirmed the accelerated influence of socio-economic status on overall and abdominal obesity and hypertension in urban Delhi (Gopalan, 1998). Hence, determining the socio-economic profile of the subjects was important to study its association with obesity and NCDs.

A structured questionnaire (Annexure I) was developed for gaining the socio-economic information comprising of variables such as age, sex, marital status, family type, education and occupation. Expecting subjects to be reluctant in revealing their absolute income, information was obtained in terms of annual income categories and ownership of assets such as residential properties (apartment or bungalow),
commercial premises (office or shop), investment properties (plot or land or farmhouse), vehicles and so on. Studies among Indians and other populations have shown that family history of diseases increases the risk in off springs (Kim et al., 2004b; Viswanathan et al., 1996). Hence, questions regarding parental history of obesity and NCD’s and self medical history were included.

3.2.2 Dietary assessment: Dietary habits of individuals vary according to socio-economic, seasonal, cultural and religious factors. Precise information on food consumption patterns of subjects through application of appropriate methodology is often needed not only for assessing the nutritional status but also for elucidating the relationship of nutrient intakes with deficiency as well as degenerative diseases. Dietary enquiries are mainly of two types, qualitative and quantitative (Bamji et al., 2003). Accordingly, dietary information was obtained by developing a food frequency questionnaire (Annexure III) and a 24 hour dietary recall questionnaire (Annexure IV). Both questionnaires were field tested before using for data collection.

a) Food frequency questionnaire (FFQ): The FFQ is one of the most economical tools used to collect comprehensive dietary data in large epidemiological studies. This method is useful for obtaining qualitative details of diet and studying the pattern of food consumption at a household level. The procedure includes assessment of the frequency of consumption of different foods – daily, weekly, fortnightly, monthly or occasionally (Bamji et al., 2003). It has been observed that a recall period of one year is unsuitable since longer the duration, more is the recall error while one week is too short a duration to get an overview of the dietary pattern. Hence, the recall period was restricted to one month in the present study since it would include the routine diet pattern along with occasional prominent events such as a festival or a function.

The FFQ had a checklist of several discriminatory food groups, namely non-vegetarian foods, milk and milk products, bakery products, sweet snacks, fried snacks, outside foods, party foods, festival foods, dry fruits etc. The prime purpose was to study the consumption of foods that are energy dense, high in fat and sugar. Lifestyle factors such as alcohol, smoking and tobacco chewing are potent determinants of ill-health, hence questions enquiring their usage were included.
b) 24 hour dietary recall: 24 hour dietary recall method is useful in obtaining quantitative details of the diet. Subjects were asked to recall a full day’s diet in a chronological order starting from breakfast to dinner. The interviewer had to skillfully obtain information on the food items consumed along with details of the ingredients used and method of cooking. Quantity of food was reported in terms of standard serving units such as one spoon, cup, plate, glass and so on. Subject were asked to recall small snacks in between meals such as a small piece of chocolate or couple of chips which could easily have been forgotten. Usually, the previous day of blood examination was considered for the 24 hour dietary recall, unless the subject was on a religious fast on the previous day or had feasted for some reason. In such a case, the day prior to the fast / feast was considered. This quantified dietary intake recall was used for estimating the average calorie and macronutrient content of a day’s diet of the subject. The database of nutritive values of commonly consumed foods published by the Biometry and Nutrition group (1999) of Agharkar Research Institute and by National Institute of Nutrition, India (Gopalan et al., 2007) were used to estimate nutrient intakes.

Due to certain inconveniences such as incompletely filled questionnaires or the inability of subjects to recall information, complete data on FFQ was available on 286 subjects, while data on 24 hour dietary recall was available for 245 subjects out of the total 302 subjects.

3.2.3 Assessment of habitual physical activity pattern: In simplest terms, obesity results from an imbalance of calorie intake and calorie output. As diet is an important determinant in the health of a person, so is physical activity. It is well documented that regularly performed physical activity reduces the risk of obesity related co-morbid conditions (WHO, 2000a).

A structured questionnaire (Annexure V) was therefore developed to gain information regarding the habitual activity pattern. It included major activity groups such as domestic, office, traveling, indoor and outdoor sports, special interests, recreational activities as well as sleep and nap patterns. These were further divided into several types of activities, e.g., the recreational activity group included TV watching, reading books and newspapers, musical instruments etc. Special interest group included activities like yoga, meditation, gardening, swimming, gym, walking, jogging etc. however, during analysis yoga and meditation were considered under
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indoor sports, while rest of the activities in this group were considered in outdoor sports. The questionnaire was field tested before using for data collection. Only one subject had not filled the entire activity questionnaire and hence data on 301 subjects was available for the analysis on physical activity. While performing combined analysis on both diet and activity, only subjects having information on all related aspects were considered.

3.2.4 Anthropometric Assessment: Nutritional anthropometry is the measurement of human body at various ages and levels of nutritional status. It is based on the concept that an appropriate measurement should reflect any morphological variation occurring due to a significant functional physiological change. The selected measurements should be simple and quick to measure, easy to reproduce and provide maximum information concerning a number of nutritional problems. Anthropometric measurements are essential to recognize both under and over nutrition, total body fat content as well as body fat distribution (Bamji et al., 2003). Techniques for various anthropometric measurements used in the present study were based on guidelines given by World Health Organization (WHO, 2008; Jelliffe, 1966)

Since the surveys were to be arranged as obesity assessment camps, a large number of subjects would have to be assessed in a short duration of time. Besides the anthropometric measurements (Annexure II), filling up of the questionnaire also had to be completed within the stipulated time. Hence, a team of at least 6-7 members was required for conducting the survey. Prior to the anthropometry rounds, training sessions were conducted for all members of the department. Each member received on-field training before his/her measurements were considered valid. Although most of the team members were well trained, an inter-observer study was undertaken to reduce investigator bias. Between investigators variation was negligible for the measurements taken with the help of digital equipments, such as weight and body fat. The variation was maximum in case of skinfold measurements (CV% for triceps: 5.5, biceps: 3.5, sub-scapular: 2.2, supra-iliac: 2.1), however it was within the permissible limits of ± 2 S.D. Investigator with the lowest standard deviation in case of skinfolds was kept constant for a particular measurement during the entire study thus reducing personal error.
a) **Weight:** Body weight is the most widely used, simplest and reproducible anthropometric measurement for the evaluation of nutritional status. It indicates body mass and is a composite of all body constituents like water, minerals, fat, protein, bone etc. Its potential is perceived not only by the health personnel, but also by the community (Bamji et al., 2003). Along with height, it is used to calculate BMI which is one of the most important indicators for assessing obesity. Body weight (kg) was recorded using electronic weighing balance (Suysan, India) which has a least count of 20g. It was ensured that the weighing scale was placed on a firm and level ground and that the zero display appeared before the subject stood on the scale. The subject was asked to remove shoes and any excessive clothing or accessories and the weight was recorded.

b) **Height:** The height of an individual is influenced both by genetic and environmental factors. Maximum growth potential of an individual is decided by hereditary factors, while the environmental factors, most important being nutrition and morbidity, determine the extent of exploitation of that genetic potential (Bamji et al., 2003). Height (cm) was recorded with a stadiometer (UNO & Co, India) which has a least count of 0.1cm. It was ensured that the stadiometer was placed on a firm and level ground. The subject was asked to step on the wooden platform bare-foot, having his feet parallel to each other. The subject’s head was kept erect and the back of the head was made to touch the upright steel rod. The horizontal metal bar was lowered gently to make contact with the top of the head. It was seen that the metal bar touches the highest point of the top of the head and the reading was noted at the top level in the window of the horizontal metal bar.

c) **Sitting height:** Sitting height is the length of the trunk and was also measured with a stadiometer (UNO & Co, India) which has a least count of 0.1cm. The equipment was kept on a sturdy table for recording the sitting height. The height of the table and the foot-rest were adjusted in order to ensure that there is a right angle formed at the knee joint. The subject was asked to sit straight on the wooden platform of the stadiometer, ensuring maximum stretch of the spine. It was ensured that the tendons of the biceps femurs of the subject do not touch the edge of the wooden platform, while the hands should be comfortably resting on his lap. Gentle upward pressure was applied by running the index finger up the spine causing the subject to
sit up straight as a reflex action. The horizontal metal bar was lowered gently to make contact with the top of the head. It was seen that the metal bar touches the highest point of the top of the head and the reading was noted at the top level in the window of the horizontal metal bar.

d) **Body Fat (%)**: Excess body fat is the hallmark of obesity. Measurement of weight or BMI alone may prove to be a false representation of health, for instance, athletes or body builders may be overweight but they have very less percentage of body fat (BF) and a high percentage of lean mass. Hence, the determination of BF (%) is of prime importance. However, highly accurate methods of BF (%) estimation such as Dual Energy X-ray Absorptiometry (DEXA), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Dual Photon Absorptiometry (DPA) and Ultrasound (US) are highly complex and expensive. For large-scale epidemiological studies, instruments based on the principle of Bio-Impedance Analysis (BIA) provide simple, safe, affordable and practical methods of evaluating three body compartments viz. fat, fat-free body mass and water (Rao, 2011).

In BIA, surface electrodes are placed on the subject’s extremities. An electrical current is passed through the electrodes, and a drop in the current is detected. The decrement in current or total body resistance is related to the complex interaction amongst a number of factors, including distance between the electrodes, volume and composition of the conducting tissues, anatomic factors, and frequency of electrical current discharged at the distal electrodes. In general, adipose tissue is a poor conductor and lean tissues, particularly the fluid compartments are good conductors. The result is that good inverse correlations are observed between resistance and total body water and fat free body mass and high positive correlations are found between resistance and total BF. Although there are small measurement errors between instruments, most of the higher quality BIA systems give similar and highly reproducible estimates of resistance (Heshka et al., 1994).

The Body Fat Analyzer manufactured by OMRON (HBF300, OMRON Corporation, Japan) based on BIA, was used for assessing BF (%). Omron has used research information from several hundred people using the underwater weighing method to develop a formula that includes five factors viz. age, weight, height, gender and electric resistance. The subject was asked to remove shoes and stand erect with feet separated at a distance of about 1 foot. Age, gender, weight and height of the
subject were entered in the machine. Subject was instructed to hold the instrument in a manner by which the middle finger of both the hands fitted in the grooves of the instrument and all other fingers folded inside forming a fist. Both the hands were stretched straight holding the equipment at heart level. After the position was appropriate, the reading was taken and the BF (%), fat mass (kg) and the grade of obesity (based on BMI) was recorded. This measurement is unsuitable for persons with body fracture, metal rods fitted in the body and also for persons below 10 years and beyond 80 years of age (Omron body fat analyzer instruction manual).

e) **Circumferences at various sites:** There is much scientific evidence to warrant that the regional distribution of BF is an important consideration in the relationship between obesity, metabolism and health (Despres et al., 1990) and hence, measurement of BF distribution is crucial in assessment of obesity related risks. One of the most important field methods of assessing BF distribution is by measuring circumferences at different sites of the body. In the present study, circumference measurements were taken at three sites using a non-stretchable fiber glass measuring tape. Results were recorded in duplicate to the nearest 0.1 cm.

**Waist circumference (WC):** Most adipose tissue (~85% of total adipose tissue mass) is located under the skin (subcutaneous fat), and a smaller amount (~15%) is located within the abdomen (intra-abdominal fat). MRI and CT are considered the gold-standard methods for determining the quantity of subcutaneous abdominal adipose tissue (SAAT) and intra abdominal adipose tissue (IAAT). However these methods are highly expensive and not affordable in large scale epidemiological studies. After adjustment for the degree of total body fatness and age, WC has been found to be a strong correlate of visceral fat (Klien et al., 2007).

WC is actually a perimeter, which provides an estimate of body girth at the level of the abdomen. Different anatomic landmarks have been used to determine the exact location for measuring WC in different clinical studies viz. 1) midpoint between the lowest rib and the iliac crest; 2) the umbilicus; 3) narrowest (minimum) or widest (maximum) WC; 4) just below the lowest rib; and 5) just above the iliac crest. The specific site used to measure WC influences the absolute WC value that is obtained. However, none of the reported studies have demonstrated an advantage of one measurement over the others (Klien et al., 2007; Ross et al., 2008).
Hence in the present study, it was decided that measurement at two sites in the abdominal region would be taken, one at the midpoint between the lowest rib and the iliac crest which was termed as WC and the other at the umbilicus which was termed as abdominal circumference. Measurements were made around the subject’s bare midriff, after the subject exhaled while standing without shoes and with both feet touching and arms hanging freely. The measuring tape was placed perpendicular to the long axis of the body and horizontal to the floor and applied with sufficient tension to conform to the measurement surface. The measurement was made ensuring that the soft tissue is not compressed during the measurement.

Abdominal circumference (AC): As mentioned above, abdominal circumference was measured at the umbilicus maintaining conditions similar to those while measuring WC.

Hip circumference (HC): Hip circumference reflects the gluteo-femoral fat deposition which is characteristic of female pattern of fat deposition. There have been contradictory reports about the protective effect of large hip circumference towards cardiovascular disease (CVD) risk (Snijder et al., 2004; Wang and Hoy, 2004). Nevertheless HC is required for calculation of Waist to Hip Ratio (WHR), which is an important marker of abdominal obesity. HC was measured around the widest part of the trochanter (buttocks) with subject standing with his feet together and arms hanging loosely by the side and palms facing inwards towards the thighs.

f) Skinfolds at various sites: Subcutaneous fat constitutes the body’s main store of energy reserves. Fat distribution in and around the body varies with age, sex, physiological status, nutritional status and ethnicity. Amount of subcutaneous fat on the trunk is believed to correlate more strongly with the risk of CVD and diabetes than the fat on the extremities (Chandalia et al., 1999). As there is a lack of simple and accurate methods of assessing BF directly, anthropometric indices such as skinfolds can be used as surrogate measures of body composition. Skinfold thickness may be taken on different trunk sites (sub-scapular, supra-iliac, abdominal etc.) or extremities (triceps, biceps, thigh, mid-calf) (Rao, 2011). Skinfold thickness is measured using a Caliper. The calipers used should have a standard contact surface (pinch area) of 20–40 mm and an accuracy of 0.1 mm. It should exert a constant
pressure of $10 \text{ g/cm}^2$ throughout the whole range of the skinfold thickness at all distances of separation of the jaws of the caliper. Some of the standard calipers are Harpenden, Lange and Best. Una calipers available in India are also found to be reliable (Bamji et al., 2003).

In the present study, Harpenden’s calipers (accuracy=0.2 mm) were used. All the skinfolds were measured on the left side of the subject at a predefined point which was marked with a pen. The skinfold should not be picked up as a ‘pinch’ but as a ‘sweep’ between the thumb and the index finger or the middle finger of the measurer over the surface of the skin from about 5–6 cm apart. The measurer initially massaged up a ‘tube’ of skinfold so as to gather all the subcutaneous fat in the fold away from the underlying muscle. While holding this fold with the left hand, jaws of the caliper were applied below the grip at the point marked for the measurement with one hand maintaining the grip on the skinfold throughout the measurement of the skinfold. Once the caliper blades were released on the skinfold tube, the measurement on the dial was taken after the pointer got stabilized.

**Triceps skinfold (TSF):** The subject was instructed to stand with his back to the observer with his arms relaxed. The tips of the acromian and the olecranon process were palpated, its length was measured and mid-point was marked. The subject was asked to bend and straighten the arm several times before taking the skinfold so as to remove underlying muscles. A vertical skinfold was picked up over the posterior surface of the triceps muscle, 1cm above the mark and the caliper measurement was recorded as per instructions mentioned above.

**Biceps skinfold (BSF):** The subject was instructed to stand facing the observer with his arms relaxed. The arm was relaxed with the palm facing forward and a vertical mark on the horizontal line was drawn from the marked mid-point. A vertical skinfold was picked up over the belly of the biceps 1cm above the line marked and the caliper measurement was recorded as per instructions mentioned above. The subject was asked to bend and straighten the arm several times before taking the skinfold so as to remove underlying muscles.

**Subscapular skinfold (SSF):** The subject was instructed to stand erect with back to the measurer and arms relaxed. The inferior angle of the scapula was determined and
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Recording of Anthropometric Measurements during the Survey

Weight

Height

Waist circumference

Abdominal circumference
Recording of Anthropometric Measurements during the Survey

Measurement of Body Fat (%)

Marking site for triceps & biceps skinfolds

Sub-scapular skinfold

Supra-iliac skinfold
the measurement was taken below that point. In obese persons, it is difficult to
determine the angle of the scapula. To locate this point, the medial border of the left
scapula was palpated and fingers were run downward along its full length until the
inferior angle was located. A diagonal skinfold was picked up immediately below the
inferior angle of the scapula with slight downward inclination and laterally along the
natural tension lines of the skin. The caliper jaw was applied at the skinfold and the
measurement on the dial was recorded (in mm) after pointer got stabilized.

**Supra-iliac skinfold (SUF):** The subject was instructed to stand erect sideways to the
measurer with arms folded. The upper border of the iliac crest was palpated and a
diagonal skinfold was taken just above the iliac crest at the mid-axillary line on the
left side. The skinfold should be slightly inclined downwards and medially along the
natural tension line of the skin for ease of pick-up. The caliper jaw was applied at the
skinfold and the measurement on the dial was recorded (in mm) after pointer got
stabilized.

### 3.3 Assessment of obesity:

Obesity is defined as a condition of excessive fat accumulation in the adipose
tissue to the extent that health may be impaired (WHO, 2000a). Assessment of overall
and abdominal obesity was performed according to the criteria described below.

#### 3.3.1 Overall obesity:

Operational definitions of overall obesity and overweight are
based on body size indicated by BMI which is closely correlated with body fatness.
The WHO consultation on obesity (WHO, 2000a) has proposed a system of
classification using BMI based on studies among Caucasians. According to this
classification, a BMI ≥ 25 kg/m² is considered as the cutoff for defining overweight
and BMI ≥ 30 kg/m² is the cutoff for defining obesity. However, it was later realized
that these standards may not be appropriate for early identification of NCD related
morbidities in certain ethnic groups, especially in the Asia-Pacific region, as among
these groups, NCDs are found to occur at lower levels of obesity (WHO, 2000b).
Based on few studies performed in the Asian subcontinent, WHO expert panel (WHO,
2000b) recommended lower ranges of BMI for the Asia-Pacific region depending on
risk factors and morbidities. According to this revised classification, a BMI ≥ 23
kg/m² may be considered as the cutoff for defining overweight and BMI ≥ 25 kg/m²
may be the cutoff for defining obesity among Asians. In the present study, we have followed the BMI classification which has been recommended for Asian population. However, these provisional recommendations may be revised by WHO in the light of further validation of studies and clinical experience.

3.3.2 Adiposity based on BF (%): A person's BF (%) is the total fat divided by the weight and consists of both the essential and storage body fat. Essential body fat is necessary for vital functions such as membrane integrity and for reproductive functions in females. Storage body fat consists of fat accumulation in adipose tissue, part of which protects internal organs in the chest and abdomen. Essential fat in men seems to be 3–8% of their body weight. When appropriate amounts of storage fat are added to the essential fat for men, it is recommended that the range of total body fat should be 15-20% of body weight. In women, the concept of essential fat must be expanded to include gender-specific fat in their breasts, pelvic region and buttocks that is apparently an evolutionary feature that provides energy during childbearing and lactation. Thus for women, the minimum levels of fatness compatible with health are based on the essential fat plus the gender-specific fat and is in the range of 12-14% of body weight with healthy levels of total fat ranging from 25-30% (WHO, 1995; Lohman et al., 1997).

Few studies have specified the ranges for BF (%) when measured by the BIA method. A particular study among urban Indian men focusing on the use of this method to determine the BF (%) have used the following categories: <10%: under body fat; 10-20%: normal body fat; 20-25%: over fat; >25%: higher body fat. These values also corresponded to the height weight tables of average values for Indian men as advised by Life Insurance Corporation of India (Singh et al., 1999). In the present study, male subjects were classified as obese if their BF (%) was above 25% (WHO, 1995; Lohman et al., 1997).

3.3.3 Abdominal obesity: The criteria used for defining the risk of NCD’s using WC by WHO (2000a) is similar to that used for BMI and have based on studies conducted among Dutch men and women. Accordingly, WC ≥ 102 cm in men and ≥ 88 cm in women was found to be associated with a substantially increased risk of metabolic complications and these cutoffs are currently used for defining abdominal obesity among Caucasian populations. However, higher risk for NCDs is seen at lower levels
of WC among Asian populations. South Asians (Indians) in particular, have high levels of abdominal adiposity and may not be considered obese by conventional BMI criteria. The WHO Asia Pacific Perspective on Obesity (WHO, 2000b) has hence suggested revised WC cutoffs of 90 cm for men and 80 cm for women to be used as interim lower values for Asians. These cutoffs are also in confirmation to suggestions by the International Diabetes Federation (IDF, 2006). In the present study we have used $WC \geq 90$ cm as the cutoff for defining abdominal obesity among men, as proposed by WHO (2000b).

The ratio of waist to hip circumference (WHR) is also used as a measure of abdominal obesity. In Caucasians, a WHR $\geq 1.0$ for men, and WHR $\geq 0.85$ for women is used to identify those with abdominal fat accumulation (WHO, 2000a). However, a recent WHO report specific to assessment of WC and WHR (WHO, 2011b) has suggested a lower cutoff of WHR (0.90) for men, while that for women continues to be the same among Asians. In the present study, WHR cutoff of $\geq 0.9$ for men has been used. However, WHO reports indicate that both WC and WHR cutoffs for defining abdominal obesity may require revision when new prospective data from the Asia-Pacific region is available.

3.4 Clinical Assessment - Blood pressure measurement:

Hypertension is one of the major NCD risk factors and its relationship with obesity is well documented. Obesity increases the risk of elevated blood pressure (BP) by multi-folds and at the same time, even a small weight loss shows immediate reduction in BP (WHO, 2000a). Hence BP measurement is one the most important criteria’s in the evaluation of NCD risk. Ideally, BP measurements are taken by medical personnel using a sphygmomanometer, but in field settings it is not always possible to have medical personnel accompanying the team. Hence, BP measurements were taken using a digital machine called the OMRON Blood Pressure monitor (OMRON T4, OMRON Corporation, Japan). The machine was calibrated on 50 subjects at a medical doctor’s clinic by measuring the BP by both Omron blood pressure monitor and a Sphygmomanometer.

The subject was asked to rest for 10 minutes prior to taking the BP measurement. Measurement was taken in sitting position with the left arm resting on the hand of the chair. An adult sized cuff was wrapped around the left arm approximately half inch above the inner side of the elbow point with a two finger
space for inflation of the cuff. The air tube of cuff was positioned in middle of the arm in line with the middle finger. The center of the arm cuff had to be at the same level as the heart of the subject as per instructions in the Omron BP monitor manual. Subject was advised to remain still and not talk during the measurement. The reading obtained was that of systolic BP (mmHg), diastolic BP (mmHg) and pulse (per min). Two readings were recorded and second reading was used in the analysis. If high BP (>140/90 mmHg) was noted, a third reading was taken after 30 minutes of rest. Measurements were recorded by trained researchers.

3.5 Estimation of bio-chemical parameters:

In the development of any deficiency disease, biochemical changes can be expected to occur prior to clinical manifestations. As clinical signs and symptoms are often non-specific, biochemical tests which can be conducted on easily accessible body fluids such as blood and urine can help to diagnose a disease (Bamji et al., 2003). Hence in the present study, blood biochemical estimations were carried out for the diagnosis of NCD risk factors such as diabetes mellitus, insulin resistance and dyslipidemia.

Estimation of fasting blood glucose (FBG) and insulin for diagnosis of diabetes and insulin resistance was performed. Serum lipids including triglycerides (TG), total cholesterol (TC) and its sub-classes viz. low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) were estimated for diagnosis of dyslipidemia. These tests were performed in an authorized pathological laboratory so as to obtain a blood report duly checked and signed by qualified medical personnel that could be given to the subjects participating in the obesity assessment survey. Subjects could take this blood report to their family physician for further consultation when required.

3.5.1 Blood collection, separation and storage: Blood specimens (10ml) of the subjects were drawn by venipuncture after ensuring a 12 hour overnight fast and consumption of normal diet on the previous day. The collected blood was distributed in four separate bulbs. For glucose estimation, 1ml of blood was put in the bulb containing the additive fluoride for preparation of fluoride plasma. For lipid estimation, 2ml of blood was put in the bulb containing no additives for preparation of serum. Remaining 7ml of blood was equally distributed in two bulbs containing the
Clinical Measurement and Blood Sample Collection

Measurement of Blood Pressure

Blood sample collection

Various activities during survey
additive ethylene diamine tetra acetic acid (EDTA) for maximum extraction of plasma that was used in the estimation of insulin and for storage in case of any further analysis. Serum was separated by centrifugation at 2000 RPM (rotations per minute) for 5 minutes, while plasma was separated by centrifugation at 2000 RPM for 10 minutes (Remi centrifuge R4C). Samples were immediately analyzed for glucose, lipid profile and insulin. About 2ml EDTA containing plasma of the subjects was stored at -20°C until further processing for any future estimations.

3.5.2 Blood parameters estimations: Methods used for the estimation of various biochemical parameters are given below.

a) Fasting blood glucose (FBG): Blood glucose level or blood sugar concentration refers to the amount of glucose present in blood and is tightly regulated in the human body. The normal blood glucose level is about 90 mg/dl. The levels rise after meals for an hour or two by a few grams and are usually lowest in the morning before the first meal of the day. Transported via the bloodstream from the intestines or liver to body cells, glucose is the primary source of energy for the body’s cells. Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycemia) or low (hypoglycemia) blood sugar. Diabetes mellitus, characterized by persistent hyperglycemia, is the most prominent disease related to failure of blood sugar regulation (Henry, 2001).

FBG was estimated using commercial kits from Liquizyme on a semi-automated biochemistry analyzer (Auto analyzer Star 21, Calenzano, Firenze, Italy). Glucose estimation was based on the GOD/POD (Glucose oxidase / Peroxidase) method. Glucose oxidase oxidizes the specific substrate β-D glucose to give gluconic acid and hydrogen peroxide. The hydrogen peroxide thus produced reacts with enzyme peroxidase present in the system to liberate oxygen. This liberated oxygen reacts with the chromogen system consisting of phenolic compounds and 4 amino antipyrine to produce quinonimine, a pink colored complex. The intensity of the color is directly proportional to the amount of glucose present in the sample and is measured spectrophotometrically at 505 nm (Henry, 2001).
b) **Total cholesterol (TC):** Cholesterol is a lipid, present in cell membranes and a precursor for steroid hormones and bile acids. It is found in blood in distinct particles called lipoproteins, containing both lipids and proteins. Lipoproteins found in humans are divided into classes according to their densities. Three major classes are found: low density lipoproteins (LDL), high density lipoproteins (HDL) and very low density lipoproteins (VLDL) (NCEP-ATP3, 2001).

TC from the serum was estimated enzymatically using commercial kits from AutoZyme\textsuperscript{NEW} on semi automated biochemistry analyzer (Auto analyzer Star 21, Calenzano, Firenze, Italy). Cholesterol estimation was based on the enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase. Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce a red quinonimine dye which has maximum absorbance at 510 nm. The intensity of the red colour is proportional to the amount of TC in the specimen (Henry, 2001).

c) **Triglycerides (TG):** TGs are transported in the blood as chylomicrons following absorption from the small intestine or as a component of VLDL if synthesized by the liver. These are a major energy reserves and are found in adipose tissue which serves as a depot or storage site for lipids. Fasting TG levels are positively associated with body weight and are considered a risk factor for atherosclerosis (NCEP-ATP3, 2001).

Fasting serum TG was estimated enzymatically using commercial kits from AutoZyme\textsuperscript{NEW} on a semi automated biochemistry analyzer (Auto analyzer Star 21, Calenzano, Firenze, Italy). TG estimation was based on the enzymatic method using lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase & peroxidase. Glycerol released from the hydrolysis of TG by lipoprotein lipase is converted by glycerol kinase into glycerol-3-phosphate which is oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red colored compound which is measured spectrophotometrically (Henry, 2001).

d) **High density lipoprotein (HDL):** HDL constitutes about 20-30% of TC and contains cholesterol and apolipoprotein AI and AII. HDL particles are able to remove
cholesterol from the arteries and transport it back to the liver for excretion or re-utilization, which is the main reason why the cholesterol carried within HDL particles is sometimes called "good cholesterol". HDL is thus thought to protect against the development of atherosclerosis (NCEP-ATP3, 2001).

Fasting serum HDL was estimated enzymatically using commercial kits from AutoZymeNEW on a semi automated biochemistry analyzer (Auto analyzer Star 21, Calenzano, Firenze, Italy). HDL estimation was based on the enzymatic method using AutoZyme HDL cholesterol precipitating reagent containing phosphotungstate/Mg$^{2+}$ combined with single step enzymatic AutoZyme cholesterol reagent. The phosphotungstate/Mg$^{2+}$ complex precipitates chylomicrons, LDL and VLDL fractions. HDL fraction remains unaffected in the supernatant. Cholesterol content of HDL fraction is assayed using AutoZyme Cholesterol (Henry, 2001).

e) Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL): LDL cholesterol constitutes about 60 to 70% of TC and contains cholesterol and apolipoprotein B. LDL particles primarily transport cholesterol into the artery wall initiating the process of formation of plaques and hence increased levels are associated with atherosclerosis. It is hence termed as the “bad cholesterol” and is the primary target for cholesterol lowering therapy. VLDL is TG-rich lipoprotein and constitutes about 10-15% of TC. Some forms of VLDL are actually partially degraded lipoproteins called VLDL remnants and these promote atherosclerosis similar to LDL (NCEP-ATP3, 2001). LDL & VLDL were calculated using the Friedwald’s equation from the TC TG & HDL values estimated enzymatically from fasting serum samples. The Friedwald’s formula was described originally by Friedwald, Fredrickson and Levy in 1972. Because most of the plasma TG are carried in VLDL, VLDL concentration is estimated as VLDL = TG / 5 (where the concentrations are expressed in mg/ dl). LDL is determined as LDL = [TC – HDL – (TG / 5)] (Henry, 2001).

f) Insulin: Insulin is a peptide hormone secreted by the β cells in islets of langerhans in the pancreas and is responsible for the metabolism and storage of carbohydrates. Insulin levels should be measured with a concomitant glucose level, since insulin secretion is regulated primarily by glucose. Disease states such as diabetes mellitus occur when insulin concentrations are inappropriate for given blood glucose levels due to insufficient secretion or resistance (Henry, 2001).
Fasting plasma Insulin was estimated by Immunoenzymetric assay using commercial kits, from INS-EASIA, Biosource. The Biosource INS-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on micro titer plates. The plates were then read on ELISA (Enzyme Linked Immuno Sorbent Assay) reader (Alpha Prime, fully automated ELISA processor, SFRI, France). Estimation of plasma insulin was introduced few months after the study started and therefore insulin values were not available for some of the subjects initially enrolled. Finally, 262 subjects out of the total 302 subjects had data on fasting plasma insulin.

### 3.6 Assessing NCD risks:
Various researchers seem to use different cutoffs for defining NCD risks or the prevalence of NCDs, for example, the two most commonly used guidelines for hypertension are by JNC VII (2003) and NCEP-ATP3 (2001). Similar is the case with other risks such as blood glucose or lipid levels. In fact some researchers (Snehalatha et al., 2009; Park et al., 2010) have also thought it appropriate to use the highest tertile or quartile values to evaluate the associations of NCD risks with adiposity. The cutoffs used by us in the study are given below.

#### 3.6.1 Hypertension:
Hypertension was defined according to the report of Seventh Joint National Committee (JNC VII) as BP >=140/90 mmHg or the intake of anti-hypertensive medication (Chobanian et al., 2003). As per the JNC VII report, treating Systolic BP and Diastolic BP to targets that are less than 140/90 mmHg is associated with a decrease in CVD complications. Information on parental history of hypertension had also been collected in the surveys.

#### 3.6.2 Diabetes and high fasting blood glucose:
After consideration of available data and recent recommendations made by other organizations, a joint WHO and International Diabetes Federation (IDF) Technical Advisory Group published the report, ‘Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications’ (WHO/IDF, 2006). In the present study, the condition of Diabetes Mellitus was defined as self-reported history of diabetes or FBG > 126 mg/dl. Impaired fasting glucose (IFG) is considered as a risk factor for further development of diabetes and CVD and hence its cutoff was considered appropriate for screening of NCD risks. Thus, the standard cutoff for IFG i.e. FBG > 110 mg/dl was used for defining high FBG and determining its prevalence in the preliminary analysis.
However, in the further analysis, since the prevalence of high insulin and high HOMA-IR was based on the respective quartile values, to maintain a uniformity, high FBG was also defined using the quartile value of 96 mg/dl.

### 3.6.3 High insulin and insulin resistance:

Insulin resistance (IR) has been routinely measured by two surrogate measures, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). Risk cutoffs for fasting plasma insulin levels have not been advised by expert committees such as WHO. However, according to available literature on diabetes, in normal individuals, fasting plasma glucose concentration is usually 70-110 mg/dl with plasma insulin concentrations usually ranging between 5-15 uU/ml. In obese individuals, basal plasma insulin is usually increased due to insulin resistance and values as high as 40-50 have been reported. In case of fasting insulin levels, physicians recommend that lower the levels, lesser the risk (Porte et al., 2003). Recent reports also suggest that although most physicians use fasting insulin levels of over 15 uU/ml to diagnose type 2 diabetes or insulin resistance, any fasting insulin level over 10 uU/ml could be a serious risk factor for diabetes. In absence of any definite cutoffs, in the present study the quartile value of insulin of 13.1 mIU/ml was used for defining high insulin.

The HOMA-IR has been developed for application in large epidemiological investigations, an alternative to the glucose clamp and is the most common surrogate measure of insulin resistance in vivo. Using HOMA-IR makes it possible to study a large number of subjects with a single glucose and insulin measurement in the fasting state (Esteghamati et al., 2010). The value of HOMA was calculated by the following equation: \[(\text{fasting insulin (µU/ml)} \times \text{fasting glucose (mmol/l)})/22.5\] and is depicted as HOMA-IR value (Matthews et al., 1985). In case of HOMA-IR, the quartile value of 2.85 was considered as a risk cutoff for defining high HOMA-IR.

### 3.6.4 Dyslipidemia:

The National Cholesterol Expert Panel – Adult Treatment Panel (NCEP-ATP) on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults established by the American Medical Association has been meeting regularly to update clinical guidelines for cholesterol testing and management. The most recent third report of the panel (NCEP-ATP3, 2001) is an evidence based and extensively referenced report that builds on the previous reports and expands the indications for
intensive cholesterol lowering therapy in practice. Accordingly to the guidelines in the report, High TC was defined as $TC \geq 200$ mg/dl; Low HDL was defined as $HDL < 40$ mg/dl; High TG was defined as $TG \geq 150$ mg/dl; High LDL was defined as $LDL \geq 130$ mg/dl; Dyslipidemia was defined as the presence of either high TC, low HDL, high TG or high LDL. Additionally, TG/HDL ratio, which is predictive of small dense LDL, a risk factor for CVD, was also considered and a cutoff value of 3 was used for defining high TG/HDL ratio (McLaughlin et al., 2003).

3.6.5 Metabolic syndrome: The clustering of CVD risk factors that includes abdominal obesity, hypertension, high FBG, high TG and low HDL is termed as the metabolic syndrome (MS). MS is known to strongly predict long-term risk of NCD related morbidity and mortality. Different definitions have been proposed for MS and hence estimates of MS vary substantially across populations depending on the criteria used. The different criteria include the ones laid down by the World Health Organization (WHO), European Group for the Study of Insulin Resistance (EGIR), NCEP-ATP3, American Association of Clinical Endocrinologists (AACE), and the International Diabetes Federation (IDF). Most of these definitions agree on the essential components, such as glucose intolerance, abdominal obesity, hypertension, and dyslipidemia, but differ in the cut-off points used for each component and the method of combining them to define MS (Mohan and Deepa, 2010).

The definition suggested by the NCEP-ATP3 uses parameters which are examined in routine clinical examination and was more appropriate for use in the present study. However, the defining levels for WC as given by the expert panel may not be applicable to Asian Indians and hence the revised WC cutoffs as suggested by WHO (2000b) were used. This modified NCEP-ATP3 definition has also been used by other investigators among Asians (Misra et al., 2005a; Ramachandran et al., 2003; Tan et al., 2004). The criteria for diagnosing MS according to NCEP-ATP3 (2001) is described below in Table 3.1.
Table 3.1 Clinical identification of MS - any 3 of the following
(NCEP-ATP3, 2001)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity</td>
<td>Waist circumference*</td>
</tr>
<tr>
<td></td>
<td>Men &gt; 102 cm</td>
</tr>
<tr>
<td></td>
<td>Women &gt; 88 cm</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥ 150 mg/dl</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men &lt; 40 mg/dl</td>
</tr>
<tr>
<td></td>
<td>Women &lt; 50 mg/dl</td>
</tr>
<tr>
<td>BP</td>
<td>≥ 130/85 mmHg</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>≥ 110 mg/dl</td>
</tr>
</tbody>
</table>

* Revised WC values (men: ≥ 90 cm; women: ≥ 80 cm) given by WHO (2000b) were used

3.7 Data management and statistical analysis:

Questionnaires were checked to ensure that all the required information was obtained from the subjects. Similarly, any obvious mistakes or absurd values for each anthropometric measurement were checked for. Data from the questionnaires was entered using the FOX-PRO database. Variables either numeric or string (character) were created for each question or measurement for entering the data in a systematic manner which could then be used for analysis. For numeric variables, data was entered as it was. Range checks were inbuilt to prevent entry of values such as those of blood parameters which fall outside the ranges specified. For string variables, the data was assigned codes for ease in data entry and analysis. In certain cases ranking was assigned, e.g., occupation was coded from 1 to 5 starting from housewife, laborer, salaried, business or professional and retired. This was to determine the socio economic status of each subject. The food frequency questionnaire was designed in a manner so as to collect the information in terms of daily, weekly or monthly basis. This was then recalculated in terms of monthly consumption of a food item. Data from the 24 hour dietary recall was entered and calculated in terms of the calorie, protein and fat intake. Information on the physical activity pattern was calculated in terms of frequency along with duration of performing each activity.
Continuous variables are reported as mean ± SD. Whenever distributions of variables were skewed, logarithmic transformations were performed. Adjustment for age was done using linear regression. Linear trends in mean values of anthropometric measurements by various categories were tested using one-way analysis of variance (ANOVA), while trends in percentages / prevalence were tested using Z test. All the differences, trends etc. were tested at 5% level of significance. Differences between mean values for groups such as obese and non obese were compared using t-test. Using logistic regression analysis, the odds ratios (OR) and 95% confidence intervals (CI) for the occurrence of NCD risk factors were computed. Multivariate logistic regression analyses (MLRA) was performed to assess NCD risk in the presence of all the confounders viz. obesity, diet and activity.

Optimal cutoff values of anthropometric measurements, viz. BMI and WC for identifying the risk of NCDs were determined using Receiver Operating Characteristic (ROC) analysis. The analysis gives the ‘sensitivity’ which measures the proportion of actual positives that are correctly identified, and the ‘specificity’ which measures the proportion of actual negatives that are correctly identified (WHO, 2011b; Rao, 2011). The true positive rate (sensitivity) is plotted against the false positive rate (1-specificity) across range of values from the diagnostic test. The decision threshold for the best trade off is the criterion value with the highest accuracy that maximizes the sum of the sensitivity and specificity (Altman, 1991). A significant area under the curve (AUC) along with sensitivity of more than 60% and specificity of more than 50% was considered appropriate. All the analysis was done using a statistical program SPSS/PC Version 11.0 for Windows (SPSS Inc. Chicago, IL). Results of the analysis are discussed in the subsequent chapters.

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